Synthetic LXR agonists increase LDL in CETP species

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Abstract **Liver X receptor (LXR) nuclear receptors regulate the expression of genes involved in whole body cholesterol trafficking, including absorption, excretion, catabolism, and cellular efflux, and possess both anti-inflammatory and antidiabetic actions. Accordingly, LXR is considered an appealing drug target for multiple indications. Synthetic LXR agonists demonstrated inhibition of atherosclerosis progression in murine genetic models; however, these and other studies indicated that their major undesired side effect is an increase of plasma and hepatic triglycerides. A significant impediment to extrapolating results with LXR agonists from mouse to humans is the absence in mice of cholesteryl ester transfer protein, a known LXR target gene, and the upregulation in mice but not humans of cholesterol 7α-hydroxylase. To better predict the human response to LXR agonism, two synthetic LXR agonists were examined in hamsters and cynomolgus monkeys. In contrast to previously published results in mice, neither LXR agonist increased HDL-cholesterol in hamsters, and similar results were obtained in cynomolgus monkeys. Importantly, in both species, LXR agonists increased LDL-cholesterol, an unfavorable effect not apparent from earlier murine studies.** **These results reveal additional problems associated with current synthetic LXR agonists and emphasize the importance of profiling compounds in preclinical species with a more human-like LXR response and lipoprotein metabolism.**—Groot, P. H. E., N. J. Pearce, J. W. Yates, C. Stocker, C. Sauermelch, C. P. Doe, R. N. Willette, A. Olzinski, T. Peters, D. d'Epagnier, K. O. Morasco, J. A. Krawiec, C. L. Webb, K. Aravindhan, B. Jucker, M. Burgert, C. Ma, J. P. Marino, J. L. Collins, C. H. Macphee, S. K. Thompson, and M. Jaye. **Synthetic LXR agonists increase LDL in CETP species.** ***J. Lipid Res.*** **2005.** 46: **2182–2191.**

Supplementary key words liver X receptor • low density lipoprotein • cholesteryl ester transfer protein • monkey • hamster • triglyceride • atherosclerosis

The liver X receptors LXRα and LXRβ (1) are ligand-activated transcription factors of the nuclear receptor superfamily that control the expression of genes involved in cholesterol homeostasis and fatty acid metabolism (2, 3). LXRα is highly expressed in liver (hence its name) but is also prevalent in adipose tissue, gut, kidney, and macrophages. LXRβ is more widely expressed and found in most tissues. Natural ligands for LXRs are oxidized derivatives of cholesterol, such as 24*S*- and 25-epoxycholesterol and 24*S*- and 22*R*-hydroxycholesterol (4–6). LXRs are intracellular cholesterol sensors that upregulate key enzymes and transporters of cholesterol metabolism and transport, such as ABCA1 and ATP binding cassette protein G1 (ABCG1) (7–9), ABCG5 and ABCG8 (10, 11), apolipoprotein E (apoE) in adipocyte and macrophages (12), and cholesteryl ester transfer protein (CETP) (13). In mice but not in primates, hepatic cholesterol 7α-hydroxylase (cyp7a) is also upregulated by LXR (5, 14, 15). LXR also affects triacylglycerol metabolism by stimulating lipogenesis and triglyceride synthesis attributable to the upregulation of sterol-regulatory element binding protein 1c (SREBP1c) and the FAS complex (16, 17). In addition, LXRs also have direct anti-inflammatory effects by downregulation of several proinflammatory genes in macrophages (18–20). Based on these data, LXR has been considered an attractive antiatherosclerosis target. Using the potent synthetic LXR agonist GW3965, our group collaborated in a study

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Abbreviations: ABCG1, ATP binding cassette protein G1; apoE, apolipoprotein E; CETP, cholesteryl ester transfer protein; cyp7a, cholesterol 7α-hydroxylase; HDL-C, high density lipoprotein-cholesterol; LXR, liver X receptor; SCD, steroyl coenzyme A desaturase; SREBP, sterol-regulatory element binding protein.

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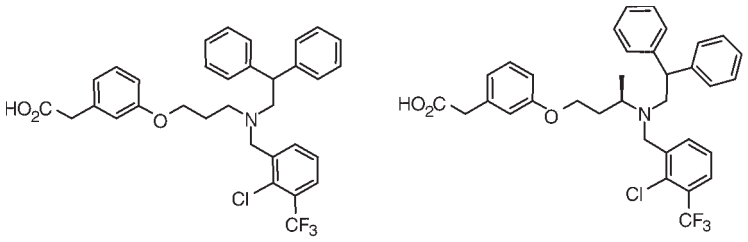

Fig. 1. Structures of synthetic liver X receptor (LXR) agonists. The use and properties of the synthetic LXR agonists GW3965 and SB742881 are described in the text.

in which antiatherosclerotic effects of LXR agonism were demonstrated in two murine models of atherogenesis, the apoE knockout and the LDL receptor knockout models (21). Antiatherosclerotic effects of LXR agonists in mice were also seen by others (22). For a more comprehensive summary of LXR, the reader is referred to several excellent reviews (2, 3, 23–28).

Despite the positive results obtained in mice, it is unknown whether pharmacological modulation of LXRs would be a successful strategy for the management of human dyslipidemia and atherosclerosis. The upregulation by LXR of genes that stimulate reverse cholesterol transport as well as the anti-inflammatory effects of LXR agonism are expected to be beneficial. However, increased lipogenesis and triglyceride synthesis (29), as seen in mice and hamsters treated with the synthetic LXR agonist T0901317, would be highly undesirable. Two proposed strategies to solve this problem are *1*) the identification of an LXRβ selective agonist, and *2*) the development of an LXR modulator with more desirable effects on target gene expression (i.e., with reduced upregulation of lipogenic genes) (24, 26, 30). For this endeavor, the identification of animal models that are predictive of the response to LXR agonists in humans is critical. Mice are unlikely to be reliable models because *1*) they lack the LXR target gene CETP, which is central in human lipoprotein metabolism, and *2*) unlike humans, their cyp7A gene is upregulated by LXR. Upregulation of CETP in humans could represent an additional liability for LXR agonists, as CETP exchanges triglyceride in apoB-containing lipoproteins for HDL-cholesterol (HDL-C), reducing HDL-C and resulting in a more atherogenic lipoprotein profile. Hamsters and primates possess a CETP gene, and in neither of these species does cyp7A seem to be an LXR target. Therefore, these species are expected to be much more representative than mice of the human response to LXR agonists.

