Pathways by Which Reconstituted High-Density Lipoprotein Mobilizes Free Cholesterol From Whole Body and From Macrophages

M. Cuchel; S. Lund-Katz; M. de la Llera-Moya; J.S. Millar; D. Chang; I. Fuki; G.H. Rothblat; M.C. Phillips; D.J. Rader

Objectives—Reconstituted high-density lipoprotein (rHDL) is of interest as a potential novel therapy for atherosclerosis because of its ability to promote free cholesterol (FC) mobilization after intravenous administration. We performed studies to identify the underlying molecular mechanisms by which rHDL promote FC mobilization from whole body in vivo and macrophages in vitro.

Methods and Results—Wild-type (WT), SR-BI knockout (KO), ABCA1 KO, and ABCG1 KO mice received either rHDL or phosphate-buffered saline intravenously. Blood was drawn before and at several time points after injection for apolipoprotein A-I, phosphatidylcholine, and FC measurement. In WT mice, serum FC peaked at 20 minutes and rapidly returned toward baseline levels by 24 hours. Unexpectedly, ABCA1 KO and ABCG1 KO mice did not differ from WT mice regarding the kinetics of FC mobilization. In contrast, in SR-BI KO mice the increase in FC level at 20 minutes was only 10% of that in control mice (P<0.01). Bone marrow-derived macrophages from WT, SR-BI O, ABCA1 KO, and ABCG1 KO mice were incubated in vitro with rHDL and cholesterol efflux was determined. Efflux from SR-BI KO and ABCA1 KO macrophages was not different from WT macrophages. In contrast, efflux from ABCG1 KO macrophages was ≈50% lower as compared with WT macrophages (P<0.001).

Conclusions—The bulk mobilization of FC observed in circulation after rHDL administration is primarily mediated by SR-BI. However, cholesterol mobilization from macrophages to rHDL is primarily mediated by ABCG1. (Arterioscler Thromb Vasc Biol. 2010;30:00-00.)

Key Words: ABCA1 ■ ABCG1 ■ apolipoprotein A-I ■ cholesterol efflux ■ reconstituted high-density lipoprotein ■ SR-BI

Administration of reconstituted high-density lipoprotein (rHDL) is associated with increase in plasma free cholesterol, suggesting that rHDL promotes cholesterol mobilization. We demonstrated that the bulk mobilization of free cholesterol observed in circulation after rHDL administration is primarily mediated by SR-BI. However, cholesterol mobilization from macrophages is primarily mediated by ABCG1.

Circulating HDL and its major protein apolipoprotein A-I (apoA-I) are generally considered to protect against atherosclerosis. The main mechanism by which HDL and apoA-I are thought to be protective is through the promotion of reverse cholesterol transport (RCT), the process by which excess cholesterol from the periphery is transported back to the liver to be further metabolized and excreted. Novel therapeutic approaches that promote RCT and hold the potential to be a major tool to reduce cardiovascular disease risks are currently being tested. One promising approach is the use of synthetic discoidal particles containing either human apoA-I or one of its variants, apoA-IMilano and phospholipids, and sometimes cholesterol,6 frequently referred as rHDL. The intravenous administration in humans of such particles has been associated with regression of coronary atherosclerosis3 and improvement in plaque characteristics,4,5 endothelial function,6 and antiinflammatory markers.7 It is also associated with a transitory increase in plasma free cholesterol (FC) in both mouse models4,9 and humans,10,11 as well as with increased cholesterol synthesis in peripheral tissues, consistent with cholesterol movement from tissues to plasma in mice,8 and increased fecal sterol excretion in humans,11,12 suggesting that rHDL is able to promote RCT. However, the molecular mechanisms by which rHDL promote cholesterol mobilization from tissues are not understood.
The first step of the RCT pathway is the efflux of FC from peripheral cells to extracellular acceptor particles. Four different mechanisms are known to contribute to this step: ABCA1-mediated efflux to apoA-I,13 SR-BI–mediated and ABCG1-mediated efflux to mature HDL,14,15 and aqueous diffusion to mature HDL.16 In vitro data suggest that rHDL can be an excellent cholesterol acceptor in the context of SR-BI–mediated and ABCG1-mediated efflux pathways, but not in the context of an ABCA1-mediated efflux pathway.17,18 The goal of this study was to investigate the molecular mechanisms underlying the FC mobilization observed after intravenous administration of rHDL and, specifically, to test the hypothesis that administration of rHDL in vivo promotes cholesterol mobilization via SR-BI–mediated and ABCG1-mediated, but not ABCA1-mediated, pathways. Because we were interested in investigating the mechanisms at both the systemic and macrophage levels, we conducted both in vivo studies in mice models and in vitro studies in primary macrophages.

