Hepatic lipase deficiency decreases the selective uptake of HDL-cholesteryl esters in vivo

Gilles Lambert,[1,]* Marcelo J. A. Amar,* Pascal Martin,* Jamila Fruchart-Najib,† Bernhard Föger,* Robert D. Shamburek,* H. Bryan Brewer, Jr.,* and Silvia Santamarina-Fojo*

Molecular Disease Branch,* National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, and Département d'Athérosclérose U325 INSERM,† Institut Pasteur, Lille, France

Abstract Recent in vitro studies have provided evidence that hepatic lipase (HL) facilitates the selective uptake of HDL cholesteryl esters (CE), but the in vivo physiological relevance of this process has not been demonstrated. To evaluate the role that HL plays in facilitating the selective uptake of HDL-CE in vivo, we studied the metabolism of [^{3}H]CEt, ^{125}I-labeled apolipoprotein (apo) A-I, and ^{131}I-labeled apoA-II-labeled HDL in HL-deficient mice. Kinetic analysis revealed similar catabolism of ^{125}I-labeled apoA-I (as well as ^{131}I-labeled apoA-II) in C57BL controls and HL deficient mice, with fractional catabolic rates (FCR) of 2.17 ± 0.15 and 2.16 ± 0.11 d^{-1} (2.59 ± 0.14 and 2.67 ± 0.13 d^{-1}, respectively). In contrast, despite similar hepatic scavenger receptor BI expression, HL-deficient mice had delayed clearance of [^{3}H]CEt compared to controls (FCR = 3.66 ± 0.29 and 4.41 ± 0.18 d^{-1}, $P < 0.05$). The hepatic accumulation of [^{3}H]CEt in HL-deficient mice (62.3 ± 2.1% of total) was significantly less than in controls (72.7 ± 3.0%), while the [^{3}H]CEt remaining in the plasma compartment increased (20.7 ± 1.8% and 12.6 ± 0.5%) ($P < 0.05$, all). In summary, HL deficiency does not alter the catabolism of apoA-I and apoA-II but decreases the hepatic uptake and the plasma clearance of HDL-CE. These data establish for the first time an important role for HL in facilitating the selective uptake of HDL-CE in vivo.—Lambert, G., M. J. A. Amar, P. Martin, J. Fruchart-Najib, B. Föger, R. D. Shamburek, H. B. Brewer, Jr., and S. Santamarina-Fojo. **Hepatic lipase deficiency decreases the selective uptake of HDL-cholesteryl esters in vivo.** *J. Lipid Res.* **2000.** 41: **667–672.**

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Hepatic lipase (HL) is a 66-kDa lipolytic enzyme synthesized primarily by the liver and attached to the vascular endothelium of the liver, ovaries, and adrenals via heparan sulfate proteoglycans (1–3). HL hydrolyzes triglycerides and phospholipids present primarily on chylomicron remnants, intermediate density lipoproteins, and high density lipoproteins (HDL) (4). In addition to its traditional role as a lipolytic enzyme, HL may serve as a ligand that mediates the interactions of lipoproteins with cell surface proteoglycans and receptors, facilitating the cellular uptake of lipoprotein particles and/or lipoprotein lipids (reviewed in ref. 5). HL has been shown to increase HDL cholesterol uptake in perfused rat livers (6, 7) and in cultured cells (8, 9). In vivo, overexpression of HL using either recombinant adenovirus (10, 11) or genetic manipulation (12, 13) significantly decreases plasma HDL concentrations. Conversely, HL deficiency in humans (14), as well as HL deficiency in rodents, induced by infusion of anti-HL antibodies (15) or by targeted disruption of the HL gene (16), led to increased plasma HDL concentrations. Despite these advances, the mechanism by which HL enhances the lipid and/or whole lipoprotein particle uptake of HDL has not been definitively established. One potential pathway involves the scavenger receptor BI (SR-BI) that mediates the selective uptake of cholesteryl esters (CE) from HDL (17). This receptor is expressed primarily in liver and steroidogenic tissues where HL is also localized (3). Although SR-BI is up-regulated in the adrenals of HL knockout female mice (18), adrenal cholesterol stores are reduced, suggesting that HL may be necessary for optimal SR-BI-mediated HDL-CE uptake. In a recent report we showed that HL enhances the selective uptake of HDL-CE via SR-BI in cultured cells by a process that requires both the ligand-binding and the lipolytic function of the enzyme (19). Using CHO cells expressing SR-BI, Collet et al. (20) showed that there was a 2-fold increase in the SR-BI-mediated selective uptake of CE from HDL incubated with HL, compared to non-treated HDL. However, the role of HL in facilitating the selective uptake of HDL-CE remains to be established in vivo.

