Both Hepatic and Extrahepatic ABCA1 Have Discrete and Essential Functions in the Maintenance of Plasma High-Density Lipoprotein Cholesterol Levels In Vivo

Roshni R. Singaraja, PhD; Miranda Van Eck, PhD; Nagat Bissada, MD; Francesca Zimetti, PhD; Heidi L. Collins, PhD; Reeni B. Hildebrand, BS; Anna Hayden; Liam R. Brunham, BS; Martin H. Kang, BS; Jean-Charles Fruchart, PhD; Theo J.C. Van Berkel, PhD; John S. Parks, PhD; Bart Staels, PhD; George H. Rothblat, PhD; Catherine Fiévet, PhD; Michael R. Hayden, MBChB, PhD

Background—Extrahepatic tissues have long been considered critical contributors of cholesterol to nascent HDL particles in the reverse cholesterol transport pathway, in which ABCA1 plays the crucial role. Recent studies, however, including both overexpression and deletion of ABCA1 selectively in the liver, have highlighted the primary role of the liver in the maintenance of HDL levels in vivo.

Methods and Results—The availability of mice with complete deletion of ABCA1 (total knockout [TKO]) and with liver-specific deletion of ABCA1 (LSKO) has enabled us to dissect the discrete roles of hepatic relative to extrahepatic ABCA1 in HDL biogenesis. Delivery of adenoviral ABCA1 resulted in selective expression of physiological levels of ABCA1 in the livers of both LSKO and TKO mice, resulting in increased HDL cholesterol (HDL-C). Expression of ABCA1 in the liver of LSKO mice resulted in plasma HDL-C levels that were similar to those in wild-type mice and significantly above those seen in similarly treated TKO mice. HDL particles from ABCA1-expressing LSKO mice were larger and contained significantly increased cholesterol compared with TKO mice. Infusion of human apolipoprotein A-I/phospholipid reconstituted HDL particles normalized plasma HDL-C levels in LSKO mice but had no effect on HDL-C levels in TKO mice.

Conclusions—Although hepatic ABCA1 appears crucial for phospholipid transport, extrahepatic tissues play an important role in cholesterol transfer to nascent HDL particles. These data highlight the discrete and specific roles of both liver and extrahepatic ABCA1 in HDL biogenesis in vivo and indicate that ABCA1 shows lipid cargo selectivity depending on its site of expression. (Circulation. 2006;114:1301-1309.)

Key Words: cholesterol ■ lipids ■ lipoproteins

A BCA1 lipidates lipid-poor apolipoprotein A-I (apoA-I) particles and thus is essential for the formation of HDL. Patients with Tangier disease harbor mutations in ABCA1 and have extremely low levels of HDL cholesterol (HDL-C). Mice with generalized deletion of their endogenous ABCA1 gene also have little or no plasma HDL-C. By contrast, overexpression of ABCA1 in transgenic mice results in increased plasma HDL-C levels. Early studies on HDL assembly have shown that apolipoproteins can remove both phospholipid and cholesterol from macrophages and generate new HDL particles. However, cells from patients with Tangier disease cannot generate new HDL particles in the presence of these apolipoproteins, showing that ABCA1 is essential for the lipidation of apolipoproteins. Together, these studies have clearly established that ABCA1 is a key protein responsible for the initial step in the reverse cholesterol transport (RCT) pathway.

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RCT is the process by which cholesterol in extrahepatic tissues is returned to the liver for excretion as bile, and HDL serves as the transport vehicle in this pathway. In the first step, cholesterol and phospholipids are effluxed from cell membranes onto apoA-I to form nascent pre–β-HDL. This cholesterol is esterified by lecithin-cholesterol acyltransferase and moves to the core of the HDL particle, generating large spherical α-HDL. Once at the liver, HDL–cholesteryl ester is cleared by the action of scavenger receptor class B type 1
In addition, in humans, HDL–cholesterol ester is transferred through the action of cholesteryl ester transfer protein onto apolipoprotein B–containing lipoproteins and taken up by the liver through the LDL receptor pathway. Thus, a central tenet to the RCT pathway hypothesis has been that extrahepatic tissues are the major contributors of cholesterol to plasma HDL.