Materials and Methods
A detailed description of animals, reagents, and methods used in the studies reported in this article are described in the supplemental materials Appendix. A brief description of the methods used is described here.

Preparation and Characterization of Discoidal Reconstituted Lipid/Protein
rHDL containing human apoA-I (~3 mg) and egg phosphatidylcholine (PC; ~7 mg) were prepared by the cholate dialysis method as previously described.19 The final rHDL particles had an average hydrodynamic diameter of ~10 nm and a protein-to-phospholipid ratio of ~1:2 (w/w). The concentration of apoA-I in rHDL is similar to that used in other animal8 and human11,12 studies.

Cholesterol Efflux Assays in Bone Marrow-Derived Macrophages
Bone marrow-derived macrophages were isolated from femurs and tibias of SR-BI knockout (KO), ABCA1 KO, and ABCG1 KO mice, and respective controls, and were cultured in DMEM supplemented with 10% fetal bovine serum and 30% L929 conditioned media as described before.20 Cellular cholesterol efflux assays were performed as previously described.20

Mice Study Protocol
Wild-type (WT) control mice were obtained from Jackson Labs. SR-BI–deficient, ABCA1-deficient, and ABCG1-deficient mice were bred in house. Mice were fed a standard chow diet ad libitum. rHDL were administered by intravenous bolus injection. Serum was obtained by retro-orbital bleeding while the mice were under isoflurane anesthesia and collected in heparinized tubes. All mice experiments were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Lipid and Lipoprotein Analysis
Total cholesterol, HDL cholesterol, FC, and phospholipid levels were measured using Wako Pure Chemical Industries reagents. The enzymatic method used to measure phospholipids selectively measures choline-containing phospholipids, ie, PC and sphingomyelin. In the context of this article, in which high doses of PC are administered into mice in the form of rHDL, we assume PC represents the majority of the phospholipids and report the results as such. Pooled plasma from each group was separated by fast protein liquid gel filtration, and total cholesterol and PC in each fraction were measured.

Kinetic Analysis
Transport and fractional catabolic rates (FCR) were calculated using the WinSAAM version 3.0.6.21 Human apoA-I, PC, and FC masses were calculated by multiplying their plasma concentrations for each mouse by the estimated plasma pool size (~3.5% of body weight).

Statistical Analysis
All values are shown as the mean±SD. A 2-tailed Student t test was used to test for statistical significance. P<0.05 was considered significant.

Results
rHDL Effectively Mobilizes Cholesterol in WT Mice In Vivo
We did not observe any changes in serum levels of either PC or FC after intravenous administration of phosphate-buffered saline in WT mice (Figure 1). In contrast, after the intravenous administration of rHDL, we observed the
SR-BI Is Required for the Rapid Mobilization of FC Observed in Circulation After the Administration of rHDL

