Liver X receptor activation promotes macrophage-to-feces reverse cholesterol transport in a dyslipidemic hamster model

Abstract  Liver X receptor (LXR) activation promotes reverse cholesterol transport (RCT) in rodents but has major side effects (increased triglycerides and LDL-cholesterol levels) in species expressing cholesteryl ester transfer protein (CETP). In the face of dyslipidemia, it remains unclear whether LXR activation stimulates RCT in CETP species. We therefore used a hamster model made dyslipidemic with a 0.3% cholesterol diet and treated with vehicle or LXR agonist GW3965 (30 mg/kg bid) over 10 days. To investigate RCT, radiolabeled 3H-cholesteryl macrophages or 3H-cholesteryl oleate-HDL were then injected to measure plasma and feces radioactivity over 72 or 48 h, respectively. The cholesterol-enriched diet increased VLDL-triglycerides and total cholesterol levels in all lipoprotein fractions and strongly increased liver lipids. Overall, GW3965 failed to improve both dyslipidemia and liver steatosis. However, after 3H-cholesteryl labeled macrophage injection, GW3965 treatment significantly increased the 3H-tracer appearance by 30% in plasma over 72 h, while fecal 3H-cholesterol excretion increased by 156% (P < 0.001). After 3H-cholesteryl oleate-HDL injection, GW3965 increased HDL-derived cholesterol fecal excretion by 64% (P < 0.01 vs. vehicle), while plasma fractional catabolic rate remained unchanged. Despite no beneficial effect on dyslipidemia, LXR activation promotes macrophage-to-feces RCT in dyslipidemic hamsters. These results emphasize the use of species with a more human-like lipoprotein metabolism for drug profiling.—Briand, F., M. Tréguier, A. André, D. Grillot, M. Issandou, K. Ouguerram, and T. Sulpice. Liver X receptor activation promotes macrophage-to-feces reverse cholesterol transport in a dyslipidemic hamster model. J. Lipid Res. 2010. 51: 763–770.

Supplementary key words  cholesteryl ester transfer protein • lipoprotein • dyslipidemia • atherosclerosis

The liver X receptors (LXR; NR1H2/3) LXRα and LXRβ are ligand-activated transcription factors of the nuclear receptor superfamily that control expression of genes involved in cholesterol homeostasis and fatty acid metabolism (1). In mice, activation of LXR has been shown to promote in vivo reverse cholesterol transport (RCT) (2, 3), a process by which cholesterol is effluxed from peripheral tissues to the liver for excretion into the bile and ultimately in the feces (4). Synthetic LXR ligands have been shown to stimulate cholesterol efflux from cultured macrophages (5, 6) and inhibit or even promote regression of atherosclerosis in mice (7, 8). Hence, studies performed in mice support LXR activation as an attractive target to prevent atherosclerosis (9).

However, concerns have been raised regarding undesired side effects such as liver steatosis, hypertriglyceridemia (10), and increased LDL-cholesterol levels in species, namely monkeys and hamsters, expressing cholesteryl ester transfer protein (CETP) (11). Despite these potentially harmful side effects, LXR agonists still represent a potential therapeutic target. For instance, indazole-based LXR modulators have been shown to reduce atherosclerosis and stimulation of hepatic triglyceride synthesis in mice and hamsters (12). Total cholesterol and LDL-cholesterol lowering effects have been recently shown in primates treated with one of those LXR modulators (13).

Although well characterized in rodents, it is actually unclear whether LXR activation would promote in vivo macrophage-to-feces RCT in species expressing CETP. This
represents an important preclinical issue before translating LXR therapies to humans, especially in the face of dyslipidemia. To address this question, we therefore developed a hamster model with both dyslipidemia and liver steatosis to assess the rate of RCT by injecting \(^3\)H-cholesterol labeled macrophages in vivo. Because this experiment gives limited information regarding HDL metabolism, we combined this method with HDL-cholesteryl ester kinetics by injecting HDL-\(^3\)H-cholesteryl oleate to better investigate the RCT process in vivo.