However, recently a more hepatocentric view of the regulation of HDL-C levels has emerged. Overexpression of adenovirally delivered ABCA1 in the liver of mice results in increased plasma HDL-C, leading to the hypothesis that liver ABCA1 is the most important contributor to plasma HDL levels. In addition, transgenic mice overexpressing ABCA1 in the liver showed increased plasma HDL-C, indicating that hepatic ABCA1 plays a major role in determining plasma HDL-C levels as well as regulating intracellular levels of hepatic cholesterol. Further evidence for the role of hepatic ABCA1 in HDL generation was demonstrated in mice with liver-specific deletion of ABCA1, in which an 80% decrease in plasma HDL-C was observed. Thus, the relative and discrete contributions of both extrahepatic and hepatic ABCA1 to the maintenance plasma HDL-C levels are not clearly delineated. In particular, whether extrahepatic tissues play an essential role in HDL biogenesis has become unclear.

Expressing ABCA1 in the liver of ABCA1−/− total knockout (TKO) mice results in mice with ABCA1 expression only in the liver, whereas expression of ABCA1 in the liver-specific ABCA1−/− (LSKO) mice results in mice with both liver and extrahepatic ABCA1 expression. Using these mice, we show that hepatic ABCA1 is essential but insufficient for the correction of the HDL-C deficiency in TKO mice and that extrahepatic ABCA1 is critical to the generation of mature HDL particles. We also conclude that a significant proportion of the cholesterol in circulating HDL is extrahepatic in origin.

### Methods

#### ABCA1 Mouse Models and Infection With Recombinant Adenovirus

TKO mice were obtained from Dr Omar Francone (Pfizer Global Research and Development, Groton, Conn) on the DBA background and were crossed to C57BL6/J mice to the N4 generation. LSKO mice were on a C57BL6/J×129 mixed background. Mice were maintained on a standard chow diet. Because both the LSKO and TKO mice were >80% C57BL6/J, C57BL6/J mice were used as wild-type controls for all experiments. All procedures on mice were approved by the institutional animal care committee. Recombinant adenoviral production and titration were performed as previously described. Preinfection blood samples were collected from the mice through saphenous vein bleeds. For infection, virus was diluted in sterile PBS. All experimental mice were preinfected with 5×10^5 plaque-forming units (pfu) of adenoaviral alkaline phosphatase (Ad-AP) delivered through the tail vein to saturate the uptake of viral particles by Kupffer cells. Three hours after the Ad-AP infection, 5×10^5 pfu of adenoaviral ABCA1 was delivered through the tail veins of the mice. Wild-type control mice were infected with the Ad-AP. Three days after infection, after a 4-hour fast, mice were euthanized, and blood and tissues were harvested. Blood was harvested by cardiac puncture, and 0.1 mL of buffer containing lecithin-cholesterol acyltransferase inhibitor (0.9% NaCl, 11 mmol/L EDTA, 110 mmol/L Tris, pH 7.4, 15 mmol/L iodoacetate acid) was added per milliliter of blood to prevent the ex vivo conversion of pre-β-HDL to mature HDL particles. After a 30-minute incubation on ice, serum was harvested and stored at 4°C.

#### In Vivo Infusion of ApoA-I/Phospholipid Discs

Six milligrams of discoidal complexes of phospholipid and apo-A-I (reconstituted HDL [rHDL]) containing human apo-A-I and phospholipid in a ratio of ~1:4.2 (wt:wt) (a kind gift from Dr Peter G. Lerch, ZLB Behring AG, Bern, Switzerland) made up in 225 μL of sterile water was infused over 5 minutes via the tail veins of the mice. Blood was collected at various time points after infusion with saphenous vein puncture and treated as described above.

#### Western Blotting

Protein lysates were generated from frozen tissues, and Western immunoblotting was performed as previously described.