We evaluated the effects of administration of rHDL in mouse models that lacked SR-BI, ABCA1, or ABCG1 to assess their role in mediating cholesterol efflux to rHDL. Baseline lipid levels for the KO mouse models and their respective controls are shown in the Table. After administration of rHDL into SR-BI–deficient mice, the human apoA-I peak at 20 minutes was lower than that observed in the control mice (85 ± 17 vs 113 ± 13 mg/dL; P = 0.051); however, by 24 hours the levels in the 2 groups of animals were similar (19 ± 9 vs 25 ± 16 mg/dL; P = 0.58). Similarly, when expressed as change from baseline, the increase in PC levels in SR-BI–deficient mice (Figure 2B) were lower than that in control mice at the 20-minute peak (201 ± 53 vs 338 ± 43 mg/dL; P = 0.01) but similar 24 hours after injection (10 ± 10 vs 4 ± 54 mg/dL; P = 0.93). The kinetic analysis of these data support the concept that the overall human apoA-I and PC clearance (FCR) and the PC transport are similar in SR-BI KO and control mice (Table). The most striking difference observed between SR-BI KO mice and their controls were the FC levels, because SR-BI–deficient mice lacked the rapid increase in response to the rHDL injection (Figure 2C). At 20 minutes after injection, changes in FC levels from baseline in SR-BI KO mice were only 10% of the changes observed in control mice (6 ± 3 vs 61 ± 13 mg/dL; P < 0.01) and increased rather slowly up to 6 hours after injection. The kinetic analysis suggests that the FCR of FC as well as the rapid input of FC into circulation

Table. Kinetic Parameters After Administration of rHDL in Mice

<table>
<thead>
<tr>
<th></th>
<th>apoA-I FCR</th>
<th>PC FCR</th>
<th>PC Trans.</th>
<th>FC FCR</th>
<th>FC Trans. AUC</th>
<th>Rapid FC Trans. AUC</th>
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<tbody>
<tr>
<td></td>
<td>pool/hr</td>
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<td>pool/hr</td>
<td>(mg/kg)*h</td>
<td>(mg/kg)*h</td>
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<tr>
<td>C57BL/6</td>
<td>0.09 ± 0.01</td>
<td>0.67 ± 0.19</td>
<td>26.16 ± 5.89</td>
<td>0.95 ± 0.22</td>
<td>182.28 ± 50.86</td>
<td>23.11 ± 5.47</td>
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<tr>
<td>SRBI WT</td>
<td>0.04 ± 0.03</td>
<td>0.50 ± 0.07</td>
<td>30.39 ± 4.88</td>
<td>1.01 ± 0.06</td>
<td>241.00 ± 49.64</td>
<td>27.96 ± 9.99</td>
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<tr>
<td>SRBI KO</td>
<td>0.04 ± 0.01</td>
<td>0.37 ± 0.20</td>
<td>33.51 ± 19.60</td>
<td>0.18 ± 0.01</td>
<td>274.59 ± 15.25</td>
<td>1.01 ± 1.75</td>
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<td>p value</td>
<td>0.88</td>
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<td>0.76</td>
<td>&lt;0.001</td>
<td>0.33</td>
<td>0.006</td>
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<td>ABCA1 WT</td>
<td>0.07 ± 0.01</td>
<td>0.51 ± 0.14</td>
<td>25.95 ± 6.89</td>
<td>1.25 ± 0.14</td>
<td>267.35 ± 39.93</td>
<td>53.86 ± 4.19</td>
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<tr>
<td>ABCA1 KO</td>
<td>0.18 ± 0.04</td>
<td>0.54 ± 0.08</td>
<td>14.94 ± 2.96</td>
<td>1.36 ± 0.16</td>
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<td>63.95 ± 12.72</td>
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<tr>
<td>p value</td>
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<td>0.73</td>
<td>0.03</td>
<td>0.36</td>
<td>0.21</td>
<td>0.18</td>
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<tr>
<td>ABCG1 WT</td>
<td>0.08 ± 0.01</td>
<td>0.40 ± 0.04</td>
<td>16.05 ± 5.31</td>
<td>1.09 ± 0.12</td>
<td>311.74 ± 28.43</td>
<td>17.73 ± 10.06</td>
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<tr>
<td>ABCG1 KO</td>
<td>0.07 ± 0.01</td>
<td>0.46 ± 0.04</td>
<td>21.21 ± 2.42</td>
<td>1.16 ± 0.15</td>
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<tr>
<td>P</td>
<td>0.13</td>
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<td>0.08</td>
<td>0.45</td>
<td>0.97</td>
<td>0.41</td>
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</table>

