Hepatic ATP-Binding Cassette Transporter A1 Is a Key Molecule in High-Density Lipoprotein Cholesteryl Ester Metabolism in Mice


Objective—Mutations in ATP-binding cassette transporter A1 (ABCA1), the cellular lipid transport molecule mutated in Tangier disease, result in the rapid turnover of high-density lipoprotein (HDL)–associated apolipoproteins that presumably are cleared by the kidneys. However, the role of ABCA1 in the liver for HDL apolipoprotein and cholesteryl ester (CE) catabolism in vivo is unknown.

Methods and Results—Murine HDL was radiolabeled with $^{125}$I in its apolipoprotein and with $[^3]$Hcholesteryl oleyl ether in its CE moiety. HDL protein and lipid metabolism in plasma and HDL uptake by tissues were investigated in ABCA1-overexpressing bacterial artificial chromosome (BAC)–transgenic (BAC*) mice and in mice harboring deletions of total (ABCA1$^{-/-}$) and liver-specific ABCA1 (ABCA1$^{L/-}$). In BAC* mice with elevated ABCA1 expression, fractional catabolic rates (FCRs) of both the protein and the lipid tracer were significantly decreased in plasma and in the liver, yielding a diminished hepatic selective CE uptake from HDL. In contrast, ABCA1$^{-/-}$ or ABCA1$^{L/-}$ mice had significantly increased plasma and liver FCRs for both HDL tracers. An ABCA1 deficiency was associated with increased selective HDL CE uptake by the liver under all experimental conditions.

Conclusions—Hepatic ABCA1 has a critical role for HDL catabolism in plasma and for HDL selective CE uptake by the liver. (Arterioscler Thromb Vasc Biol. 2006;26:1821-1827.)

Key Words: ABCA1 ■ HDL ■ selective uptake ■ cholesteryl ester
ABCA1–bacterial artificial chromosome (BAC)-transgenic

To investigate the role of ABCA1 in HDL metabolism, plasma cholesterol in mice with overexpression of ABCA1 was addressed in some studies,5 the role of ABCA1 in HDL metabolism of other organs has not been investigated. In this study, the function of ABCA1 in plasma metabolism of 2 major components of the HDL particle (ie, apolipoproteins and CE) was addressed. Besides, the role of ABCA1 in HDL uptake by tissues and in particular by the liver was investigated. It is shown that ABCA1 is essential for the catabolism of HDL-associated apolipoproteins and lipids and for the selective uptake of HDL-associated CE, in particular by the liver.

In addition, although catabolism by liver and kidneys were addressed in studies,5 the role of ABCA1 in HDL metabolism of other organs has not been investigated. In this study, the function of ABCA1 in plasma metabolism of 2 major components of the HDL particle (ie, apolipoproteins and CE) was addressed. Besides, the role of ABCA1 in HDL uptake by tissues and in particular by the liver was investigated. It is shown that ABCA1 is essential for the catabolism of HDL-associated apolipoproteins and lipids and for the selective uptake of HDL-associated CE, in particular by the liver.

**Methods**

For Methods, please see the online supplement, available at http://atvb.ahajournals.org.

**Results**

Supplemental Figures I, II, III, and IV are available online at http://atvb.ahajournals.org

**Plasma Cholesterol in Mice With Overexpression of ABCA1**

To investigate the role of ABCA1 in HDL metabolism, ABCA1–bacterial artificial chromosome (BAC)-transgenic (BAC⁺) mice were used that express ABCA1 in the liver and in other tissues.10,11 BAC⁺ animals showed an increase in plasma total cholesterol (T-C; \( P < 0.0001; n = 6 \)) and HDL-C compared with WT littermate controls (Table 1).

Because untreated BAC⁺ mice had quantitatively only a mild increase in ABCA1 protein expression (supplemental Figure I) and in plasma HDL-C (Table), these animals were fed the liver X receptor (LXR) agonist T0901317 to increase ABCA1 and HDL-C.11 Feeding this compound to BAC⁺ mice further increased ABCA1 protein expression (supplemental Figure I). The LXR agonist yielded an additional and significant increase in plasma T-C of 69% (\( P < 0.0001; n = 7 \)) and in HDL-C of 81% (\( P < 0.0001; n = 7 \)) compared with WT controls (Table).