#### Plasma Lipid and Lipoprotein Analysis

The distribution of lipids in plasma lipoproteins was assessed as described. Plasma (50 μL) from each mouse was fractionated with the use of a Superose 6 column (3.2×30 mm, Smart-system, Pharmacia, Uppsala, Sweden). Cholesterol, triglyceride, and phospholipid contents in the effluent were determined with the use of colorimetric assays (Roche Diagnostics, Basel, Switzerland), with the efficiency of recovery from the column taken into account. Human apo-A-I in fast protein liquid chromatography (FPLC) fractions was quantified by sandwich ELISA with the use of rabbit anti-human apo-A-I antibody (Calbiochem 178422, Calbiochem, San Diego, Calif) and peroxidase-conjugated mouse anti-human apo-A-I antibody (Calbiochem 178472).

Unfractionated plasma and lipoprotein lipid (total cholesterol, free cholesterol, triglycerides) concentrations were determined by enzymatic assays with the use of commercially available reagents (BioMerieux, Lyon, France, for total cholesterol RTU and triglycerides PAP 1000; Wake Chemicals GmbH, Neuss, Germany, for free cholesterol). Unfractionated plasma levels of apo-A-I were determined by immunonephelometry with the use of mouse-specific antibodies developed in rabbits. HDL (density=1.063 to 1.21 g/mL) fraction was isolated from serum by sequential ultracentrifugation. It was assayed for its protein and lipid (esterified cholesterol, free cholesterol, triglyceride, and phospholipid) content.

#### Lipoprotein Particle Analysis by NMR

Proton NMR spectra of each plasma specimen (0.2 mL) were acquired in replicates with the use of an automated 400-MHz lipoprotein analyzer at LipoScience (Raleigh, NC), and the lipid methyl signal envelope was decomposed computationally to give the amplitudes of the contributing signals of 16 (cholesteromicrons, 6 VLDL, 1 IDL, 3 LDL, 5 HDL) lipoprotein subclasses. Conversion factors relating these signal amplitudes to subclass concentrations expressed in cholesterol mass concentration units were then applied. The 16 measured subclasses were grouped for analysis into the following 10 subclass categories: large VLDL (60 to 200 nm), medium VLDL (35 to 60 nm), small VLDL (27 to 35 nm), IDL (23 to 27 nm), large IDL (21.3 to 23 nm), medium LDL (19.8 to 21.2 nm), small LDL (18.3 to 19.7 nm), large HDL (8.8 to 13 nm), medium HDL (8.2 to 8.8 nm), and small HDL (7.3 to 8.2 nm). IDL and LDL subclass diameters are consistent with both electron microscopy and LDL lipid compositional data. Weighted average VLDL, LDL, and HDL particle sizes (nm diameter) were computed as the sum of the diameter of each subclass multiplied by its relative mass percentage as estimated from the amplitude of its methyl NMR signal.

#### Measurement of Cholesterol Efflux

Cholesterol efflux via SR-B1 with the use of FusAH cells and via ABCA1 with the use of J774 cells was determined as previously described.
Liver-Specific Expression of Normal ABCA1 Levels

We and others have previously determined that the overexpression of ABCA1 in wild-type mice with the use of adenovirus increases plasma HDL-C levels. However, these studies did not investigate the contribution of extrahepatic ABCA1 to plasma HDL-C levels. It should also be noted that in these studies, ABCA1 was expressed at levels much higher than physiologically normal murine ABCA1 levels. To determine the specific contribution of hepatic and extrahepatic ABCA1 to circulating HDL levels, it is important to express normal levels of exogenous ABCA1 in the liver but not extrahepatic tissues of recipient mice. Adenoviral delivery of ABCA1 in the livers of TKO and LSKO mice resulted in levels of ABCA1 protein equivalent to those of wild-type mice specifically in the livers (Figure 1A) of recipient mice. No expression of adenoviral ABCA1 was observed in spleen (Figure 1B) and in other tissues from the TKO mice. The LSKO mice have endogenous ABCA1 in every tissue except the liver. Controlling for equivalent hepatic ABCA1 levels in these models allows for specific assessment of the role of extrahepatic ABCA1 to HDL biogenesis in vivo. Protein and lipid data were assessed 3 days after infection, a time point previously shown to coincide with maximum expression of exogenous protein.

