Objective—The ability of high-density lipoprotein (HDL) particles to accept cholesterol from peripheral cells, such as lipid-laden macrophages, and to transport cholesterol to the liver for catabolism and excretion in a process termed reverse cholesterol transport (RCT) is thought to underlie the beneficial cardiovascular effects of elevated HDL. The liver X receptors (LXRs; LXRα and LXRβ) regulate RCT by controlling the efflux of cholesterol from macrophages to HDL and the excretion, catabolism, and absorption of cholesterol in the liver and intestine. Importantly, treatment with LXR agonists increases RCT and decreases atherosclerosis in animal models. Nevertheless, LXRs are expressed in multiple tissues involved in RCT, and their tissue-specific contributions to RCT are still not well defined.

Approach and Results—Using tissue-specific LXR deletions together with in vitro and in vivo assays of cholesterol efflux and fecal cholesterol excretion, we demonstrate that macrophage LXR activity is neither necessary nor sufficient for LXR agonist–stimulated RCT. In contrast, the ability of LXR agonists primarily acting in the intestine to increase HDL mass and HDL function seems to underlie the ability of LXR agonists to stimulate RCT in vivo.

Conclusions—We demonstrate that activation of LXR in macrophages makes little or no contribution to LXR agonist–stimulated RCT. Unexpectedly, our studies suggest that the ability of macrophages to efflux cholesterol to HDL in vivo is not regulated by macrophage activity but is primarily determined by the quantity and functional activity of HDL. (Arterioscler Thromb Vasc Biol. 2014;34:1650-1660.)

Key Words: atherosclerosis ▪ lipoproteins, HDL ▪ macrophages

Cardiovascular disease (CVD) is a leading cause of death globally, and it is well established that elevated levels of cholesterol in the blood are a major contributor to disease development. Excess plasma cholesterol accumulates in macrophages lodged in blood vessel walls which along with an associated inflammatory response initiate the formation of atherosclerotic lesions. Statin therapy is highly effective for lowering disease-causing low-density lipoprotein (LDL) cholesterol, thereby reducing morbidity and mortality associated with CVD. Nevertheless, the residual risk for major cardiac events remains high for patients receiving LDL-lowering therapies prompting the search for complementary therapeutic approaches. Epidemiological studies have demonstrated that levels of high-density lipoprotein (HDL) cholesterol are inversely associated with CVD suggesting the potential therapeutic benefit of raising HDL. Recent clinical trials with cholesteryl ester transfer protein (CETP) inhibitors and niacin, however, have failed to demonstrate clinical benefits of increasing HDL cholesterol. The clinical trial results have led to the suggestion that HDL functionality, rather than the absolute mass of HDL cholesterol, may be a more accurate indicator for CVD risk. The ability of HDL to promote cholesterol efflux from macrophage foam cells within atherosclerotic lesions was one of its earliest recognized functions. Importantly, cholesterol efflux from foam cells has been shown to increase macrophage egression and to reduce lesion burden in animal models of CVD. Measuring the dynamic rate of macrophage cholesterol efflux, therefore, may be a better predictor of the antiatherogenic effects of novel HDL-targeted therapies.

The movement of cholesterol from peripheral cells such as macrophages to HDL constitutes the first step in a process termed reverse cholesterol transport (RCT). HDL-derived cholesterol is then trafficked to the liver where it is catabolized or excreted to the bile. Recent studies have also described hepatic-independent pathways for cholesterol secretion. Studies in animal models indicate that measurements of RCT can strongly predict the effect of genetic and pharmacological manipulations on atherosclerosis. Similarly, in humans, an inverse relationship has been uncovered between the ability of patient sera to accept cholesterol from macrophages in vitro and measurements of carotid intima–media thickness with cholesterol acceptor capacity being a strong predictor of coronary disease status. The use of in vitro measurements of plasma cholesterol acceptor activity for predicting CVD as well as the proteins/particles...
in human sera responsible for accepting cholesterol, however, remain controversial.20,21

Integral to the regulation of RCT are the liver X receptors (LXRs), LXRα (NR1H3) and LXRβ (NR1H2), which are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. Studies using genetic knockouts and synthetic agonists have defined important roles for LXRs in the control of cholesterol homeostasis and fatty acid metabolism.22–24 Treatment of animals with LXR agonists results in changes in gene expression promoting the efflux of cholesterol from peripheral cells such as macrophages, the secretion of cholesterol from the liver, and the inhibition of cholesterol absorption in the intestine.22 Importantly, the endogenous ligands for LXRs are oxidized forms of cholesterol (oxysterols) that increase coordinately with intracellular cholesterol levels, thus allowing these receptors to act as sensors to maintain appropriate cholesterol levels throughout the body.25,26 At the molecular level, LXRs control macrophage cholesterol efflux by regulating expression of genes encoding the ATP-binding cassette (ABC) transporters ABCA1 and ABCG1 as well the gene encoding apolipoprotein E.22 Upregulation of ABCA1 and ABCG1 results in increased transfer of intracellular cholesterol to HDL particles, and genome-wide association studies have linked both transporters to HDL cholesterol levels in humans.27,28 Mutations in the human ABCA1 gene results in a genetic syndrome referred to as Tangier disease. Patients with Tangier disease characteristically present with little or no HDL and massive accumulation of cholesterol in lymph tissues and are at increased risk for atherosclerosis.19,29,30 LXR also regulates expression of ABCG5 and ABCG8, 2 half-transporters that dimerize to form an additional cholesterol transporter.31,32 Expression of ABCG5/ABCG8 is largely restricted to the liver and intestine, where these proteins function to promote the excretion of cholesterol (liver) and limit cholesterol absorption (intestine).33 Genetic deletion of ABCG5/8 or deletion of LXRα in the liver largely blocks the ability of LXR agonists to stimulate fecal excretion of cholesterol.34,35 Thus, activation of LXRs promotes a net movement of cholesterol from the periphery out of the body. Not surprisingly, LXR agonists decrease atherosclerosis in animal models of CVD.34,36–38

Treatment with LXR agonists also increases plasma HDL cholesterol,34,39 suggesting that LXRs can regulate RCT in both a cell autonomous fashion, by controlling the transporters required to mobilize intracellular cholesterol, as well as in a nonautonomous fashion by regulating the amount of cholesterol acceptor in plasma. Interestingly, the ability of LXR agonists to increase HDL cholesterol levels is largely mediated by the induction of ABCA1 expression in the intestine.34,40 Not unexpected then is the observation that an intestinal-specific LXR agonist increases RCT.41 Although LXR agonists seem to act in macrophages, the liver, and the intestines to stimulate RCT, studies using genetic knockouts indicate that macrophages are the major site of LXR agonist–dependent antiatherogenic activity.38,42,43 The atherosclerosis studies therefore led us to question the tissue-specific contributions of LXRs to the regulation of RCT. Combining in vivo measurements with tissue-selective knockouts, we show that the ability of LXRs to regulate HDL quantity and activity is a major driver of RCT. In contrast, macrophage LXR activity is neither necessary nor sufficient. Furthermore, our studies suggest that the ability of macrophages to efflux cholesterol to HDL in vivo is primarily determined by the quantity and functional activity of HDL in the surrounding environment.

