Liver ABCA1 Deletion in LDLrKO Mice Does Not Impair Macrophage Reverse Cholesterol Transport or Exacerbate Atherogenesis

Xin Bi, Xuewei Zhu, MyNgan Duong, Elena Y. Boudyguina, Martha D.Wilson, Abraham K. Gebre, John S. Parks

Objective—Hepatic ATP binding cassette transporter A1 (ABCA1) expression is critical for maintaining plasma high-density lipoprotein (HDL) concentrations, but its role in macrophage reverse cholesterol transport and atherosclerosis is not fully understood. We investigated atherosclerosis development and reverse cholesterol transport in hepatocyte-specific ABCA1 knockout (HSKO) mice in the low-density lipoprotein (LDL) receptor KO (LDLrKO) C57BL/6 background.

Approach and Results—Male and female LDLrKO and HSKO/LDLrKO mice were switched from chow at 8 weeks of age to an atherogenic diet (10% palm oil, 0.2% cholesterol) for 16 weeks. Chow-fed HSKO/LDLrKO mice had HDL concentrations 10% to 20% of LDLrKO mice, but similar very low-density lipoprotein and LDL concentrations. Surprisingly, HSKO/LDLrKO mice fed the atherogenic diet had significantly lower (40% to 60%) very low-density lipoprotein, LDL, and HDL concentrations (50%) compared with LDLrKO mice. Aortic surface lesion area and cholesterol content were similar for both genotypes of mice, but aortic root intimal area was significantly lower (20% to 40%) in HSKO/LDLrKO mice. Although macrophage ^{3}H-cholesterol efflux to apoB lipoprotein–depleted plasma was 24% lower for atherogenic diet–fed HSKO/LDLrKO versus LDLrKO mice, variation in percentage efflux among individual mice was <2-fold compared with a 10-fold variation in plasma HDL concentrations, suggesting that HDL levels, per se, were not the primary determinant of plasma efflux capacity. In vivo reverse cholesterol transport, resident peritoneal macrophage sterol content, biliary lipid composition, and fecal cholesterol mass were similar between both genotypes of mice.

Conclusions—The markedly reduced plasma HDL pool in HSKO/LDLrKO mice is sufficient to maintain macrophage reverse cholesterol transport, which, along with reduced plasma very low-density lipoprotein and LDL concentrations, prevented the expected increase in atherosclerosis. **(*Arterioscler Thromb Vasc Biol*. 2013;33:2288-2296.)**

Key Words: atherosclerosis ■ cardiovascular diseases ■ cholesterol ■ lipids ■ lipoproteins

Atherosclerosis-associated cardiovascular disease (CVD) is the leading cause of death worldwide.[1] The inverse relationship between plasma high-density lipoprotein (HDL) levels and CVD risk has made raising HDL levels a popular potential therapeutic target for CVD prevention. ATP binding cassette transporter A1 (ABCA1) belongs to a large family of the ATP binding cassette transporters.[2] ABCA1 mediates cellular free cholesterol (FC) and phospholipid efflux to apolipoprotein A-I (apoA-I), resulting in the formation of nascent HDL that undergoes subsequent maturation to become plasma HDL.[2] The critical role of ABCA1 in HDL formation was established when it was found to be the genetic defect in Tangier disease, a disorder in which HDL levels are <5% of normal.[3–5] Studies with animal models have also documented the essential function of ABCA1 in maintaining plasma HDL levels.[6–8]

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Despite the well-established role of ABCA1 in HDL formation, its effect on atherogenesis is less clear. Premature atherosclerosis has been reported in some, but not all, people with Tangier disease.[9] Common genetic variants in ABCA1 have been reported to influence the risk and severity of CVD[10–12]; however, low HDL caused by loss-of-function mutations in ABCA1 does not contribute to increased risk of CVD in the general population.[13] Controversial results also exist in studies with mouse models. Overexpression of human ABCA1 (hABCA1) in the liver and macrophages of B6 mice resulted in an antiatherogenic lipid profile and lower aortic atherosclerosis, whereas in apoE knockout (KO) mice, overexpressing hABCA1 increased atherosclerosis with minimal effect on plasma lipids.[14] Physiological overexpression of a

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full-length hABCA1 containing bacterial artificial chromosome in apoE KO and low-density lipoprotein (LDL) receptor KO (LDLrKO) mice both revealed an atheroprotective role of ABCA1.[15,16] Significantly larger lesions occurred in ApoE or LDLrKO mice transplanted with bone marrow from mice with total body ABCA1 deficiency.[17–20] In contrast, total body ABCA1 deficiency in apoE KO or LDLrKO mice did not result in increased atherosclerosis compared with control mice.[18] The complex relationship between global ABCA1 expression and atherosclerosis susceptibility observed in humans and mouse models of atherosclerosis was at least partially attributed to reduction in atherogenic lipoproteins concomitant with ABCA1 deficiency, or to the use of different promoters for transgenic overexpression.