apoA-I, human apolipoprotein A-I; AUC, area under the curve; FC, free cholesterol; FCR, fractional catabolic rate; KO, knockout mice; PC, phosphatidylcholine; trans, transport; WT, control wild-type mice.

Values for C57BL/6 mice are shown for comparison.

P values refer to comparison between the wild-type control mice (WT) and the knockout (KO) mice.

Figure 2. Human apoA-I (A), PC (B), and FC (C) levels in serum from SR-BI KO (n = 3) and control (n = 4) male mice before and 20 minutes and 2, 6, and 24 hours after intravenous administration of rHDL. PC and FC levels are expressed as change from baseline. See Materials and Methods for details. Solid line, KO mice; dashed line, control mice.
were markedly reduced in the SR-BI–deficient mice as compared with their controls \((P<0.01; \text{Table})\).

**ABCA1 and ABCG1 Are Not Required for the Rapid Mobilization of FC Observed in Circulation After the Administration of rHDL**

After administration of rHDL, the serum concentration of human apoA-I in ABCA1-deficient mice was similar to that in control mice 20 minutes after injection but declined more rapidly over time (Figure 3A), consistent with the known increase in apoA-I catabolism in ABCA1 deficiency. Kinetic analysis showed that the human apoA-I FCR was significantly faster in ABCA1 KO mice as compared with WT mice \((P=0.002; \text{Table})\), explaining the more rapid decline in concentration over time. Changes in the serum concentrations of PC in ABCA1 KO mice after rHDL injection were similar to those observed in control mice (Figure 3B). Kinetic analysis showed that the PC FCR observed in ABCA1-deficient mice was similar to that in control mice, whereas PC transport into the circulation was significantly lower \((P=0.03; \text{Table})\). The increase in FC levels was similar to that observed in control mice at the earlier time points; however, a modest but significant decrease in FC concentrations was observed 6 hours after injection (Figure 3C). Kinetic analysis showed that after rHDL injection, FC FCR and transport into circulation in ABCA1-deficient mice were similar to those observed in control mice (Table).

After administration of rHDL into ABCG1 KO mice, the rapid appearance of human apoA-I and increase in PC and FC levels (Figure 4) were similar to those observed in control mice. Kinetic analysis showed that the human apoA-I and FC FCR observed in ABCG1-deficient mice were similar, and that the slightly but significantly increased PC FCR \((P=0.01)\) was balanced by a tendency of PC transfer to increase \((P=0.08)\) as compared to control mice (Table).

**ABCG1 Contributes to the Mobilization of FC From Primary Macrophages Incubated in Presence of rHDL**

To directly assess the mechanisms, if any, responsible for cholesterol mobilization from macrophages after the administration of rHDL, we conducted in vitro studies using bone marrow-derived macrophages from SR-BI, ABCA1,
Discussion

Administration of rHDL is associated with increased FC in plasma in humans\textsuperscript{10,11} and mice,\textsuperscript{8} and with increased fecal cholesterol excretion,\textsuperscript{11,12} increased ex vivo cholesterol efflux ability,\textsuperscript{7} and improvement of atherosclerosis burden\textsuperscript{8} in humans, suggesting that administration of rHDL promotes RCT. The main objective of these studies was to evaluate the molecular mechanisms underlying the FC mobilization observed after the administration of rHDL at both systemic and macrophage levels.