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

Results

Macrophage LXR Is Not Necessary for LXR Agonist–Dependent RCT

LXR activity in the liver and the macrophage is thought to contribute to RCT,44 but the relative contribution of LXR at these sites has not been well defined. To determine the contribution of macrophage LXR to RCT, we injected bone marrow–derived macrophages that had been loaded with 3H-cholesterol in vitro into the peritoneal space of mice and followed the movement of macrophage-derived cholesterol to the plasma and ultimately to the feces as described by Naik et al.45 For these studies, we used C57BL/6J (LXRα+) and Lxrα−/−/Lxrβ−/− (DKO) mice in the C57BL/6J background to generate 3 groups of animals: LXR+ macrophage introduced into LXR+ mice (referred to as MacLXR+/LXR+), LXR+ macrophage introduced into DKO mice (referred to as MacLXR+/DKO), and DKO macrophages introduced into LXR+ mice (referred to as MacDKO/LXR+). For the RCT experiments, age-matched male mice were treated with vehicle or the LXR agonist T0901317 (10 milligram/kilogram [mpk]) daily by oral gavage for 3 days before injection. After injection of radiolabeled macrophage, mice continued to be treated with vehicle or agonist for the duration of the experiment (for a total of 5 doses) and the appearance of 3H-sterol was quantitated in the plasma at 6, 24, and 48 hours after injection. At completion of the experiment (48 hours), the amount of 3H-sterol in the feces and liver was determined. In preliminary experiments, we found that LXR activation (eg, rise in plasma triglycerides) can be observed after 3 doses of T0901317 at 10 mpk and that the plasma concentrations of T0901317 are similar between C57BL/6J and Lxrα−/−/Lxrβ−/− mice and ≥10 times above the reported EC50 (data not shown).

As expected, agonist treatment of MacLXR+/LXR+ mice stimulates the appearance of macrophage-derived cholesterol in plasma over the time course and in the feces at
48 hours (Figure 1A and 1B). When LXR is present only in macrophages (MacLXR+/DKO), however, the amount of macrophage-derived cholesterol in the plasma and feces is significantly decreased (Figure 1A and 1B). Similarly, the ability of T0901317 to increase the accumulation of macrophage-derived cholesterol in the plasma of MacLXR+/DKO mice is decreased by 70% (Figure 1A), and agonist-stimulated fecal excretion is completely blocked in these animals (Figure 1B). Quantification of ABCA1 mRNA levels in macrophage re-extracted from the peritoneal space at completion of the experiment demonstrates that placing LXR+ macrophages into DKO mice does not impair macrophage LXR transcriptional activity (Figure 1C). In contrast to the decreased RCT observed in the MacLXR+/DKO mice, selective deletion of LXR in macrophages (MacDKO/LXR+) has little or no effect on either the accumulation of 3H-cholesterol in the plasma or the feces (Figure 1B). Quantification of ABCA1 mRNA levels in macrophage re-extracted from the peritoneal space at completion of the experiment demonstrates that placing LXR+ macrophages into DKO mice does not impair macrophage LXR transcriptional activity (Figure 1C). In contrast to the decreased RCT observed in the MacLXR+/DKO mice, selective deletion of LXR in macrophages (MacDKO/LXR+) has little or no effect on either the accumulation of 3H-cholesterol in the plasma or the feces (Figure 1B). Little or no differences among the groups are seen when hepatic levels of 3H-sterols were examined (Figure 1 in the online-only Data Supplement). To further address the contribution of macrophage LXR activity to the ability of LXR agonists to increase the accumulation of macrophage-derived cholesterol in the plasma, we examined 3H-cholesterol levels in vehicle- and T0901317-treated MacLXR+ and MacDKO/LXR+ mice at 30, 60, and 90 minutes after introducing radiolabeled macrophage into the peritoneal space. As shown in Figure 1D, pretreatment of mice with T0901317 significantly increases 3H-cholesterol in the plasma by 60 minutes. Even at these short time points, however, the LXR genotype of the macrophages has no effect on the response to agonist treatment. The observation that LXR macrophage activity does not seem to play a role in the accumulation of 3H-cholesterol in the plasma in vivo is consistent with studies in vitro demonstrating that ABCA1 expression and cholesterol efflux is actually slightly increased in \( \text{Lxr}^{-/-}\) macrophages.46 In the absence of agonists, LXRs repress transcription by interacting with corepressors and this activity is lost on genetic deletion.46 A similar upregulation of ABCA1 expression is observed in DKO macrophages recovered from the peritoneal space of LXR+ mice after in vivo RCT experiments (Figure 1C).