Plasma Lipids in Adeno-ABCA1–Infected TKO and Adeno-ABCA1–Infected LSKO Mice

Basal levels of plasma HDL-C were 5.9±1.1 mg/dL in the LSKO, 3.6±1.0 mg/dL in the TKO, and 38.0±1.3 mg/dL in the wild-type mice. After the delivery of adenoviral ABCA1, plasma HDL-C was increased in LSKO mice to levels indistinguishable from wild-type levels (adeno-ABCA1–infected LSKO [Ad-LSKO], 38.2±1.4 mg/dL; wild-type, 38.0±1.3 mg/dL; n=6; P=0.500; Figure 2 and Table). By contrast, the expression of similar amounts of ABCA1 in the livers of the TKO mice resulted in only a partial increase in plasma HDL-C levels (adeno-ABCA1–infected TKO [Ad-TKO], 20.2±9.2 mg/dL; wild-type, 38.0±1.3 mg/dL; n=6; P=0.010; Figure 2 and Table).

Plasma phospholipids followed a similar trend, with the adeno-ABCA1–expressing LSKO mice showing near normalization (wild-type, 99.7±25.4 mg/dL versus Ad-LSKO, 65.3±40.4 mg/dL; n=6; P=0.032), whereas TKO mice showed only a partial increase (wild-type, 99.7±25.4 mg/dL versus Ad-TKO, 23.8±36.3 mg/dL; n=6; P=0.008) (Figure 3 and Table). ApoA-I levels were also increased to wild-type levels in the adeno-ABCA1–expressing LSKO mice but not in TKO mice (Table). After adeno-ABCA1 infection, plasma total cholesterol levels returned to wild-type levels in the LSKO mice but were only 56% of wild-type in the TKO mice.

Results

Delivery of Human ABCA1 by Adenovirus Results in Liver-Specific Expression of Normal ABCA1 Levels

Figure 1. ABCA1 expression specifically in the livers of Ad-ABCA1–treated mice. Three days after injection, tissues were harvested, and protein lysates were prepared. Blots were immunodetected with antibodies specific for ABCA1. GAPDH was used as a loading control. A, Western blots of liver lysates after injection of LSKO and TKO mice with 5.0×10⁸ pfu Ad-ABCA1 showing ABCA1 protein in the liver at levels similar to those of wild-type (wt) mice. B, Western blots of spleen lysates from the Ad-ABCA1–treated LSKO and TKO mice showing no increase in ABCA1 in the spleen of infected mice compared with wild-type, indicating that the adenoviral infection was specific to the liver.

Statistical Analyses

All statistical analyses were performed with the use of the Mann-Whitney test. Values are median ± interquartile range.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.
Plasma Lipid and Lipoprotein Levels in LSKO and TKO Mice

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<td>WT</td>
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<td>6.7±1.7</td>
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Values are median±interquartile range. WT indicates wild-type.

**Figure 2.** Plasma cholesterol levels in mice infected with Ad-ABCA1. Blood samples were drawn after a 4-hour fast 3 days after infection with 5×10⁶ pfu per mouse of adenoviral ABCA1. Plasma from individual mice was loaded onto a Superose 6 column, and fractions were collected. Fractions 3 to 7 represent VLDL, fractions 8 to 14 LDL, and fractions 15 to 19 HDL. A. The distribution of cholesterol in the different lipoproteins in TKO and LSKO mice is shown. B. The levels of VLDL and LDL-C are shown. Values represent the mean±SD of at least 6 mice. Plasma levels of HDL-C were indistinguishable in the LSKO mice compared with wild-type (WT) controls. However, plasma HDL-C levels were significantly lower in the TKO mice than in controls. No significant differences in LDL-C and a mild decrease in VLDL-C in LSKO compared with TKO were observed.

**Characterization of Lipid Particles From Adeno-ABCA1–Infected LSKO and TKO Mice**

Because plasma HDL-C levels were elevated to wild-type levels in the Ad-LSKO but not TKO mice, we next compared HDL particle size and composition in these different groups.
of mice. Plasma from the Ad-LSKO and TKO mice were subjected to NMR analysis to determine the size and concentration of HDL, LDL, and VLDL particles. A significant increase in average HDL particle size in sera from the infected LSKO mice was observed compared with particles from TKO mice (Figure 4A), indicating that further maturation of HDL particles occurred in the presence of extrahepatic ABCA1 in the LSKO mice (Ad-LSKO, 11.9 ± 0.4 nm diameter versus Ad-TKO, 10.3 ± 0.7 nm diameter; n = 3 pools of 2; P = 0.029). No significant difference in the size of the VLDL or LDL particles was observed in both the LSKO and TKO mice (Figure 4B), showing that the activity of ABCA1 was not altering the composition of non-HDL particles. The mass concentration of cholesterol in the large HDL particles from infected LSKO mice was significantly higher than that from TKO mice (Figure 4C), indicating that extrahepatic ABCA1 contributes to the addition of cholesterol to the maturing HDL particles.