In the present study, we demonstrate that HL deficiency in mice *1*) does not alter the catabolism of apoA-I and apoA-II but *2*) decreases the hepatic uptake and the plasma clearance of HDL-CE. These findings provide for

Abbreviations: HL, hepatic lipase; HDL, high-density lipoprotein; apo, apolipoprotein; SR-BI, scavenger receptor class B type I; CE, cholesteryl ester; CEt, cholesteryl ether; ELISA, enzyme linked immunosorbent assay; FCR, fractional catabolic rate; PR, production rate.

[1] To whom correspondence should be addressed.

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the first time direct evidence for a role of HL in facilitating the selective uptake of HDL-CE in vivo.

MATERIAL AND METHODS

Animals and diet

HL knockout mice (16) were backcrossed to strain C57BL/6 for at least 6 generations. Control C57BL/6 mice were obtained from Charles River (Wilmington, MA). Both control and homozygous HL knockout mice were 3 months old at the beginning of the study.

Measurement of plasma lipids, apolipoproteins and lipoproteins

Plasma total cholesterol and triglycerides (Sigma, St. Louis, MO), as well as free cholesterol and phospholipids (Wako, Osaka, Japan) were measured in 12-μl aliquot of plasma using commercial kits and the Hitachi 911 automated chemistry analyzer (Boehringer-Mannheim, Indianapolis, IN). HDL-cholesterol was determined after heparin–$CaCl_2$ precipitation of apoB-containing lipoproteins (21). Mouse apoA-I and A-II were quantitated by a sandwich enzyme-linked immunosorbent assay (ELISA) as previously described (22). Lipoproteins from pooled plasma samples (100 μl) were separated by gel filtration using two Superose 6HR 10/30 columns in series (FPLC) (Pharmacia Biotech Inc., Piscataway, NJ) at 0.3 ml/min with PBS buffer containing 0.02% EDTA and 0.04% sodium azide.

Labeling of HDL with [^{3}H]CEt

HDL from both control and HL knockout mice were labeled with [^{3}H]cholesteryl-palmityl-ether (CEt), as previously described (23). Homogeneous labeling of HDL was ascertained by superimposing radioactivity and chemistry profiles obtained by FPLC. More than 99% of the label co-migrated with CEt on thin-layer chromatography. The radioactivity of ^{3}H was quantitated in a Tri-Carb 2500 TR liquid scintillation counter (Packard, Downers Grove, IL).

Labeling of HDL with ^{125}I-labeled-apoA-I and ^{131}I-labeled-apoA-II

Lyophilized mouse apoA-I and apoA-II were iodinated by a modification of the iodine monochloride method (24). Approximately 0.5 mol of iodine was incorporated per mole of protein. Four μg of ^{125}I-labeled mouse apoA-I and ^{131}I-labeled mouse apoA-II were mixed with HDL ($1.063 < d < 1.21$) from both C57BL and HL knockout mice (250 μg of protein). Labeled HDL was dialyzed extensively against PBS containing 0.01% EDTA at 4°C. To assess the distribution of apoA-I and apoA-II amongst the different lipoproteins, 50 μl of plasma from either control or HL knockout mice was mixed with either ^{125}I-labeled apoA-I or ^{131}I-labeled apoA-II (10^4 cpm each) and the radiolabeled lipoproteins were separated by FPLC. Radioactivity (^{125}I /^{131}I) was measured in the eluted fractions, using a Cobra γ-counter (Packard, Downers Grove, IL).