MATERIALS AND METHODS

Animals and diet

All animal protocols were approved by the local ethical committee (Comité régional d’éthique de Midi-Pyrénées). Male Golden Syrian hamsters (91–100 g, 6 weeks old) were housed in plastic cages (6–8 animals/cage) containing wood shavings and maintained in a room with a 12-h light cycle with free access to food and water. Animals were adapted to these conditions and fed a rodent chow diet (Purina chow #5001, Research Diets, NJ) for 1 week. This rodent chow diet was defined as the control diet.

Hamsters were then placed on either the control chow diet \((n = 8)\) or a chow diet supplemented with 0.3% cholesterol \((n = 8)\), pelleted from Research Diets, NJ. The diet was continued for 4 weeks and hamster body weight was monitored weekly. After 4 weeks of diet, hamsters were fasted overnight, blood was monitored using a glucometer (Roche Diagnostics, Meylan, France), and a blood sample (1 ml) was collected by retro-orbital bleeding and under isoflurane anesthesia. Animals were then euthanized by cervical dislocation, exsanguinated, and liver was harvested, weighed, flash-frozen in liquid nitrogen, and stored at \(-80^\circ\text{C}\) until biochemical analysis.

In another experiment, hamsters were fed a cholesterol-enriched diet during 4 weeks and then treated orally for a total of 10 days with either vehicle \((a \text{ saline solution of } 0.5% \text{ hydroxypropyl methylcellulose, twice daily})\) or LXR agonist GW3965 \(30 \text{ mg/kg, twice daily}\) over 72 h. Blood was collected by retro-orbital bleeding and under isoflurane anesthesia. Animals were then euthanized by cervical dislocation, exsanguinated, and liver was harvested, weighed, flash-frozen in liquid nitrogen, and stored at \(-80^\circ\text{C}\) until biochemical analysis.

In a third experiment, hamsters were fed a cholesterol-enriched diet during 4 weeks and then treated orally for a total of 10 days with either vehicle \((a \text{ saline solution of } 0.5% \text{ hydroxypropyl methylcellulose, twice daily})\) or LXR agonist GW3965 \(30 \text{ mg/kg, twice daily}\). After 7–8 days of treatment, hamsters underwent in vivo macrophage-to-feces RCT or HDL-cholesteryl ester kinetics as described below.

Another group of hamsters fed the cholesterol-enriched diet were kept treated by vehicle \((n = 8)\) or GW3965 \((n = 8)\) until the end of the treatment period \((10 \text{ days})\) to collect blood by retro-orbital bleeding \((6 \text{ h postgavage, nonfasting conditions})\). Plasma was isolated and kept frozen at \(-80^\circ\text{C}\) until biochemical analysis. After being euthanized by cervical dislocation, liver and the medium part \((\text{jejunum})\) of the small intestine were harvested. Jejunum segment was isolated using the 1:3:2 \((\text{duodenum-jejunum-ileum})\) lengths ratio method \((14)\). Harvested tissues were weighed and flash-frozen in liquid nitrogen and then stored at \(-80^\circ\text{C}\) until liver lipids assays. An aliquot of each tissue was also stored at \(-80^\circ\text{C}\) in RNAlater (Invitrogen) for hepatic and intestinal gene expression.

Biochemical analysis

Total cholesterol, triglycerides, and free fatty acids were assayed using commercial kits (Biomérieux, Marcy l’Étoile, France; Wako Chemicals, Richmond, VA). HDL-cholesterol was determined using the phosphotungstate/MgCl\(_2\) precipitation method. Non-HDL cholesterol levels were then determined by subtracting HDL-cholesterol values from total plasma cholesterol. Plasma CETP, phospholipid transfer protein (PLTP), and lecithin:cholesterol acyl transferase (LCAT) activities were measured by fluorescence using commercial kits (Roarbiomedical, New York, NY) as well as hepatic lipase (HL) activity (ProGen, Heidelberg, Germany). Fast protein liquid chromatography lipoprotein profiles (total cholesterol and triglycerides) using pooled plasma were performed as previously described \((15)\). Hepatic cholesterol, triglycerides, and fatty acid levels were determined from liver homogenate incubated with deoxycholate \((10)\).