Because LXR agonists regulate many genes in lipid metabolism,12 ABCA1⁻⁻ mice were fed T0901317 to determine whether the increase in HDL-C observed in the presence of the compound was mediated through ABCA1. Indeed, no change in plasma HDL-C was observed in treated ABCA1⁻⁻ mice (Table). This result indicates that ABCA1 is responsible for HDL-C elevation in response to LXR agonist treatment.

**HDL Catabolism in Plasma in Mice With Overexpression of ABCA1**

After injection of \( ^{125} \text{I}- \text{TC-}[^{3} \text{H}] \text{CEt-HDL} \), the catabolism of this preparation was investigated in WT, BAC⁺, and BAC⁺ mice fed the LXR agonist.15 The plasma fractional catabolic rates (FCRs) for both HDL tracers were decreased in BAC⁺ mice compared with WT (Figure 1A). This decrease was not statistically significant, presumably because of the low levels of ABCA1 transgene expression in BAC⁺ mice. However, a significant decrease in plasma FCRs was observed in the LXR agonist-treated BAC⁺ mice (\( ^{125} \text{I}: \text{WT}, 68.9 \pm 5.7 \) versus LXR agonist–fed BAC⁺, \( 51.2 \pm 5.2 \) pools × 10³ × h⁻¹, \( n = 7 \), \( P = 0.0001; \) \( ^{3} \text{H}[\text{CEt}: \text{WT}, 157.1 \pm 13.5 \) versus LXR agonist–fed BAC⁺, \( 118.2 \pm 11.9 \) pools × 10³ × h⁻¹, \( n = 7 \), \( P = 0.0001; \) Figure 1A). If the difference in plasma FCRs is calculated

![Figure 1](http://atvb.ahajournals.org/)
showed the major changes in HDL tracer internalization in 21.2 versus LXR agonist–fed BAC compared with WT, even when BAC treated BAC uptake of both tracers (Figure 1D). The most dramatic fall in the adrenal FCRs for [3H]CEt or 125I-TC nor HDL CE substantially higher than those for [3H]CEt, and this observation is consistent with a role of the kidneys in HDL apolipoprotein catabolism.14 In this organ, the tissue uptake of 125I-TC/[3H]CEt-HDL was explored 24 h after injection in mice.9 This selective CE removal were significantly different in BAC mice (125I: WT, 28.8±4.0 versus BAC+, 25.9±1.5 pools×10^3 × h^-1, n=5, P=NS; [3H]CEt: WT, 114.9±21.2 versus BAC+, 100.5±11.4 pools×10^3 × h^-1, n=6, P=NS). This decrease in hepatic HDL tracer uptake was significantly different in LXR agonist–treated BAC+ mice (125I: WT, 28.8±4.0 versus LXR agonist–fed BAC+, 20.9±1.9 pools×10^3 × h^-1, n=6, P=0.00005; [3H]CEt: WT, 114.9±21.2 versus LXR agonist–fed BAC+, 78.3±14.0 pools×10^3 × h^-1, n=6, P=0.0009). Hepatic HDL selective CE uptake (ie, the difference between [3H]CEt and 125I-TC) decreased in BAC+ mice (WT, 86.1±21.5; BAC+, 74.5±11.5; P=NS) and was significantly lower in LXR agonist–treated BAC+ animals (WT, 86.1±21.5; LXR agonist–fed BAC+, 57.4±14.2; P=0.01; Figure 1B). As expected, quantitatively, the highest rates of HDL tracer uptake from all tissues investigated were observed in the liver.

In adrenals, a trend toward a decrease in FCR for [3H]CEt in BAC+ mice (Figure 1C) was observed. However, neither the adrenal FCRs for [3H]CEt or 125I-TC nor HDL CE selective uptake were significantly different in BAC+ mice compared with WT, even when BAC+ mice were fed the LXR agonist.