HDL metabolic studies

Mice were anesthetized by intraperitoneal injection of 11 μl/g of body weight of 2.5% Avertin (Aldrich, Milwaukee, IL). [^{3}H]CEt-labeled HDL (5×10^5 dpm; specific activity 40 dpm/ng HDL-CE) and ^{125}I-labeled apoA-I/^{131}I-labeled apoA-II-labeled HDL (10^6 dpm; specific activities 50 dpm/ng apoA-I and 55 dpm/ng apoA-II) were injected into the exposed saphenous veins of mice, using a 1-cc insulin syringe (Becton Dickinson, Franklin Lakes, NJ). Prior to each blood collection, mice were briefly anesthetized with methoxyflurane (Pitman-Moore, Mundelein, IL). Blood samples were collected in capillary tubes coated with heparin (Scientific Products, McGaw Park, IL) from the retro-orbital plexus and centrifuged at 2500 *g* for 20 min at 4°C. Plasma was removed, aliquoted, and immediately frozen. The plasma disappearance curves were generated by dividing the plasma radioactivity at each time point by the radioactivity of the injected HDL. More than 98% of the injected dose was present in the plasma at the 1-min time point. The fractional catabolic rates (FCR) were determined from the area under the curves using a multiexponential curve-fitting technique on the SAAM program as previously described (25). ApoA-I and apoA-II production rates (PR) were calculated using the formula: $PR = [FCR\ (d^{-1}) \times \text{plasma volume (ml)} \times \text{plasma apolipoprotein concentration } (\mu g/ml)] / [\text{body weight (g)}]$. Plasma volume was estimated as 3.16% of body weight. The lipoprotein distribution of [^{3}H]CEt, ^{125}I-labeled apoA-I and ^{131}I-labeled apoA-II was assessed by FPLC analysis of plasma samples at the 1-min and 10-h time points. After the 10-h blood sample was collected, a subset of mice injected with [^{3}H]CEt-HDL were exsanguinated (1.2 ml blood each) and killed by cervical dislocation. Liver, adrenals, ovaries, heart, lungs, kidneys, and spleen were harvested. CEt was extracted in 20 volumes of chloroform–methanol, 2:1 (v/v). Phases were separated by the addition of water and aliquots of the lower organic phase were counted. Mean recoveries at 10 h were greater than 90% of the injected doses.

Quantification of hepatic SR-BI expression

Livers (0.5 g wet weight) from a subset of control and HL knockout mice were harvested, cut into small pieces, and immediately transferred in 5 ml of PBS 1× containing a protease inhibitor cocktail P2714 (Sigma, St Louis, MO). The suspensions were homogenized and centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was centrifuged in a TI-60 rotor at 40,000 rpm using a L8-70M ultracentrifuge (Beckman, Palo Alto, CA) for 1 h at 4°C. Pellets were resuspended in NuPAGE LDS sample buffer (1× final concentration). Immunoblot analysis of SR-BI expression was performed as previously described (19).

Statistical analysis

Results are expressed as mean ± SEM. The statistical significance of the differences of the mean between two groups is evaluated using the Student's *t*-test. P values < 0.05 are considered to be statistically significant.