RNA extraction and gene expression analysis

Tissue for mRNA analysis was homogenized, and RNA was isolated using Trizol reagent \((\text{Invitrogen})\) according to the manufacturer’s instructions. Real-time quantitative PCR analysis was performed as follows: 1 µg of total RNA was reverse transcribed using 100 units of Moloney Murine Leukemia-Virus reverse transcriptase \((\text{Invitrogen})\). Real time quantitative PCR was performed on the 7000 Sequence Detection System with SYBR green \((\text{Eugentech, Angers, France})\). The reaction contained 10 ng of reverse transcripted total RNA, 500 nM forward and reverse primers, and 5× Sybr green Mix. Primer sequences are available on request. All reactions were performed at least in triplicate and cyclophilin RNA amplification was used as a reference. In all PCR assays and for each primer set, expression of a control cDNA was included as inter-run calibrator.

In vivo macrophage-to-feces RCT

Preparation of J774 cells and in vivo RCT study were performed as previously described \((16)\) with minor modifications. J774 cells obtained from the American Type Culture Collection \((\text{Manassas, VA})\) were grown in suspension in RPMI/HEPES supplemented with 10% FBS and 0.5% gentamicin in suspension in Nalgene Teflon flasks. Cells were radiolabeled with 5 µCi/ml \(\text{^3H-cholesterol and cholesteryl loaded with } 50 \mu\text{g/ml oxidized LDL (kindly provided by Marine Goffinet from Cerenis Therapeutics) over } 48 \text{ h. Radiolabeled cells were then washed with RPMI/HEPES and equilibrated for } 4 \text{ h in fresh RPMI/HEPES supplemented with } 0.2% \text{ BSA and gentamicin. Cells were pelleted by low speed centrifugation and resuspended in MEM/HEPES prior to injection into hamsters.}\)

\(^3\)H-cholesterol-labeled and oxidized LDL-loaded J774 cells \((2.5 \times 10^7 \text{ cells containing } 10 \times 10^2 \text{ dpm in } 0.5 \text{ ml minimum essential medium})\) were injected intraperitoneally into individually caged hamsters. Animals had free access to food and water and were treated twice daily with vehicle \((n = 6)\) or LXR agonist GW3965 \((30 \text{ mg/kg twice daily})\) over 72 h. Blood was collected by retro-orbital bleeding and under isoflurane anesthesia at 24, 48, and 72 h to measure radioactivity released into the plasma \((10 \mu\text{l counted in a liquid scintillation counter})\). Hamsters were then sacrificed by cervical dislocation and liver was harvested from each animal. An approximately 50 mg piece of liver was homogenized using an ultrasound probe in 500 µl water then 300 µl were counted in a liquid scintillation counter. Feces were collected over 72 h and were stored at \(4^\circ\text{C}\) before extraction of cholesterol and bile acid.

Fecal cholesterol and bile acid extraction was performed as previously described \((16)\). The total feces collected from 0 to 72 h were weighed and soaked in Millipore water \((1 \text{ ml water/100 mg feces})\) overnight at \(4^\circ\text{C}\). The following day, an equal volume of absolute ethanol was added, and the mixtures were homogenized. To extract the \(^3\)H-cholesterol and \(^3\)H-bile acid fractions, 1 ml of the homogenized samples was combined with 1 ml ethanol and 200 µl NaOH. The samples were saponified at 95°C for 1 h and cooled to room temperature and then \(^3\)H-cholesterol was extracted 2 times with 3 ml hexane. The extracts were pooled, evaporated, resuspended in toluene, and then counted in a liquid
scintillation counter. To extract \(^3\)H-bile acids, the remaining aqueous portion of the feces was acidified with concentrated HCl and then extracted 2 times with 3 ml ethyl acetate. The extracts were pooled together, evaporated, resuspended in ethyl acetate, and counted in a liquid scintillation counter.

Results were expressed as a percent of the radioactivity injected recovered in plasma, liver, and feces. The plasma volume was estimated as 3.5% of the body weight.

In vivo \(^3\)H-cholesterol oleate-HDL kinetics

A plasma pool was obtained from normocholesterolemic hamsters maintained on a control diet. Hamster HDL (d = 1.07–1.21) were then isolated by ultracentrifugation as previously described (17). After extensive dialysis against saline, HDL were labeled with \(^3\)H-cholesterol oleate in the presence of lipoprotein deficient serum collected from rabbit plasma, as already described (17). The labeled HDL were reisolated by ultracentrifugation (d = 1.07–1.21) then extensively dialyzed prior injection.

