Tissue-Specific Liver X Receptor Activation Promotes Macrophage Reverse Cholesterol Transport In Vivo

Tomoyuki Yasuda, Didier Grillot, Jeffery T. Billheimer, François Briand, Philippe Delerive, Stephane Huet, Daniel J. Rader

Objective—We previously reported that a systemic liver X receptor (LXR) agonist promoted macrophage reverse-cholesterol transport (mRCT) in vivo. Because LXR are expressed in multiple tissues involved in RCT (macrophages, liver, intestine), we analyzed the effect of tissue-specific LXR agonism on mRCT.

Methods and Results—In initial studies, the systemic LXR agonist GW3965 failed to promote mRCT in a setting in which LXR was expressed in macrophages but not in liver or intestine. To evaluate the effect of LXR activation specifically in small intestine on mRCT, wild-type mice were treated with either intestinal-specific LXR agonist (GW6340) or systemic LXR agonist (GW3965). Both GW3965 and GW6340 significantly promoted excretion of $[^3H]$-sterol in feces by 162% and 52%, respectively. To evaluate the requirement for macrophage LXR activation, we assessed the ability of GW3965 to promote mRCT in wild-type mice using primary macrophages deficient in LXR$\alpha/\beta$ vs wild-type macrophages. Whereas GW3965 treatment promoted fecal excretion compared with vehicle, its overall ability to promote mRCT was significantly attenuated using LXR$\alpha/\beta$ knockout macrophages.

Conclusion—We demonstrate that intestinal-specific LXR agonism promotes macrophage RCT in vivo and that macrophage LXR itself plays an important, but not predominant, role in promoting RCT in response to an LXR agonist. (Arterioscler Thromb Vasc Biol. 2010;30:781-786.)

Key Words: reverse cholesterol transport • high density lipoproteins • liver X receptor • tissue specific

Reverse-cholesterol transport (RCT) is a physiological process that helps to prevent accumulation of excess cholesterol in peripheral tissues.1 Efflux of cholesterol from vessel wall macrophages is thought to be a critical first step by which the RCT pathway protects against atherosclerosis.2 Liver X receptors (LXR) are nuclear receptors activated by endogenous oxysterols that play an important role in the control of cellular and whole-body cholesterol homeostasis.3 The main target tissues of LXR agonists relevant to RCT are macrophages, liver, and small intestine. LXR agonists increase ATP-binding cassette transporters A1 (ABCA1) and G1 (ABCG1) expression in macrophages, resulting in increased cholesterol efflux,4 ABCG5/85 and cholesterol 7 $\alpha$-hydroxylase (CYP7A1)$^6$ expression in liver,$^5,6$ resulting in increased sterol excretion into bile, and ABCG5/8 expression in small intestine,$^5$ resulting in increased cholesterol excretion in feces. We developed an assay that specifically traces RCT from the macrophage to the feces in vivo7 and previously reported that a systemic LXR agonist promoted macrophage RCT in vivo.7 However, the relative roles of LXR agonism in macrophages, liver, and intestine in RCT are not known. Therefore, we tested the effects of tissue-specific LXR agonism on macrophage RCT.

Materials and Methods
Phosphate-buffered saline and RPMI/HEPES were purchased from Invitrogen. [1,2-$^3$H]Cholesterol and cholesteryl oleate (cholesteryl-[1,2,6,7-$^3$H]) were purchased from Perkin-Elmer Life Science. Hydroxypropyl methyl cellulose (HMPC K100) was purchased from Shin-etsu. Other reagents without citation in this article were purchased from Fisher Scientific. A systemic LXR$\alpha/\beta$ agonist (GW3965) and an intestine-specific LXR$\alpha/\beta$ agonist (GW6340) were obtained from GlaxoSmithKline Medicinal Chemistry Department. In vitro GW3965 is highly specific for LXR receptors,$^8$ and GW6340 is the ester form of GW3965. GW6340 is described in a patent (PCT/US01/27622; GW6340=example 4; http://www.wipo.int/pctdb/en/). The structure of the intestinal-specific LXR agonist GW6340, a close analog of GW3965, is shown in Supplemental Figure 1 (available online at http://atvb.ahajournals.org). This compound has a pEC50=7 in vitro potency in LXR transactivation assay measuring ABCA1 promoter activity, a value comparable to the potency of GW3965. The dose for GW6340 was chosen to achieve a similar intestinal gene induction compared to GW3965 (Figure 2B).