SB742881 is a newly identified potent dual LXR agonist that is structurally similar to GW3965 (J. P. Marino et al., unpublished data). Here, we report on the effects of both GW3965 and SB247881 on plasma lipids and lipoprotein cholesterol and apolipoproteins in hamsters and cynomolgus monkeys. In addition, the expression of various LXR target genes in different hamster tissues and a partial fatty acid composition analysis of hamster serum triglycer-

TABLE 1. Primers and probes used for quantitative real-time PCR

mRNA	Sequence Detection Primers	Probe
ABCA1	F: GGGACACACAGGCAGCATC R: AATGAAGAACCCCTCGCCC	TCTTGATTTTGTCACTGCTACACTGGCAGG
ABCG1	F: AGGCCAGCGGAAGCG R: AAAGAACATGACAGGAGGGTTATTG	TGGCCATCGCACTCGAGCTGG
ABCG5	F: GAAGTGGCAGATGCTGCTGG R: CATCCCAAGCAGCACGAGAG	TTGTATCATGAGGCTGCCAGATTCGGA
ABCG8	F: AACGACTTCCGAGACCTGCC R: GGGCTGATGTGTCCGTGAAG	TCCTTTACTATGGCCACGATGCCAAGC
ANGPTL3	F: CAAGCAACTCTCAAGTGTTTAATGTG R: ACCCATCTTTTCGGTGTTGAA	ACTGCGACACCCAATCAGGTAGTCCATG
CETP	F: ACGGCCACCCACCTTGA R: GGGAGGTCCTCAGAGATGTTCTT	TCCCATCACAAGGGCCATTTCGTCTA
FAS	F: CGGTCTGGAAAGCTGAAGGATC R: CGGAGTGAGGCTGGGTTGA	CCAAGCAGGCACACACAATGGACCC
GAPDH	F: GAACATCATCCCTGCATCCA R: CCAGTGAGCTTCCCGTTCA	CTTGCCCACAGCCTTGGCAGC
LDLr	F: GCCGGGACTGGTCAGATG R: ACAGCCACCATTGTTGTCCA	GCACTCATTGGTCCTGCAGTCCTTGAT
LPL	F: TCATCGTGGTGGATTGGCT R: CCAGCTTGGTGTATCCAGCA	TCGGGCCCAGCAACACTATCCAGT
SCD1	F: CCGCCATCCTGCTGATGT R: AAGCTTCACCCCAGAAATACCA	TTTCATCCTGCCCACGTTTG
SREBP1c	F: GAAGCCATGGATTGCACGT R: AATCAAACAGGCCAGGGAAGT	TGCTCCAGCTCATCAACAACCAAGACA

ABCG1, ATP binding cassette protein G1; ANGPTL3, angiopoietin-like-3; CETP, cholesteryl ester transfer protein; F, forward; LDLr, low density lipoprotein receptor; R, reverse; SCD1, steroyl coenzyme A desaturase 1; SREBP1c, sterol-regulatory element binding protein 1c.

ides and free fatty acids are reported. The results show that, in addition to an increase of plasma triglycerides, both LXR agonists increase plasma LDL-C and apoB. LXR subtype-selective agonists or LXR modulators may be required to dissociate positive antiatherosclerosis mechanisms from undesirable effects on plasma lipids/lipoproteins obtained with current dual LXRα/β agonists.

MATERIALS AND METHODS

LXR compounds

GW3965, described previously (31), and SB742881 are structurally similar synthetic dual LXR agonists discovered within GlaxoSmithKline (**Fig. 1**). SB742881 is twice as potent as GW3965 in LXRα peptide recruitment assays (84 vs. 175 nM, respectively), equipotent (20–25 nM) in LXRβ peptide recruitment assays, and equally efficacious in transient transfection and cholesterol efflux assays in cultured cells (data not shown).

Hamster dosing, plasma lipid and lipoprotein, and hepatic triglyceride measurement

All animal experiments were performed under GlaxoSmithKline Animal Care and Use Committee guidelines. Male Golden Syrian hamsters (n = 8/group) were obtained from Charles River Laboratories. Animals were orally dosed twice daily for 7 days with either vehicle (a saline solution of 0.5% hydroxypropyl methylcellulose) or with the LXR agonist SB742881A (1, 3, 10, and 30 mg/kg) or GW3965 (30 mg/kg) in vehicle. Body weights were measured on days 5 and 8 of the study. Approximately 5 h after the morning dose on the 8th day, hamsters were anesthetized and terminal blood samples were obtained via cardiac puncture. Lipoproteins were separated by size exclusion on a silica gel column (Tosch Corp.) with 0.15 M NaCl as the mobile phase using an Agilent Technologies 1100 series HPLC apparatus with LC 3D ChemStation software. The cholesterol content of the fractions was measured continuously using Biomerieux reagents (BMX 61219). Serum total cholesterol was measured using the same reagent, and triglycerides were measured using Biomerieux reagents (BMX 61236). Lipids were extracted using a modified Folch, Lees, and Sloane Stanley procedure (32) from a discrete section of a major liver lobe, and triglyceride content per gram of tissue was quantitated using Sigma GPO reagent. For each parameter, means ± SEM for all hamsters within each treatment group were calculated. Parametric one-way ANOVA with Fisher's least significant difference post hoc tests was used to calculate significant differences.

Hamster tissue isolation and quantitative gene expression measurement

After blood collection, hamsters were killed by cervical dislocation. Peritoneal macrophages were isolated by lavage of the peritoneal cavity with 10 ml of PBS and collected by centrifugation for 10 min in a LAS Macros centrifuge. Cell pellets were lysed in RLT buffer (Qiagen) with 1% mercaptoethanol, and cell pellets were frozen at −20°C before isolation of total RNA. Small intestines (from below the stomach to the start of the cecum) and liver (a discrete section of a major lobe) were weighed, cut into smaller pieces, and added to 30 and 10 ml, respectively, of RNALater. After cooling to 4°C, samples were frozen at −20°C before isolation of total RNA. Small intestine samples were pulverized in liquid nitrogen, homogenized in Trizol (Invitrogen), and extracted with chloroform, and total RNA was precipitated by isopropanol. Macrophage and liver total RNAs were prepared using the Qiagen RNeasy Mini kit according to the manufacturer's instructions. Total RNA from four like samples (i.e., macrophage, liver) from each treatment group (n = 8) were pooled, giving two combined samples per tissue for the vehicle and LXR agonist treatment groups. Approximately 2.6 μg of total RNA was pretreated with Dnase 1 (Promega) before the generation of cDNA using random 9mers and Moloney murine leukemia virus reverse transcriptase (Invitrogen) according to the manufacturers' instructions. Real-time PCR was conducted in an Applied Biosystems 7700 Sequence Detection System in 45 μl reactions with 12.5 ng of cDNA and 300 nM of the primers and probes listed in **Table 1**.