RESULTS

The plasma lipids, lipoproteins, and apolipoproteins of the C57BL and HL knockout mice used in the present study are summarized in **Table 1**. Compared to age- and sex-matched C57BL control animals, HL-knockout mice had 2- to 2.6-fold higher plasma concentrations of total cholesterol, phospholipids, cholesteryl esters as well as HDL-cholesterol and 1.35-fold higher apoA-I and apoA-II plasma concentrations ($P < 0.01$, all), but similar levels of fasting plasma triglycerides. To assess the lipoprotein distribution of apoA-I and apoA-II in both mouse models, radiolabeled ^{125}I-labeled apoA-I and ^{131}I-labeled apoA-II were added to plasma from both control and HL knockout mice and the lipoproteins were separated by FPLC. Greater than 98% of the radiolabeled apoA-I and apoA-II were found to be associated with lipoproteins eluting in the

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TABLE 1. Plasma lipids, lipoproteins, and apolipoproteins of C57BL and HL knockout female mice

	C57BL (n = 15)	HL-KO (n = 15)
	mg/dL	*mg/dL*
TC	60 ± 5	144 ± 34[a]
CE	43 ± 4	113 ± 28[a]
TG	73 ± 4	72 ± 14
PL	117 ± 6	236 ± 51[a]
HDL-C	58 ± 6	122 ± 12[a]
ApoA-I	53 ± 10	73 ± 6[a]
ApoA-II	23 ± 1	31 ± 5[a]

[a] $P < 0.01$ compared to C57BL.

HDL elution volume range (i.e., 25–31 ml) for both C57BL and HL knockout mice (data not shown).

To evaluate the effects of HL on the selective uptake of HDL-CE in vivo, we performed a series of kinetic analyses of ^{125}I-labeled apoA-I, ^{131}I-labeled apoA-II, and [^{3}H]CEt-labeled HDL in C57BL and HL knockout mice. Plasma was collected at multiple time points after the initial injection of radiolabeled HDL and the plasma decay of ^{125}I-labeled apoA-I, ^{131}I-labeled apoA-II, and [^{3}H]CEt were ascertained (**Fig. 1 A–C**). Compared to C57BL controls, the plasma clearance of [^{3}H]CEt HDL was delayed in HL-deficient mice (FCR = 4.41 ± 0.18 and 3.66 ± 0.29d^{-1}, respectively, $P < 0.05$). In contrast, the catabolism of both ^{125}I-labeled apoA-I labeled HDL (FCR = 2.17 ± 0.15 and 2.16 ± 0.11 d^{-1}) and ^{131}I-labeled apoA-II-labeled HDL (FCR = 2.59 ± 0.14 and 2.67 ± 0.13 d^{-1}) were similar ($P > 0.5$) in C57BL and HL knockout mice, respectively. ApoA-I and apoA-II production rates were increased in HL knockout mice (50 ± 3.6 and 38.5 ± 2.1 μg/g·d) compared to controls (36.2 ± 1.9 and 27.8 ± 1.4 μg/g·d, respectively). FPLC analysis of plasma samples at 1 min and 10 h (Fig. 1 D–F) after injection demonstrated that greater than 98% of [^{3}H]CEt, ^{125}I-labeled apoA-I and, ^{131}I-labeled apoA-II remained associated with HDL. Thus, the absence of HL in HL knockout mice significantly decreases the clearance of HDL-CE without altering HDL whole particle catabolism.

HL deficiency in mice also markedly decreased the hepatic accumulation of [^{3}H]CEt derived from HDL (**Table 2**). Ten hours after injection of [^{3}H]CEt-labeled HDL, the [^{3}H]CEt present in the liver of HL knockout mice (62.3 ±