**HDL Levels and Adipose Activity Drive LXR Agonist–Dependent RCT**

LXR agonists are known to increase HDL cholesterol predominantly by increasing expression of ABCA1 in the intestine.44 Consistent with an LXR agonist–dependent increase in HDL cholesterol (Table), plasma from T0901317-treated C57BL/6J (LXR+) mice has increased cholesterol acceptor activity in vitro when 3H-cholesterol–loaded RAW264.7 cells are used as donor macrophages. The effect of agonist, however, is lost when plasma from DKO animals is used (Figure 2A). To further address the contribution of HDL to macrophage efflux, a similar series of in vitro efflux experiments were performed using fast liquid protein chromatography (FPLC)–purified HDL particles (Figure 2B). For experiments with FPLC-purified HDL, peak HDL fractions were pooled (Figure II in the online-only Data Supplement) and normalized by the amount of apolipoprotein A1 (APOA1) as determined by Western blotting (Figure IIIA in the online-only Data Supplement). Using APOA1 as a relative measure for particle number, HDL from agonist-treated C57BL/6J mice accepts greater amounts of macrophage cholesterol compared with DKO mice (Figure 2B).
experiments show that LXR agonist treatment increases both HDL mass and HDL function. During the course of in vivo RCT experiments, it is likely that macrophage-derived 3H-cholesterol incorporates into cells and tissues throughout the body. Thus, along with increasing the HDL cholesterol acceptor activity of HDL, LXR agonists may also increase the amount of cholesterol in plasma by promoting efflux from other tissues via transcriptional upregulation of ABCA1, ABCG1, and apolipoprotein E. To address the possible contributions of different tissues to LXR agonist–stimulated RCT, radiolabeled LXR+ macrophages were introduced into vehicle- and T0901317-treated LXR+ mice (Mac LXR+/LXR+) and multiple tissues were harvested at 48 hours postinjection to determine whether agonist treatment promotes a net loss in tissue-associated 3H-sterols. As shown in Figure 2C, a significant agonist-dependent decrease is observed in white adipose tissue (gonadal fat pad) suggesting that fat tissue may make an important contribution to LXR-stimulated accumulation of cholesterol in the plasma and feces. T0901317-dependent changes in 3H-sterol levels were not observed in other tissues (Figure 2C). Importantly, the decrease in adipose 3H-sterol levels could result from increased LXR transcriptional activity in fat cells, the improved acceptor activity of HDL, or both.

### Diet-Dependent Regulation of Liver LXR Activity and RCT

We have previously determined under severe hyperlipidemic conditions (Ldlr−/− mice on Western diet) that liver-specific LXR activity is decreased compared to control mice, and that this decrease in LXR activity is mediated by high levels of liver triglyceride levels. To further investigate this observation, we examined the effects of LXR agonist treatment on liver LXR activity in vivo. As shown in Figure 2D, treatment with T0901317 significantly increases liver LXR activity in both control and lipogenic mice, indicating that LXR agonist treatment can increase liver LXR activity even in the presence of high levels of triglycerides. This finding has important implications for the potential use of LXR agonists in the treatment of hyperlipidemia, as it suggests that these agents may be effective even in the presence of high levels of triglycerides, which is often a limiting factor in the use of these agents in clinical practice.
deletion of LXRα impairs the accumulation of macrophage-derived cholesterol in both the plasma and the feces. To further investigate the contribution of liver LXR activity to RCT, liver-specific knockout LXRα (LivKO) mice and floxed littermate controls (carrying the floxed LXRα allele without albumin Cre recombinase) were placed on a standard chow diet with or without 0.2% cholesterol. LXRα is the major LXR subtype expressed in the liver, and the ability of T0901317 to increase plasma triglycerides and to induce expression of hepatic ABCG5, ABCG8, and ABCA1 is significantly impaired in LivKO mice (Table and Figure IV in the online-only Data Supplement). After 4 weeks on diet, plasma total cholesterol increases 30% to 50% in both LivKO and littermate control groups fed the 0.2% cholesterol diet (Table). Consistent with published data, the 0.2% cholesterol diet also significantly increases hepatic cholesterol in LivKO mice attributable to impaired fecal excretion and decreased bile acid synthesis (Figure VA in the online-only Data Supplement). Hepatic triglycerides, however, are not increased (Figure VB in the online-only Data Supplement), and the increase in hepatic cholesterol measured in LivKO mice does not result in a significant increase in liver damage (Figure VC and VD in the online-only Data Supplement), markers of inflammation, or markers of endoplasmic reticulum stress (data not shown). For the final week of the diet treatment (week 4), mice were treated with vehicle or T0901317 and RCT was measured in vivo as in previous experiments by introducing radiolabeled LXR+ macrophages. On a standard chow diet, the appearance of 3H-cholesterol in the plasma of T0901317-treated LivKO and littermate controls is significantly increased at 24 and 48 hours (Figure 3A), indicating that liver LXRα activity is not required for agonists to increase the accumulation of 3H-cholesterol in the plasma. On the contrary, the ability of LXR agonists to increase fecal sterol excretion is completely lost in LivKO mice (Figure 3B), a result consistent with decreased agonist-dependent regulation of ABCG5 and ABCG8 in the livers of these animals (Figure IV in the online-only Data Supplement). Interestingly, exposure to the 0.2% cholesterol diet impairs both LXR agonist–dependent plasma and fecal cholesterol accumulation in LivKO mice relative to controls (Figure 3C and 3D). Thus, dietary cholesterol uncovers a critical role for hepatic LXR activity in controlling the accumulation of macrophage-derived cholesterol in plasma. The ability of LXR agonists to increase HDL cholesterol levels in LivKO mice is also sensitive to dietary cholesterol (Figure 4A and Table) despite similar increases in the intestinal mRNA levels of ABCA1 (Figure VI in the online-only Data Supplement). Furthermore, a dietary cholesterol-dependent decrease in cholesterol acceptor activity is also observed when FPLC-purified HDL particles isolated from T0901317-treated LivKO mice are compared with HDL particles from littermate controls in vitro (Figure 4B; see Figures II and IIIC and IIID in the online-only Data Supplement for FPLC profiles and APOA1 levels). The reason(s) why the cholesterol enriched diet impairs the ability of LXR agonist treatment to increase HDL mass and function remains to be determined. Nevertheless, the failure of T0901317 to modulate HDL levels and functional activity in cholesterol-fed LivKO mice supports the hypothesis that the ability of LXR agonists to promote the accumulation of macrophage-derived cholesterol in plasma is largely derived from systemic effects on HDL and independent of macrophage LXR activity.