For each gene and tissue, expression relative to the GAPDH housekeeping gene was calculated as difference in threshold cycles for target and reference genes (ΔCt), and the average of these two values for the two pooled samples from vehicle-treated hamsters was set to 1. For each of the treatment groups, the ΔCt for each gene and tissue was similarly calculated, and the fold

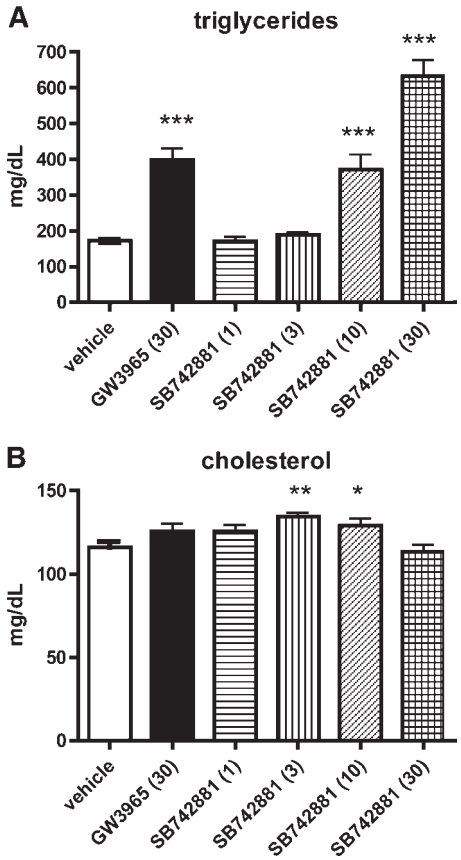

Fig. 2. Effect of LXR agonists on plasma lipids in Golden Syrian hamsters. Hamsters on a normal diet were orally dosed twice daily for 7 days with 0.5% hydroxypropyl methylcellulose vehicle, GW3965 (30 mg/kg), or SB742881 (1, 3, 10, or 30 mg/kg) (n = 8/group). Plasma triglycerides (A) and cholesterol (B) were determined 5 h after the last dose as described in Materials and Methods. White bars, vehicle treatment; black bars, GW3965 treatment; horizontal stripes, SB742881 1 mg/kg; vertical stripes, SB742881 3 mg/kg; diagonal stripes, SB742881 10 mg/kg; hatched bars, SB742881 30 mg/kg. Significant differences between vehicle and LXR agonist treatment groups are indicated. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ as calculated with Fisher's least significant difference post hoc tests. Error bars represent ± SEM.

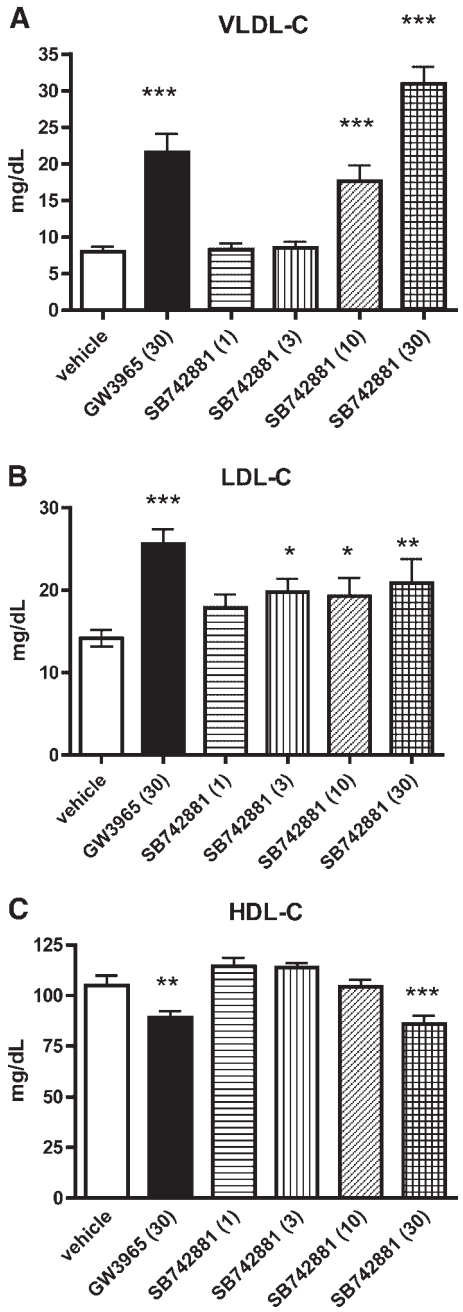

Fig. 3. Effect of LXR agonists on the distribution of plasma lipoprotein cholesterol in Golden Syrian hamsters. Hamsters on a normal diet were orally dosed twice daily for 7 days with 0.5% hydroxypropyl methylcellulose vehicle, GW3965 (30 mg/kg), or SB742881 (1, 3, 10, or 30 mg/kg) (n = 8/group). Plasma obtained 5 h after the last dose was fractionated by HPLC, and the cholesterol content of each fraction was determined. The summed cholesterol contents of the VLDL fractions (A), LDL fractions (B), and HDL fractions (C) are shown. White bars, vehicle treatment; black bars, GW3965 treatment; horizontal stripes, SB742881 1 mg/kg; vertical stripes, SB742881 3 mg/kg; diagonal stripes, SB742881 10 mg/kg; hatched bars, SB742881 30 mg/kg. Significant differences between vehicle and LXR agonist treatment groups are indicated. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ as calculated with Fisher's least significant difference post hoc tests. VLDL-C, very low density lipoprotein-cholesterol. Error bars represent ± SEM.

change relative to vehicle was calculated from the ΔΔCt values by the relative Ct method as specified by Applied Biosystems. Parametric one-way ANOVA with Fisher's least significant difference post hoc tests was used to calculate significant differences.

Fatty acid compositional analysis of polar and nonpolar plasma lipids

Golden Syrian hamsters were orally dosed once daily with 0.5% hydroxypropyl methylcellulose vehicle or with GW68395A (10 mg/kg) or SB742881 (3, 10, or 30 mg/kg) in vehicle (n = 6/group) for 7 days. Approximately 5 h after the last dose, hamsters were anesthetized and terminal blood samples were obtained via retro-orbital bleeding. Aliquots of plasma from vehicle- and LXR agonist-treated animals were pooled, and the pooled samples were subjected to TrueMass® quantitative lipid metabolite analysis (Lipomics Technologies, Inc., West Sacramento, CA).