Subsequent studies with hepatocyte-specific ABCA1 KO (HSKO) mice suggested a major role for the liver in maintaining systemic HDL levels, leading to investigation of the role of hepatic ABCA1 in atherogenesis.[6] Joyce et al[21] found that liver-specific overexpression of ABCA1 in LDLrKO mice led to increased atherosclerosis, presumably because of increased plasma concentrations of apoB-containing lipoproteins (apoB Lp) concomitant with a significant increase in plasma HDL. A more recent study of hepatic ABCA1 deletion in chow-fed apoE KO mice showed a significant increase in early-stage atherosclerosis.[16] However, several issues were not addressed in that study. First, only early atherosclerosis was examined; mice consumed a chow diet for 12 weeks, and total plasma cholesterol (TPC) concentrations were relatively low (250–400 mg/dL). Thus, the effect of hepatocyte deletion of ABCA1 on more advanced atherosclerosis is unknown. Second, HDL cholesterol (HDL-C) concentrations are quite low in apoE KO mice (≈29 mg/dL). Therefore, the difference between apoE KO and HSKO-apoE KO strains may be too small to differentially affect atherosclerosis. Third, apoE has atheroprotective roles at the level of the arterial wall that are absent in the apoE KO background.[22] Finally, no in vivo reverse cholesterol transport (RCT) studies were performed.

To address these gaps in knowledge, we investigated the influence of hepatic ABCA1 expression on RCT and development of more advanced atherosclerotic lesions in LDLrKO mice. Our results suggest a minimal impact of hepatic ABCA1 deletion on in vivo macrophage RCT and atherogenesis development in atherogenic diet–fed LDLrKO mice, although plasma HDL concentrations were markedly reduced in HSKO/LDLrKO mice compared with LDLrKO mice. This surprising outcome likely resulted from the significant reduction in atherogenic lipoproteins (ie, very low-density lipoprotein [VLDL] and LDL) observed in diet-fed HSKO/LDLrKO mice, as well as sufficient HDL to mediate RCT.

Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

Hepatocyte ABCA1 Deletion Reduces Plasma Lipids

To induce atherosclerosis development, mice were switched from chow to an atherogenic diet at 8 weeks of age for 16 weeks. After 2 weeks of diet feeding, TPC and FC increased for both groups of mice, but values were significantly lower in HSKO/LDLrKO mice throughout the 16-week study (area under curve; $P<0.01$; Figure 1A and 1B). A consistent trend in plasma triglyceride (TG) concentrations was not observed (Figure 1A and 1B). We previously reported that HDL cholesterol (HDL-C) levels in chow-fed HSKO mice were ≈80% lower than wild-type mice.[6,23] Fast protein liquid chromatography (FPLC) fractionation of plasma lipoproteins showed that chow-fed HSKO/LDLrKO mice had significant reductions in HDL-C (1.74 versus 0.21 mmol/L in males; 67.3 versus 8.1 mg/dL in males, $P<0.0001$; 1.50 versus 0.28 mmol/L in females; 58.0 versus 10.9 mg/dL in females, $P<0.0001$), contributing to the lower TPC levels in HSKO/LDLrKO versus LDLrKO mice (4.48 versus 2.68 mmol/L in males; 173.1 versus 103.6 mg/dL in males, $P<0.0001$; 5.17 versus 3.44 mmol/L in females; 199.7 versus 133 mg/dL in females, $P=0.0004$). VLDL-C and LDL-C concentrations were similar between genotypes (Figure 1C). The less pronounced hyperlipidemia in atherogenic diet–fed HSKO/LDLrKO mice was mainly attributed to lower VLDL-C (14.43 versus 6.08 mmol/L in males; 557.9 versus 235.0 mg/dL in males, $P=0.0050$; 16.49 versus 10.05 mmol/L in females; 637.7 versus 388.7 mg/dL in females, $P=0.0283$) and LDL-C levels (16.19 versus 11.65 mmol/L in males; 626.2 versus 450.5 mg/dL in males, $P=0.0221$; 11.80 versus 10.32 mmol/L in females; 456.3 versus 399.0 mg/dL in females, $P=0.3441$), whereas HDL-C concentrations remained significantly different between genotypes (2.0 versus 1.05 mmol/L in males; 77.4 versus 40.5 mg/dL in males, $P=0.0007$; 0.98 versus 0.36 mmol/L in females; 37.9 versus 14.0 mg/dL in females, $P<0.0001$; Figure 1D). These data document the critical role of hepatocyte ABCA1 in maintaining the plasma HDL-C pool in hyperlipidemic mice and demonstrate a potential role for hepatic ABCA1 in regulating plasma apoB-containing lipoprotein concentrations during atherogenesis.

VLDL Catabolism Is Increased in HSKO/LDLrKO Mice

To determine the explanation for reduced VLDL and LDL concentrations in atherogenic diet–fed HSKO/LDLrKO mice, we investigated VLDL production and catabolism. VLDL TG production was measured after in vivo inhibition of TG lipolysis with intravenous Triton administration to fasted mice. The rate of hepatic VLDL TG mass accumulation during Triton block of lipolysis was similar for both genotypes of mice (Figure IA and IB in the online-only Data Supplement). Similarly, in a separate experiment, secretion rates of TG and radiolabeled apoB were not significantly different between groups (data not shown). We next investigated VLDL particle turnover using ^{125}I-VLDL from LDLrKO mice, which was indistinguishable in chemical composition from HSKO/LDLrKO mouse VLDL (Table I in the online-only Data Supplement). VLDL particles were enriched in cholesterol ester (41% to 43%) at the expense of TG (5%), typical of β-VLDL,[24] likely because of the prolonged residence time in plasma in the absence of active LDLr.[25] VLDL particle turnover, measured as decay of ^{125}I-VLDL apoB clearance from plasma, was more rapid in HSKO/LDLrKO versus LDLrKO recipient mice (area under curve; $P<0.05$; Figure II in the online-only Data Supplement), suggesting that the lower VLDL-C concentrations in HSKO/