Figure 3. In vivo reverse cholesterol transport in chow and 0.2% cholesterol diet–fed liver-specific knockout liver X receptor–α (LivKO) mice. 3H-cholesterol and acetylated low-density lipoprotein–loaded C57BL6J bone marrow–derived macrophages (BMMs) were injected into floxed or LivKO mice fed standard chow (A and B) or 0.2% cholesterol–supplemented diet (C and D). Animals were treated for 3 days with or without 10 mg/kg T0901317 (n=6/group) before BMM injection, and the amount of 3H-sterol in plasma (A and C) and feces (B and D) was determined as described in the Materials and Methods in the online-only Data Supplement. Mice continued to receive vehicle or T0901317 treatment for the duration of the experiment. Data are mean±SEM. *Statistically significant difference between vehicle- and T0901317-treated animals of the same genotype (P≤0.05%). †Statistically significant difference between floxed and LivKO mice with the same treatment (P≤0.05%). CPM indicates counts per minute.
Our results indicate that LXR activation can improve the cholesterol acceptor activity of HDL, and this effect is influenced by liver LXR activity in a diet-dependent fashion. As an initial characterization of HDL particle composition, we measured phospholipid levels in the FPLC-purified HDL fractions. Phospholipids are the major components by mass of HDL, and several studies suggest that HDL phospholipid levels are a better predictor of cholesterol efflux than other HDL parameters. As shown in Figure 4C and 4D, T0901317 treatment increases the amount of total phospholipids associated with purified HDL particles (normalized by APOA1 levels) from standard chow–fed floxed and LivKO mice (Figure 4C). The increase in HDL phospholipid levels is consistent with studies demonstrating that LXR agonist treatment increased HDL particle size. The effect of agonist treatment on HDL phospholipid levels, however, is lost in 0.2% cholesterol diet–challenged LivKO animals (Figure 4D). Phospholipid transfer protein is an HDL-bound protein that plays a major role in regulating HDL size and phospholipid composition through its phospholipid transfer activity. Phospholipid transfer protein mRNA levels have been shown to be regulated by LXR; however, we did not detect significant differences in plasma phospholipid transfer protein activity between floxed and LivKO mice on either dietary condition (Table I in the online-only Data Supplement).

CETP Decreases Macrophage-Derived Cholesterol in Plasma

To test the hypothesis that LXR-dependent regulation of HDL levels and activity plays a major role in driving the accumulation of macrophage-derived cholesterol in plasma, we took advantage of the observation that LXR agonist–dependent increases in HDL cholesterol are lost in CETP transgenic mice. CETP facilitates the transfer of cholesterol esters from HDL to apolipoprotein B–containing particles, thereby decreasing HDL cholesterol levels. Importantly, the transgene is under control of the human CETP promoter which has been shown to be directly regulated by LXR in human cells and in transgenic mice (Figure VIIA and VIIB in the online-only Data Supplement). Indeed, treatment of CETP transgenic mice with T0901317 decreases HDL cholesterol by ≈25% and raises the amount of cholesterol associated with apolipoprotein B–containing lipoprotein particles (Figure 5A and 5B and Table). To determine the effect of CETP expression on RCT in vivo, CETP transgenic mice and littermate controls were treated with vehicle or T0901317 and injected with 3H-cholesterol–labeled C57BL/6J (LXR+) bone marrow–derived macrophages as described in previous experiments. Consistent with a critical role for HDL in promoting the accumulation of macrophage-derived cholesterol in plasma, the amount of 3H-cholesterol in this compartment at 24 and 48 hours is significantly reduced in CETP transgenic mice and the ability of T0901317 to increase plasma cholesterol accumulation is lost (Figure 5C). Similarly, unfractionated plasma and FPLC-purified HDL particles from T0901317-treated CETP transgenic mice do not exhibit increased efflux activity as is observed in nontransgenic controls (Figure 5D and 5E). The ability of LXR agonists to increase HDL phospholipids, however, is not impaired in CETP transgensics (Figure VIIIC in the online-only Data Supplement). Taken together, the RCT and in vitro efflux experiments indicate that LXR-dependent
upregulation of CETP expression counters the ability of agonists to enhance the appearance of macrophage-derived cholesterol in the plasma. In contrast to the inhibitory effect of CETP expression on the accumulation of macrophage-derived cholesterol in plasma, LXR agonist treatment increases fecal 3H-sterol levels in both CETP transgenic and littermate controls (Figure 5F). Interestingly, CETP expression also results in a significant increase in fecal bile acids in vehicle-treated animals (Figure VIID in the online-only Data Supplement). Increased bile acid synthesis has previously been reported in CETP transgenic mice.57,58 Little or no difference was observed in hepatic 3H-cholesterol levels among the groups (data not shown). Thus, as observed with the LXRα liver-specific knockout (LivKO) mice, it is possible to functionally sever the transfer of macrophage-derived cholesterol to the plasma from subsequent fecal excretion.

Discussion

The discovery that LXR agonists can promote macrophage cholesterol efflux in vitro via direct regulation of the genes encoding ABCA1, ABCG1, and apolipoprotein E22,59 suggested a simple hypothesis for the cardioprotective effect of LXR activation based on promoting cholesterol transfer from macrophage foam cells to HDL, the first step in the RCT pathway. This hypothesis is supported by the finding that macrophage LXR activity is required for the antiatherogenic activity of LXR agonists.60 Combining in vitro cholesterol efflux measurements, in vivo RCT assays, and tissue-specific LXR knockouts, we now demonstrate that the ability of LXR agonists to stimulate RCT in vivo defined as the transfer of macrophage-derived cholesterol to the feces is largely independent of macrophage LXR activity (Figure 6). Thus, macrophage LXRs are neither necessary nor sufficient for LXR agonists to increase RCT, at least when measured in an acute assay during a 48-hour time course. Additionally, our studies suggest that it is the ability of LXR agonists to increase HDL biogenesis and to improve HDL functional activity that is largely responsible for stimulating the appearance of macrophage-derived cholesterol in plasma (Figure 6). The LXR agonist used in
these studies, T0901317, has been reported to modulate other
nuclear receptors, at least in vitro.60–62 Therefore, the possi-
bility that another nuclear receptor, such as the pregnane X
receptor, contributes to the activity of this molecule in vivo
cannot be ruled out. All the activities of T0901317 measured
in this work, however, are lost in cells and animals that are
deficient in LXRs.

On a standard mouse chow diet, the ability of LXR agonists
to stimulate the accumulation of macrophage-derived choles-
terol in plasma is independent of LXR activity in both mac-
rophages and the liver. Previous studies have determined that
LXR agonists increase HDL cholesterol by inducing ABCA1
expression in the intestine.34,40,63 Consistent with an important
role for intestinal LXR activity in regulating RCT is the find-
ing that selective activation of LXRs in the intestine using
either a poorly absorbed intestine-specific LXR agonist41 or
intestine-specific transgenic overexpression of a hyperactive
LXR (VP16-LXRα)64 increases RCT when measured using
assays similar to those described in this work. Furthermore,
our studies indicate that intestinal LXR activation can increase
the cholesterol acceptor activity of HDL particles (Figure 6)
most likely by increasing the production of immature nascent
particles that have been shown to be preferred cholesterol
acceptors.55–63 Interestingly, this work also describes a poten-
tial role for LXR activity in white adipose in regulating cho-
lesterol trafficking.