Cynomolgus monkey studies

The fasting lipid profile of male cynomolgus monkeys was examined for 3 weeks (one sample per week) to eliminate outliers and to allocate animals to treatment groups. An average of these values was used for each parameter as a baseline value for each monkey. Two separate studies were performed, comparing vehicle (1% hydroxypropyl methylcellulose) treatment (n = 4) to once daily oral dosing with either 10 mg/kg GW3965 (n = 5) or 10 mg/kg SB742881 (n = 5). For all monkeys, 17 h fasting sera samples were obtained before dosing on days 0, 3, 7, and 14 (GW3965 study) and on days 0, 7, 14, and 21 (SB742881 study) and after washout. Analyses of serum lipids, lipoproteins, and apolipoproteins were performed on an Olympus AU640 chemistry analyzer (Olympus America, Inc., Diagnostic Systems Group, Melville, NY). ApoA-I and apoB were quantitated using end

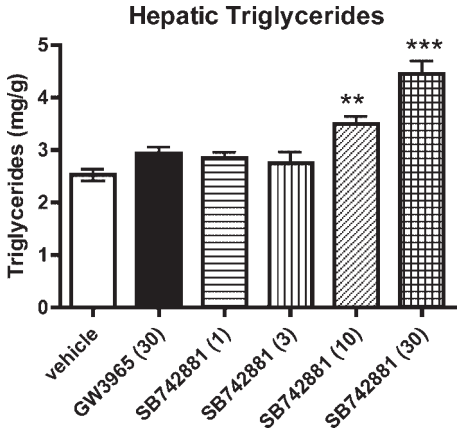

Fig. 4. Effect of LXR agonists on hepatic triglycerides in Golden Syrian hamsters. Hamsters on a normal diet were orally dosed twice daily for 7 days with 0.5% hydroxypropyl methylcellulose vehicle, GW3965 (30 mg/kg), or SB742881 (1, 3, 10, or 30 mg/kg) (n = 8/group). Lipids were extracted using a modified Folch, Lees, and Sloane Stanley procedure (32) from a discrete section of a major liver lobe, and triglyceride content per gram of tissue was quantitated using Sigma GPO reagent. White bars, vehicle treatment; black bars, GW3965 treatment; horizontal stripes, SB742881 1 mg/kg; vertical stripes, SB742881 3 mg/kg; diagonal stripes, SB742881 10 mg/kg; hatched bars, SB742881 30 mg/kg. Significant differences between vehicle and LXR agonist treatment groups are indicated. *** $P < 0.001$, ** $P < 0.01$ as calculated with Fisher's least significant difference post hoc tests. Error bars represent ± SEM.

point turbidimetric immunoassays as specified by the manufacturer (Wako Diagnostics, Richmond, VA). Triglyceride and total cholesterol were quantitated using assays from Olympus America, whereas LDL-C and HDL-C were determined with EZ LDL (Trinity Biotech, St. Louis, MO) and HDL Direct Liquid Select (Equal Diagnostics, Exton, PA) reagents. For each monkey at each time point, the percentage change from the pretreatment average baseline value of each parameter was calculated. For each treatment study and parameter combination, the treatment minus vehicle contrast was estimated for each time point. These estimates and their raw significance levels were determined using repeated-measures analysis with SAS Proc Mixed. The adjusted significance levels were calculated for the multiple time point contrasts using a Bonferroni adjustment within each parameter and treatment study combination.

RESULTS

LXR compounds

The structure of GW3965 has been described previously (33), and this compound has been used in a variety of in vivo and in vitro studies. SB742881 differs from GW3965 in having a methyl substitution on the propanolamine linker (Fig. 1). Compared with GW3965, SB742881 is twice