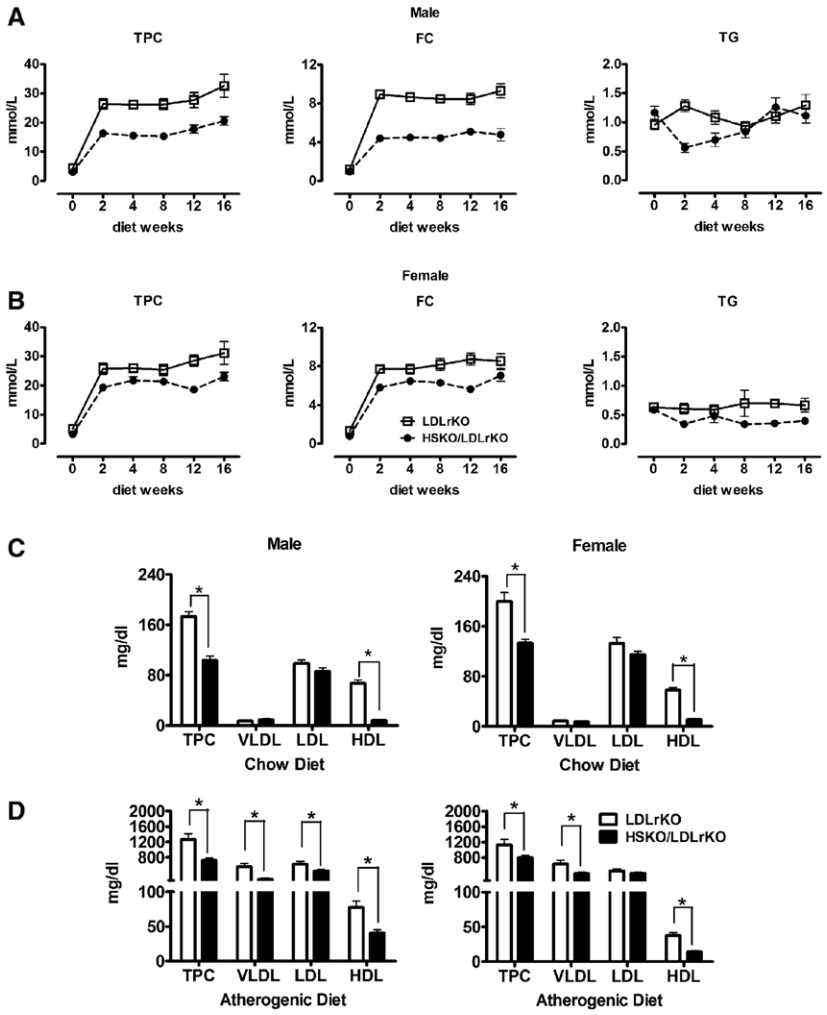

Figure 1. Plasma lipid and lipoprotein concentrations during atherosclerosis progression. Fasting (4 hours) plasma total cholesterol (TPC), free cholesterol (FC), and triglyceride (TG) concentrations were measured by enzymatic assays in male (**A**) and female (**B**) mice of the indicated genotype during a 16-week atherosclerosis progression phase (n=9–13). Plasma lipoprotein cholesterol distributions of mice before (**C**; chow fed at 7–9 weeks of age) or after (**D**) 16 weeks of atherogenic diet consumption were determined after FPLC fractionation of plasma (n=9–19). Data expressed as mean±SEM. **P*<0.05. HDL indicates high-density lipoprotein; HSKO, hepatocyte-specific ABCA1 knockout; LDL, low-density lipoprotein; LDLrKO, LDL receptor KO; and VLDL, very low-density lipoprotein.

LDLrKO mice were attributable to increased VLDL particle catabolism. Expression of hepatic genes involved in VLDL catabolism was similar for HSKO/LDLrKO and LDLrKO mice (Figure IIC in the online-only Data Supplement). However, plasma apoE levels were lower in atherogenic diet–fed HSKO/LDLrKO mice in addition to the anticipated reduction in apoA-I levels attributable to low plasma HDL concentrations (Figure IID in the online-only Data Supplement). Furthermore, most of the plasma apoE as well as apoA-I migrated in the HDL size range (8–10 nm) on nondenaturing gradient gels (Figure IIE and IIF in the online-only Data Supplement). Given these results, we speculate that lower VLDL-C levels in HSKO/LDLrKO mice were likely a result of decreased competition by apoE-containing plasma HDL for hepatic VLDL uptake, resulting in increased removal of VLDL particles from plasma in HSKO/LDLrKO versus LDLrKO mice.

Effect of Hepatocyte ABCA1 Deletion on Hepatic and Biliary Lipids

To address whether hepatocyte ABCA1 ablation impacts liver lipid metabolism, we measured hepatic and biliary lipid levels. Unlike our previous study in which similar hepatic lipid content was observed for chow-fed HSKO versus wild-type mice,[23] atherogenic diet–fed HSKO/LDLrKO mice had lower (significant in female mice) hepatic total cholesterol (TC), FC, and cholesterol ester concentrations relative to LDLrKO mice (Figure 2A and 2B). Hepatic TG and phospholipid concentrations were similar between the 2 genotypes (Figure 2A and 2B). However, there was no significant difference in biliary TC, phospholipid, and bile acid concentrations or molar percentage composition between the 2 genotypes (Figure IIIA–IIID in the online-only Data Supplement), and fecal cholesterol excretion was similar (Figure IIIE in the online-only Data Supplement). To investigate whether the decreased liver cholesterol content was associated with transcriptional regulation concomitant with ABCA1 ablation, we measured expression of genes involved in hepatic lipid metabolism. SREBP1c was significantly downregulated, and several other genes (HMGCoA synthase, ACC1) showed downward trends in expression in HSKO/LDLrKO mouse liver, suggesting decreased de novo lipogenesis (Figure 2C). Liver expression of several LXR target genes was similar in HSKO/LDLrKO and LDLrKO mice (Figure 2C).