In the kidneys, ABCA1 overexpression decreased the uptake of both tracers (Figure 1D). The most dramatic fall in renal 125I-TC organ-FCR was detected in the LXR agonist–treated BAC+ mice. The renal uptake rates for 125I-TC were substantially higher than those for [3H]CEt, and this observation is consistent with a role of the kidneys in HDL apolipoprotein catabolism.14 HDL uptake by brain, heart, lungs, spleen, stomach, intestine, and carcass was also explored (data not shown). Organ FCRs for both HDL tracers of these tissues were not significantly changed in BAC+ and in BAC+ mice fed the LXR agonist compared with WT littermates.

HDL Catabolism in Plasma in Mice With Total Deficiency of ABCA1

Because ABCA1 overexpression decreased HDL catabolism, it was hypothesized in analogy to TD patients that in mice, the HDL decay is decreased in the absence of ABCA1. To address this question, mice with an induced deficiency of ABCA1 (ABCA1−/−) were used.13 ABCA1−/− mice harboring the human ABCA1 (ABCA1−/−) were included in this study because any ABCA1-mediated differences in HDL catabolism between ABCA1−/− and WT mice should be reversed when ABCA1 is re-expressed.13 Plasma T-C and HDL-C of these animals are presented in the Table. The complete absence of ABCA1 resulted in a large increase in plasma FCRs of both HDL tracers when compared with WT (125I: WT, 81.5±11.6 versus ABCA1−/−, 355.0±71.0 pools×10^3 × h^-1, n=6, P=0.0002; [3H]CEt: WT, 177.8±74.9 versus ABCA1−/−, 1273.9±203.5 pools×10^3 × h^-1, n=6, P=0.00001; Figure 2A). Selective CE removal from HDL was increased in ABCA1−/− mice (WT, 96.3±36.7 versus ABCA1−/−; 918.9±215.6; n=6; P<0.0001; Figure 2A). When rescued by BAC+, the plasma FCRs for both HDL tracers were significantly decreased to near those of WT mice (125I: ABCA1−/−, 355.0±71.0 versus BAC+/−, 140.3±93.1 pools×10^3 × h^-1, n=6, P<0.0001; [3H]CEt: ABCA1−/−, 1273.9±203.5 versus BAC+/−, 411.5±293.8 pools×10^3 × h^-1, n=6, P<0.0001), as were the rates of selective CE uptake from HDL (ABCA1−/−, 918.9±215.6 versus BAC−/−, 278.2±308.2; n=6; P=0.0009). Thus, plasma HDL catabolism and HDL-selective CE uptake by all tissues of the mice are increased in the absence of ABCA1, and this effect is reversed on ABCA1 expression.

HDL Uptake by Tissues in Mice With Overexpression of ABCA1

Tissue uptake of 125I-TC/[3H]CEt-HDL was explored 24 h after injection in mice.9,13 Liver, adrenals, and kidneys showed the major changes in HDL tracer internalization in these experiments (Figure 1). Remarkably, these tissues have high levels of ABCA1 expression.2 The liver is the principal site of HDL catabolism in rodents, and this is confirmed here (Figure 1B).9 In this organ, the uptake of both HDL-associated tracers decreased in BAC+ mice (125I: WT, 28.8±4.0 versus BAC+, 25.9±1.5 pools×10^3 × h^-1, n=5, P=NS; [3H]CEt: WT, 114.9±21.2 versus BAC+, 100.5±11.4 pools×10^3 × h^-1, n=6, P=NS). This decrease in hepatic HDL tracer uptake was significantly different in LXR agonist–treated BAC+ mice (125I: WT, 28.8±4.0 versus LXR agonist–fed BAC+, 20.9±1.9 pools×10^3 × h^-1, n=6, P=0.00005; [3H]CEt: WT, 114.9±21.2 versus LXR agonist–fed BAC+, 78.3±14.0 pools×10^3 × h^-1, n=6, P=0.0009). Hepatic HDL selective CE uptake (ie, the difference between [3H]CEt and 125I-TC) decreased in BAC+ mice (WT, 86.1±21.5; BAC+, 74.5±11.5; P=NS) and was significantly lower in LXR agonist–treated BAC+ animals (WT, 86.1±21.5; LXR agonist–fed BAC+, 57.4±14.2; P=0.01; Figure 1B). As expected, quantitatively, the highest rates of HDL tracer uptake from all tissues investigated were observed in the liver.