Animals
Wild-type C57BL/6 mice were obtained from the Jackson Laboratory (Bar Harbor, Me), and LXRe/β double-knockout (LXR DKO) mice were obtained from Taconic (Germantown, NY). Mice were fed a standard chow diet (laboratory diet 5010; 4.8% fat and 267 ppm cholesterol) ad libitum before and during the study. For plasma lipid

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From Institute for Translational Medicine and Therapeutics and Cardiovascular Institute (T.Y., J.T.B., F.B., D.J.R.), University of Pennsylvania School of Medicine, Philadelphia, Pa; GlaxoSmithKline Research Center (D.G., P.D., S.H.), Les Ulis, France.
Correspondence to Daniel J. Rader, MD, University of Pennsylvania School of Medicine, 654 BRBII/III, 421 Curie Boulevard, Philadelphia, PA 19104. E-mail rader@mail.med.upenn.edu
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781
analyses, animals were fasted for 4 hours and then bled from the retro-orbital plexus. All animals were housed according to guidelines of the Institutional Animal Care and Usage Committee of the University of Pennsylvania. All protocols were considered and approved by the Institutional Animal Care and Usage Committee.

**Lipid Analysis**

Total cholesterol, high-density lipoprotein cholesterol (HDL-C), phospholipid, and triglyceride levels were measured with the use of diagnostic reagents from Thermo Scientific. Pooled plasma from each group (150 μL) was separated by fast protein liquid chromatography gel filtration. Total cholesterol in each fraction was measured with the total cholesterol E kit from Wako Pure Chemicals.

**Real-Time Polymerase Chain Reaction**

Total RNA was isolated from mouse tissue using TRIzol Reagent from Invitrogen. The cDNA was produced from total RNA with reverse-transcription using high-capacity cDNA reverse-transcription kit from Applied Biosystems. Quantification of mRNA expression using TaqMan assay systems was performed by ABI 7300 real-time polymerase chain reaction system from Applied Biosystems. All reagents necessary for performing a TaqMan reverse-transcription polymerase chain reaction assay including primers and probes were purchased from Applied Biosystems and used according to the manufacturer’s instructions. 9

**Macrophage RCT Study**

Experiments were performed in wild-type mice and LXR DKO mice. For each experiment, each group was dosed with 30 mg/kg body weight GW3965, GW6340, or vehicle (HPMC K100 0.5% in saline 10 mmol/L EDTA; pH 7.4), HDL was exchange-labeled with [3H]-cholesterol, and loaded with 25 μmol/L GW3965. The systemic LXR agonist GW3965, or vehicle for 10 days with GW3965 or vehicle before injection of cholesterol-labeled J774 macrophages, and they continued to be gavaged during the 48-hour experiment. At day 5, plasma HDL-C levels were increased in GW3965-treated wild-type mice (111 ± 12 vs 73 ± 10 mg/dL) but did not change in GW3965-treated LXR DKO mice (Supplemental Table I, available online at http://atvb.ahajournals.org). [3H]-Cholesterol counts in plasma of GW3965-treated mice showed a trend toward an increase in wild-type mice (Figure 1A), which was not observed in LXR DKO mice (Figure 1B). There was no difference in [3H]-cholesterol in the liver at the end of the 48-hour time period among 4 groups (Figure 1C). GW3965 treatment promoted significantly greater fecal excretion of total tracer and cholesterol tracer but not bile acid tracer (Figure 1D) in wild-type mice but not in LXR DKO mice. These data suggest that LXR activation in macrophages alone is insufficient to promote RCT and that LXR activation in the liver or the intestine is also required for promoting macrophage RCT.