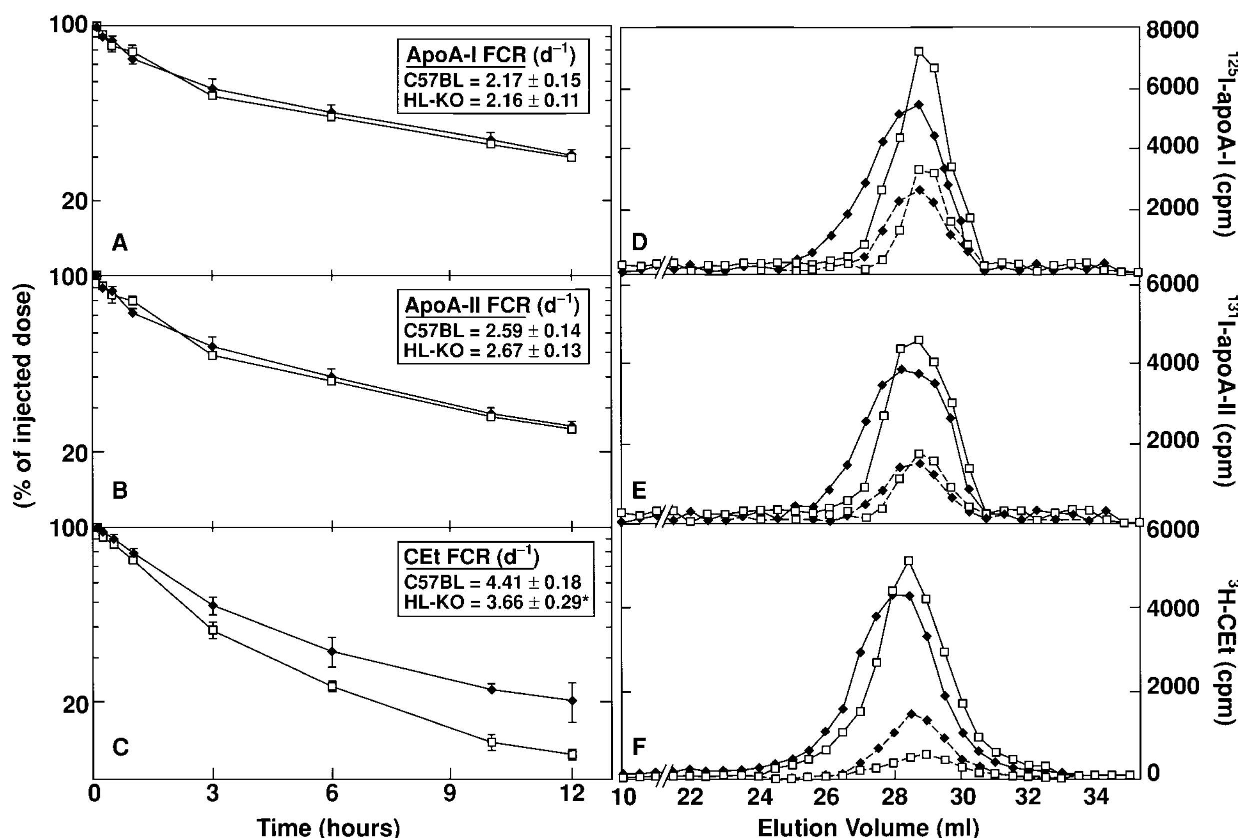

Fig. 1. Kinetic analysis of ^{125}I-labeled apoA-I, ^{131}I-labeled apoA-II, and [^{3}H]CEt in C57BL (□) and HL knockout (◆) female mice. ^{125}I-labeled apoA-I and ^{131}I-labeled apoA-II-labeled HDL was injected into 5 HL-knockout and 5 C57BL control mice. [^{3}H]CEt-labeled HDL was injected into 10 HL-knockout and 10 C57BL control mice. The plasma disappearance curves (panels A–C) were generated by dividing the plasma radioactivity at each time point by the radioactivity of the injected material. The FCR (inset within panels A–C) were determined from the area under the curves using a multiexponential curve fitting technique on the SAAM program. The plasma disappearance of ^{125}I-labeled apoA-I (panel D), ^{131}I-labeled apoA-II (panel E), and [^{3}H]CEt (panel F) was further assessed by FPLC analysis of plasma samples at the 1-min (straight lines) and 10-h (dotted lines) time points; * $P < 0.05$.

