Pharmacological Activation of Liver X Receptors Promotes Reverse Cholesterol Transport In Vivo

Snehal U. Naik, PhD; Xun Wang, PhD; Jaqueline S. Da Silva, PhD; Michael Jaye, PhD; Colin H. Macphee, PhD; Muredach P. Reilly, MD; Jeffrey T. Billheimer, PhD; George H. Rothblat, PhD; Daniel J. Rader, MD

Background—Liver X receptors (LXRs) are ligand-activated transcription factors involved in the control of lipid metabolism and inflammation. Synthetic LXR agonists have been shown to inhibit the progression of atherosclerosis in mice, but the mechanism is uncertain. LXR agonism upregulates the genes encoding ATP binding cassette transporters A1 (ABCA1) and G1 (ABCG1) in macrophages, thus promoting efflux of cholesterol; it also upregulates liver and intestinal ABCG5 and ABCG8, helping to promote biliary and fecal excretion of cholesterol. Thus, LXR agonism may inhibit atherosclerosis through promotion of reverse cholesterol transport (RCT) in vivo, but this has not been proven. We previously described an in vivo method to trace the movement of cholesterol from 3H-cholesterol–labeled J774 macrophages into plasma, into liver, and ultimately into the bile and feces as free cholesterol or bile acids. In the present study we used this approach to test the hypothesis that administration of the synthetic LXR agonist GW3965 would increase the rate of macrophage RCT in vivo.

Methods and Results—Three different mouse models—wild-type C57BL/6 mice, LDLR/apobec-1 double knockout mice, and human apolipoprotein (apo)B/cholesteryl ester transfer protein (CETP) double transgenic mice—were treated with either vehicle or GW3965. Mice were injected intraperitoneally with 3H-cholesterol–labeled and cholesterol-loaded macrophages and monitored for the appearance of 3H-tracer in plasma, liver, and feces. Administration of GW3965 significantly increased the levels of 3H-tracer in plasma and feces in all 3 mouse models.

Conclusions—These results demonstrate that administration of the LXR agonist GW3965 increases the rate of RCT from macrophages to feces in vivo. (Circulation. 2006;113:90-97.)

Key Words: cholesterol ■ lipids ■ lipoproteins ■ reverse cholesterol transport

Liver X receptors (LXRs) are transcription factors that belong to the ligand-activated nuclear hormone receptor superfamily. Both LXRα and LXRβ are bound and activated by oxysterols and play an important role in the control of lipid homeostasis. Activation of LXRs induces expression of several genes implicated in the regulation of lipid metabolism and cholesterol transport. Two relevant LXR target genes are the ATP-binding cassette transport proteins ABCA1 and ABCG1, which are important for the efflux of excess cellular cholesterol from cells to acceptors such as lipid-poor apolipoprotein A-I (apoA-I) and mature HDL, respectively. Genetic deficiency of ABCA1, ABCG1, or both LXRα and LXRβ is associated with macrophage cholesterol accumulation in mice. Furthermore, synthetic LXR agonists have been shown to inhibit the development of atherosclerosis in mouse models of atherosclerosis. However, the mechanism of these antiatherogenic effects is uncertain, and LXR agonists have never been shown to promote macrophage-to-feces reverse cholesterol transport (RCT) in vivo. In fact, it is possible that the antiatherogenic effects of LXR agonists could be due mainly to the established antiinflammatory effects of LXR activation. To directly test the hypothesis that LXR agonism promotes macrophage RCT in vivo, we utilized a newly developed assay.

Methods

Animals

Wild-type C57BL/6 mice were obtained from the Jackson Laboratory (Bar Harbor, Maine), and apolipoprotein B (apoB)/cholesteryl ester transfer protein (CETP) double transgenic mice were obtained from Taconic (Germantown, Md). LDLR/apobec double knockout mice were bred in-house (originally from Genentech Inc, San Francisco, Calif).
Francisco, Calif). Mice were fed a standard chow diet ad libitum before and during the study. For plasma lipid 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.