To test the hypothesis that agonist-dependent increases in
HDL mass and function drive the accumulation of mac-
rophage-derived cholesterol in plasma during RCT assays,
we took advantage of the observation that the ability of LXR
agonists to raise HDL cholesterol is lost in CETP trans-
genic mice.53,56 CETP, an enzyme that transfers cholesterol
esters from HDL to apolipoprotein B–containing lipoprotein
particles in exchange for triglycerides, is not expressed in
rodents, but the human gene used in this study is regulated
by LXRs.55,56,68 Importantly, CETP activity in the plasma is
increased after LXR agonist treatment, HDL levels are low-
ered, and plasma cholesterol accumulation measured during
RCT assays is decreased. The cholesterol acceptor activity
of unfractionated plasma and FPLC-purified HDL from
T0901317-treated CETP transgenic mice is also reduced
relative to nontransgenic controls. Finally, the conclusion
that increasing CETP activity impairs HDL particle func-
tion is consistent with reports that inhibition of CETP activ-
ity improves the cholesterol acceptor activity of human HDL
particles.69 Taken together, the data support the hypothesis
that the ability of LXR agonists to increase the accumula-
tion of macrophage-derived cholesterol in plasma is primarily
determined by the quantity and quality of the HDL particles.
Nevertheless, in CETP transgenic animals, LXR agonist treat-
ment still increases fecal excretion of macrophage-derived
cholesterol. Therefore, we cannot rule out the possibility that
CETP expression decreases the levels of macrophage-derived
cholesterol in plasma by increasing hepatic clearance via
receptors for apolipoprotein B–containing particles. Similar
to CETP expression, Bi et al45 found that liver-specific dele-
tion of Abca1 reduces plasma HDL levels and decreases
plasma accumulation of 3H-cholesterol in RCT assays without
altering fecal sterol excretion. Bi et al45 suggest that the small
plasma HDL pool that remains in the liver Abca1 knockout
mice may be quantitatively sufficient to mediate the transpor-
t of macrophage-derived cholesterol to the liver for excretion.
Our study with CETP transgenic mice together with the work
of Bi et al45 raises the possibility, at least under these exper-
imental conditions, that the appearance of macrophage-derived
cholesterol in the plasma is a not a rate-limiting step for fecal
cholesterol excretion.

In contrast to CETP transgenic expression, liver-specific
deletion of LXRα (LivKO) has little or no effect on the accu-
mulation of macrophage-derived cholesterol in plasma (on a
standard chow diet) but strongly inhibits LXR agonist–stimu-
lated fecal cholesterol excretion (Figure 6). Thus, our analysis
of CETP transgenic and LXRα LivKO mice indicates that it
is possible to functionally separate plasma cholesterol accumu-
lation from fecal excretion. Plasma cholesterol accumula-
tion is primarily controlled by the ability of LXRs to regulate
the quantity and quality of HDL, whereas fecal excretion is
controlled by LXR-dependent regulation of hepatic ABCG5
and ABCG8 levels, allowing a single transcription factor
pair (LXRα and LXRβ) to coordinate cholesterol movement
throughout the body. These results raise the question regard-
ing the potential therapeutic benefit of regulating either mac-
rophage cholesterol efflux or fecal excretion independently.
Current therapeutic approaches for atherosclerotic CVD all
involve reducing LDL cholesterol in the blood. Therefore,
if increasing fecal cholesterol excretion ultimately reduces
plasma LDL levels, one might predict a therapeutic benefit.
On the contrary, APOA Milano and other APOA1-derived
peptides have been shown to increase macrophage cholesterol
efflux and to improve cardiovascular end points, although it
not clear that the beneficial effects of these agents are depen-
dent on promoting cholesterol efflux.70,71 Future studies that,
for instance, combine macrophage-selective overexpression
of ABCA1 with LXR liver-specific knockouts may be a way
to address the therapeutic benefits of increased macrophage
efflux in the absence of fecal cholesterol excretion.

Interestingly, the contribution of liver LXR activity to RCT
can be influenced by the cholesterol content of the diet. As
described above, on a standard mouse chow diet, knocking out
LXRα in the liver has little or no effect on the accumulation of
macrophage-derived cholesterol in plasma while completely
eliminating agonist-stimulated fecal excretion (Figure 6).
When cholesterol (0.2%) is added to the diet, however, LXR
agonist–dependent plasma cholesterol accumulation is sig-
nificantly decreased in LivKO mice. The absence of agonist-
dependent accumulation of macrophage-derived cholesterol
in plasma when cholesterol is included in the diet correlates
with the inability of agonist treatment to increase HDL cho-
lesterol and improve the acceptor capacity of purified HDL in
LivKO mice under these conditions. LXR agonist treatment
still increases ABCA1 expression in the intestines of LivKO
on the 0.2% cholesterol diet, and the reason(s) why HDL cho-
lesterol levels are not increased in these mice remains to be
determined. Compared with littermate floxed controls on the
0.2% cholesterol diet, LivKO mice have increased hepatic
cholesterol levels although we did not detect any evidence for
increased hepatic inflammation, endoplasmic reticulum stress, or liver damage in these mice. We and others have shown that the ability of LXR agonists to increase HDL levels in LXR−/− mice is lost under severe hyperlipidemic conditions such as Ldlr−/− or Apoe−/− mice on Western diets.34,36,37,39,73 Thus, the ability of LXR agonists to regulate HDL metabolism can be influenced by dietary cholesterol levels. Interestingly, Kalaany et al6 demonstrated that Lxra−/−/Lxrβ−/− mice are resistant to high-fat diet–induced obesity; however, this resistance is only observed when the high-fat diet also contains cholesterol. These observations raise the possibility that hepatic cholesterol accumulation leads to the generation of a paracrine signal that can influence lipid metabolism in other tissues.