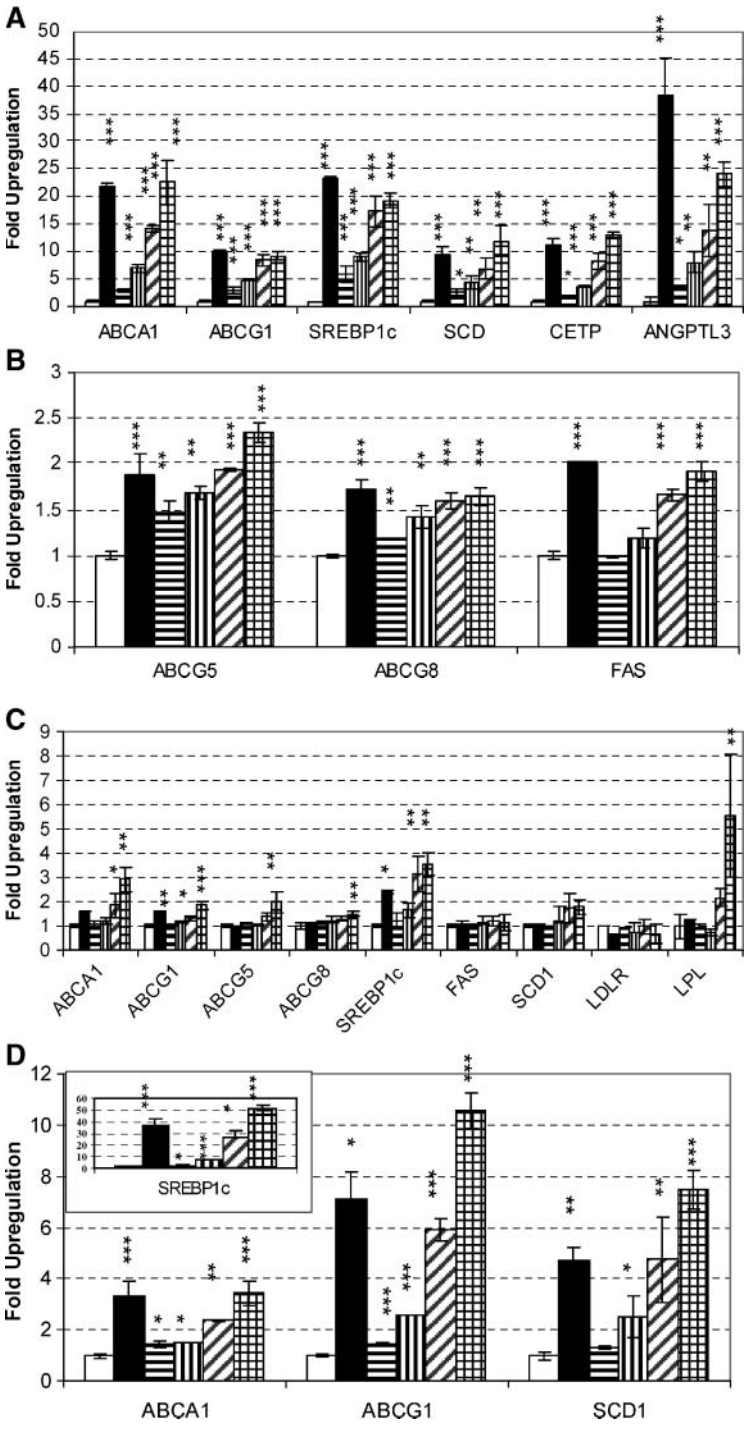

Fig. 5. Effect of LXR agonists on gene expression in small intestines (A, B), liver (C), and peritoneal macrophages (D) of Golden Syrian hamsters. Hamsters on a normal diet were orally dosed twice daily for 7 days with 0.5% hydroxypropyl methylcellulose vehicle, GW3965 (30 mg/kg), or SB742881 (1, 3, 10, or 30 mg/kg) (n = 8/group). Gene expression was measured by real-time PCR as described in Materials and Methods. White bars, vehicle treatment; black bars, GW3965 treatment; horizontal stripes, SB742881 1 mg/kg; vertical stripes, SB742881 3 mg/kg; diagonal stripes, SB742881 10 mg/kg; hatched bars, SB742881 30 mg/kg. Significant differences between vehicle and LXR agonist treatment groups are indicated. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ as calculated with Fisher's least significant difference post hoc tests. Error bars represent the range of expression levels.

as potent in LXRα peptide recruitment assays (84 vs. 175 nM, respectively), equipotent in LXRβ peptide recruitment assays (20–25 nM), and equally efficacious in transient transfection and cholesterol efflux assays in cultured cells (data not shown).

Effect of synthetic LXR agonists in hamsters

Hamster plasma lipids and lipoproteins and hepatic triglycerides. Treatment of hamsters on a normal diet for 7 days with the synthetic dual LXR agonist GW3965 (30 mg/kg) increased plasma triglycerides 230% relative to vehicle. At 1 and 3 mg/kg, the structurally related compound SB742881 had no effect on plasma triglycerides; however, at 10 and 30 mg/kg, plasma triglycerides were dose-dependently increased 215–367% relative to vehicle-treated hamsters (**Fig. 2A**). In contrast to the pronounced effects on plasma triglycerides, no effect on plasma total cholesterol was observed with high doses of either compound, although intermediate doses (3–10 mg/kg) of SB742881 slightly increased total plasma cholesterol (Fig. 2B). To examine the effect of LXR agonist treatment on the cholesterol content of different lipoproteins, hamster plasma was fractionated by HPLC.

The effects of LXR agonists on plasma VLDL-C (**Fig. 3A**) were consistent with the observed increases in plasma triglycerides. Thus, VLDL-C in the two lowest dose SB742881 treatment groups was essentially unchanged relative to that of the vehicle group. At doses of 10 and 30 mg/kg, SB732881 dose-dependently increased VLDL-C by 220% and 386%, respectively, relative to vehicle. The 270% increase in VLDL-C by 30 mg/kg GW3965 was intermediate between the effects of the two highest doses of SB742881, which nicely parallels the relative effects of these treatments on plasma triglycerides.

LDL-C was increased by both LXR agonists (Fig. 3B). GW3965 (30 mg/kg) increased LDL-C by 180% (from 14.2 to 25.6 mg/dl) compared with vehicle-treated hamsters, whereas SB742881 dose-dependently increased LDL-C to 147% (20.9 mg/dl) of the vehicle treatment group value. In contrast to the clear dose-dependent effects of LXR agonists on LDL-C, significant changes in HDL-C were observed only at the 30 mg/kg dose of both compounds, where SB742881 and GW3965 reduced HDL-C compared with the vehicle group by 8% and 15%, respectively. Neither compound had any effect on body weight development (data not shown), although analysis of the livers at the end of the study showed an increase in triglyceride content in the higher dose groups of SB742881 (10 and 30 mg/kg) (**Fig. 4**). Surprisingly, triglyceride content was not increased in livers of hamsters dosed with GW3965, consistent with the lesser potency of this compound compared with SB742881 and the relative effects of these compounds on hepatic gene expression (see below).

Hamster gene expression. The expression of LXR target genes in various tissues and cells was examined to allow correlation with the observed changes in lipids and lipoproteins. Among the tissues and cells examined (peritoneal macrophages, small intestine, and liver), upregulation of LXR target genes by both LXR agonists was consistently strongest in small intestine, as seen earlier in mice (24). This may be attributable in part to the generally lower level of basal expression of LXR target genes relative to housekeeping genes in intestine compared with other tissues examined (data not shown). ABCA1 and ABCG1 were strongly upregulated in the small intestine by 30 mg/kg GW3965 and dose dependently by 3–30 mg/kg SB742881 (**Fig. 5A**), whereas ABCG5 and ABCG8 were also upregulated by both compounds but to a lesser extent (Fig. 5B). Like the ABC transporters, some lipogenic genes [e.g., SREBP1c and steroyl coenzyme A desaturase (SCD1)] were strongly upregulated by the LXR agonists in small intestine, whereas increases in FAS expression were smaller. Other LXR target genes whose expression was strongly upregulated by both LXR agonists in intestine included CETP and angiopoietin-like-3, a relatively recently discovered LXR target gene encoding a secreted protein that inhibits LPL activity (34, 35).