The day before the in vivo experiment, catheters were inserted into the carotid artery (blood sampling) and into the jugular vein (injection) under isoflurane anesthesia. Catheters were kept patent with NaCl 0.9%. To prevent blood circulation and coagulation inside the catheter, a small volume of heparin (500 IU/ml) and glycerol (1 g/ml) was injected at the catheter extremity.

On the day of the experiment, the hamsters were weighed and placed into individual cages. Animals had free access to food and water and were treated twice daily with vehicle (n = 6) or LXR agonist GW3965 (30 mg/kg twice daily) in hamsters fed the chow + 0.3% cholesterol diet over 48 h. The \(^3\)H-cholesterol oleate-labeled HDL (≈ 2 million dpm) were injected intravenously and blood samples (150 µL) were collected at time (injection) under isoflurane anesthesia. Catheters were kept patent with NaCl 0.9%. To prevent blood circulation and coagulation measurement in a liquid scintillation counter (10 µL plasma immediately centrifuged and stored at 4°C until radioactivity was counted in a liquid scintillation counter.

RESULTS

Cholesterol-enriched diet induces dyslipidemia and hepatic steatosis in hamsters

Golden Syrian hamsters were fed a control chow or chow + 0.3% cholesterol diet for 4 weeks. As shown in Table 1, the chow + 0.3% cholesterol diet had no effect on body weight. However, there was a slight but significant 17% increase in blood glucose levels after an overnight fast (P < 0.001). The cholesterol-enriched diet increased total cholesterol plasma levels by 119% (P < 0.001). Both the HDL-cholesterol and non-HDL-cholesterol levels were increased (76 and 233%, respectively, P < 0.001 for both), which led to a 19% lower HDL-cholesterol-total cholesterol ratio (P < 0.001). Plasma triglycerides increased by 31% with the cholesterol-enriched diet (P < 0.05), while free fatty acids remained unchanged. The cholesterol-enriched diet increased CETP, PLTP, and LCAT activity by 33, 81, and 8%, respectively (P < 0.01 for all). There was no change regarding HL activity.

The diet-induced dyslipidemia was confirmed with lipoprotein cholesterol profiles analyzed by fast protein liquid chromatography (pooled plasma from overnight fasted hamsters), with higher total cholesterol levels in VLDL, LDL, and HDL fractions (Fig. 1A) as well as higher VLDL-triglycerides (Fig. 1B). No change was detectable regarding LDL and HDL-triglycerides. Moreover, liver harvested after 4 weeks of diet showed a 66% increase in organ weight (0.027 ± 0.001 vs. 0.045 ± 0.001 g/g body weight, P < 0.001, data not shown). As shown in Fig. 1C, chow + 0.3% cholesterol diet strongly increased hepatic cholesterol, triglycerides, and fatty acids by 535, 168, and 437%, respectively (P < 0.001 for all).

Overall, these data indicate that chow + 0.3% cholesterol diet provided over 4 weeks induces dyslipidemia and liver steatosis in golden Syrian hamsters.

LXR agonist GW3965 fails to improve dyslipidemia and hepatic steatosis in hamsters fed a cholesterol-enriched diet

We then investigated the effects of the LXR agonist GW3965 (30 mg/kg twice daily) in hamsters fed the chow + 0.3% cholesterol diet. After 10 days of treatment, blood was collected 6 h after drug dosing (nonfasting conditions) to assay plasma lipids. As shown in Table 2, there was a slight 11% decrease in total cholesterol (P < 0.05 vs. vehicle). There was a similar trend for HDL-cholesterol levels, although the 17% decrease did not reach significance (P = 0.06). When lipoprotein profiles were performed (pooled plasma from nonfasted hamsters), the decrease in HDL-cholesterol was more evident, while total cholesterol levels were slightly lower in LDL fractions (Fig. 2A). Meanwhile, calculated non-HDL-cholesterol and the HDL-cholesterol-total cholesterol ratio remained unchanged (Table 2). The main effect of GW3965 on plasma lipids was a significant 52% increase in plasma triglycerides (Table 2). This increased was seen in only the VLDL
fractions (Fig. 2B). Treatment with GW3965 increased CETP activity by 22% (measured at 6 h postdosing), but not significantly (Table 2). There was no significant effect on liver total cholesterol, triglycerides, or fatty acids (Fig. 2C). Except for VLDL-triglycerides, these data suggest that LXR activation with GW3965 has no major effects on plasma and liver lipids in dyslipidemic hamsters.