We next quantified protein, phospholipid, free cholesterol, and cholesterol ester in HDL isolated by ultracentrifugation. When normalized to HDL protein levels, no significant differences in phospholipid concentrations were observed in the HDL particles from either the LSKO or TKO mice (Figure 4D), although a trend toward increased phospholipids in the HDL from LSKO mice was evident. HDL particles from the LSKO mice had significantly increased free cholesterol and cholesterol ester content compared with HDL from TKO mice (free cholesterol: Ad-LSKO = 0.10 ± 0.07 versus Ad-TKO = 0.06 ± 0.01, percent composition normalized to protein; n = 6; P = 0.026; cholesterol ester: Ad-LSKO = 0.16 ± 0.11 versus Ad-TKO = 0.08 ± 0.04, percent composition normalized to protein; n = 6; P = 0.007) (Figure 4D), adding further evidence for a role for extrahepatic ABCA1 in the maturation of the HDL particle.

Serum From Adeno-ABCA1–Expressing LSKO Mice Is a Better Cholesterol Efflux Acceptor Than Serum From TKO Mice

ABCA1 is vital for the initial lipidation of lipid-free apoA-I, which prevents the premature turnover of apoA-I and degradation in the kidney. Once lipidated by ABCA1, HDL particles can then take up lipid by both ABCA1-dependent and -independent mechanisms. To determine the ability of serum from the Ad-LSKO and TKO mice to act as acceptors for effluxed cholesterol, we performed cholesterol efflux assays using Fu5AH rat hepatoma cells and cAMP-treated J774 mouse macrophage cells. Fu5AH cells have very low expression of ABCA1 but have high levels of SR-B1 expres-
When added to radiolabeled Fu5AH cells, the serum from the adeno-ABCA1–expressing LSKO mice was able to elicit more cholesterol efflux (Figure 5A), indicating that the particles lipidated by ABCA1 are better acceptors of cholesterol through ABCA1-independent mechanisms. J774 cells have very little endogenous ABCA1. However, when stimulated with cAMP, a large increase in ABCA1 RNA and protein is observed. When serum from the LSKO and TKO mice was added to radiolabeled J774 cells both without (Figure 5B) and with (Figure 5C) cAMP treatment, the serum from LSKO mice again elicited more cholesterol efflux than serum from TKO mice. This further indicates that the HDL particles from the LSKO mice are better able to act as acceptors for ABCA1-independent efflux. This is compatible with data showing that ABCG1 and SR-B1 efflux lipids specifically to more mature HDL particles,27,28 as seen in the LSKO mice.

Plasma Lipids in LSKO and TKO Mice Infused With rHDL

Thus far, our data suggest that ABCA1 in the liver generates an early, phospholipidated nascent HDL particle that acquires further lipids through the action of extrahepatic ABCA1, resulting in the maturation of the nascent HDL to larger, cholesterol-rich HDL. We hypothesized that if the role of ABCA1 in the liver was to generate these phospholipidated nascent HDL particles, the infusion of phospholipidated apoA-I rHDL particles into LSKO mice should result in the restoration of plasma HDL-C to wild-type levels, whereas in the TKO mice, infusion of these particles should not result in further HDL maturation because of the absence of extrahepatic ABCA1.