**Cell Culture**

J774 cells obtained from the American Type Culture Collection (ATCC; Manassas, Va) were grown in suspension in RPMI/HEPES supplemented with 10% FBS and 0.5% gentamicin. Cells were cultured in Nalgene Teflon flasks (Fisher Scientific) and radiolabeled with 5 μCi/mL 3H-cholesterol and cholesterol loaded with 25 μg/mL acetylated LDL (acLDL). Forty-eight hours later, cells were washed with RPMI/HEPES and equilibrated for 4 hours in fresh RPMI/HEPES supplemented with 0.2% BSA and gentamicin. For the initial study in wild-type mice, J774 cells were treated with either dimethyl sulfoxide (DMSO) (vehicle) or 10 μM LXR agonist GW3965 during the equilibration period. For other studies, cells were grown in medium and not pretreated. Before injection into mice, cells were spun down and resuspended in RPMI/HEPES. The LXR agonist GW3965 (EC50, 190 nmol/L) was obtained from GlaxoSmithKline, King of Prussia, Pa, and has been described previously.15

**In Vivo Studies**

**Wild-Type Mice**

Before the initial experiment, twenty-four 6- to 8-week-old male C57BL/6 mice were divided into 4 groups so that the mean plasma cholesterol levels between the 4 groups were similar. Two of the groups were dosed with 10 mg/kg GW3965 twice a day for 10 days by oral gavage. Control animals (the remaining 2 groups) received vehicle (0.5% methylcellulose). Before injection of J774 cells, animals were once again bled to measure changes in plasma lipid levels and to analyze plasma LXR agonist concentrations.

On the day of injection, animals were caged individually with unlimited access to food and water. 3H-Cholesterol–labeled and acLDL-loaded J774 cells (typically 4.5 × 10^6 cells containing 7.5 × 10^5 counts per minute [cpm] in 0.5 mL minimum essential medium) were injected intraperitoneally as described previously.14 Both vehicle-treated and LXR agonist–treated mice were injected with either vehicle (DMSO) or LXR agonist–treated J774 cells. Thus, the 4 groups of mice were the control group (−/−), vehicle-treated mice injected with either vehicle/DMSO or LXR agonist–treated J774 cells. Blood was collected at 6, 24, and 48 hours, and plasmas were used for liquid scintillation counting (LSC). Feces were collected continuously from 0 to 48 hours and stored at 4°C before extraction of cholesterol and bile acid. Animals continued to receive vehicle or drug during the 48-hour RCT study. At study termination (48 hours after injection), mice were exsanguinated and perfused with cold PBS, and portions of the liver were removed and flash-frozen for lipid extraction and gene expression analysis. A half-inch piece of the small intestine was also dissected out, flushed with cold, sterile PBS, and flash-frozen for gene expression analysis. Two additional independent studies (n = 6 per group) were performed in wild-type mice that did not include pretreatment of cells but only treatment of the mice with GW3965 or vehicle.

**LDLR/apobec Double Knockout Mice**

The procedure for the experiments in which LDLR/apobec double knockout mice (9-month-old female mice) were used was essentially the same as that for the wild-type mouse experiment except that the J774 cells were not treated with GW3965 in these experiments. The 2 groups (n = 8 per group) represent vehicle- and GW3965-treated mice only. Before injection of J774 cells, animals were once again bled to measure changes in plasma lipid levels and to analyze LXR agonist concentrations. This was done with the use of a method based on protein precipitation/dilution with acetonitrile (containing a mass spectral internal standard) followed by LC/MS/MS analysis employing positive-ion Turbo IonSpray Ionization. The average plasma GW3965 concentration was 1.3 μmol/L, which was 7-fold above the EC50 (190 nmol/L) for the compound.15

**ApoB/CETP Double Transgenic Mice**

The procedure for the experiments in which apoB/CETP double transgenic mice (6- to 8-week-old male mice) were used was essentially the same as that for the LDLR/apobec double knockout mouse experiment. The 2 groups (n = 6 per group) represent vehicle- and GW3965-treated mice only.