Bone marrow transplantation experiments and overexpression studies indicate that macrophages are the site of LXR agonist–dependent antiatherogenic activity.38,42,43 The studies described in this work, however, indicate that macrophage LXR activity does not play a significant contribution to RCT. Similarly, using LvKO mice in a severe hyperlipidemic environment (Ldlr−/−+Western diet), we demonstrated that LXR agonists can reduce atherosclerosis without increasing RCT.34 Kappus et al74 also reached an analogous conclusion in a recent study using mice with myeloid-specific double knockout of Abca1 and Abcg1. Together, these observations suggest that although hematopoietic LXR expression is required for the beneficial effects of LXR agonists, an increase in RCT or macrophage efflux is not. LXR activation inhibits nuclear factor κB signaling suggesting decreased inflammation as an obvious mechanism for LXR-dependent antiatherogenic activity.75,76 A dominant role for anti-inflammatory activity as the beneficial effect of LXR activation on atherosclerosis has important implications for the potential therapeutic use of LXR agonists. In particular, in vitro experiments have suggested that LXR agonists can have proinflammatory activities in human macrophages57 in contrast to the anti-inflammatory effects measured in rodents. Additionally, as described above, preclinical studies examining the antiatherogenic activity of LXR ligands generally have been performed under severe hyperlipidemic conditions where the ability of LXR agonists to increase HDL mass is lost.34,37,78 Because human CVD patients do not usually present with the supraphysiologic plasma cholesterol levels observed in genetic mouse models, the ability of LXR agonists to stimulate RCT may be maintained in humans and could be therapeutic. As we observe in CETP transgenic mice, however, the ability of LXR agonists to increase HDL cholesterol seems to be lost in nonhuman primates that express CETP.79,80

Recent clinical trials with niacin7 and CETP inhibitors8 have called into question the hypothesis that raising HDL cholesterol has beneficial effects on human CVD. The clinical trials together with experiments suggesting that the cholesterol acceptor activity of HDL isolated from patients can be a more accurate measurement of CVD risk have led to the proposal that assessing HDL function may be more relevant than measurements of HDL cholesterol mass.9,15,20 Along with increasing the levels of HDL cholesterol, LXR agonist treatment also increases the cholesterol acceptor activity of HDL particles that were normalized by the quantity of APOA1. HDL particles are heterogeneous in size and composition making it difficult to discern the LXR-dependent modifications that improve cholesterol acceptor activity. Nevertheless, our initial analysis of HDL particle composition found increased levels of phospholipids (normalized to APOA1) in the HDL particles purified from agonist-treated animals. The phospholipid:APOA1 ratio in HDL has been shown to be an important determining factor in predicting macrophage efflux. Studies using mice and rats expressing human APOA1 indicate that the prime component of HDL that modulates cholesterol efflux is HDL phospholipid.11,12 Furthermore, the correlation between macrophage cholesterol efflux and HDL phospholipid in human sera is stronger than with any other measured lipoprotein parameter, including HDL cholesterol, APOA1, and triglycerides.48 CETP expression, however, seems to impact HDL function without modulating phospholipid levels, suggesting that multiple components of HDL can influence particle function. LXRs likely regulate multiple pathways that modulate HDL activity, and future studies using detailed lipidomic and proteomic approaches can be used to further define the LXR-dependent changes in HDL composition that regulate HDL particle function. These studies that define particle function may open the door to new therapeutic approaches for targeting HDL.

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Disclosures
None.

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by the quantity and functional activity of high-density lipoprotein. Macrophages to efflux cholesterol to high-density lipoprotein in vivo is not regulated in a cell autonomous fashion but is primarily determined primarily acting in the intestine seems to underlie the ability of LXR agonists to stimulate RCT in vivo. Our studies suggest that the ability of the periphery out of the body. Using tissue-specific LXR deletions, we demonstrate that macrophage LXR activity is neither necessary nor the efflux of cholesterol from macrophages and the excretion of cholesterol from the liver resulting in a net movement of cholesterol from α The liver X receptors (LXRs), LXR and LXR, are important regulators of cholesterol transport. Treatment with LXR agonists promotes the efflux of cholesterol from macrophages and the excretion of cholesterol from the liver resulting in a net movement of cholesterol from the periphery out of the body. Using tissue-specific LXR deletions, we demonstrate that macrophage LXR activity is neither necessary nor sufficient for LXR agonist-stimulated RCT. In contrast, the ability of LXR agonists to increase high-density lipoprotein mass and function primarily acting in the intestine seems to underlie the ability of LXR agonists to stimulate RCT in vivo. Our studies suggest that the ability of macrophages to efflux cholesterol to high-density lipoprotein in vivo is not regulated in a cell autonomous fashion but is primarily determined by the quantity and functional activity of high-density lipoprotein.
Macrophage-Independent Regulation of Reverse Cholesterol Transport by Liver X Receptors