**In Vivo HDL Turnover Studies**

Wild-type C57BL/6 were fed a control or LXR agonist GW3965-supplemented (10 mg/kg/d) chow diet ad libitum for 2 weeks. GW3965 was grinded and added to the chow diet at 100 mg/kg diet (Research Diets). HDL was prepared from pooled human plasma by sequential ultracentrifugation (density 1.063 < density < 1.21 g/mL). After extensive dialysis against dialysis buffer (0.15 mol/L NaCl, EDTA 1 mmol/L; pH 7.4), HDL was exchange-labeled with cholesterol olate as previously described. 13 After labeling, the HDL was re-isolated by ultracentrifugation (density 1.063 < density < 1.21 g/mL), extensively dialyzed, filter-sterilized, and stored at 4°C until injection. [3H]-Cholesterol olate-labeled HDL (1–2 million cpm/animal) was injected intravenously via tail veins into mice (n = 5–6/group). Blood samples were drawn by retro-orbital bleeding at 2 minutes, 1 hour, 3 hours, 6 hours, 9 hours, 24 hours, and 48 hours (∼25 μL at each time point). Feces were collected continuously from 0 to 48 hours and stored at 4°C before extraction of cholesterol and bile acids. At study termination (48 hours after injection), mice were exsanguinated, perfused with ice-cold phosphate-buffered saline, and liver samples were collected for analysis. Plasma decay curves for both tracers were normalized to radioactivity at the initial 2-minute time point after tracer injection. To measure [3H]-tracer recovery in liver, lipid extraction was performed according to the procedure of Bligh and Dyer. 7 Briefly, a 50-μg piece of tissue was homogenized in water, and then lipids were extracted with a mixture of chloroform/methanol 2:1 (vol/vol). The lipid layer was collected, evaporated, resuspended in toluene, and counted in a liquid scintillation counter. Results were expressed as a percentage of the injected dose. The injected dose was calculated by multiplying the initial plasma counts (2-minute time point) by the estimated plasma volume (3.5% of the body weight).

**Statistical Analyses**

All values are presented as mean ± SD. Comparisons between control and agonist-treated mice were made with the use of the Student t test (2-tailed) and ANOVA with the use of GraphPad Prism Software.

**Results**

**LXR Agonist in Macrophages Alone Is Insufficient to Substantially Promote RCT in the Absence of Hepatic and Intestinal LXR Expression**

To test whether activation of LXR in macrophages is, by itself, sufficient to promote macrophage RCT in the absence of LXR in all other tissues, wild-type and LXR DKO mice were gavaged for 10 days with GW3965 or vehicle before injection of cholesterol-labeled J774 macrophages, and they continued to be gavaged during the 48-hour experiment. At day 5, plasma HDL-C levels were increased in GW3965-treated wild-type mice (111 ± 12 vs 73 ± 10 mg/dL) but did not change in GW3965-treated LXR DKO mice (Supplemental Table I, available online at http://atvb.ahajournals.org). [3H]-Cholesterol counts in plasma of GW3965-treated mice showed a trend toward an increase in wild-type mice (Figure 1A), which was not observed in LXR DKO mice (Figure 1B). There was no difference in [3H]-cholesterol in the liver at the end of the 48-hour time period among 4 groups (Figure 1C). GW3965 treatment promoted significantly greater fecal excretion of total tracer and cholesterol tracer but not bile acid tracer (Figure 1D) in wild-type mice but not in LXR DKO mice. These data suggest that LXR activation in macrophages alone is insufficient to promote RCT and that LXR activation in the liver or the intestine is also required for promoting macrophage RCT.