Impact of Hepatic ABCA1 on Atherosclerosis Development

To investigate the impact of hepatocyte ABCA1 deficiency on atherosclerosis development in LDLrKO mice, 3

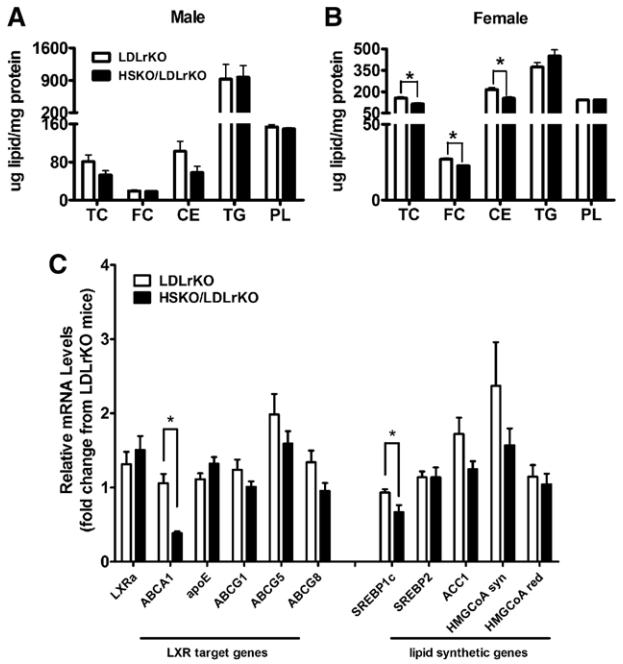

Figure 2. Hepatic lipid content and gene expression. Lipid content was determined using detergent-based enzymatic assays of hepatic lipid extracts from 4-hour–fasted male (**A**) or female (**B**) mice after 16 weeks of atherogenic diet consumption (n=7–12). **C**, Hepatic gene expression in male mice (n=7). Data were expressed in mean±SEM. **P*<0.05. CE indicates cholesterol ester; FC, free cholesterol; LDLrKO, low-density lipoprotein receptor KO; TC, total cholesterol; and TG, triglyceride.

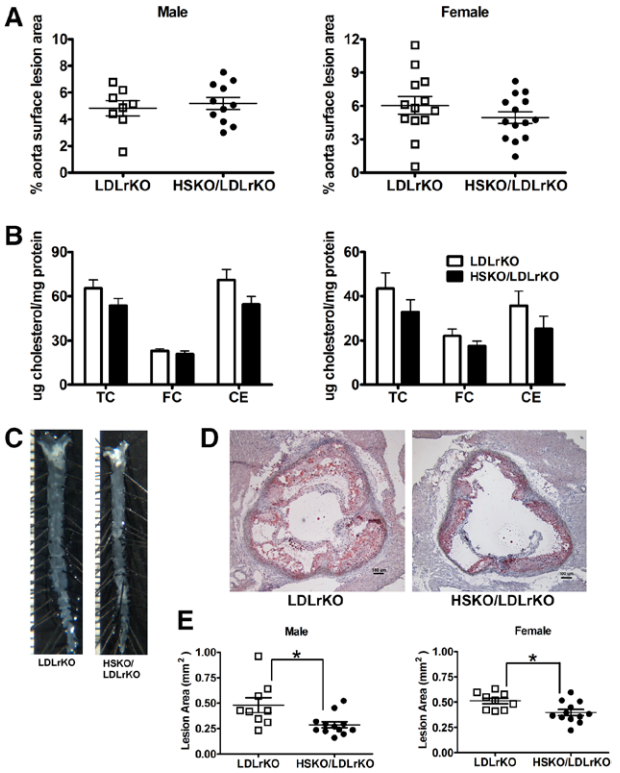

Figure 3. Atherosclerosis evaluation. **A**, Aorta surface lesion area was expressed as the percentage of total aorta surface area. **B**, Aortas were lipid extracted for quantification of total cholesterol (TC) and free cholesterol (FC) content using gas-liquid chromatography. Cholesterol ester (CE) content was calculated as (TC-FC)×1.67. **C**, Representative en face aorta from a low-density lipoprotein receptor KO (LDLrKO; **left**) and hepatocyte-specific ABCA1 knockout (HSKO)/LDLrKO (**right**) mouse. **D**, Representative LDLrKO (**left**) and HSKO/LDLrKO (**right**) mouse aortic root sections stained with Oil Red O. **E**, Aortic root lesion area. Each point represents the average lesion area of 3 sections per mouse. Horizontal lines denote the mean±SEM for each genotype. **P*<0.05.