**Plasma Lipid Analysis**

Plasma HDL cholesterol was measured on a Cobas Fara with the use of Sigma Diagnostic reagents. Pooled plasma (160 μL) from mice was analyzed by fast protein liquid chromatography (FPLC) gel filtration (Amersham Pharmacia Biotech) on 2 Superose 6 columns. The cholesterol concentrations in the FPLC fractions were determined by an enzymatic assay (Wako Pure Chemical Industries Ltd). The 3H-tracer distribution across the FPLC profile was determined by LSC.

**Tissue Lipid Extraction**

Tissue lipids were extracted according to the procedure of Bligh and Dyer.16 Results for liver were expressed as percentage of counts per minute injected (whole organ). Briefly, a 50-mg 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 LSC.

**Fecal Cholesterol and Bile Acid Extraction**

The total feces collected from 0 to 48 hours were weighed and soaked in Millipore water (1 mL water per 100 mg feces) overnight at 4°C. The next day, an equal volume of ethanol was added, and the samples were homogenized. A 200-μL aliquot of each homogenized fecal sample was counted in a LSC to establish the 'H-total sterols. To extract the 'H-cholesterol and 'H-bile acid fractions, 2 mL of the homogenized samples was combined with 2 mL ethanol, a known amount of '4C-choleic acid as an internal standard, and 400 μL NaOH. The samples were saponified at 95°C for 2 hours and cooled to room temperature, and then 'H-cholesterol was extracted 3 times with 9 mL hexane. The extracts were pooled, evaporated, resuspended in toluene, and then counted in a LSC. To extract 'H-bile acids, the remaining aqueous portion of the feces was acidified with concentrated HCl and then extracted 3 times with 9 mL ethyl acetate. The extracts were pooled together, evaporated, resuspended in ethyl acetate, and counted in a LSC.

**RNA Extraction and Gene Expression Analysis**

An ∼30- to 50-mg piece of tissue was homogenized, and RNA was isolated with the use of Trizol reagent according to the manufacturer’s instructions. Real-time quantitative polymerase chain reaction (PCR) assays were performed with an Applied Biosystems 7300 sequence detector. Briefly, 5 μg of total RNA was reverse transcribed with the use of an Applied Biosystems High Capacity cDNA archive kit according to the package instructions. Each 25-μL amplification reaction contained 100 ng cDNA, 900 nmol/L forward primer, 900 nmol/L reverse primer, 200 nmol/L fluorescent probe, and 2× universal PCR master mix. Primer and probe sequences are available on request.

**Statistical Analysis**

Values are presented as mean ± SEM. 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
GW3965 Treatment Increased Macrophage RCT in Wild-Type Mice
In the first RCT study in wild-type mice, the experimental design involved treatment of animals with either vehicle or GW3965; in addition, the \(^3\)H-cholesterol–labeled J774 cells were also pretreated ex vivo with either DMSO (vehicle) or 10 \(\mu\)mol/L GW3965. The expression of ABCA1 in the J774 cells after treatment with 10 \(\mu\)mol/L GW3965 for 4 hours was increased 4.0±0.3-fold compared with DMSO-treated cells. However, there was no significant difference in \(^3\)H-tracer levels in plasma, liver, or feces of the untreated animals injected with cells pretreated with GW3965 compared with vehicle-treated cells.

Mice were gavaged for 10 days with GW3965 or vehicle before injection of J774 cells and continued to be gavaged during the 48-hour experiment. To confirm the effect of LXR agonist treatment, gene expression in liver of vehicle- and GW3965-treated wild-type mice was assessed by quantitative PCR (Figure 1A). Expression of ABCA1 mRNA increased significantly by 2-fold; expression of ABCG1 (3-fold), ABCG5 (2-fold), ABCG8 (1.7-fold), CYP7A1 (2-fold), and SREBP1c (3-fold) were also significantly increased. In the small intestine, expression of ABCA1 (14-fold), ABCG1 (7-fold), ABCG5 (1.3-fold), ABCG8 (1.8-fold), and SREBP1c (7-fold) were significantly increased in response to LXR agonist treatment (Figure 1B). These results demonstrate that GW3965 treatment induced hepatic and intestinal expression of the expected LXR-regulated genes.