In liver, the effects of LXR agonist treatment on LXR target gene expression were much more modest compared with those in small intestine (Fig. 5C). The greatest effects were observed for SREBP1c expression, which was upregulated 2.4-fold by 30 mg/kg GW3965 and dose dependently with SB742881 to a maximum of 3.5-fold at the 30 mg/kg dose. Small increases in hepatic ABCA1 and ABCG1 expression were obtained with GW3965 and dose

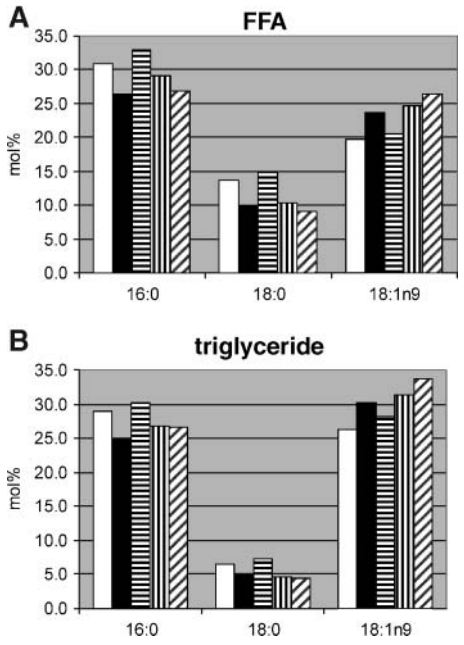

Fig. 6. Plasma free fatty acids and triglyceride composition suggests enhanced steroyl coenzyme A desaturase activity in LXR agonist-treated hamsters. Pooled sera from Golden Syrian hamsters orally dosed for 7 days with 0.5% hydroxypropyl methylcellulose, GW3965 (10 mg/kg), or SB742881 (1, 3, or 10 mg/kg) (n = 6/group) were subjected to TrueMass (Lipomics, West Sacramento, CA) quantitative lipid metabolite analysis. The mol% of palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1n9) in plasma free fatty acids (A) and triglycerides (B) is plotted for each treatment group. White bars, vehicle; black bars, GW3965; horizontal stripes, SB742881 1 mg/kg; vertical stripes, SB742881 3 mg/kg; diagonal stripes, SB742881 10 mg/kg. Oleic acid is the direct delta-9 desaturation product of stearic acid (18:0).

dependently with SB742881. Of note, at the same dose (30 mg/kg), SB742881 upregulated to a greater extent than GW3965 six of the nine genes examined in liver, consistent with the high hepatic expression of LXRα and the greater potency of SB742881 versus GW3965 for LXRα. Effects on other genes, including ABCG5, ABCG8, FAS, SCD, low density lipoprotein receptor, and LPL, when observed were very small and/or not clearly dose dependent; therefore, they were not considered reliable. In peritoneal macrophages, expression of ABCA1, ABCG1, SCD1 (Fig. 5D), and particularly SREBP1c (Fig. 5D, inset) was increased by GW3965 and dose dependently by SB742881.

Compositional analysis of hamster plasma free fatty acids and triglycerides. The observed upregulation of SREBP1c by both synthetic LXR agonists and its relationship with SB742881 dose correlates well with measured increases in VLDL-C and plasma triglycerides in LXR agonist-treated hamsters. This result supports the concept of SREBP1c as a master regulator of lipogenic gene expression. SCD is both an LXR (36) and SREBP1c (31, 37) target gene, and as shown in Fig. 5, it can be strongly upregulated by LXR agonists in hamsters. SCD catalyzes the rate-limiting step in the synthesis of unsaturated fatty acids. The principal product of SCD is oleic acid (18:1n9), formed by the desaturation of stearic acid (18:0). To validate the upregulation of SCD in vivo, hamsters were treated with LXR agonists and both neutral and polar plasma lipids were subjected to fatty acid compositional analysis (TrueMass®). For this study, hamsters (n = 6/group) were dosed once daily with GW3965A (10 mg/kg) or with SB742881 (3, 10, or 30 mg/kg) for 7 days, and a single pooled sample from each group was analyzed. Consistent with the observed upregulation of SCD, we observed an increased mol% of oleic acid in both plasma free fatty acids (**Fig. 6A**) and triglycerides (Fig. 6B) with both the single dose of GW3965 and dose dependently with SB742881. In concert with the increased mol% of C:18 unsaturated fatty acid in the plasma free fatty acid and triglyceride fractions in LXR agonist-treated hamsters, the mol% of the saturated C:18 fatty acid stearic acid was decreased by LXR activation. The effects of LXR agonist treatment on the mol% of palmitic acid (16:0) in both free fatty acids and triglycerides closely resembled the effects observed with stearic acid. The increased fraction of the unsaturated fatty acid, oleic acid, together with decreases in the fractions of both saturated 16:0 and 18:0 fatty acids, provide biochemical support for an increase of fatty acid synthesis and desaturation by LXR agonists.

Effects of synthetic LXR agonists in cynomolgus monkeys

To test whether the effects of synthetic LXR agonists on lipids and lipoproteins observed in hamsters reflect the consequences of LXR agonism in higher species, experiments were conducted in cynomolgus monkeys dosed once daily for 2 or 3 weeks with 10 mg/kg GW3965 (**Fig. 7**) or SB742881 (**Fig. 8**), respectively. Neither synthetic LXR agonist increased plasma triglycerides (Figs. 7A, 8A), whereas