Our in vivo results are consistent with the hypothesis that SR-BI, but not ABCA1 or ABCG1, is responsible for most of the increase in FC levels observed in circulation after the administration of rHDL. Relative to WT mice, we observed an increased response to rHDL in SR-BI KO mice, with a significantly lower peak in FC levels soon after rHDL administration (Figure 2C). The kinetic analysis also supports the concept that SR-BI plays a critical role in the rapid early efflux of FC into circulation in response to rHDL injection, as demonstrated by the reduction of the rapid FC transport observed in the SR-BI KO mice, as well as a role in FC uptake, as demonstrated by the reduction in FC FCR observed in the SR-BI KO mice. These results are consistent with the known role of liver SR-BI in HDL metabolism in mice,\textsuperscript{22–24} the ability of SR-BI to mediate bidirectional cholesterol flux between cells and HDL,\textsuperscript{16} and the ability to promote cholesterol efflux from a variety of cell types to mature HDL\textsuperscript{25} and to rHDL.\textsuperscript{26,27}

Although we measured cholesterol concentrations in blood cells over time in animals injected with rHDL and excluded the possibility that blood cells are a major source of the increased serum FC observed in our studies (data not shown), we did not assess which tissues are the primary sources of the FC mobilization in response to rHDL infusion. SR-BI is abundantly expressed in the liver,\textsuperscript{28} and it is possible that a considerable proportion of the FC mobilized in response to rHDL is derived from this organ. Based on the data presented by Alam et al,\textsuperscript{8} the liver is one of the biggest contributors to the FC mobilization in response to rHDL injection. However, we did not observe which tissues are the primary sources of the FC mobilization in response to rHDL infusion. Therefore, we cannot determine with certainty which tissues are the primary sources of the FC mobilization in response to rHDL infusion.

The results of the in vivo studies suggest that ABCA1-mediated and ABCG1-mediated mechanisms do not contribute substantially to the amount of FC mobilized after rHDL administration, at least at the earlier time points. It is, however, interesting to notice that the liver contributes significantly to the FC mobilization in response to rHDL infusion. The liver is one of the biggest contributors to the FC mobilization in response to rHDL infusion, and it is possible that a considerable proportion of the FC mobilized in response to rHDL is derived from this organ. Based on the data presented by Alam et al,\textsuperscript{8} the liver is one of the biggest contributors to the FC mobilization in response to rHDL injection. However, we did not observe which tissues are the primary sources of the FC mobilization in response to rHDL infusion. Therefore, we cannot determine with certainty which tissues are the primary sources of the FC mobilization in response to rHDL infusion.
rHDL and to the improvement in the atherosclerotic burden that may be associated with the administration of rHDL in humans, and animals. Because administration of rHDL is being considered for the treatment of atherosclerosis, we were interested in assessing the ability of rHDL in promoting cholesterol efflux specifically from macrophages and the underlying mechanisms. We conducted in vitro studies using bone marrow-derived macrophages isolated from SR-BI KO, ABCA1 KO, and ABCG1 KO mice and their respective controls, as described, and showed that rHDL promotes FC efflux from primary macrophages by an ABCG1-mediated pathway, whereas SR-BI seems not to play a significant role. Although SR-BI is expressed in macrophages and a protective role of macrophage SR-BI has been suggested from studies performed using bone marrow transplantation, these results are consistent with our previously published data in which we have shown that macrophage SR-BI does not play a dominant role in either FC efflux from macrophages or macrophage RCT, contrary to the key role played by macrophage ABCG1.

Our data in primary macrophages support the hypothesis that rHDL promote efflux from macrophages via an ABCG1-mediated mechanism (Figure 5C). ABCG1 is highly expressed in macrophages and mediates macrophage cholesterol efflux to mature HDL and rHDL. Although its role in atherosclerosis is still debated, its role in controlling cholesterol homeostasis in macrophages is well-supported by published data and our results suggest that an ABCG1-mediated mechanism is at least partly responsible for the improved atherosclerotic burden observed in previous studies after administration of rHDL.