**An Intestinal-Specific LXR Agonist Increased Macrophage RCT in Wild-Type Mice**

To determine the role of intestinal LXR activation in promoting RCT, we used an intestinal-specific LXR agonist, GW6340. Wild-type mice were gavaged with GW6340, the systemic LXR agonist GW3965, or vehicle for 10 days before injection of cholesterol-labeled J774 macrophages, and they continued to be gavaged during the 48-hour experiment. To confirm the intestinal-specific effect of GW6340, gene expression in liver and small intestine of vehicle, GW3965-treated, and GW6340-treated wild-type mice were assessed by quantitative polymerase chain reaction (Figure 2A, 2B). GW3965-treated mice had significantly increased LXR target gene expression in liver and small intestine, whereas GW6340-
treated mice had increased LXR target gene expression exclusively in small intestine. In addition, treatment with GW3965 significantly increased hepatic triglyceride content, whereas treatment with GW6340 did not (Supplementary Table II). These observations confirm that, in vivo, GW6340 is an intestinal-specific LXR agonist and, unlike the systemic LXR agonist GW3965, does not modulate hepatic gene expression or triglyceride content.

At day 5, plasma HDL-C levels were significantly higher in GW3965-treated mice compared with vehicle-treated mice (100 ± 16 vs 57 ± 7 mg/dL; Supplemental Table III). Whereas there was a trend toward an increased HDL-C level in GW6340-treated mice, it was not significant (Supplemental Table III). In the fast protein liquid chromatography analysis, GW3965 substantially increased cholesterol in the usual HDL fraction as well as in a large HDL fraction, whereas GW6340 modestly increased cholesterol in the usual HDL fraction (Figure 3A). The [3H]-cholesterol counts in plasma after J774 macrophage injection, expressed as a percentage of total [3H]-counts injected, were significantly increased in GW3965-treated mice compared with control mice, whereas they trended higher but were not significantly different in the GW6340-treated mice compared with the vehicle-treated mice (Figure 3C). The [3H]-cholesterol in plasma tracked closely with the cholesterol mass in plasma lipoprotein distribution and were consistent with HDL [3H] counts highest in GW3965-treated mice, next highest in GW6340-treated mice, and lowest in vehicle-treated mice (Figure 3B). There were no differences in [3H]-cholesterol in the liver at the end of 48-hour time period among the 3 groups (Figure 3D). As expected, GW3965-treated mice excreted a significantly 162% higher amount of macrophage-derived tracer in the feces compared with the control mice (Figure 3E). Interestingly, GW6340-treated mice also excreted significantly more (52%) macrophage-derived tracer in the feces compared with the control mice (Figure 3E). Thus intestine-specific LXR activation promotes macrophage RCT, although it may be less effective than systemic LXR activation.

**Macrophage LXR Contributes to the Ability of a Systemic LXR Agonist to Promote Macrophage RCT**

We tested whether macrophage LXR is critical for the ability of a systemic LXR agonist to promote RCT. As expected, in
BMM from wild-type mice, both GW3965 and acetylated low-density lipoprotein treatment increased cholesterol efflux, whereas in BMM from LXR DKO mice GW3965 and acetylated low-density lipoprotein treatment had no effect on efflux ex vivo (Supplemental Figure II). Next, we evaluated RCT in vivo using primary BMM from LXR DKO and wild-type mice. Wild-type mice were gavaged for 10 days with GW3965 or vehicle before injection of BMM from either wild-type or LXR DKO mice and continued to be gavaged during the 48-hour experiment. At day 5, plasma HDL-C levels were increased in GW3965-treated mice (Supplemental Table IV). The [3H]-cholesterol counts in plasma were significantly increased by GW3965 treatment in mice injected with wild-type BMM (Figure 4A). In contrast, there was no effect of GW3965 treatment on plasma [3H]-cholesterol counts in mice injected with LXR DKO BMM (Figure 4B). There was no difference in [3H]-cholesterol in the liver at the end of the 48-hour time period among 4 groups (Figure 4C). GW3965 treatment significantly increased fecal excretion of total tracer in mice injected with wild-type BMM but not in mice injected with LXR DKO BMM (Figure 4D). Excretion of cholesterol tracer was markedly increased by GW3965 in wild-type BMM and was increased, modestly but significantly, by GW3965 in LXR DKO BMM (Figure 4D). In contrast, GW3965 had no effect on fecal excretion of bile acid tracer in either group (Figure 4D). These data confirm that macrophage LXR is an important determinant of the ability of a systemic LXR agonist to promote macrophage RCT, but that LXR agonist promotes the fecal excretion of macrophage-derived cholesterol even in the absence of macrophage LXR.