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TABLE 2. [^{3}H]CEt organ distribution, 10 h after infusion of [^{3}H]CEt-HDL in C57BL and HL knockout mice

	C57BL (n = 6)	HL-KO (n = 6)
	% of injected dose	
Plasma	12.6 ± 0.5	20.7 ± 1.8[a]
Liver	72.7 ± 3.0	62.3 ± 2.1[a]
Adrenals	0.40 ± 0.12	0.36 ± 0.11
Ovaries	0.38 ± 0.03	0.31 ± 0.07
Lungs	1.59 ± 0.06	1.22 ± 0.13
Kidneys	1.48 ± 0.09	1.70 ± 0.07
Heart	0.71 ± 0.08	0.70 ± 0.08
Spleen	0.40 ± 0.03	0.31 ± 0.07

[a] $P < 0.01$ compared to C57BL.

2.1% of total) was significantly less ($P < 0.01$) than the [^{3}H]CEt present in the livers of C57BL control mice (72.7 ± 3.0% of total). Conversely, the [^{3}H]CEt remaining in the plasma compartment was increased in HL knockout mice compared with controls (20.7 ± 1.8 and 12.6 ± 0.5% of total, respectively, $P < 0.05$). The sum of the [^{3}H]CEt present in the plasma and liver accounted for more than 80% of the injected dose in both groups. In contrast to the liver and plasma, no difference in the accumulation of [^{3}H]CEt in the kidneys, spleen, heart, lungs, adrenals, and ovaries between the two study groups was detected (Table 2). This indicates that the decrease in HDL-CEt plasma clearance and the hepatic accumulation of HDL-CEt in HL knockout mice is due to decreased selective hepatic uptake of CEt from HDL.

To rule out the possibility that the decreased hepatic selective uptake of HDL-CE observed in HL knockout mice is secondary to reduced expression of SR-BI in the liver, we measured the expression of SR-BI in both C57BL and HL-deficient mice by immunoblot analysis of liver extracts. **Figure 2** illustrates representative data from endogenous SR-BI expression in C57BL and HL-deficient mice matching for body and liver weights, age, and sex. Similar amounts of endogenous hepatic SR-BI expression were found in both HL knockout and C57BL controls.

DISCUSSION

Recent studies have demonstrated that HL enhances the selective uptake of HDL-CE via SR-BI in cultured cells

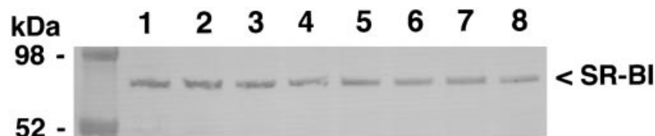

Fig. 2. Immunoblot analysis of SR-BI hepatic expression in C57BL and HL knockout female mice. Mouse liver extracts (10 μg protein) were analyzed by polyacrylamide gel electrophoresis followed by immunoblot analysis using a rabbit polyclonal antibody recognizing the 82-kDa mouse SR-BI. Lanes 1,2,5,6 HL-knockout mice; lanes 3,4,7,8 C57BL mice.

(19, 20). For maximum efficiency, this process requires both the lipolytic function and the ligand-binding role of the enzyme (19). Thus, HL-mediated HDL lipolysis and ligand interaction with SR-BI may be essential for the selective uptake of HDL-CE. However, the physiological relevance of this HL function in HDL metabolism remains to be established in vivo. Here, we examine the potential role of HL in facilitating the selective uptake of HDL-CE in vivo by performing a series of autologous HDL metabolic studies in HL knockout and C57BL control mice. HL knockout mice represent an ideal animal model to address this question because, unlike previous reports using antibodies to inhibit HL activity (15), HL knockout mice have a complete deficiency of HL, precluding the action of endogenous HL either as a lipolytic enzyme or as a ligand.

Our findings demonstrate that HL deficiency in HL-knockout mice significantly delays the plasma clearance of [^{3}H]CEt HDL. This data is consistent with previous rat liver perfusion data, which have shown that the uptake of CE from reconstituted HDL is decreased when the livers are HL depleted with heparin (6, 7). Similarly, in vitro studies have demonstrated that HL expression enhances the selective uptake of HDL cholesteryl esters in CHO cells (8) and in McArdle cells where this process has been proposed to be largely mediated by interactions with heparan sulfate proteoglycans (9).