measurements of atherosclerosis were made. En face aortic surface lesion area (Figure 3A and 3C) and aortic cholesterol content (Figure 3B) were similar for HSKO/LDLrKO and LDLrKO mice, although there was a trend toward reduced aortic cholesterol content in HSKO/LDLrKO mice. Furthermore, cross-sectional analysis of Oil red O–stained aortic root sections revealed significantly smaller lesions in both female (0.51 versus 0.40 mm^2; P=0.0141) and male (0.48 versus 0.29 mm^2; P=0.0341) HSKO/LDLrKO mice versus their LDLrKO counterparts (Figure 3D and 3E), suggesting that deletion of hepatocyte ABCA1 expression may actually protect against more advanced atherosclerotic lesion development in the aortic root. Additional support for this concept was obtained with additional analysis of lesion complexity; aortic root sections stained with Masson's trichrome showed less necrosis, acute inflammation, and adventitial inflammation in lesions of HSKO/LDLrKO versus LDLrKO mice fed the atherogenic diet for 16 weeks (Figure IVA in the online-only Data Supplement). In a separate experiment to evaluate very early stages of aortic atherosclerosis (ie, 5 weeks atherogenic diet feeding), we observed similar aortic cholesterol content and aortic root lesion area between genotypes (Figure IVB and IVC in the online-only Data Supplement). Taken together, unlike previous findings in apoE KO mice,[16] the absence of hepatic ABCA1 did not accelerate early-stage atherogenesis in LDLrKO mice and seemed to protect against late-stage, more advanced atherosclerosis.

Role of Hepatocyte ABCA1 in Macrophage RCT In Vivo

One atheroprotective mechanism proposed for HDL is the transport of excess macrophage cholesterol to the liver for excretion (ie, RCT).[26] To determine whether the large reduction of plasma HDL in HSKO/LDLrKO mice diminished RCT, we performed in vivo macrophage RCT assays. ^{3}H-cholesterol–radiolabeled J774 macrophages were injected into the peritoneal cavity of HSKO/LDLrKO or LDLrKO mice after 5 weeks of atherogenic diet feeding. The plasma ^{3}H–cholesterol closely tracked with lipoprotein cholesterol mass (Figure 4A and 4B). The amount of ^{3}H tracer in plasma 48 hours after injection was significantly lower in HSKO/LDLrKO mice, likely reflecting the lower levels of plasma lipoproteins in these mice (Figure 4C). However, the tracer levels in the liver, bile, and feces were similar between the 2 groups (Figure 4D–4F), suggesting that in vivo macrophage RCT was not impaired in HSKO/LDLrKO mice, despite the much lower HDL-C in these mice. A similar outcome was obtained using radiolabeled bone marrow–derived macrophages injected into mice fed the atherogenic diet for 16 weeks (Figure V in the online-only Data Supplement). We

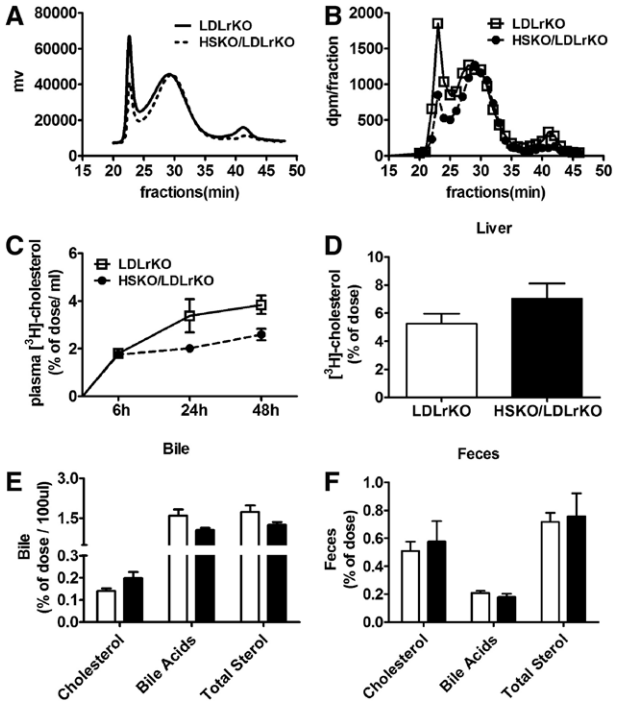

Figure 4. Macrophage RCT. ^{3}H–cholesterol radiolabeled, cholesterol-loaded J774 macrophages were injected into the peritoneal cavity of mice fed the atherogenic diet for 5 weeks. Plasma cholesterol mass (**A**) and ^{3}H-cholesterol (**B**) distribution were determined after FPLC separation of plasma. Plasma ^{3}H–cholesterol radiolabel at different time points (**C**) and in liver (**D**), bile (**E**), and feces (**F**) 48 hours after injection. Data are expressed as mean±SEM, n=7 to 8. *P<0.05. HSKO indicates hepatocyte-specific ABCA1 knockout; and LDLrKO, low-density lipoprotein receptor KO.