Mice treated with GW3965 had 54% higher HDL cholesterol levels than vehicle-treated mice. The increase in HDL cholesterol was also evident when lipoproteins were separated by size by FPLC (Figure 1C). The plasma \(^3\)H-cholesterol levels in GW3965-treated mice were significantly higher at 6, 24, and 48 hours than those in vehicle-treated mice (Figure 1D) regardless of whether the cells were pretreated with GW3965. The appearance of \(^3\)H-tracer in plasma of treated mice was rapid, with a >3-fold increase at 6 hours compared with the control group, and remained substantially higher than in control mice at all time points. Furthermore, the \(^3\)H-cholesterol in plasma tracked closely with the cholesterol mass in plasma lipoproteins (Figure 1C). The \(^3\)H-tracer levels in liver in the 2 groups of GW3965-treated mice were not different compared with the control animals (Figure 1E). However, mice treated with the LXR agonist had 2.5-fold significantly higher \(^3\)H-total fecal sterol excretion than vehicle-treated control mice (Figure 1F). This increase was largely due to an increase in fecal \(^3\)H-cholesterol excretion, but there was a trend toward an increase in fecal \(^3\)H-bile acid excretion as well.

The ability of an LXR agonist to increase RCT in wild-type mice was studied in 2 additional independent experiments that were similar in design and size except that cells were not pretreated with GW3965, and thus there were only 2 groups: mice treated with GW3965 or vehicle. Analysis of the data from these 3 independent experiments showed that GW3965-treated mice had an average increase in plasma macrophage-derived \(^3\)H-cholesterol (at 48 hours) of approximately 2-fold, in fecal \(^3\)H-total sterol excretion of 1.9-fold, in fecal \(^3\)H-cholesterol of 2.5-fold, and in fecal \(^3\)H-bile acid of 1.4-fold compared with vehicle-treated mice. All of these differences, with the exception of the \(^3\)H-bile acid, were statistically significant \((P<0.05)\).

GW3965 Treatment Increased Macrophage RCT in LDLR/apobec-1 Double Knockout Mice
LDLR/apobec-1 double knockout mice have elevated LDL cholesterol and apoB-100 levels and develop extensive atherosclerosis on a low-fat chow diet, thus more closely resembling human pathophysiology. To study the effect of GW3965 on RCT in a hypercholesterolemic setting, we performed studies in this murine model. In this series of experiments, female mice were gavaged with either vehicle or GW3965 for 10 days before assessment of RCT. The J774 cells were not pretreated with GW3965 in these experiments.

To confirm the effect of LXR agonist treatment, gene expression in livers of vehicle- and LXR agonist–treated LDLR/apobec-1 double knockout mice was assessed by quantitative PCR. Expression of ABCA1 increased significantly by 1.6-fold in LXR-treated mice (Figure 2A); in addition, expressions of ABCG1 (2-fold), ABCG5 (3-fold), ABCG8 (2-fold), and CYP7A1 (3-fold) were also significantly increased.

Plasma FPLC analysis showed a relatively minimal effect of GW3965 on plasma lipoprotein levels (Figure 2B). GW3965-treated mice had a modest but significant 14% higher plasma \(^3\)H-cholesterol level at 48 hours \((P<0.05)\) (Figure 2C). The \(^3\)H-cholesterol in plasma of these mice tracked closely with the cholesterol mass in plasma lipoproteins (Figure 2B). The level of tracer in the liver of LXR agonist–treated mice at 48 hours was not different than that of vehicle-treated mice (data not shown). However, GW3965-treated LDLR/apobec-1 double knockout mice excreted 1.9-fold more \(^3\)H-total sterol and \(^3\)H-cholesterol into feces \((P<0.05)\) over 48 hours than control mice (Figure 2D). The \(^3\)H-bile acids in LXR-treated mice were also significantly higher than in vehicle-treated mice (1.8-fold; \(P=0.01\)).