To confirm that activation of LXR in the liver and intestine promotes the fecal excretion of HDL-derived cholesterol, we performed a [3H]-HDL-cholesteryl-ester kinetics study with GW3965-treated mice. Compared to the control group treated with vehicle, GW3965 treatment did not alter the rate of plasma turnover of [3H]-HDL-cholesteryl-ester (Figure 5A) or the amount of [3H]-cholesterol in the liver (Figure 5B), but it significantly increased the fecal cholesterol excretion of the HDL-derived cholesterol (Figure 5C). These data confirm the macrophage RCT study results and suggest that LXR agonism promotes RCT, at least in part, by accelerating the rate by which HDL-C is targeted for biliary and fecal excretion.

Discussion

In these studies, we provide evidence that LXR in all 3 major LXR-expressing tissues, macrophages, liver, and intestine, play a role in promoting macrophage RCT in response to LXR activation. We show that that ability of an LXR agonist to promote macrophage RCT requires LXR expression in tissues other than just the macrophage. We provide the first evidence to our knowledge that an intestine-specific LXR agonist promotes macrophage RCT in vivo. Finally, we show that macrophage LXR is an important, but not the sole, component of the ability of a systemic LXR agonist to promote RCT and that an LXR agonist promotes the fecal excretion of HDL-derived cholesterol. These observations are potentially relevant to therapeutic targeting of LXR for reduction of atherosclerotic vascular disease.
The small intestine plays a critical role in cholesterol homeostasis. It regulates cholesterol absorption and excretion and also produces apolipoprotein A-I and nascent HDL. Intestine-specific ABCA1 deficiency resulted in a 30% reduction of plasma HDL levels, whereas a systemic LXR agonist significantly increased plasma HDL levels even in liver-specific ABCA1-deficient mice. These data indicate that intestinal ABCA1 is a major determinant of plasma HDL-C levels. In this study, we used an intestine-specific LXR agonist GW6340 to address the question of how intestine-specific LXR agonist influences macrophage RCT and showed that it significantly promoted macrophage RCT. GW6340 significantly upregulated ABCA1, ABCG5, and ABCG8 expression in the small intestine but not in the liver. Fast protein liquid chromatography analysis suggested that it modestly increased cholesterol mass and macrophage-derived cholesterol tracer in the HDL fraction, providing 1 possible mechanism by which it promoted macrophage RCT. In addition, ABCG5/8 promotes cholesterol excretion in the small intestine. Recently, Veen et al reported that direct intestinal secretion of plasma cholesterol significantly contributes to fecal neutral sterol loss in mice. This transintestinal cholesterol excretion was upregulated by a LXR agonist and impaired in ABCG5-deficient mice. Thus, the intestine-specific LXR agonist might stimulate transintestinal excretion of plasma cholesterol partially via activating expression of the ABCG5/ABCG8 heterodimer. Therefore, the intestine-specific LXR agonist may promote RCT through at least 2 independent pathways: increasing intestinal HDL production and promoting intestinal excretion of HDL-derived cholesterol.