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organ site for HDL catabolism under any experimental conditions (Figure 2).

In ABCA1<sup>−/−</sup> mice, the ABCA1 deficiency had no impact on HDL catabolism by brain, heart, lungs, spleen, stomach, intestine, and carcass (data not shown).

**HDL Catabolism in Plasma in Mice With Liver-Specific Deficiency of ABCA1**

To address the role of hepatic ABCA1 in HDL metabolism in more detail, liver-specific ABCA1 knockout (ABCA1<sup>−/−L/−</sup>) mice were used. These animals show a substantial (P=0.0002) decrease in plasma HDL-C compared with WT (Table).

ABCA1<sup>−/−L/−</sup> mice had significantly increased plasma FCRs for both HDL tracers (125I: WT, 73.9±14.0 versus ABCA1<sup>−/−L/−</sup>, 125.7±55.9 pools×10<sup>3</sup> × h<sup>−1</sup>, n=6, P=0.03; [3H]CET: WT, 125.6±33.2 versus ABCA1<sup>−/−L/−</sup>, 339.7±145.4 pools×10<sup>3</sup> × h<sup>−1</sup>, n=6, P=0.003; Figure 3A). Besides, selective CE removal from HDL by tissues increased in ABCA1<sup>−/−L/−</sup> rodents (WT, 51.7±36.0 versus ABCA1<sup>−/−L/−</sup>, 214.0±155.9; n=6; P=0.02).
HDLCatabolism in Mice With Adenovirus-Mediated ABCA1Expression in the Liver

To address the role of hepatic ABCA1 for HDL metabolism, ABCA1 was delivered by adenovirus (Ad-ABCA1) to ABCA1−/− (supplemental Figure II) and to ABCA1−/− (supplemental Figure III) mice.4 After Ad-ABCA1 injection, plasma T-C and HDL-C of ABCA1−/− and ABCA1−/− mice significantly increased (ABCA1−/−: T-C=9.3±4.8, HDL-C=3.9±1.3 and Ad-ABCA1–treated ABCA1−/−: T-C=39.8±11.6, HDL-C=19.2±4.3, mg/dL, n=6; ABCA1−/−: T-C=10.3±4.8, HDL-C=6.5±2.4 and Ad-ABCA1–treated ABCA1−/−: T-C=51.7±5.3, HDL-C=38.3±1.3 mg/dL, n=6). In contrast, no change in plasma cholesterol was observed in mice injected with the control adenovirus. 

125I-TC/[3H]CET-HDL metabolism was investigated in ABCA1−/− and ABCA1−/− mice injected with the control virus or with Ad-ABCA1 (supplemental Figures II and III). For comparison, WT mice were included in these studies. In both groups of ABCA1-deficient mice, ABCA1 expression decreased the plasma FCRs for 125I-TC and [3H]CET significantly (P<0.001). Similarly, selective CE removal from HDL ([3H]CET-125I-TC) by the liver decreased significantly (P<0.05) because of Ad-ABCA1 injection.

Similarly, selective CE removal from plasma HDL ([3H]CET-125I-TC) by the liver decreased significantly (P<0.05) because of Ad-ABCA1 injection. In adrenals and kidneys of ABCA1−/− and ABCA1−/− mice, Ad-ABCA1 injection decreased the organ FCRs for 125I-TC and for [3H]CET close to those of WT (supplemental Figures II and III).