To investigate this hypothesis, we infused particles with a 1:4.2 (wt:wt) ratio of apoA-I/phospholipid into tail veins of LSKO and TKO mice. Mice were bled at 0, 6, and 24 hours.
after infusion, and plasma lipoproteins were separated by FPLC. In the rHDL-administered LSKO and TKO mice, minimal plasma lipids were present at the 0-hour time point (LSKO, $4.1 \pm 7.7$ mg/dL; TKO, $6.4 \pm 4.6$ mg/dL). At 6 hours after rHDL injection, an increase in both VLDL-C and HDL-C was observed in both sets of mice (LSKO, VLDL-C = $6.0 \pm 1.9$ mg/dL, HDL-C = $8.8 \pm 1.8$ mg/dL; TKO, VLDL-C = $9.5 \pm 5.7$ mg/dL, HDL-C = $7.1 \pm 2.6$ mg/dL; VLDL $P = 0.171$; HDL $P = 0.100$). This increase was independent of ABCA1 because the TKO mice have no ABCA1. However,

Figure 5. Efflux of cholesterol to serum from Ad-ABCA1–infected mice. Serum from 4 LSKO and TKO mice was used for the assessment of its ability to elicit cholesterol efflux. A, Cholesterol efflux to serum from LSKO mice was higher than that elicited by serum from TKO mice. Efflux of cholesterol from J774 cells (B) and J774 cells treated with cAMP (C) was higher to serum from LSKO compared with TKO mice.

Figure 6. Plasma lipoprotein and apolipoprotein levels in LSKO and TKO mice infused with human apoA-I/phospholipid rHDL particles. Wild-type (wt), LSKO, and TKO mice (n = 6) were infused over 5 minutes with 6 mg of rHDL particles through their tail veins. Plasma was obtained at various time points after infusion. FPLC separation of lipoprotein fractions and cholesterol distribution in plasma from mice 0 (A), 6 (B), and 24 hours (C) after infusion of rHDL are shown. Distribution of human apoA-I in lipoprotein fractions 0 (D), 6 (E), and 24 hours (F) after rHDL infusion is shown. Quantification of phospholipid in lipoprotein fractions 0 (G), 6 (H), and 24 hours (I) after rHDL infusion is shown. Infusion of rHDL resulted in a normalization to wild-type levels of plasma HDL-C, HDL/apoA-I, and HDL/phospholipid in the LSKO mice but not the TKO mice.
at 24 hours after infusion of rHDL, a specific and significant increase in plasma HDL-C was observed in LSKO mice (Figure 6A, 6B, 6C). In contrast, in the TKO mice given rHDL, no significant changes were observed in plasma HDL-C levels at 24 hours after rHDL infusion (LSKO, 11.0±2.8 mg/dL; TKO, 2.8±3.0 mg/dL; P=0.014; n=3 pools of 2). A similar trend was observed with human apoA-I, which was indistinguishable from wild-type in the LSKO mice and virtually absent in the TKO mice at the 24-hour time point (Figure 6D, 6E, 6F). Plasma phospholipids also followed a similar trend, with the LSKO mice given rHDL showing a significant increase in plasma phospholipid, whereas the TKO mice showed no significant difference in plasma phospholipid (Figure 6G, 6H, 6I). These data confirm that ABCA1 in the liver has an important role in the generation of early HDL particles. However, the presence of extrahepatic ABCA1 is essential for the further lipidation and maturation of the early HDL particles.

Discussion

In this study we have shown that ABCA1 in the liver is essential but not sufficient for the maintenance of plasma HDL-C levels in vivo and that extrahepatic ABCA1 contributes significantly to circulating HDL-C levels. Early studies by Glomset and colleagues indicated that cholesterol acquired by extracellular tissues from de novo synthesis or lipoproteins is returned to the liver for excretion as bile by a process termed RCT. HDL is assumed to be the vehicle for cholesterol in the RCT pathway. However, recent data have highlighted the vital role of the liver in plasma HDL metabolism and indicated that the liver is the main source of cholesterol in plasma HDL.

We designed experiments to dissect the specific and discrete roles of hepatic and extrahepatic ABCA1 by selectively expressing ABCA1 in the livers of mice with either generalized deletion of ABCA1 (TKO) or mice with liver-specific deletion of ABCA1 (LSKO). Expression of ABCA1 in the livers restored plasma HDL-C to wild-type levels in LSKO but not in TKO mice, despite the similar levels of hepatic ABCA1, showing that extrahepatic tissues are crucial contributors of cholesterol to HDL particles in vivo.