We previously reported that ABCA1-deficient and ABCG1-deficient macrophages showed a significant reduction of macrophage-derived fecal cholesterol excretion. LXR agonist treatment is known to upregulate ABCA1 and ABCG1 and cholesterol efflux from macrophages. These data suggest that macrophage LXR is crucial for the ability of an LXR agonist to promote RCT. We show here that macrophage-specific LXR deficiency resulted in a significant blunting, but not abolishment, of the ability of the systemic LXR agonist GW3965 to promote RCT. However, even in mice injected with labeled macrophages lacking LXR, GW3965-treated mice still increased fecal cholesterol excretion compared with vehicle group. Once cholesterol efflux occurred from macrophages to plasma through an LXR-independent pathway, activated LXR in liver and small intestine likely promoted cholesterol excretion from plasma to feces. Using HDL labeled with cholesteryl ester we confirmed that GW3965 promoted the fecal excretion of HDL-derived cholesterol in a macrophage-independent manner.

Some systemic LXR agonists activate SREBP-1c in the liver, leading to elevated plasma triglycerides and fatty liver. This has slowed the clinical development of LXR agonists as a potential treatment for atherosclerosis. Based on our studies, we suggest that intestinal-specific LXR agonism,
Figure 5. WT mice were fed the experimental diets for 2 weeks and HDL turnover studies were performed as described in Materials and Methods (n=6/group). A, The change of [3H]-HDL-cholesteryl-ester in plasma. B, [3H]-Cholesteryl in liver. C, The [3H] tracer distribution in feces. **P<0.01 vs control group. Data are expressed as mean±SD.

which avoids undesired effects of activating hepatic LXR, might be expected to promote macrophage RCT and thus potentially might be antiatherogenic.

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Disclosures

Didier Grillot and Stephane Huet are employees of GlaxoSmithKline. Philippe Delerive was previously employed by GlaxoSmithKline.

References

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Supplemental Methods

**Cholesterol efflux study with bone marrow macrophages**

Bone marrow macrophages (BMMs) were isolated from femurs and tibias and cultured in Dulbecco modification of Eagle media (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 30 % L929 conditioned medium for 7 days\(^1\). BMMs were grown in 12-well Petri dishes, labeled with 5 μCi/ml [3H]-cholesterol, and loaded with or without 25 μg/ml acetylated LDL (acLDL). Twenty-four hours later, cells were washed and equilibrated overnight in DMEM-0.2% bovine serum albumin (BSA) with or without 10 μM GW3965. The cells were washed with PBS and cholesterol efflux was initiated by the addition of DMEM-0.2 % BSA with or without 25 μg/mL HDL3. After 4 hours incubation, aliquots of the medium were filtered by MultiScreen-HV filter plates from Millipore to remove the floating cells and the supernatants were counted in a liquid scintillation counter (LSC). The cells were extracted with 2-propanol, evaporated under nitrogen gas, resuspended in toluene, and counted in an LSC. Cholesterol efflux was expressed as percentage of total tritium radioactivity presented in the cells plus the effluxed medium.

**Hepatic Triglyceride and Cholesterol Measurements**

Livers were perfused and homogenized in saline using 3 ml/g liver. The homogenates were diluted 5X with PBS and lipids solubilized at 37 degrees for 5 minutes in 1 % deoxycholate for triglyceride or 0.25 % deoxycholate for cholesterol. Triglyceride and cholesterol levels were measured with the use of diagnostic reagents from Thermo Scientific.

**Reference**

Supplemental legends

Supplemental Figure I. Structure of systemic LXR agonist GW3965 and intestine-specific LXR agonist GW6340.

Supplemental Figure II. BMMs from wild-type (WT) or LXR DKO mice were labeled with [3H]-cholesterol and loaded with or without 25 μg/ml acetylated LDL (acLDL) for 24 hours. Cells were equilibrated for 18 hours in the presence or absence of 1 μM (GW3965). Cholesterol efflux was determined in the absence (A) or presence of HDL3(25 μg/ml) (B) for 4 hours. Data are expressed as mean ± SD; n = 3. **P < 0.01.
Table I. Effects of 5-day GW3965 treatment on plasma lipid levels