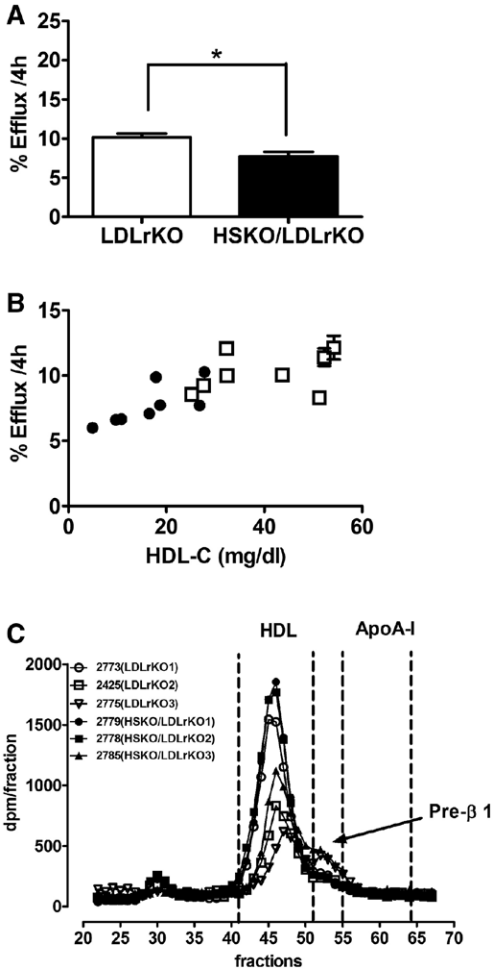

Figure 5. Plasma cholesterol efflux capacity. **A**, Cholesterol efflux to apoB lipoprotein–depleted plasma. J774 macrophages were radiolabeled with ^{3}H-cholesterol for 24 hours and then incubated for 4 hours with medium containing 2.8% plasma from low-density lipoprotein receptor KO (LDLrKO) or hepatocyte-specific ABCA1 knockout (HSKO)/LDLrKO mice fed an atherogenic diet (n=8). An aliquot of medium and cellular lipid extract was taken for scintillation counting to determine percentage cholesterol efflux during the 4-hour incubation. *P<0.05. **B**, Percentage cholesterol efflux was plotted against each animal's plasma high-density lipoprotein cholesterol (HDL-C) level. Open symbols=LDLrKO; closed symbols=HSKO/LDLrKO. **C**, Apo-B–depleted plasma medium 4 hours after cholesterol efflux underwent FPLC separation; radioactivity of each fraction was counted by scintillation counting.

also measured cholesterol content of resident peritoneal macrophages in atherogenic diet–fed mice and observed no significant difference between the 2 genotypes, although there was a trend toward decreased cholesterol in HSKO/LDLrKO mice (Figure VI in the online-only Data Supplement). Collectively, these data suggest that in vivo macrophage RCT is not impaired in HSKO/LDLrKO mice, despite significantly lower plasma steady-state HDL-C levels.

Plasma Cholesterol Efflux Capacity

In vivo RCT results suggested that the plasma HDL pool in HSKO/LDLrKO mice was sufficient to maintain normal cholesterol efflux from macrophages for ultimate excretion into feces. One possible explanation for this outcome could be a fraction of mouse plasma HDL that is highly efficient at effluxing macrophage FC, compensating for low plasma HDL levels in RCT in HSKO/LDLrKO mice. For example, preβ 1 seems to be the preferred acceptor for ABCA1-mediated FC efflux in human plasma, but its concentration is typically <10% of total HDL.[27] To examine this possibility, we measured the ability of apoB lipoprotein–depleted plasma from atherogenic diet–fed HSKO/LDLrKO and LDLrKO mice to efflux ^{3}H-cholesterol from cholesterol-loaded macrophages and observed a significant reduction (24%) in HSKO/LDLrKO versus LDLrKO plasma (10.14% versus 7.75%, P=0.0067; Figure 5A). However, analysis of individual animal HDL-C concentrations versus percentage of efflux values (Figure 5B) demonstrated <2-fold variation in percentage efflux values compared with a 10-fold variation in plasma HDL-C concentrations, suggesting that HDL-C concentration, per se, is not a primary determinant of plasma efflux capacity.

After the cholesterol efflux experiment, we fractionated a subset of individual plasma-containing efflux media using an FPLC column capable of separating plasma HDL particles, preβ 1 HDL, and lipid-free apoA-I from one another (Figure VII in the online-only Data Supplement). The distribution of ^{3}H-cholesterol between the main HDL peak (fractions 41–50) and preβ 1 HDL elution region (fractions 51–55) did not reveal

a disproportionate amount of ^{3}H-FC in the preβ 1 peak in HSKO/LDLrKO versus LDLrKO plasma (Figure 5C), and the percentage of ^{3}H-FC in the preβ 1 peak relative to the entire HDL elution region (ie, fractions 41–55) was 20.9±3.4% and 20.7±5.2%, respectively (n=6/genotype). In addition, apoA-I Western blot analysis of plasma separated by agarose gel electrophoresis did not show an increase in preβ 1 HDL for HSKO/LDLrKO compared with LDLrKO mice (Figure VIID in the online-only Data Supplement). Taken together, these results suggest that HSKO/LDLrKO mice do not compensate for reduced plasma HDL levels with an increase in amount or cholesterol efflux efficiency of preβ 1 HDL to maintain in vivo RCT at levels comparable with LDLrKO mice.

Discussion

Hepatocyte ABCA1 plays a crucial role in HDL biogenesis,[2] but its role in atherogenesis is less clear. In the current study, we addressed this gap in knowledge by performing atherosclerosis and in vivo macrophage RCT studies in atherogenic diet–fed HSKO mice crossed into the LDLrKO background. Compared with LDLrKO (control) mice, HSKO/LDLrKO mice had reduced TPC, primarily because of a 40% to 50% reduction in VLDL and LDL, and similar or reduced atherosclerosis. Furthermore, in vivo macrophage RCT to feces was similar for both genotypes of mice, although efflux of macrophage ^{3}H-FC was significantly reduced in apoB lipoprotein–depleted plasma from HSKO/LDLrKO mice. In agreement with the in vivo RCT data, resident peritoneal macrophage cholesterol content, biliary lipid composition, and fecal cholesterol mass were similar for mice with or without hepatocyte ABCA1 expression. These results suggest that the markedly reduced plasma HDL pool in HSKO/LDLrKO mice is sufficient to maintain macrophage RCT, which, together with decreased VLDL and LDL levels, prevented the expected increase in atherosclerosis.