LXR agonist GW3965 has significant effects on genes involved in cholesterol metabolism

The effects of LXR agonist GW3965 on liver and intestine gene expression are shown in Fig. 3. In the liver (Fig. 3A), there was a 69, 164, and 878% increase in ATP binding cassette protein A1, G5, and G8 mRNA expression, respectively \( (P < 0.001 \text{ vs. vehicle for all}) \), while G1 expression was unexpectedly decreased by 74% with GW3965 treatment \( (P < 0.001) \). The reason for this lower hepatic ABCG1 expression remains unclear. Expression of cholesterol 7\( \alpha \)-hydroxylase A1 was 85% lower as well \( (P < 0.001) \). The ACAT2 expression increased by 56% \( (P < 0.001) \). As expected, a huge 1551% increase in hepatic sterol-regulatory element binding protein 1c expression was observed with GW3965 treatment \( (P < 0.001) \). As shown in Fig. 3B, the same effect was seen in the intestine \( (2117\% \text{ increase, } P < 0.001) \). The expected effect of GW3965 on ATP binding cassette protein A1 and G1 intestinal expression was observed \( (1235 \text{ and } 2551\% \text{ increase, respectively, } P < 0.001 \text{ for both}) \). Effects of LXR activation on genes involved in intestinal cholesterol absorption were also observed with a 159 and 105% increase in ABCG5 and G8 mRNA expression, respectively \( (P < 0.001 \text{ for both}) \), while Niemann Pick C1-Like 1 expression was decreased by 34% \( (P < 0.02) \). No effect was seen on ACAT2 expression.

These data indicate that LXR agonist GW3965 has significant effects on the expression of genes involved in cholesterol metabolism.

LXR agonist GW3965 promotes macrophage-to-feces RCT in dyslipidemic hamsters in vivo

To investigate whether LXR activation promotes RCT, radiolabeled macrophages were injected in dyslipidemic hamsters treated with vehicle or LXR agonist GW3965. As shown in Fig. 4A, plasma tracer appearance was \( \sim 30\% \) higher in hamsters treated with the LXR agonist at 24 h \( (P < 0.01 \text{ vs. vehicle}) \), 48 h, and 72 h \( (P < 0.05 \text{ for both}) \) after injection. As shown in Fig. 4B, \( ^3\text{H} \)-tracer recovery was 27% higher in the liver of hamsters treated with GW3965 at 72 h \( (P < 0.05) \). The \( ^3\text{H} \)-tracer recovery was also measured in feces after chemical extraction (Fig. 4C). Interestingly, there was a significant 156% increase in the \( ^3\text{H} \)-free sterols fecal excretion for hamsters treated with the LXR agonist GW3965. No significant change was seen in the bile acids fraction. Overall, these data show that LXR activation promotes macrophage-to-feces RCT in dyslipidemic hamsters in vivo.

LXR agonist GW3965 promotes HDL-derived fecal cholesterol excretion despite no effect on plasma FCR

To test whether LXR agonist GW3965 affects HDL metabolism and HDL-derived cholesterol fecal excretion, hamsters were then injected with \( ^\text{H} \)-cholesterol oleate-labeled HDL. As shown in Fig. 5A, GW3965 had no effect on plasma decay curve. Expectedly, plasma FCR, calculated with the SAAMII software, remained unchanged (Fig. 5A). Free sterols and bile acids were extracted from feces