Hepatic Scavenger Receptor Class B Type I in Mice With Modified ABCA1 Expression

Hepatic HDL selective CE uptake is decreased in BAC+ and increased in ABCA1−/− and in ABCA1−/− mice. Scavenger receptor class B type I (SR-BI) is an HDL receptor that mediates selective lipid uptake.13,16 Therefore, SR-BI expression in liver lysates isolated from WT, BAC+, ABCA1−/−, and ABCA1−/− mice was assessed by immunoblotting. In BAC+ mice treated with the LXR agonist, hepatic SR-BI expression decreased compared with WT (supplemental Figure I). In contrast, in ABCA1−/− (supplemental Figure IV) and in ABCA1−/− mice (supplemental Figure IV), no increase in SR-BI expression was observed compared with WT liver. Analogously in BAC−/−, ABCA1−/− mice, there was no change in hepatic SR-BI (supplemental Figure IV).

Discussion

This study reinforces that ABCA1 has a dominant effect on plasma HDL-C levels. A deficiency of ABCA1 is associated with low and a high expression of this protein with increased HDL-C. The low HDL-C in liver-specific ABCA1-deficient mice and the normalized HDL-C after the adenovirus-mediated transfer of ABCA1 to the liver point to a major role of hepatic ABCA1 as primary molecule that determines HDL-C in vivo.

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Similarly, selective CE removal from plasma HDL ([3H]CET-125I-TC) by the liver decreased significantly (P<0.05) because of Ad-ABCA1 injection.
catabolism that was observed here is in line with another study using ABCA1-transgenic mice.17 With respect to whole-body HDL catabolism, quantitatively, the highest HDL tracer uptake rates were detected in the liver, and this was true for BAC+ and for WT mice. These results show that the liver is the dominant site for HDL catabolism in mice with physiological and induced ABCA1 overexpression.

These BAC+ mice with transgenic ABCA1 expression display an increase in plasma HDL-C. This rise and the decreased HDL catabolism by issues suggests that the ABCA1-mediated delay in HDL catabolism is a mechanism that contributes to the increased plasma HDL-C. However, ABCA1 is a key molecule in the formation of HDL particles as well.15 Therefore, it seems possible that ABCA1 has a dual effect on both HDL catabolism and synthesis. Both mechanisms presumably contribute to the ABCA1-dependent increase in plasma HDL-C.

HDL catabolism in mice with total or liver-specific ABCA1 deficiency was accelerated. The stimulated decay of both HDL tracers yielded an increase in selective CE uptake from plasma HDL by tissues. With respect to the increased decay of 125I-labeled apolipoproteins, these experiments agree with previous studies in ABCA1−/− mice5 and in TD patients.6 The selective CE clearance from plasma HDL was increased in ABCA1−/− and ABCA1−/−L mice. Similarly, HDL holo-particle catabolism, as represented by 125I-TC, is stimulated in both models. With respect to specific tissues, liver, adrenal, and kidney uptake of HDL tracers were significantly increased in both ABCA1-deficient animal models, and this resulted in upregulated rates of hepatic and adrenal-selective CE uptake. With respect to whole-body HDL catabolism, again, the HDL uptake rates were highest in the liver. These results provide evidence that ABCA1 in the liver plays an essential role in whole-body HDL metabolism by delaying HDL turnover and by diminishing selective CE uptake from HDL. For ABCA1−/−L mice, it has been suggested that the increased catabolism of HDL particles and of HDL apolipoproteins occurs in the kidneys and in the liver.5 This conclusion is confirmed in this study. Besides the previously described increase in HDL protein uptake in the liver of ABCA1−/−L mice, it is demonstrated here that the ABCA1-deficient murine liver displays a substantial increase in selective HDL CE uptake as well.5

ABCA1-deficient mice have very low HDL-C in plasma.5,15 Previously, it was suggested that this finding is attributable to a decreased HDL synthesis.15 However, in this study, HDL catabolism is accelerated in ABCA1-deficient mice. Therefore, the mechanism(s) underlying the low plasma HDL presumably is both attributable to a decrease in HDL synthesis and an increase in HDL catabolism.