Atherogenic diet–fed HSKO/LDLrKO mice displayed similar en face aortic lesion area and cholesterol content, but reduced aortic root lesion size relative to LDLrKO mice (Figure 3). The fact that development of atherosclerosis in the aortic root precedes that in the whole aorta and is typically more advanced[28] may suggest that hepatic ABCA1 deficiency is protective in more advanced stages of atherosclerosis, perhaps by preserving RCT during extended periods of hyperlipidemia (see below). Only 1 other study has investigated the role of hepatocyte ABCA1 deficiency in atherogenesis.[16] In that study, aortic lesion size and cholesterol content were significantly increased in HSKO/apoE KO versus apoE KO mice fed chow for 12 weeks, supporting an antiatherogenic role for hepatic ABCA1. There are several differences between the studies that may explain the divergent outcomes. First, chow-fed apoE KO mice had modest hypercholesterolemia (TPC=250–400 mg/dL) and early atherosclerotic lesions, with low amounts of aortic cholesterol,[16] whereas atherogenic diet–fed LDLrKO mice in the present study had much higher TPC concentrations (≈1000 mg/dL) and more aortic cholesterol accumulation (≈60-fold more TC). In a follow-up study of very early atherosclerosis (5 weeks atherogenic diet feeding), we again observed no difference in aortic cholesterol content between HSKO/LDLrKO and LDLrKO mice (Figure IVB in the online-only Data Supplement), although aortic TC levels were 4- to 6-fold less than mice fed the atherogenic diet for 16 weeks (compare Figure 3B with Figure IVB in the online-only Data Supplement), but 10-fold higher than aortic TC levels of HSKO/apoE KO mice fed chow for 12 weeks.[16] VLDL and LDL concentrations were significantly lower in the HSKO/apoE KO versus apoE KO mice, but the magnitude of the reduction (45 mg/dL for VLDL; 60 mg/dL for LDL) was much less than HSKO/LDLrKO mice (250–300 mg/dL for VLDL-C; 60–170 mg/dL for LDL-C) compared with LDLrKO mice. Although the percentage reduction in HDL-C compared with their respective controls was similar for both studies (≈50%), the absolute HDL-C values were much lower in HSKO/apoE KO mice compared with HSKO/LDLrKO mice, which may have been low enough to limit RCT; however, macrophage RCT was not measured in the Brunham study. Finally, apoE expression is atheroprotective independent of plasma lipoprotein concentrations.[29–31] One or more of these differences may explain why hepatocyte ABCA1 deletion was neutral or atheroprotective in LDLrKO mice and atherogenic in apoE KO mice.

Epidemiological studies have documented an inverse association between plasma HDL-C concentrations and coronary heart disease (CHD), supporting the role of HDL as an antiatherogenic lipoprotein.[32] The protective role of HDL in CHD is primarily attributed to RCT, but HDL also inhibits lipoprotein oxidation, inflammation, and hematopoiesis and maintains endothelial function, all of which are atheroprotective.[33,34] However, recent studies have challenged the assumption that raising HDL-C levels will uniformly translate into reductions in CHD.[35,36] Other studies have suggested the HDL particle number and size (ie, subfraction distribution) are better predictors of CHD risk than HDL-C[37–39] and that HDL function may be more important in preventing CHD than HDL-C.[40,41] Animal studies have shown a more consistent association between increased atherosclerosis and decreased macrophage RCT than with reduced HDL-C.[42] In support of the concept that HDL function may be more important than HDL-C in determining CHD risk, we show that a substantial reduction of plasma HDL-C in atherogenic diet–fed HSKO/LDLrKO mice did not significantly affect aortic atherogenesis, in vivo macrophage RCT, or fecal cholesterol excretion. These results are compatible with the idea of a small, dynamic HDL pool that efficiently removes cholesterol from arterial macrophage foam cells and rapidly transports it to the liver for excretion without a detectable increase in plasma HDL-C. Pre-β 1 HDL is a preferential acceptor for macrophage cholesterol efflux via ABCA1 and may function accordingly, although it is generally <10% of total HDL mass.[27] However, a large HDL pool would not be necessary for this mechanism to be operational because aortic cholesterol ester mass in atherogenic diet–fed HSKO/LDLrKO mice is <1% of the steady-state plasma cholesterol pool. Overall, these observations support the concept that HDL quality or function may be a better predictor of atheroprotection than HDL-C, per se.

A recent study by the Rader laboratory supports our results that a marked decrease in plasma HDL did not affect

macrophage RCT.[43] They found that pharmacological inhibition of ABCA1 by probucol resulted in an 80% reduction of plasma HDL-C in chow-fed wild-type mice, but macrophage RCT was unaffected, although the flux of ^{3}H-cholesterol from plasma HDL into the liver and feces was increased. In addition, probucol treatment of SR-BI KO mice reduced plasma HDL-C by 63% and stimulated macrophage RCT. Based on these combined results, Yamamoto et al[43] suggested that hepatocyte ABCA1 may normally function to counterbalance RCT by mobilizing FC from hepatocytes back into plasma to help maintain plasma HDL-C. However, probucol, an antioxidant drug with a long history of clinical use, has broad pharmacological properties (including effects relevant to whole body lipid metabolism) and is not specific for ABCA1.[44] Here, we show that specific genetic deletion of hepatocyte ABCA1 supports the conclusions of Yamamoto et al.[43] Results from both studies suggest that the plasma HDL pool remaining after whole body pharmacological inhibition of ABCA1 or genetic deletion of hepatocyte ABCA1, although small, is quantitatively sufficient or functionally efficient to mediate macrophage cholesterol transport back to the liver for excretion. As discussed above, maintaining macrophage RCT in the face of considerable reductions in the plasma HDL pool seems to result in atheroprotection during long periods of hyperlipidemia when advanced atherosclerosis development is ongoing (Figure 3E).