GW3965 Treatment Increased Macrophage RCT in apoB/CETP Double Transgenic Mice
Mice lack CETP (an LXR target gene), and CETP-mediated transfer of cholesteryl ester from HDL to apoB-containing lipoproteins may play a role in RCT. Therefore, we performed additional studies in human apoB/CETP double transgenic mice as another approach to using mice that more closely resemble the human physiology. Gene expression analysis in livers of the GW3965-treated mice with the use of quantitative PCR also confirmed that both ABCA1 and ABCG1 were significantly upregulated by the LXR agonist (2.5- and 2.7-fold, respectively), whereas the CYP7A1 levels did not change significantly (1.1-fold) compared with vehicle-treated mice.

Plasma HDL cholesterol levels in mice treated with GW3965 increased compared with vehicle-treated mice as determined by direct measurement as well as by FPLC analysis (Figure 3A). \(^3\)H-Cholesterol–labeled, cholesterol-loaded J774 cells were injected into the mice after 10 days of
GW3965 or vehicle treatment. GW3965-treated mice had higher plasma $^3$H-cholesterol levels at all time points studied (Figure 3B): 30% higher at 6 hours, 42% higher at 24 hours, and 48% higher at 48 hours. The $^3$H-cholesterol in plasma of these mice also tracked closely with the cholesterol mass in plasma lipoproteins (Figure 3A). The level of tracer in the liver of LXR agonist–treated mice trended toward an increase of 22%, although this was not statistically significant compared with vehicle-treated mice (data not shown). However, GW3965-treated mice excreted 41% more $^3$H-total sterol and 64% more $^3$H-cholesterol into feces ($P<0.05$) over 48 hours than control mice (Figure 3C). The $^3$H-bile acids in LXR-
treated mice were not significantly different compared with control (Figure 3C). A similar experiment in the same mouse model but in which mice were administered the LXR agonist in chow diet also promoted RCT to a similar extent (27% increase in fecal total sterol excretion compared with vehicle-treated mice; P<0.05; data not shown).
Discussion

We report for the first time direct proof that administration of a synthetic LXR agonist increases the reverse transport of cholesterol from macrophages to feces in vivo. We show that mice treated with the synthetic LXR agonist GW3965 have significantly higher macrophage-derived \(^3\)H-cholesterol in plasma and feces over 48 hours than vehicle-treated mice in 6 independent experiments in 3 different mouse models. Although previous studies have shown that administration of an LXR agonist to mice increased fecal neutral sterol mass excretion, the source of the increased fecal cholesterol was unknown. Our studies are the first to show promotion of RCT specifically from macrophages to feces, a pool that is too small to measure by approaches based solely on measurement of mass. Thus, promotion of macrophage RCT may be an important mechanism by which LXR agonism reduces atherosclerosis in mice.

Our assay allowed us to manipulate either the injected macrophages or the animals themselves with the LXR agonist. We hypothesized that pretreatment alone of the macrophages before injection would upregulate cholesterol efflux pathways and increase macrophage RCT. Our data, however, suggest that LXR agonist pretreatment of cells alone did not increase macrophage RCT. An explanation for this could be that once the cells were injected into the untreated mice, the pharmacological effect of pretreatment on macrophage gene expression was rapidly lost. It is likely that to test the role of macrophage gene products in this in vivo assay, more stable alterations of gene expression in the macrophages will be required. In contrast, the impact of treating the animals with the LXR agonist had a much greater effect on macrophage RCT. It is likely that 10 days of treatment with GW3965 results in substantial drug levels in peritoneal fluid and that on injection of the labeled J774 cells, they are immediately exposed to therapeutic concentrations of drug, which results in upregulation of ABCA1, ABCG1, and potentially other genes involved in cholesterol efflux (like apoE), thus promoting increased efflux. An important factor in the promotion of macrophage RCT by GW3965 is likely to be the enhancement of cholesterol efflux from the injected macrophages, which is reflected in the increased plasma tracer levels at all time points.