Other pathways, particularly aqueous diffusion, must also mediate cholesterol efflux in presence of rHDL because efflux is preserved, albeit reduced, in ABCG1-deficient macrophages. It is also reasonable to think that after remodeling in vivo with a generation of lipid-poor apoA-I, rHDL may also promote ABCA1-mediated macrophage cholesterol efflux. Contrary to the role that ABCG1 has in mediating cholesterol efflux in macrophages, ABCA1-mediated cholesterol mobilization does not appear to contribute significantly to the bulk of FC mobilized soon after HDL mobilization. This is probably attributable to the fact that the mass of cholesterol mobilized by macrophages via ABCG1 is a small part of the overall cholesterol tissue pool.

One possible limitation of our in vivo studies is the large difference in HDL levels in the different mouse models. The difference in pool size could potentially affect how the different mouse models responded to rHDL administration. However, we do not expect an effect of pool size on FC mobilization, because we believe there are sufficient phospholipids in the rHDL to allow for ample FC uptake.

In conclusion, we demonstrate that SR-BI, and not ABCA1 or ABCG1, is responsible for the bulk of the rapid FC mobilization to the circulation (primarily nonmacrophage) that is observed after intravenous administration of rHDL. In contrast, ABCG1, and not SR-BI or ABCA1, is primarily responsible for FC mobilization to rHDL from macrophages. Because macrophage cholesterol efflux is arguably more relevant to atherosclerosis than efflux from nonmacrophage tissues, these results call into question the concept of using serum FC as a surrogate for the antiatherosclerotic efficacy of rHDL infusion.

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The authors thank Margaret Nickel, Vinh Nguyen, and Aisha Wilson for outstanding technical support.

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Disclosure
None.

References


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Pathways by which reconstituted HDL mobilizes free cholesterol from whole body and from macrophages

M. Cuchel: rHDL mobilizes cholesterol via different pathways

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¹Institute of Translational Medicine and Therapeutics, University of Pennsylvania School of Medicine, ²Division of Gastroenterology, Hepatology and Nutrition, Children’s Hospital of Philadelphia, Philadelphia, PA
**Materials.** Egg phosphatidylcholine (PC) was purchased from Avanti Polar Lipids. ApoA-I was isolated from human plasma HDL as described before\(^1\). [1,2-\(^3\)H]-cholesterol was purchased from NEN Life Science Products. Bovine serum albumin (BSA), HEPES, and all reagents, solvents and chemicals were obtained from Fisher Scientific or as specified in the text. Phosphate-buffered saline (PBS), Eagle’s minimal essential medium (MEM) and RPMI were purchased from Mediatech. Fetal bovine serum (FBS), bovine calf serum (CS), enzymes and antibiotics for cell culture were obtained from Sigma. Compound CP113, 818 was a gift from Pfizer. Tissue culture flasks were obtained from Corning. All filtration products were obtained from Millipore. Other materials and reagents were obtained as noted.