The similar atherosclerosis outcome in aortas of HSKO/LDLrKO and LDLrKO mice and the aortic root atheroprotection in HSKO/LDLrKO mice may be partially ascribed to the significant reduction in plasma VLDL and LDL levels in atherogenic diet–fed HSKO/LDLrKO mice (Figure 1D). Whole body ABCA1 KO mice in LDLrKO or apoE KO backgrounds also had similar extent of atherosclerosis that was attributed to reduced plasma apoB Lp concentrations.[18] Altered apoB Lp metabolism is also a feature of Tangier disease, in which plasma LDL levels are ≈50% normal attributable to a 2-fold increase in the fractional catabolic rate of LDL.[45] Chow-fed HSKO mice demonstrated a 50% lower plasma LDL concentration, a 2-fold increase in ^{125}I-LDL tracer removal rate from plasma, and a 2-fold increase in hepatic LDLr expression relative to wild-type mice, suggesting that increased hepatic LDLr expression may be responsible for the decreased plasma LDL concentrations.[23] Crossing chow-fed HSKO mice into the LDLrKO background normalized (ie, increased) plasma LDL concentrations to those of chow-fed LDLrKO mice, lending additional support that increased hepatic LDLr expression is the key mediator of reduced plasma LDL in chow-fed HSKO mice (Figure 1C). However, atherogenic diet–fed HSKO/LDLrKO mice exhibited diminished VLDL-C and LDL-C relative to LDLrKO mice, which was attributable to increased VLDL catabolism (Figure II in the online-only Data Supplement) with no difference in VLDL production (Figure I in the online-only Data Supplement). Because these mice lack functional LDLr, increased catabolism of VLDL had to be mediated through an LDLr-independent pathway. Hepatic LDLr–related protein mRNA (Figure IIC in the online-only Data Supplement) and protein levels (data not shown) were similar between groups, suggesting the involvement of other potential pathways, such as heparan sulfate proteoglycans.[46] However, expression of hepatic lipoprotein catabolic genes, such as SR-BI, syndecan 1, hepatic lipase, lipoprotein lipase, and apoC3, were similar between genotypes (Figure 2B). Van Eck et al[24] have shown that HDL can effectively compete for hepatic uptake of βVLDL particles via SR-BI dependent- and independent-mediated mechanisms. In the absence of functional LDLr, VLDL residence time in plasma is significantly prolonged,[25] allowing alternate hepatic VLDL particle uptake pathways to predominate. We show that the pool of apoE-enriched HDL, a likely competitor for alternate hepatic VLDL particle uptake pathways, is diminished in atherogenic diet–fed HSKO/LDLrKO mice (Figure IIF in the online-only Data Supplement). Based on our combined results and the previous studies by Van Eck et al,[24] we propose that the increased VLDL particle catabolism in HSKO/LDLrKO mice is mediated through decreased competition for hepatic uptake of VLDL by apoE-containing HDL. Regardless of the exact mechanism, hepatic ABCA1 expression seems to be an important regulator of plasma apoB Lp as well as HDL levels, both of which modulate atherosclerosis progression.

In conclusion, we investigated the impact of hepatocyte ABCA1 deletion on relatively advanced atherosclerosis and macrophage RCT during disease progression. Unexpectedly, we found that hepatocyte ABCA1 deletion did not exacerbate lesion development in atherogenic diet–fed LDLrKO mice, and was atheroprotective in the aortic root, likely because of reduced apoB Lp levels and maintenance of macrophage RCT, despite a large reduction in plasma HDL-C. Our results are also compatible with the idea proposed by Yamamoto et al[43] that hepatic ABCA1 may normally recycle a significant amount of plasma HDL-C removed by the liver back into plasma to maintain the plasma HDL-C pool. If true, these findings would result in a paradigm shift because decreased hepatic ABCA1 expression, resulting in lower plasma HDL-C, may actually increase RCT and reduce CHD risk by reducing the recycling of hepatic cholesterol back into plasma through nHDL formation by ABCA1.

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Disclosures

None.

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Significance

Hepatocyte ATP binding cassette transporter A1 (ABCA1) plays a pivotal role in maintaining plasma high-density lipoprotein (HDL) levels, but its impact on macrophage reverse cholesterol transport and atherogenesis is less clear. In this study, we show the importance of hepatic ABCA1 in regulating both plasma HDL and apoB lipoprotein metabolism under hyperlipidemic conditions. Despite a 50% reduction in plasma HDL cholesterol in the absence of hepatic ABCA1, atherosclerosis was not worsened, likely because of the maintenance of in vivo macrophage reverse cholesterol transport and the concomitant paradoxical 40% to 50% reduction in plasma very low-density lipoprotein and low-density lipoprotein levels. In addition, macrophage cholesterol efflux to apoB lipoprotein–depleted plasma varied <2-fold compared with a 10-fold variation in plasma HDL cholesterol concentrations, supporting the concept that steady-state HDL cholesterol concentration is not the primary determinant of plasma cholesterol efflux capacity. Therapeutic interventions targeting hepatic ABCA1 expression to alleviate cardiovascular burden should take into consideration that paradoxical effects on apoB lipoprotein metabolism may oppose atheroprotection.