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Stimulation of Lipogenesis by Pharmacological Activation of the Liver X Receptor Leads to Production of Large, Triglyceride-rich Very Low Density Lipoprotein Particles*

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The oxysterol-activated liver X receptor (LXR) provides a link between sterol and fatty acid metabolism; activation of LXR induces transcription of lipogenic genes. This study shows that induction of the lipogenic genes Srebp-1c, Fas, and Acc1 upon administration of the synthetic LXR agonist T0901317 to C57BL/6J mice (10 mg/kg/day, 4 days) is associated with massive hepatic steatosis along the entire liver lobule and a 2.5-fold increase in very low density lipoprotein-triglyceride (VLDL-TG) secretion. The increased VLDL-TG secretion was fully accounted for by formation of larger (129 ± 9 nm versus 94 ± 12 nm, a 2.5-fold increase of particle volume) TG-rich particles. Stimulation of VLDL-TG secretion did not lead to elevated plasma TG levels in C57BL/6J mice, indicating efficient particle metabolism and clearance. However, T0901317 treatment did lead to severe hypertriglyceridemia in mouse models of defective TG-rich lipoprotein clearance, i.e. APOE*3-Leiden transgenic mice (3.2-fold increase) and ApoE—/—/LDLr—/— double knockouts (12-fold increase). Incubation of rat hepatoma McA-RH7777 cells with T0901317 also resulted in intracellular TG accumulation and enhanced TG secretion. We conclude that, in addition to raising high density lipoprotein cholesterol concentrations, pharmacological LXR activation in mice leads to development of hepatic steatosis and secretion of atherogenic, large TG-rich VLDL particles.

The nuclear liver X receptor α (LXRα; NR1H3) and LXRβ (NR1H2) are involved in the control of cholesterol and fatty acid metabolism. LXRα is expressed mainly in the liver whereas LXRβ is ubiquitously expressed (1, 2). Activated LXRα heterodimerize with the retinoid X receptor (NR2B1) and bind to a LXR response element, consisting of two hexameric nucleotide direct repeats separated by four nucleotides, to induce gene transcription (1, 2). Oxysterols constitute the physiological ligands for LXR. The most potent LXR-activating oxysterols are 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, and 24(S,25)-epoxycholesterol (3). Activation of LXR in macrophages results in increased expression of genes encoding ATP-binding cassette (ABC) cholesterol transporters ABCA1 (4–7) and ABCG1 (8, 9) and apolipoprotein E (apoE) (9). ABCA1, ABCG1, and apoE are involved in cholesterol efflux from macrophages toward high density lipoproteins (HDL), which is considered the critical first step of reverse cholesterol transport. In the liver, LXR is involved in transcriptional control of Cyp7a1, encoding a critical enzyme in the conversion of cholesterol into bile acids (3, 10), as well as in transcriptional control of ABCG5/ABCG8 (11, 12), ABC transporters implicated in biliary cholesterol excretion. Induction of intestinal Abca1, Abcg5, and Abcg8 expression upon LXR activation is thought to reduce the efficiency of cholesterol absorption and hence to accelerate fecal cholesterol disposal (4). The physiological role of LXR in the control of cholesterol metabolism and the possibility of pharmacological interventions aimed at prevention of atherosclerosis via this regulatory system have recently been reviewed extensively (13–17).

In addition to cholesterol transport genes, LXR has been reported to control genes that encode proteins involved in de novo lipogenesis. Induced transcription has been reported for the gene encoding the sterol-regulatory element-binding protein-1c (SREBP-1c) (18–20), the transcription factor that regulates expression of various lipogenic genes, including those encoding acetyl-CoA carboxylase (ACC) and fatty acid synthase (21). Apart from indirect actions through SREBP-1c, LXR also directly influences transcription of Fas (22) and of genes encoding lipoprotein lipase (23), cholesterol ester transfer protein (24), and stearyl-CoA desaturase-1 (25).