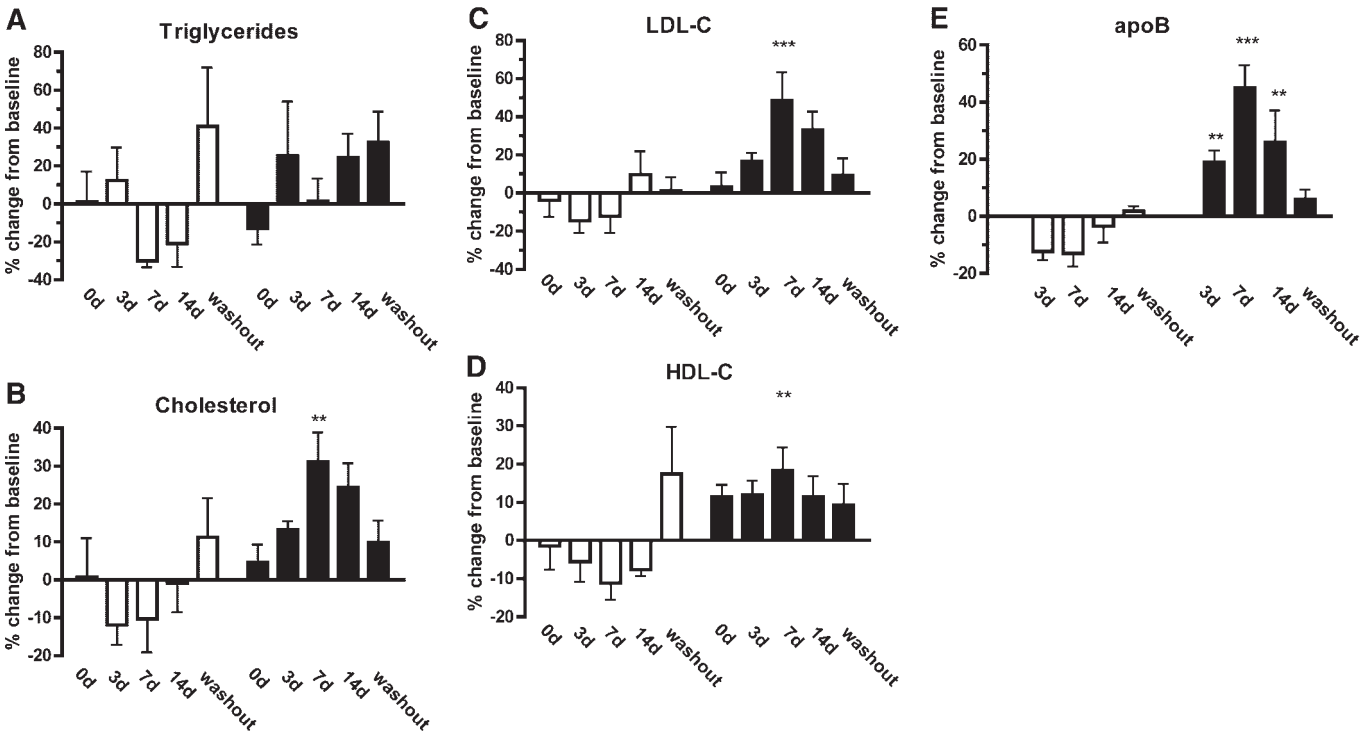

Fig. 7. Changes in plasma lipids, lipoprotein cholesterol, and apolipoprotein B (apoB) in cynomolgus monkeys treated with the LXR agonist GW3965. Monkeys were dosed daily for 2 weeks with vehicle (white bars) or 10 mg/kg GW3965 (black bars). Plasma samples were obtained before dosing (baseline), at 0, 3, 7, and 14 days of dosing, and after a 14 day washout period. The data represent means ± SEM of the percentage change from the baseline value of each measure. A: Triglycerides. B: Total cholesterol. C: LDL-C. D: HDL-C. E: ApoB. Changes in apoB represent differences from day 0, because baseline apoB levels were not obtained. *** $P < 0.001$, ** $P < 0.01$. *P* values have Bonferroni adjustments within each panel. Error bars represent ± SEM.

both compounds significantly increased total cholesterol at least at one time point during treatment (Figs. 7B, 8B). The increase in plasma total cholesterol by both compounds is primarily attributable to an increase of plasma LDL-C (Figs. 7C, 8C). HDL-C was increased at 7 days in GW3965-treated animals (Fig. 7D) but not at any time point with SB742881 treatment (Fig. 8D). In concert with the increase of plasma LDL-C by both compounds, apoB levels were also increased significantly in LXR agonist-treated monkeys at multiple time points (Figs. 7E, 8E).

DISCUSSION

In hamsters, the novel dual LXRα/β agonist SB742881 dose-dependently increased plasma VLDL and LDL-C, with small but significant decreases in plasma HDL-C. Plasma triglycerides were also increased quite dramatically. Similar changes in plasma lipids were seen with GW3965, the parent compound of SB742881. These data suggest that LXR agonists in hamster increase hepatic triglyceride and VLDL synthesis, as observed previously in mice. This increase in hepatic triglyceride synthesis was accompanied by 38% and 78% increases in hepatic triglyceride content with 10 and 30 mg/kg doses of SB742881, respectively. Consistent with this supposition, we saw a dose-dependent increase in mRNA for SREBP1c and SCD1 in liver, small intestine, and peritoneal macrophages. An increase in SCD1 is expected to result in an increased conversion of stearate C18:0 to oleate C18:1, and measurements of fatty acid species in the triglyceride and free fatty acid fractions in plasma indeed demonstrated a dose-dependent increase in the molar fraction of C18:1, with a concomitant decrease in molar fractions of saturated precursor C16 and C18 fatty acids, consistent with an upregulation and increased activity of (hepatic) SCD1.

Interestingly, a dose-dependent increase in mRNA for CETP in small intestine was also observed (up to 13-fold), and the parallel increase in plasma LDL-C and decrease in plasma HDL-C concentration could be explained by the combination of higher plasma VLDL concentration and CETP activity. Such a relationship between VLDL and CETP and the associated effects on LDL and HDL are known to exist in human and other CETP species (for a recent review, see 38). Theoretically, further upregulation of CETP by LXR agonists may even exaggerate the CETP-mediated exchange of neutral lipids between HDL and apoB-containing lipoproteins. These unfavorable shifts in lipoprotein profile in hamsters and monkeys treated with dual LXRα/β agonists could be predictive of their pharmacology in humans.

But on a positive note, LXR agonists are expected to upregulate ABCA1 and other ABC transporters that may facilitate cellular cholesterol efflux and hepatic and intestinal sterol excretion. In our hamster studies, GW3965 and SB742881 upregulated the ABC transporters ABCA1 and ABCG1 in liver, small intestine, and peripheral macrophages as well as ABCG5 and ABCG8 in small intestine. Although these changes may favor reverse cholesterol transport and an increase in fecal sterol excretion, their effect on plasma HDL (an expected increase) was