Mice with a total ABCA1 deficiency yielded qualitatively identical results as animals with a liver-specific lack of this protein. This is true for both HDL plasma FCRs and organ FCRs. Besides, adenovirus-mediated hepatic ABCA1 expression in ABCA1−/− and in ABCA1−/−L mice or transgene-induced ABCA1 expression reversed the changes in HDL metabolism induced by a deficiency of this protein. Also, these results provide evidence that ABCA1 in the liver plays a dominant role in HDL homeostasis in vivo.

The question has to be addressed whether the increase in HDL catabolism in the absence of ABCA1 is caused by the decreased HDL plasma pool in ABCA1−/− and ABCA1−/−L mice. Patients with TD have an almost complete lack of HDL in plasma, and radiolabeled HDL is rapidly cleared from the circulation.6,7 However, this rapid HDL catabolism was independent of HDL pool size because it did not change despite the acute infusion of HDL to bring plasma HDL concentrations back to near normal.7 In TD patients, only the apolipoprotein moiety of HDL was labeled.7 Therefore, the fate of HDL CE in the face of a diminished HDL pool was unknown. However, another study injected HDL radiolabeled in its unesterified and esterified cholesterol moieties into apoA-I−/−-deficient (apoA-I−/−) mice with low plasma HDL-C levels and found that compared with WT, there was no difference in the turnover of HDL lipids in apoA-I−/− mice.18 These observations suggest that the HDL pool size does not affect HDL turnover rates. In summary, it is unlikely that an altered HDL pool size of ABCA1-deficient mice affected the result of this study.

A mechanism by which ABCA1 presumably can alter HDL catabolism in the liver is through the HDL receptor SR-BI.16 Overexpression of SR-BI decreases plasma HDL-C, accelerates HDL catabolism, and increases selective CE uptake by the liver.19 In contrast, an induced deficiency of SR-BI increases plasma HDL-C, delays HDL catabolism, and decreases hepatic selective CE uptake.13 In BAC+ mice, hepatic SR-BI expression was reduced, and this observation is consistent with the diminished selective HDL CE uptake by the liver in metabolic studies. However, in ABCA1−/− and in ABCA1−/−L mice, the hepatic expression of SR-BI was similar as in WT controls, although selective HDL CE uptake by the liver was substantially upregulated in vivo. This lack of regulation of SR-BI is consistent with a previous report.20 One possibility that might explain these discrepancies is an SR-BI−independent mechanism for HDL lipid uptake; such a pathway could mediate hepatic HDL CE uptake at least in part.13 Besides, a novel HDL receptor has been defined that may play a role in HDL degradation as well.21

In summary, ABCA1 has a substantial impact on HDL metabolism in plasma and by tissues in mice. ABCA1 overexpression retards the catabolism of HDL in plasma and decreases selective HDL CE uptake by the liver. In contrast, an ABCA1 deficiency accelerates HDL catabolism in plasma and increases HDL-selective CE uptake by the liver. All experiments are in line with a key function of ABCA1 in the liver for HDL metabolism in vivo. Besides, the liver also quantitatively has a dominant function in HDL catabolism in mice with a deficiency or a high level of ABCA1 expression.

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For Acknowledgments, please see the online supplement.

**Disclosures**

None.

**References**

Singaraja et al. ABCA1 and HDL Cholesteryl Ester Metabolism in Mice


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Online supplement

**Hepatic ABCA1 is a key molecule in HDL cholesteryl ester metabolism in mice**


**Methods**

**Mice**

Mice transgenic for the human ABCA1 gene (BAC⁺) were described (1,2) and were pure C57BL6/J. ABCA1 knockout (ABCA1⁻⁻) (3) and liver-specific ABCA1 knockout (ABCA1⁻⁻/⁻⁻) (4) mice were obtained from Dr. Omar L. Francone (Pfizer Global Research) and Dr. John S. Parks (Wake Forest University) respectively, and were approximately 80% C57BL6/J. Littermate controls of the same sex and age were used in all studies. In some cases, mice were fed orally the LXR agonist T-0901317 (Tularik®, Sigma) or the solvent for 4 days at a dose of 10 mg/kg/day (2). All mice were maintained on chow diet.