In contrast to our findings with [^{3}H]CEt HDL, the catabolism of ^{125}I-labeled apoA-I and ^{131}I-labeled apoA-II-labeled HDL was similar between control and HL knockout mice indicating that the major difference in the plasma clearance of [^{3}H]CEt HDL between the two mouse models involved a selective uptake pathway. Despite similar apoA-I and apoA-II FCRs, the plasma concentrations of apoA-I and apoA-II were increased 1.35-fold in HL knockout compared to control mice. FPLC analysis revealed that greater than 98% of ^{125}I-labeled-apoA-I and ^{131}I-labeled-apoA-II remained associated with HDL during the metabolic study, ruling out the possibility that a significant proportion of either apoA-I or apoA-II was catabolized with non-HDL lipoproteins. Based on the FCRs and plasma pool size, the production rates for apoA-I and apoA-II were increased in HL knockout mice compared to controls, suggesting the presence of feedback regulation of apoA-I and apoA-II expression in HL knockout mice. Interestingly, overexpression of recombinant HL by 30-fold using adenovirus enhanced the catabolism of not only HDL cholesterol but also of apoA-I and apoA-II (26), suggesting that in contrast to our findings in HL knockout mice, overexpression of HL enhances both whole particle catabolism as well as selective uptake of HDL-CE. It is important to emphasize, however, that the HDL ($1.063 < d < 1.210$) used in the present study, excluded the large HDL_1 particles. It is possible that decreased clearance of apoA-I and/or apoA-II from large HDL_1 was not detected in the study.

Our combined results indicate that the effect of HL on HDL metabolism in vivo is mediated, at least in part, by a selective uptake pathway. Thus HL deficiency decreases

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the plasma clearance of HDL-CE, without significantly altering the catabolism of apoA-I and apoA-II. Furthermore, the [^{3}H]CEt remaining in the plasma compartment is increased and the hepatic accumulation of [^{3}H]CEt derived from HDL is decreased in HL knockout mice compared to controls, indicating that the hepatic transfer of HDL-CE and thus the process of reverse cholesterol transport is impaired in the absence of HL in vivo.

Our data also demonstrated that most of the radiolabeled HDL-CEt was present in the liver and plasma, with only minor accumulation (<5% of total [^{3}H]CEt counts) detected in the other organs tested (i.e., lungs, kidneys, adrenals, ovaries, spleen, heart). We found no significant difference in the uptake of [^{3}H]CEt between HL-deficient and C57BL control mice in these organs. These findings contrast with those reported by Wang et al. (18) who reported that the cholesterol stores, as determined by quantitation of total tissue cholesterol, were depleted in the adrenals of HL-deficient female mice despite up-regulation of SR-BI. The fact that our study measures CEt rather than total cholesterol accumulation and that the quantity of [^{3}H]CEt present in adrenals is small (<0.4% of total) may, at least partially, explain the discrepant findings.

It appears that the action of HL on HDL facilitates the selective uptake of HDL-CE in tissues where SR-BI and HL co-localize (i.e., liver and steroidogenic tissues). We and others have previously shown that HL and SR-BI synergistically enhance the selective uptake of HDL-CE in transfected cells (19, 20), suggesting that SR-BI-mediated selective uptake of HDL-CE is the metabolic pathway impaired in the liver of HL knockout mice. In our report, similar levels of SR-BI expression were observed in the livers of HL knockout and control mice, ruling out the possibility that the decreased hepatic selective uptake of HDL-CE observed in HL knockout mice might result from decreased hepatic expression of SR-BI.

In summary, the present study demonstrates that HL deficiency decreases the plasma clearance and the hepatic uptake of HDL-CE without significantly changing the catabolism of apoA-I and apoA-II. These findings establish for the first time direct in vivo evidence to support a role of HL in facilitating the hepatic selective uptake of HDL-CE and thus modulating reverse cholesterol transport and provide new insights into the role of HL in HDL metabolism.

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