To validate the effect of an LXR agonist on RCT in a relevant hypercholesterolemia model that develops atherosclerosis, we extended our studies to the LDLR/apobec-1 double knockout mouse model. Our studies demonstrated that administration of GW3965 to these mice also significantly increased macrophage RCT to an extent similar to that seen in wild-type mice. Finally, LXR\(\beta\)s also regulate various enzymes involved in lipoprotein remodeling, including CETP.\(^{13}\) CETP promotes the net transfer of cholesteryl esters from HDL to apoB-containing particles in exchange for triglycerides and has been suggested to be important for RCT in humans.\(^{21}\) Mice do not have plasma CETP activity, posing a major difference between mice and humans with regard to HDL metabolism and RCT. In light of this, we also studied the effect of GW3965 on macrophage RCT in apoB/CETP double transgenic mice, which exhibit a humanlike lipoprotein distribution.\(^{23}\) Treatment of the apoB/CETP double transgenic mice with GW3965 resulted in a significant increase in macrophage RCT, suggesting that an LXR agonist promotes the rate of macrophage RCT in vivo even in the presence of CETP expression. However, it is interesting to note that the magnitude of the increase in macrophage RCT with GW3965 was considerably less than that seen in wild-type and LDLR/apobec-1 double knockout mice. Whether this is because of the presence of CETP or due to other differences among these models has yet to be determined.

Although one major effect of the LXR agonist on RCT was likely at the level of the injected macrophages, several hepatic genes involved in hepatic cholesterol metabolism and RCT are also known to be upregulated by LXR,\(^{23,24}\) an observation confirmed in our studies. Upregulation of hepatic ABCG5/8 very likely promoted the rate of excretion of \(^3\)H-cholesterol into the bile, thus promoting overall RCT. It is notable that despite the substantial increase in plasma \(^3\)H-cholesterol levels and fecal \(^3\)H-sterol excretion, the livers of LXR agonist–treated mice at 48 hours generally had no increase in tracer compared with control mice. This is consistent with a scenario whereby treatment with the LXR agonist increased not only the flux of \(^3\)H-cholesterol into the liver but also the transport of \(^3\)H-sterol out of the liver into bile, resulting in a net increase in hepatic \(^3\)H-sterol at the 48-hour time point. In 2 of the 3 models we studied, hepatic expression of CYP7A1 was also upregulated by GW3965. Although fecal \(^3\)H-bile acids were increased in both, the effect was modest. Interestingly, in CETP/apoB transgenic mice, hepatic CYP7A1 mRNA was not increased in the GW3965-treated mice, and fecal \(^3\)H-bile acids were also not increased. Because LXR agonists do not upregulate expression of the human CYP7A1 gene, the results in mice with regard to effects on bile acid synthesis should be extrapolated with caution. In any case, it is likely that the effect of the LXR agonist in the liver contributed to the overall increase in macrophage RCT seen in these experiments. Studies of macrophage RCT with LXR agonists in LXR\(\alpha/\beta\)-deficient mice would help to address the relative importance of the effect of the LXR agonist on injected macrophages versus the liver in promoting macrophage RCT.

Recent data suggest that the intestine may play a role in directly excreting plasma-derived cholesterol into the feces, thus serving as a liver-independent pathway for RCT.\(^{25}\) Indeed, an LXR agonist was shown to increase fecal excretion of neutral steroids independent of biliary steroid secretion.\(^{26}\) In this paradigm, treatment with GW3965, by upregulating intestinal expression of genes such as ABCG5/G8, may have promoted direct intestinal transport of HDL \(^3\)H-cholesterol into the lumen, thus contributing to the overall increase in fecal \(^3\)H-sterol excretion and macrophage RCT.