For adenovirus-mediated expression of ABCA1 in the liver, 5x10⁸ pfu/mouse of control adenovirus were injected through the tail vein in order to saturate the uptake of viral particles by Kupffer cells (5,6). Three hours later, 5x10⁸ pfu/mouse of adenovirus harboring human ABCA1 (Ad-ABCA1) or the respective control virus were tail vein injected. Three days later, HDL metabolism was investigated in these rodents. All animal protocols were approved by the institutional animal care committees.

**Isolation and radiolabeling of HDL**

HDL (d = 1.063-1.21 g/ml) was isolated from WT mice (C57BL6/J) by ultracentrifugation and labeled with ¹²⁵I-Tyramine Cellobiose (¹²⁵I-TC) in its protein and
[^3]H]Cholesteryl Oleyl Ether ([^3]H)CEt) (Amersham) in its lipid moiety (7,8). Both tracers are intracellularly trapped after uptake and are not released from tissues (8).

**HDL metabolism in mice**

Plasma decay rates for ^125^I-TC-/[^3]H]CEt-HDL. Mice were fasted for 4 hours and injected with ^125^I-TC-/[^3]H]CEt-HDL via the tail vein (9,10). Plasma decay data were obtained by drawing periodic blood samples (30 µl per time point) at 0.16, 0.5, 2, 6, 20 and 24 hours after injection. Animals were fasted throughout the 24 hour study period but had unlimited access to water. Aliquots of plasma were directly assayed for ^125^I radioactivity and[^3]H] was analyzed after lipid extraction (11). Computer analysis was used to fit a least-squares multiexponential curve to each set of plasma decay data and to calculate plasma-fractional catabolic rates (plasma-FCR) (7,12).

Tissue analysis of ^125^I-TC-/[^3]H]CEt-HDL uptake. Tissue sites of uptake of HDL-associated ^125^I-TC and[^3]H]CEt were determined 24 hours after injection of ^125^I-TC-/[^3]H]CEt-HDL when both tracers were predominantly cleared from plasma (9,10). Finally the animals were anesthetized, the abdomen and chest were opened, and a catheter was inserted into the heart. The inferior vena cava was cut, and the mice were perfused with saline (50 ml per animal). After perfusion, liver, adrenals, kidneys, brain, heart, lungs, spleen, stomach, intestine and carcass were harvested and homogenized. Homogenates from each tissue and from carcass were directly assayed for ^125^I radioactivity and aliquots were analyzed for[^3]H] after lipid extraction (11).

Total radioactivity recovered from all tissues and from carcass of each mouse was calculated (9). The fraction of total tracer uptake attributed to a specific organ was calculated as the radioactivity recovered in that organ divided by the total radioactivity
recovered from all tissues and carcass. Thus the % of recovered extravascular radioactivity in tissues is determined 24 h after injection of labeled HDL.

To allow direct comparison of the specific activities of the various tissues in HDL uptake and to directly compare the rates of uptake of the apolipoprotein component and the CE moiety of HDL in tissues, the data are expressed as organ-fractional catabolic rates (organ-FCR) (8,9). These rates refer to the whole organ.

The organ-FCR is calculated as follows:

\[
\text{Organ-FCR in tissue } X = \text{Plasma-FCR} \times \left(\frac{\text{Fraction[%] of Total Body Tracer Recovery in Tissue } X}{100}\right)
\]

These organ-FCR’s represent the fraction of the plasma pool of either HDL tracer cleared by an organ per hour (8).

Selective HDL CE uptake is calculated as the difference in organ-FCR’s between \(^{3}\text{H}\)CEt and \(^{125}\text{I}\)-TC (8,9).