**Preparation and Characterization of Discoidal Reconstituted Lipid/Protein.**

rHDL containing human apoA-I and egg PC were prepared by the cholate dialysis method\(^2\). Briefly, the desired amount of egg PC in CHCl\(_3\) was added to a 15 ml glass tube and dried under nitrogen, then under high vacuum. Subsequently, the egg PC was hydrated in TRIS-buffered saline (TBS), pH 7.4, vortexed to generate multilamellar vesicles, and incubated with the desired amount of sodium cholate at 37 °C for 1.5 h to generate mixed detergent-PC micelles. The solution was mixed every 15 min during the incubation until completely clear. Next, apoA-I freshly dialyzed from 6 M guanidine HCl in TBS was added to the lipid-detergent mixture at an egg PC to apoA-I ratio of 2.5:1 (w/w; equivalent to a molar ratio of 100:1). The lipid-detergent-apo A-I complexes were then further incubated for 1 h at 37 °C. Sodium cholate was removed from the dispersion by extensive dialysis at 4 °C against TBS. To obtain a homogenous
population of rHDL particles the preparation was fractionated on a gel filtration column (60 X 1.6-cm Superdex 200 HR column, Amersham Pharmacia Biotech)\(^3\) as necessary. rHDL complexes were isolated, concentrated, dialyzed into phosphate-buffered saline and used within 2 days of preparation. The final particle compositions were determined by analyzing for PC, using an enzymatic assay kit (Wako Chemicals) and protein, using either the Lowry procedure\(^4\) or the absorbance coefficient at 280 nm. The final rHDL particles had an average hydrodynamic diameter of approximately 10nm and a protein to phospholipid to protein ratio of about 2:1(w/w).

**Cholesterol efflux assays in primary macrophages.** Bone marrow-derived macrophages (BMM) were isolated from femurs and tibias of SR-BI\(^{-}\), ABCA1\(^{-}\) and ABCG1-KO mice and respective controls, and cultured in DMEM supplemented with 10% FBS and 30% L929 conditioned media as described before\(^5\). Cells were labeled with \(^{3}\text{H}\)-cholesterol (2 μCi/mL, Amersham). In the experiments with BMMs from ABCA1 KO, ABCG1 KO animals or corresponding controls, BMMs were loaded with cholesterol using acLDL (25 ug/ml) during labeling. In the experiments with BMMs from SR-BI KO or corresponding control animals, cells were labeled in the absence of acLDL to avoid downregulation of SR-BI expression. After labeling step, all cells were washed and equilibrated overnight either in DMEM containing BSA (2mg/ml). For the cholesterol efflux, medium containing 25 μg/mL rHDL, 25 μg/mL HDL3 or 10 μg/ml free apoA-I was added to BMM cells. After 4 hours, aliquots of the medium were removed and the \(^{3}\text{H}\)-cholesterol released was measured by liquid scintillation counting.
The \(^{3}\text{H}\)-cholesterol present in the cells was determined by extracting the cell lipids with isopropanol and measured by liquid scintillation counting.

**Mouse protocol.** Wild type (WT) C57BL/6, DBA/J and B6;129S2/J mice were obtained from The Jackson Laboratories (Bar Harbor, ME). ABCA1 (DBA/1-Abca1/J) knock out (KO), SR-BI (B6;129S2-Scarb1\(^{tm1Kri}/J\)) KO and ABCG1KO (kindly donated by Dr. Marcelo Amar of NIH/NHLBI) mice were bred in house. Mice were fed a standard chow diet ad libitum. Mice (~3-6 months old) received 200 \(\mu\)l of either PBS containing rHDL or PBS alone as a bolus intravenous injection via tail vein. The dose of apoA-I was approximately 3 mg (range 3.0-3.5 mg/200 \(\mu\)l) and the dose of egg PC was approximately 5 mg (range 4.9-5.8 mg/200 \(\mu\)l). Blood was sampled via retro-orbital bleeding using heparinized glass tubes before and 20 min, 2h, 6h, 24h after injection. Experiments were performed at least twice for each strain of mice. All procedures were approved by the University of Pennsylvania Animal Care Committee.

**Lipid and lipoproteins assays.** Plasma total cholesterol, HDL cholesterol, FC, phospholipids and apoA-I levels were measured on a Cobas Fara (Roche Diagnostics System, Inc) using Wako Pure Chemical Industries reagents. The enzymatic method used to measure phospholipids selectively measures choline-containing phospholipids, i.e. PC and sphingomyelin. In the context of this paper, where high doses of PC are administered into mice in the form of rHDL, we assume that the contribution of sphingomyelin to the total phospholipids levels in serum is minor, and report the phospholipids levels as PC. Pooled plasma from each time point was separated using
FPLC gel filtration (Amersham Pharmacia Biotech) on 2 Superose 6 columns as described previously\(^6\) for determination of cholesterol and PC profiles.