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**TABLE 2. Plasma lipids in nonfasted hamsters fed a chow + 0.3% cholesterol diet after 10 days of treatment with vehicle or LXR agonist GW3965 twice daily**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>GW3965</th>
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<tbody>
<tr>
<td>Total cholesterol (g/l)</td>
<td>3.33 ± 0.14</td>
<td>2.95 ± 0.13*</td>
</tr>
<tr>
<td>HDL-cholesterol (g/l)</td>
<td>2.19 ± 0.05</td>
<td>1.82 ± 0.11</td>
</tr>
<tr>
<td>Non-HDL-cholesterol (g/l)</td>
<td>1.14 ± 0.17</td>
<td>1.14 ± 0.22</td>
</tr>
<tr>
<td>HDL-cholesterol/total cholesterol</td>
<td>0.66 ± 0.02</td>
<td>0.62 ± 0.03</td>
</tr>
<tr>
<td>Triglycerides (g/l)</td>
<td>0.97 ± 0.05</td>
<td>1.48 ± 0.12*</td>
</tr>
<tr>
<td>CETP activity (pmol/h/µl)</td>
<td>10.0 ± 1.41</td>
<td>12.2 ± 0.93</td>
</tr>
</tbody>
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Mean ± SEM, n = 8 per group, *P < 0.05 vs. vehicle.
collected over 48 h after tracer injection. As shown in Fig. 5B, GW3965 significantly increased by 64% fecal free sterols excretion ($P < 0.01$). There was no change in fecal bile acids. These data indicate that LXR activation has no effect on plasma FCR but promotes HDL-derived cholesterol fecal excretion in dyslipidemic hamsters in vivo.

DISCUSSION

The present results demonstrate that LXR activation with GW3965 promotes macrophage-to-feces RCT in a hamster model with both dyslipidemia and liver steatosis. Because plasma FCR after $[^3]H$-cholesterol oleate-labeled HDL injection was not changed, LXR activation likely had major effects on macrophage cholesterol efflux, biliary cholesterol excretion, and inhibition of intestinal cholesterol absorption. Overall, this led to an enhanced macrophage- and HDL-derived cholesterol fecal excretion, which respectively increased by 156 and 64%.

To better perform preclinical profiling of compounds affecting RCT (e.g., LXR agonists), we aimed to develop a nutritional model with a similar lipoprotein metabolism compared with humans. Among small animals, the hamster does express CETP and HL, which potentially forms small dense LDL (18), and has no hepatic apolipoprotein B48 production (19). Thus, the hamster has been useful to test the effects of CETP inhibition (20), activation of LXR (21, 11), farnesoid X receptor (22), and peroxisome proliferator activated receptor $\alpha$ (23) on lipoprotein metabolism. Moreover, hamsters easily develop dyslipidemia when fed an atherogenic diet (23, 24) and insulin resistant/diabetic states can be reached when diets are supplemented with fructose (25) or high percentage of fat (23).

In the present study, a chow+0.3% cholesterol diet was used to mainly induce dyslipidemia with limited effects on...
studies in hamsters have suggested that metabolic disturbance induced by dietary cholesterol might be related to LXR activation (25). One might also speculate that the 0.3% cholesterol might increase LDL-cholesterol levels through a lower hepatic LDL-receptor expression, because LXR has been shown to induce the E3 ubiquitin ligase Inducible Degrader of the LDLR in mice (26). Interestingly, treatment with the LXR agonist TO901317 in hamster disregulates hepatic insulin signaling, which dramatically leads to higher triglyceride-rich VLDL particles production (27). The present data suggest that similar effects may have occurred in our hamster model.

Because biochemical parameters (e.g., HDL-cholesterol levels) are not fully predictive regarding the rate of RCT, we then used a combination of in vivo RCT and HDL-cholesteryl ester kinetic experiments. Despite the maintained insulin resistance/diabetes. As expected, hamsters fed a chow + 0.3% cholesterol diet had a significant decrease in the HDL-cholesterol-total cholesterol ratio and increased triglycerides levels as well as CETP activity.

Based on our hamster model, we then investigated whether LXR activation affects lipoprotein metabolism and RCT in light of the metabolic disorders aforementioned. As previously described in normal hamsters (11), the main GW3965 side effect was the increased plasma triglyceride levels, a likely consequence of the strong increase in sterol-regulatory element binding protein 1c mRNA expression seen in both liver and intestine. Despite the increase in VLDL-triglycerides, it is likely that other LXR side effects (increased LDL-cholesterol and CETP activity) have been partly offset by the dyslipidemic state already induced upon the 0.3% cholesterol diet. Very recent