**Immunoblots**

Protein lysates were prepared from livers, separated by SDS-PAGE under non-reducing conditions, transferred to polyvinylidene fluoride (PVDF) membranes (Millipore) and probed with anti-SR-BI (Novus Biochemicals), anti-ABCA1 (13) and anti-GAPDH (Chemicon) antibodies. GAPDH was used as a loading control.

Densitometric scanning was performed using Quantity One software (BioRad).

**Calculations and Statistics**

All statistical analyses were performed using Students t-test. Values are means ±SD. ns = not significant at p=0.05.
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References for methods/Online supplement


Supplemental Figure legends (online)

Figure I. ABCA1 and SR-BI expression in the liver of WT, BAC^+ and BAC^+ mice fed the LXR agonist

Protein lysates (40 µg per lane) from the respective murine livers were immunoblotted for ABCA1, SR-BI and GAPDH. Three independent similar blots yielded qualitatively identical results.

Figure II. Plasma decay and uptake of ^125I-TC-/[^3H]CEt-HDL by liver, adrenals and kidneys in WT, ABCA1^+/+ and ABCA1^+/− mice injected with Ad-ABCA1

WT and ABCA1^+/+ mice were injected with a control virus or Ad-ABCA1 as described in Methods. 72 hours later, ^125I-TC-/[^3H]CEt-HDL was injected in these animals. Then blood was harvested periodically for 24 hours and tissues were finally collected. Plasma and tissue samples were analyzed for both HDL tracers. Plasma (A), liver (B), adrenal (C), and kidney (D) FCR’s for ^125I-TC (^125I) and [^3H]CEt (^3H) and selective HDL CE uptake (^3H – ^125I) were calculated. Values are means ± SD. n=6 mice per group.

Figure III. Plasma decay and uptake of ^125I-TC-/[^3H]CEt-HDL by liver, adrenals and kidneys in WT, ABCA1^L/L and ABCA1^L/L mice injected with Ad-ABCA1

WT and ABCA1^L/L mice were injected with a control virus or Ad-ABCA1 as outlined in Methods. 72 hours later, ^125I-TC-/[^3H]CEt-HDL was administered intravenously to these animals. Thereafter blood was harvested periodically for 24 hours and tissues collected. Plasma and tissue samples were analyzed for both HDL tracers. Plasma (A), liver (B), adrenal (C) and kidney (D) FCR’s for ^125I-TC (^125I) and [^3H]CEt (^3H) and selective HDL CE (^3H – ^125I) uptake were calculated. Values are means ± SD. n=6 mice per group.
Figure IV. ABCA1 and SR-BI expression in the liver of WT, ABCA1<sup>+/−</sup>, BAC<sup>+</sup>ABCA1<sup>+/−</sup> and ABCA1<sup>L/L</sup> mice

Protein lysates (40 µg per lane) from the respective murine liver were immunoblotted for ABCA1, SR-BI and GAPDH. In each case, at least three independent similar blots yielded qualitatively identical results.
Figure I

**Ratio ABCA1 : GAPDH**

WT  | BAC⁺  | BAC⁺ + LXR agonist
---  | ------|---------------------
0,62 | 0,66  | 0,70
0,66 | 0,70  | 0,74
0,70 | 0,74  | 0,78
0,74 | 0,78  |

**Ratio SR-BI : GAPDH**

WT  | BAC⁺  | BAC⁺ + LXR agonist
---  | ------|---------------------
0,2  | 0,4   | 0,6
0,4  | 0,8   | 1,0
0,6  | 1,2   | 1,4
Figure II

A  Plasma-FCR

B  Liver-FCR

C  Adrenal-FCR

D  Kidney-FCR

* p<0.05  ** p<0.001  *** p<0.0001
Figure IV

A

ABCA1
SR-BI
GAPDH

WT  ABCA1−/−  BAC⁺  ABCA1−/−

B

ABCA1
SR-BI
GAPDH

WT  ABCA1−/−

BAC⁺  ABCA1−/−

Ratio SR-BI : GAPDH

WT  ABCA1−/−  BAC⁺  ABCA1−/−

A