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Supplemental Figure I. Liver $^3$H sterol levels. The amount of $^3$H-sterol in the livers of animals at the conclusion of the RCT experiment were determined as described in Material and Methods. Data are mean ± SEM.
Supplemental Figure II. FPLC profiles. A) C57bl6/J; B) DKO; C) Lxrα<sup>fl/fl</sup> albumin-CRE− (Floxed) on chow diet; D) Lxrα<sup>fl/fl</sup> albumin-CRE+ (LivKO) on chow diet; E) Floxed on 0.2% cholesterol diet for 4 weeks; and F) LivKO on 0.2% cholesterol diet for 4 weeks were treated with vehicle or T0901317 (10 mpk) for 5 days, plasma was pooled, subjected to FPLC and the cholesterol content of each fraction was measured as described in Materials and Methods. (n=5-6/group) The three fractions containing the greatest amounts of cholesterol were pooled (green circles) and used in additional experiments.
Supplemental Figure III. APOA1 protein levels in FPLC-purified HDL. Peak HDL fractions from FPLC separations were pooled and APOA1 protein levels were measured by Western blot (n = 4-6/group). Quantification of APOA1 levels was carried out as described in Materials and Methods. Vehicle treated C57BL6/J, Floxed and CETP− was set as 100%. All the samples in each panel were run on the same gel.
Supplemental Figure IV. Hepatic ABC transporter expression in LivKO mice. Total RNA was extracted from liver tissue at the completion of in vivo RCT assays and mRNA levels of ABCG5 (A), ABCG8 (B) and ABCA1 (C) were measured by quantitative real-time PCR. Data are mean ± SEM. *Statistically significant difference between vehicle and T0901317-treated animals of the same genotype ($p \leq 0.05\%$). †Statistically significant difference between Floxed and LivKO with the same treatment ($p \leq 0.05\%$).
Supplemental Figure V. Effect of 0.2% cholesterol diet on hepatic lipids and liver enzymes. At the completion of the in vivo RCT study hepatic cholesterol, hepatic triglycerides, AST activity and ALT activity were determined as described in Materials and Methods. Data are mean ± SEM, (n=5-6/group). *Statistically significant difference between vehicle and T0901317-treated animals of the same genotype (p ≤ 0.05%). †Statistically significant difference between Floxed and LivKO with the same treatment (p ≤ 0.05%).
Supplemental Figure VI. Intestine Abca1 mRNA Levels in chow and 0.2% cholesterol diet fed LivKO animals. Total RNA was isolated from intestines at the completion of in vivo RCT studies and ABCA1 mRNA levels were measured by quantitative real-time PCR as described in Materials and Methods. Data is mean ± SEM, (n=5-6/group). *Statistically significant difference between vehicle and T0901317-treated animals of the same genotype and diet conditions ($p \leq 0.05\%$). #Statistically significant difference between chow and 0.2% cholesterol diet fed mice of the same genotype and treatment ($p \leq 0.05\%$).
Supplemental Figure VII. LXR agonists increase CETP activity. CETP− and CETP+ mice were treated vehicle or 10 mg/kg T0901317 for 5 days (n=5/group). A) Total RNA was isolated from liver tissue and the mRNA levels of CETP was measured by quantitative real-time PCR as described in Materials and Methods. B) CETP activity in plasma was determined by fluorometric assay as described in Materials and Methods. C) Total phospholipids in FPLC purified HDL from CETP− and CETP+ mice treated for 5 days with vehicle or T0901317 (10 mpk). HDL phospholipid levels were normalized by HDL APOA1 protein levels as determined by Elisa. D) At completion of the in vivo RCT experiment fecal sterols were extracted and the amount of 3H-cholesterol and 3H-bile acids were determined as described in Materials and Methods. Data are mean ± SEM. *Statistically significant difference between vehicle and T0901317-treated animals of the same genotype (p ≤ 0.05%). †Statistically significant difference between CETP− and CETP+ with the same treatment (p ≤ 0.05%). ND = not detected.
**Supplemental Table I. Plasma PLTP Activity**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Strain</th>
<th>Drug Treatment</th>
<th>PLTP Activity (nmole transferred/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow Floxed</td>
<td></td>
<td>Vehicle</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T0901317</td>
<td>4.3 ± 0.3*</td>
</tr>
<tr>
<td>Chow LivKO</td>
<td></td>
<td>Vehicle</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T0901317</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>0.2% Cholesterol Floxed</td>
<td></td>
<td>Vehicle</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T0901317</td>
<td>3.3 ± 0.2*</td>
</tr>
<tr>
<td>0.2% Cholesterol LivKO</td>
<td></td>
<td>Vehicle</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T0901317</td>
<td>3.2 ± 0.3*</td>
</tr>
</tbody>
</table>

*Statistically significant difference between vehicle and T0901317-treated animals of the same genotype (n = 4-6, \( p \leq 0.05\% \)). Data are mean ± SEM.
MATERIALS AND METHODS

**Reagents.** LXR agonist T0901317 was purchased from Cayman Chemical. $^3$H-cholesterol was purchased from Perkin Elmer. $^{14}$C-Cholic acid was purchased from American Radiolabeled Chemicals Inc.

**Animal experiments.** All animal experiments were approved by the Institutional Animal Care and Research Advisory Committee of the University of Virginia. Lxr$^\alpha-/-$ and Lxr$^\beta-/-$ and Lxr$^\alpha^{fl/fl}$/albumin-Cre mice have been described previously. Male CETP transgenic mice (The Jackson Laboratory) were bred with female C57BL6/J (The Jackson Laboratory) to generate CETP transgenic (CETP$^+$) and CETP$^-$ littermate controls. All animals were housed in a temperature-controlled environment with 12-hour light/12-hour dark cycles. Age-matched mice had free access to water and were fed standard rodent chow (TD 7001, Harlan Teklad) or a 0.2% cholesterol diet (TD 07798, Harlan Teklad). For 0.2% cholesterol diet experiments, animals were switched from standard chow to cholesterol diet at 8 weeks of age and experiments carried out following 4 weeks of diet. Animals were treated with vehicle (80% polyethylene glycol, 20% Tween-80) or LXR agonist T0901317 (10 mg per kg of body weight) by oral gavage once per day in the morning. Samples were drawn or experiments were initiated 3 hours after the 3$^{rd}$ dose (in vivo RCT studies) or 5$^{th}$ dose (plasma analysis and FPLC studies).

**Plasma Analyses.** Blood was collected into EDTA-coated tubes (Starstedt). Plasma was separated by centrifugation and assayed for total cholesterol (Thermo Scientific), triglycerides (Pointe Scientific), CETP activity (Sigma Aldrich), PLTP activity (Roar Biomedical Inc.), aspartate aminotransferase (AST) activity (Pointe Scientific) activity, alanine aminotransferase (ALT) activity (Thermo Scientific) and APOA1 levels (Novatein Biosciences). Plasma lipoprotein levels in pooled plasma samples (n=4-6/group) were analyzed by FPLC using a Superose G6 10/300 GL column (GE Healthcare), and assayed for total cholesterol per fraction by enzymatic analysis (Thermo Scientific). FPLC purified fractions containing peak HDL-cholesterol (n=3 fractions) were pooled for in vitro experiments. FPLC purified HDL was assayed for total phospholipid by colorimetric kit (Wako).

**Western blotting.** FPLC purified HDL samples (12.5 μl) in triplicate were resolved on 12% SDS-polyacrylamide gels and transferred to PVDF membranes (Millipore). Membranes were then hybridized with goat anti–mouse APOA1 antibody (Abcam 7614) at a 1:1000 dilution, followed by secondary antibody incubation at a 1:5,000 dilution. APOA1 protein was detected by chemiluminescence and quantitated with ImageQuant software.

**Liver cholesterol and triglycerides.** Liver samples (0.1 g) were homogenized in 2 ml Folch (chloroform/methanol, 2:1, v/v) with a polytron homogenizer. The organic phase was separated and then dried under nitrogen. Samples were reconstituted in isopropanol:Triton-X100 (9:1 v/v) and aliquots subjected to colorimetric enzymatic assays for total cholesterol (Thermo Scientific) or triglycerides (Pointe Scientific).