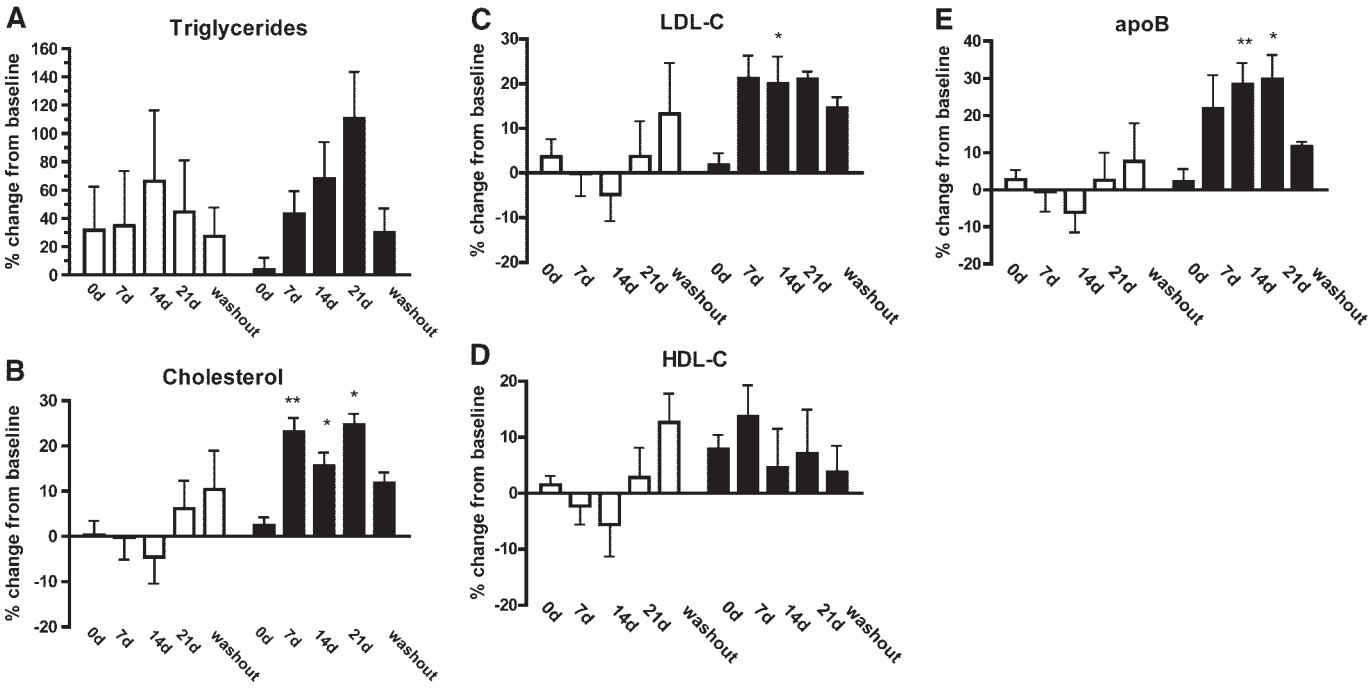

Fig. 8. Changes in plasma lipids, lipoprotein cholesterol, and apoB in cynomolgus monkeys treated with the LXR agonist SB742881. Monkeys were dosed daily for 3 weeks with vehicle (white bars) or 10 mg/kg SB742881 (black bars). Plasma samples were obtained before dosing (baseline), at 7, 14, and 21 days of dosing, and after a 14 day washout period. The data represent means ± SEM of the percentage change from the baseline value of each measure. A: Triglycerides. B: Total cholesterol. C: LDL-C. D: HDL-C. E: ApoB. ** $P < 0.01$, * $P < 0.05$. *P* values have Bonferroni adjustments within each panel. Error bars represent ± SEM.

not evident in hamster, probably because of an overruling influence of the aforementioned effects on VLDL synthesis and CETP.

To further investigate the complex relationship between LXR activation and plasma lipoproteins, SB742881 and GW3965 were evaluated in a second CETP species, cynomolgus monkeys. In 14–21 day dosing studies in monkeys with 10 mg/kg/day compounds versus vehicle, both LXR agonists increased plasma total cholesterol, LDL-C, and apoB during the course of treatment. Although LDL-C was increased only at a single time point in each study, the apoB increase was much more consistent. This may reflect the generation of smaller LDL and/or cholesterol enrichment of VLDL (possibly via CETP). It is worth noting that in neither study was LDL-C increased at the final time point; thus, it is possible that the LDL-C increase is transient. Similar transient effects of LXR agonists on plasma triglycerides in the mouse have been reported (21). Despite our findings that both compounds increased triglycerides in hamsters, neither compound increased plasma triglycerides in cynomolgus monkeys. This may be attributable to the more efficient metabolism and clearance of triglyceride-rich particles in monkeys versus hamsters. Alternatively, this may reflect methodological differences, as the hamsters were not fasted before sampling, whereas the monkeys were. A separate study with SB742881 in cynomolgus monkeys confirmed the dose dependence of the effects reported here (unpublished data). HDL-C was increased after 7 days of treatment in GW3965-treated monkeys; however, we believe that this result is not attributable to the LXR agonist for the following reasons: *1*) significance was calculated by comparing time-matched LXR agonist-treated versus vehicle-treated monkeys, and changes in HDL-C are only significant because HDL-C decreased in the vehicle group; and *2*) no HDL-C increase was observed with the closely related compound SB742881. Furthermore, neither LXR agonist increased HDL-C in hamsters (this study) or apoA-I in monkeys (unpublished data). No change in CETP levels (as measured by ELISA) was detected in monkeys treated with SB742881 (data not shown; samples from the GW3965 study were not available). Thus, at least in monkeys, the background level of CETP rather than an increased level resulting from LXR agonism appears to account for the results obtained.

Our results in two CETP species, cynomolgus monkeys and hamsters, support the concept that dual LXRα/β agonists increase plasma LDL-C and apoB and call into question the future of agents of this class as therapeutics for the treatment of dyslipoproteinemia and atherosclerosis in humans. However, given the proven antiatherosclerosis efficacy of LXR agonists in mice and their well-understood anti-inflammatory and reverse cholesterol transport-stimulatory activities in a variety of species, it is difficult to give up on LXR as a therapeutic target for human atherosclerosis. To progress, LXR agonists capable of selective upregulation of reverse cholesterol transport genes such as ABC transporters versus lipogenic genes will be required. An additional beneficial feature of such compounds would be if the anti-inflammatory properties seen with current LXR agonists could be maintained. Two strategies have been proposed for the development of such desirable LXR compounds: *1*) LXRβ selective agonists; and *2*) LXR modulators. The concept that LXRβ selective agonists would lack the lipogenic properties of dual LXR agonists is based largely on data from knockout mice, which show reduced plasma triglycerides and hepatic lipogenic gene expression in LXRα but not LXRβ knockout mice (14, 39). However, cocrystal structures of LXR with synthetic and endogenous agonists reveal complete conservation between contact residues in the ligand binding pockets of LXRα and LXRβ (40–43, reviewed in 44). Therefore, the development of LXR subtype selective agents may be an arduous task. Tissue- or gene-selective LXR modulators may be more promising, based on precedents from another nuclear receptor, estrogen (45, 46). Indeed, it was demonstrated recently that GW3965 is much less lipogenic than T0901317 in mice and differentially recruits transcription cofactor peptides in vitro (47). Together with the present results, these data suggest that an even more favorable modulator profile than that of GW3965 is required for human therapeutic use. The steroidal compound *N,N*-dimethyl-3β-hydroxycholenamide was recently shown to possess an LXR modulator-like profile in vitro and in vivo in mice (48). Whether additional liabilities of such putative LXR modulators will be revealed in species that more closely reflect human lipoprotein metabolism, such as hamsters and monkeys, remains to be seen. Our results, showing that the dual LXR agonists GW3965 and SB742881 increase LDL-C in CETP species, emphasize the importance of such models for the evaluation of the next generation of LXR agents.

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