![Fig. 4. Effects of GW3965 LXR agonist on macrophage-to-feces RCT. Hamsters on a chow + 0.3% cholesterol diet were orally treated twice daily for 7 days with vehicle (0.5% hydroxypropyl methylcellulose) or GW3965 (30 mg/kg) and then injected with $^3$H-cholesterol labeled macrophages. Animals were treated with vehicle or GW3965 during the 72 h experiment. Time course of $^3$H-cholesterol distribution in plasma of hamsters of each group was established (A) after injection of radiolabeled macrophages. Liver (B) and fecal (C) $^3$H-tracer recovery in hamsters of vehicle group (solid bars) and GW3965 group (gray bars) were also monitored. Data are expressed as percent of dpm injected ± SEM (n = 6 per group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. vehicle).](image)

![Fig. 5. Effects of GW3965 LXR agonist on HDL-cholesteryl esters kinetics and HDL-derived fecal cholesterol excretion. Hamsters on a chow + 0.3% cholesterol diet were orally treated twice daily for 8 days with vehicle (0.5% hydroxypropyl methylcellulose) or GW3965 (30 mg/kg) and then injected with $^3$H-cholesterol oleate labeled HDL. Animals were treated with vehicle or GW3965 during the 48 h experiment. After injection of radiolabeled HDL, plasma decay curves (A) of vehicle- (solid plots) and GW3965-treated hamsters (gray plots) were analyzed and fitted curves (dashed line) and FCR were calculated by SAAMII software. Fecal $^3$H tracer recovery (B) were detected in hamsters treated with vehicle (solid bars) or GW3965 LXR agonist (gray bars). Values are means ± SEM (n = 6 per group, ** $P < 0.01$ vs. vehicle).](image)
or somewhat aggravated dyslipidemic state, LXR activation with GW3965 significantly promoted macrophage-to-feces RCT in vivo. After radiolabeled macrophage injection, the first stimulated step by LXR activation was likely the macrophage cholesterol efflux, which led to the ∼30% increase in 3H-cholesterol plasma appearance at all time points. This increase was not related to a lower removal of cholesterol esters originally contained in HDL, because plasma FCR remained unchanged upon LXR activation. One might also speculate that more cholesterol effluxed from the macrophage reached the liver for further excretion into the bile rather than accumulation in hepatocyte. Actually, ABCG5 and G8, known targets of LXR (28), were strongly increased and may have directly promoted biliary cholesterol excretion, as described in mice (29). Besides increased biliary cholesterol excretion, inhibition of intestinal cholesterol absorption might have been the other crucial mechanism for promoting macrophage- and HDL-derived cholesterol fecal excretion. Recently, inhibiting intestinal cholesterol absorption in mice with ezetimibe (16, 30) or PPARδ agonist (16) has been shown to promote macrophage-to-feces RCT. In mice, activation of LXR reduces intestinal cholesterol absorption, because intestinal ABCG5/G8 (28, 29) and Niemann Pick C1-Like 1 (31) are targeted by LXR as well. In the present study, similar effects were found upon GW3965 treatment, which would have contributed to reduce intestinal reabsorption of macrophage- and HDL-derived cholesterol.

Although biliary excretion and intestinal absorption may have been the main contributors to higher cholesterol fecal excretion, LXR agonist GW3965 could have stimulated a novel pathway for RCT, namely transintestinal cholesterol excretion (32). This nonbiliary route, which mediates the direct cholesterol excretion from plasma to the intestinal lumen, has been shown to be stimulated by LXR activation in Mdr2 knock-out mice, a model of biliary cholesterol excretion deficiency (33). To address whether this occurs in hamsters, hamsters, hamsters, and primates (13), still represent a potential therapeutic target. In this goal, LXR modulators with favorable pharmacological profiles, as recently shown in hamsters and primates (13), still represent a potential therapy to treat cardiovascular diseases.  

In conclusion, the present data demonstrate that LXR agonist GW3965 promotes macrophage-to-feces RCT in dyslipidemic hamsters in vivo. These results suggest that LXR agonists would be likely to promote RCT in humans with dyslipidemia and emphasize the relevance of LXR as a therapeutic target. In this goal, LXR modulators with favorable pharmacological profiles, as recently shown in hamsters and primates (13), still represent a potential therapy to treat cardiovascular diseases.

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