**Quantitative real-time PCR analysis.** Total RNA was extracted from ~100 mg pieces of tissue (liver and small intestine) using a microbead tissue homogenizer and PureZOL (Bio-Rad)/chloroform extraction. Total RNA was isolated from aqueous phase using an RNeasy kit (QIAGEN). RNA was treated with DNase I and reverse transcribed into cDNA with random hexamers using a High-Capacity cDNA Reverse Transcription kit (Life Technologies). RT-qPCR reactions contained 25 ng of cDNA, 385 nM of each primer, and 6.25 μl of SYBR Green Supermix (Bio-Rad) and were carried out in triplicate using a Bio-Rad MyiQ instrument. Relative mRNA levels were calculated using the comparative Ct method and normalized to cyclophilin. Oligonucleotides used for real-time PCR are listed in Supplemental Table 1.
**Cholesterol efflux experiments.** RAW264.7 cells were plated in 96-well plates (2 x 10^5 cells/well) and 24 hours later labeled with 1 µCi/ml ^3H^-cholesterol (PerkinElmer) in DMEM plus 1% FBS media for 18-24 hours. Radiolabeled cells were washed with pre-warmed PBS and incubated for 18-24 hours with serum-free media containing 0.03% pooled plasma (n=5-7 animals/group) or with 25% FPLC-purified HDL normalized to APOA1 levels as determined by Western blotting as cholesterol acceptors. For each individual efflux experiment using FPLC purified HDL, triplicate samples of all the HDL samples being compared were quantitated on the same western blot. Following incubation with acceptors, media was collected and radioactivity was measured by liquid scintillation counting. Cells were washed with PBS and lysed in 100 µl of 0.2 N NaOH, and radioactivity in cell lysis was quantitated by liquid scintillation counting. Cholesterol efflux was expressed as percentage of cpm in the medium divided by the total counts (cpm_{media}/(cpm_{media} + cpm_{cell})). Acceptor-dependent efflux was determined by subtracting the efflux of vehicle cells cultured without acceptor. APOA1 (10 µg/ml) or HDL (15 µg/ml) was included as positive control.

**In vivo RCT.** In vivo RCT experiments were carried out as described by Naik et al. Animals were on either chow or 0.2% cholesterol diet as indicated. Three days prior to and for the duration of the experiment mice were gavaged with vehicle or T0901317 (10 mpk). Bone marrow derived macrophages were loaded with 25 µg/ml acetylated LDL and 5 µCi/ml ^3H^-cholesterol for 48 hours in vitro. Cholesterol-loaded cells were injected into the peritoneal cavity of mice (~4.5 x 10^5 cells/mouse, 3 x 10^5 cpm, n=6/group), which were housed individually for the duration of the experiment. Blood was collected at 6, 24, and 48 hours after injection, and the ^3H^-cholesterol in triplicate plasma samples (10 ul aliquots) was determined by scintillation counting. Frozen livers, testes, quadriceps muscle, and gonadal fat pads were ground with mortar and pestle and lipids extracted from a single (fat and testes) or duplicate (liver and muscle) 100 mg samples by standard Folch extraction. Lipids were resuspended in 1ml of liquid scintillation fluid and the ^3H^-cholesterol levels in triplicate 200 ul aliquots was determined by scintillation counting. Feces was collected at 48 hours, homogenized in 50% ETOH by polytron homogenizer and ^3H-sterol levels determined by scintillation counting in 200 ul aliquots in triplicate. To measure ^3H-cholesterol and ^3H-bile acid in feces, 2 mL of homogenized samples was combined with 2 mL ethanol, 0.03µCi of ^14C-cholic acid as an internal standard, and 400 µL NaOH. The samples were saponified at 95°C for 2 hours, cooled to room temperature and cholesterol separated from bile acids by extracting 2 times with 6 mL hexane. The extracts were pooled, evaporated, resuspended in toluene and ^3H-cholesterol levels were determined by scintillation counting. To extract bile acids, the remaining aqueous fraction after the hexane extractions was acidified with concentrated HCl and then extracted 2 times with 6 mL ethyl acetate. The extracts were pooled together, evaporated, resuspended in ethyl acetate, and ^3H-bile acids levels were determined by scintillation counting and normalized to the recovery of ^14C-cholic acid.

To measure gene expression in recovered BMMs, 48 hours after ^3H-macrophage injection the peritoneal cavity of the mouse was flushed with PBS and cells were collected. Recovered cells were lysed with PureZOL (Bio-Rad), extracted with chloroform, and total RNA was isolated from the aqueous layer using an RNeasy kit (QIAGEN). Quantitative RT-PCR was carried out as described above.

**Statistics.** A normal distribution of the data for each experiment was first confirmed using D’Agostin-Pearson omnibus normality tests. Results were then analyzed by 2-way ANOVA with Tukey’s post-test using GraphPad Prism (GraphPad Software). For the time course experiments 2 way ANOVA with repeated measures was used. Comparisons that appeared to be significant (p ≤ 0.05) were further examined by non-parametric Mann-Whitney tests (comparisons of two
groups) or Kruskal-Wallis tests (more than 2 groups). Only comparisons that were considered significant ($p \leq 0.05$) by both ANOVA and non-parametric testing were reported.

Supplemental Table 1. Oligonuclotides for real-time PCR.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence 1</th>
<th>Sequence 2</th>
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</thead>
<tbody>
<tr>
<td>Mouse cyclophilin</td>
<td>5’CGATGACAGGCCCTTGG 3’</td>
<td>5’TCTGCTGTCTTTGGAACTTTGTC 3’</td>
</tr>
<tr>
<td>Mouse ABCA1</td>
<td>5’ GCTCTCAGGTGGGATGCAG 3’</td>
<td>5’GGCTCGTCCAGAATGACAAC 3’</td>
</tr>
<tr>
<td>Mouse ABCG1</td>
<td>5’ ATCTGAGGGATCTGGCTCTGA 3’</td>
<td>5’CCTGATGCCACTTCCATGA 3’</td>
</tr>
<tr>
<td>Human CETP</td>
<td>5’GGCCAATCAAGTATGGGTTG-3’</td>
<td>5’ACAGACACGTTCTGAATGGGAGA-3’</td>
</tr>
<tr>
<td>Mouse ABCG8</td>
<td>5’TGCCACACCTCCACATGTCCGCTCTGA 3’</td>
<td>5’ATGAAGGCCCCCCGAGTAAGGAGTAGA 3’</td>
</tr>
<tr>
<td>Mouse ABCG5</td>
<td>5’ ATGAAGGCCGGCAGTAAGGAGTAGA 3’</td>
<td>5’GGCAGGTTTTTCGATGAACTG 3’</td>
</tr>
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</table>

References