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<tr>
<th>Mice</th>
<th>Drug</th>
<th>Total cholesterol</th>
<th>HDL cholesterol</th>
<th>Triglyceride</th>
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<tr>
<td>Wild type</td>
<td>Vehicle</td>
<td>96.8 ± 9.6</td>
<td>73 ± 9.7</td>
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<td></td>
<td>GW3965</td>
<td>142.8 ± 14.6 **</td>
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<tr>
<td>LXR KO</td>
<td>Vehicle</td>
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<td>68.8 ± 11</td>
<td>33.3 ± 9.9&lt;sup&gt;§§&lt;/sup&gt;</td>
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<td></td>
<td>GW3965</td>
<td>98.5 ± 15.1</td>
<td>65.3 ± 23.6</td>
<td>33 ± 7.4&lt;sup&gt;§§&lt;/sup&gt;</td>
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</table>

Values are means ± SD in mg per 100 mL plasma. In each group, n=6 samples were determined. See Methods for details on diets and lipid analysis. **p<0.01 vs vehicle group. §§p<0.01 vs wild type mice.
Table II . Effects of 12-day GW3965 and GW6340 treatment on hepatic lipid levels

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<th>Drug</th>
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<td>3.11 ± 0.42</td>
<td>4.3 ± 0.5</td>
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<tr>
<td>Wild type</td>
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<tr>
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<td>GW3965</td>
<td>2.91 ± 0.33</td>
<td>7.14 ± 1.03*</td>
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</table>

Values are means ± SD in mg per g liver. In each group, n=5 samples were determined. See Methods for details on lipid analysis. **p<0.01 vs vehicle and GW6340 group.
Table III. Effects of 5-day GW3965 and GW6340 treatment on plasma lipid levels

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<th>Mice</th>
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<th>HDL cholesterol</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
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<td>89 ± 4.3</td>
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<tr>
<td>Wild type</td>
<td>GW6340</td>
<td>107.9 ± 23.5</td>
<td>62.5 ± 10.5</td>
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<tr>
<td>Wild type</td>
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<td>131.5 ± 15.7</td>
<td><em>100.3 ± 15.9</em>*</td>
<td>51.3 ± 10.4</td>
</tr>
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</table>

Values are means ± SD in mg per 100 mL plasma. In each group, n=5 samples were determined. See Methods for details on diets and lipid analysis. **p<0.01 vs vehicle group. §§p<0.01 vs GW6340 group
Table IV. Effects of 5-day GW3965 treatment on plasma lipid levels

<table>
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<th>Drug</th>
<th>Total cholesterol</th>
<th>HDL cholesterol</th>
<th>Triglyceride</th>
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<td><strong>WT</strong></td>
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<td>82.3 ± 4.6</td>
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<td><strong>WT</strong></td>
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<td>132.2 ± 9.4</td>
<td>107.7 ± 8.6</td>
<td>49.7 ± 12.7</td>
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<tr>
<td>LXR DKO</td>
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<td>87.5 ± 6.8</td>
<td>71 ± 7.1</td>
<td>42.2 ± 14.2</td>
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<tr>
<td>LXR DKO</td>
<td>GW3965</td>
<td>130.0 ± 6.7</td>
<td>105.2 ± 5.2</td>
<td>46.5 ± 8.4</td>
</tr>
</tbody>
</table>

Values are means ± SD in mg per 100 mL plasma. In each group, n=6 samples were determined. See Methods for details on diets and lipid analysis. WT and LXR DKO indicates BMMs from wild-type mice and LXR DKO mice, respectively. **p<0.01 vs vehicle group.
Supplemental Figure I

**GW3965**
LXR transactivation pEC50=6.7

**GW6340**
LXR transactivation pEC50=7
Supplemental Figure II

A. BSA

- FC efflux (% of cpm in FC/4h)
- WT
- LXR KO

B. HDL3 specific efflux

- FC efflux (% of cpm in FC/4h)
- WT
- LXR KO

Legend:
- control
- +GW3965
- +acLDL

** indicates significance.