**Kinetic analysis.** Transport and fractional catabolic rates (FCR) were calculated using the WinSAAM version 3.0.6\(^7\). Human apoA-I mass (in mg) was calculated by multiplying the plasma concentration of apoA-I (in mg/L) for each mouse by the estimated plasma pool size (in L, approximately 3.5% of body weight). The human apoA-I FCR was calculated by fitting a monoexponential curve to the human apoA-I mass in plasma at each timepoint. PC mass (in mg) in plasma was calculated by multiplying the plasma concentration of PC (in mg/L) for each mouse by the estimated plasma pool size (in L, approximately 3.5% of body weight). The PC FCR was calculated by fitting the total PC mass in plasma at each timepoint to a compartmental model consisting of a single compartment. The endogenous PC transport rate through plasma was calculated by multiplying the baseline PC mass, determined prior to injection of rHDL, by the PC FCR. FC mass (in mg) in plasma was calculated by multiplying the plasma concentration of FC (in mg/L) of each mouse by the estimated plasma pool size (in L, approximately 3.5% of body weight). The FCR for FC was calculated by fitting the total FC mass in plasma at each timepoint to a compartmental model consisting of a single compartment. The FC transport rate, which varied with time, consisted of two components. The major component was directly proportional to the plasma PC concentration at the corresponding time. This constant was very similar in all mouse strains studied, ranging between 0.11 to 0.15 mg FC/mg PC/h. The second component, representing the initial rapid transport of FC into plasma, was described by a function that rapidly decreased
over time. The area under the curve (AUC) was determined by integration of the equations describing FC transport between 0 and 24 hours.

**Statistical analysis.** Data are presented as mean ± SD. The t-test was used to test the difference between 2 groups and a p value of <0.05 was considered significant.
References


Supplemental Table 1. Serum lipid levels for mice at baseline

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<th>TC (mg/dl)</th>
<th>FC (mg/dl)</th>
<th>PC (mg/dl)</th>
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<td>C57BL6 (n=12)</td>
<td>74 ± 9</td>
<td>17 ± 3</td>
<td>150 ± 19</td>
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<td>SRBI-control (n=4)</td>
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<td>20 ± 4</td>
<td>208 ± 36</td>
</tr>
<tr>
<td>SRBI-KO (n=3)</td>
<td>234 ± 32</td>
<td>155 ± 9</td>
<td>248 ± 29</td>
</tr>
<tr>
<td>ABCA1-control (n=4)</td>
<td>67 ± 3</td>
<td>9 ± 1</td>
<td>140 ± 5</td>
</tr>
<tr>
<td>ABCA1-KO (n=4)</td>
<td>5 ± 3</td>
<td>2 ± 1</td>
<td>34 ± 9</td>
</tr>
<tr>
<td>ABCG1-control (n=5)</td>
<td>123 ± 22</td>
<td>41 ± 8</td>
<td>233 ± 29</td>
</tr>
<tr>
<td>ABCG1-KO (n=5)</td>
<td>83 ± 4</td>
<td>29 ± 3</td>
<td>155 ± 9</td>
</tr>
</tbody>
</table>

TC, total cholesterol; FC, free cholesterol; PC, phosphatidylcholine; control, wild type control mice on same background as knock out mice; KO, knock out
Supplemental Figure 1. FPLC profiles of serum of C57BL/6 female mice at baseline and after administration of rHDL. See methods section for details. A: Phosphatidylcholine (PC) concentrations at 0 and 20 min. B: Total cholesterol concentrations at 0 (gray line, solid diamonds) and 20 min (black solid line, square symbols). C: Total cholesterol (black solid line and symbols) and free cholesterol (gray solid line and symbols) concentration 20 minutes after rHDL injection.