Because expression of ABCA1 in LSKO but not TKO mice resulted in increased plasma HDL-C levels despite similar hepatic ABCA1 levels, we predicted that HDL from LSKO mice would be larger and contain more cholesterol compared with HDL from TKO mice. Indeed, HDL particles from LSKO mice were larger and had significantly increased free and esterified cholesterol content compared with HDL from TKO mice. This finding further indicated that extrahepatic ABCA1 was essential for the addition of cholesterol and maturation of HDL particles. However, HDL from both mice had similar protein/phospholipid ratios, suggesting that hepatic ABCA1 has a major role in the phospholipidation of lipid-free apoA-I and that the ABCA1-mediated phospholipidation of lipid-poor apoA-I is largely independent of extrahepatic ABCA1 activity.

These data suggest that the major role of hepatic ABCA1 is to phospholipidate the lipid-free apoA-I generated in the liver and thereby generate early pre-β-HDL particles. If this hypothesis were correct, the delivery of nascent HDL particles to mice should compensate for the lack of hepatic ABCA1 by providing a particle similar to that normally generated by hepatic ABCA1, and thus HDL levels in the LSKO mice would be expected to reach wild-type levels, whereas TKO mice missing extrahepatic ABCA1 would be expected to still have reduced HDL-C levels. Indeed, injection of apoA-I/phospholipid rHDL discs into LSKO and TKO mice resulted in a significant increase in plasma HDL-C in the LSKO but not the TKO mice, providing further support for our finding that ABCA1 in the liver generates early HDL particles that then mature into large HDL particles through the addition of cholesterol mediated by extrahepatic ABCA1.

Our studies provide support for and delineate the specific role for extrahepatic tissues in the RCT pathway. These data indicate that although hepatic ABCA1 is necessary for the biogenesis of early HDL particles, the addition of cholesterol to these particles occurs predominantly in extrahepatic tissues. Our data are consistent with the RCT model in which an immature HDL particle is the recipient for cholesterol delivered from extrahepatic tissues. This suggests that ABCA1 has 2 different roles depending on the tissues in which it is expressed. In the liver, it primarily transports phospholipids to lipid-poor apoA-I, and in extrahepatic tissues, it transports mainly cholesterol to these phospholipidated apoA-I particles. This is compatible with the concept that ABCA1 shows lipid cargo selectivity on the basis of its site of expression. It is also possible that ABCA1 promotes the transport of phospholipids and cholesterol without discriminating between them but that the specific cargo is influenced by the particular lipid milieu in the different tissues. In the liver, the plasma membrane microdomains from which ABCA1 transports lipids may be phospholipid rather than cholesterol enriched, whereas in extrahepatic tissues this may be reversed. Thus, ABCA1 may transport mainly phospholipids from the liver and cholesterol from other tissues. Further studies on the mechanism of action of ABCA1, especially in relation to its localization in specific membrane subdomains, and the characterization of the lipid compositions of these domains should help to elucidate the underlying mechanisms influencing the cargo selectivity of ABCA1.

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Disclosures

Dr Rothblat received research support from Pfizer for assays of cholesterol flux, as well as honoraria from Esperon. Dr Rothblat also served on the Torcetrapib Advisory Board. Dr Michael Hayden received honoraria from Pfizer. The remaining authors report no conflicts.


CLINICAL PERSPECTIVE

The discovery that ABCA1 plays a critical role in HDL biogenesis and maintenance of HDL levels in vivo has led to numerous investigations directed at answering the question of which tissues are crucial to this process. Initially, it was thought that extrahepatic tissues were important for maintenance of HDL levels in vivo. However, the genetic experiments in mice with overexpression or underexpression of ABCA1 in the liver led to a hepatocentric view of HDL biogenesis. In this study, which takes advantage of different genetically manipulated animals in different tissues, the authors have demonstrated that both hepatic and extrahepatic tissues are necessary for the maintenance of HDL levels in vivo. Hepatic cells appear to be crucial for transport of phospholipids, whereas extrahepatic tissues contribute mainly cholesterol to the nascent HDL particle. Any approach to increasing HDL levels in humans by raising ABCA1 levels should optimally target ABCA1 in both hepatic and extrahepatic tissues.
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