The clinical development of LXR agonists has been slowed by hepatic steatosis and elevations in plasma triglycerides and LDL cholesterol in preclinical models.\(^{27,28}\) In the present study we demonstrate that administration of the synthetic LXR agonist GW3965 to mice substantially increased macrophage RCT without inducing substantial hepatic steatosis. Similar to the observation of an increase in LDL cholesterol in CETP-expressing species by Groot et al,\(^{28}\) our studies with...
GW3965 in the apoB/CETP double transgenic mouse model also resulted in an increase in LDL cholesterol. Despite this, our studies in 3 different mouse models provide some hope that selective LXR modulators may be successful in promoting macrophage RCT without causing adverse lipid-related consequences and therefore might be able to be advanced into the clinic as a novel therapeutic approach for atherosclerosis.

Macrophages play a central role in innate immunity, and LXRs have been demonstrated to inhibit the production of such macrophage inflammatory mediators as inductive NO synthase, cyclooxygenase-2, and interleukin-6 in the arterial wall, thereby preventing the development of atherosclerosis in various murine animal models. Whether the overall beneficial effect of a synthetic LXR agonist in preventing the progression of atherosclerosis is a result of modulation of RCT, inhibition of inflammation, or a balance of the 2 processes remains to be determined.

In summary, we report for the first time direct proof that administration of a synthetic LXR agonist promotes RCT of macrophage-derived cholesterol in vivo. These studies suggest that promotion of macrophage RCT by LXR agonism contributes to the mechanism by which this approach reduces atherosclerosis in mice and may be an effective strategy in humans.

Acknowledgments
Drs Naik and Wang were supported by a National Research Service Award training grant (T32 HL07971). This work was supported in part by grant P01 HL22633 from the National Heart, Lung, and Blood Institute. Dr Rader is a recipient of a Clinical Scientist Award in Translational Research from the Burroughs Wellcome Fund and an Alternative Drug Discovery Initiative Award funded by GlaxoSmithKline. We are indebted to Aisha Faruqi, Archana Vemulapalli, David Chang, Anna DiFlorio, and Linda Morrell for expert technical assistance. We also acknowledge Christopher Evans and Harvey Fries for analysis of the GW3965 plasma samples.

Disclosure
Drs Jaye and Macphee are full-time employees of GlaxoSmithKline. Dr Rader has served as a consultant to and has research funding from GlaxoSmithKline.

References
Liver X receptors (LXRs) play an important role in lipid metabolism and inflammation by modulating the expression of genes involved in these processes. Furthermore, synthetic LXR agonists have been shown to inhibit the development of atherosclerosis in mouse models of atherosclerosis; however, the mechanism by which this occurs is unclear. In the present study we demonstrate for the first time direct proof that administration of a synthetic LXR agonist substantially increases reverse cholesterol transport of macrophage-derived cholesterol in vivo. Our studies, in which we used a novel method to trace the movement of cholesterol from macrophages into liver and ultimately into bile and feces in 3 different LXR agonist–treated mouse models, provide direct proof that administration of a synthetic LXR agonist promotes reverse cholesterol transport in vivo. Moreover, our study provides further insight into the possibility that selective LXR modulators might be a novel therapeutic approach for the treatment of atherosclerosis in humans and that promotion of reverse cholesterol transport could be one of the mechanisms by which this is achieved.
Pharmacological Activation of Liver X Receptors Promotes Reverse Cholesterol Transport In Vivo
Snehal U. Naik, Xun Wang, Jaqueline S. Da Silva, Michael Jaye, Colin H. Macphee, Muredach P. Reilly, Jeffrey T. Billheimer, George H. Rothblat and Daniel J. Rader

Circulation. 2006;113:90-97; originally published online December 19, 2005; doi: 10.1161/CIRCULATIONAHA.105.560177
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/113/1/90

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/