Recently, the availability of synthetic LXR agonists, e.g. T0901317, has provided more insight into metabolic consequences of LXR activation. Oral administration of T0901317 to C57BL/6 mice increased HDL cholesterol concentrations in plasma, probably related to induction of the Abca1 and Abcg1 expression (18). In addition to this potentially beneficial effect, treatment with LXR agonists also led to a marked increase in hepatic triglyceride (TG) content (18). In Lxrα/Lxrβ double
knockout mice, hepatic steatosis did not occur upon treatment with high does of the agonist (18). In wild type mice, but not in Lxra knockout mice, hepatic TG accumulation was also observed upon feeding a high cholesterol diet (10). Hepatic steatosis is thought to be a result of increased fatty acid synthesis caused by induction of lipogenic genes. De novo lipogenesis, in turn, is thought to contribute to regulation of hepatic very low density lipoprotein (VLDL) production (26), but data on VLDL production upon LXR activation are not available. In this study, we examined the effects of T0901317 treatment on VLDL-TG production rate in relation to steady state plasma TG levels in mice. Our results demonstrate that administration of the synthetic LXR agonist resulted in an increased hepatic production rate of VLDL-TG, caused by formation of large TG-rich particles. In wild type mice, increased VLDL-TG production was compensated for by efficient metabolism and clearance, and no effects on fasting TG levels were seen. However, in mice with impaired particle clearance (apoE/H11002-Leiden transgenics) treatment with the LXR agonist resulted in a marked hypertriglyceridemia.

**EXPERIMENTAL PROCEDURES**

**Animals**—Male C57BL/6J mice (Harlan, Horst, The Netherlands), were housed in a light- and temperature-controlled facility. The animals were fed a commercially available lab chow (RMH-B, Hope Farms BV, Woerden, The Netherlands), containing 6.2% fat and ~0.01% cholesterol (w/w), and water ad libitum. Male transgenic mice expressing the human APOE*3-Leiden gene (27, 28) and apoE/H11002/LDLr double knockout mice (29) were bred at the animal facility of the Gausius Laboratory (Leiden, The Netherlands) and were fed the same standard diet. The mice received humane care, and experimental procedures were in accordance with local guidelines for use of experimental animals.

**Plasma and Liver Tissue Sampling**—C57BL/6J mice received 10 mg/kg LXR agonist T0901317 (kindly donated by Organon Laboratoires, Bo, The Netherlands), or its solvent by gavage daily for 4 days. T0901317 was dissolved in Me2SO and Chremophor (both from Sigma) in 5% mannitol/water, to a final concentration of 2.5 mg/ml. On the morning of the fifth day, a large blood sample was collected by cardiac puncture and centrifuged. The obtained plasma was stored at –20°C until analysis. The liver was quickly removed, weighed, and frozen in separate portions for RNA isolation and lipid analysis. Parts of the liver were frozen in isopentane and used for microscopic examination.

**In Vivo VLDL Triglyceride Production Rate**—C57BL/6J wild type mice received T0901317 or the solvent only exactly as described. On the fifth day, after a fasting period of 10 h, mice received an injection of 120,000 rpm in a Optima TM LX tabletop ultracentrifuge (Beckman Coulter, Fullerton, CA). Particle diameters were calculated from the volume fractions of the submicron particle analyzer (Nicomp Particle Sizing Systems, Santa Barbara, CA). VLDL triglyceride production rate was calculated from the slope of the triglyceride concentration versus time curve (30). The large blood sample was used for isolation of VLDL.

**Hepatic RNA Isolation and Measurement of mRNA Levels by Real-time PCR** (Taqman)—Total RNA was isolated from ~30 mg of liver tissue with the TRIzol method (Invitrogen, Paisley, United Kingdom). RNA was converted to single stranded cDNA by a reverse transcription procedure with Moloney murine leukemia virus-RT (Roche Diagnostics, Mannheim, Germany) according to the protocol of the manufacturer using random primers. cDNA levels were measured by real-time PCR using the ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA).

For the PCR amplification studies, an amount of cDNA corresponding to 20 ng of total RNA was amplified using the qPCR core kit (Eurogentec, Seraing, Belgium) essentially according to the protocol of the manufacturer and optimized for amplification of the particular gene using the appropriate forward and reverse primers (Invitrogen) and a template-specific 3‘-TAMRA (6-carboxytetramethylrhodamine)/5‘-FAM (6-carboxyfluorescein)-labeled double dye oligonucleotide probe (Eurogentec). In the same experiments, calibration curves were run on serial dilutions of cDNA solution and only dilutions resulting in series containing 4, 2, 1, 0.5, 0.25, 0.125, 0.062, and 0.031 × cDNA present in the assay incubation. The data obtained were processed using the software program ABI Sequence Detector (version 1.6.3; System Applied Biosystems, Foster City, CA). All quantified expression levels were within the linear part of the calibration curves and were calculated by using these curves. PCR results were normalized to β-Arnt mRNA levels. The sequences of the primers and probes used in this study are listed in Table I.

**Histology**—Liver histology was examined on frozen liver sections after Oil-Red-O staining for neutral lipids by standard procedures.

**Plasma Lipid Analyses**—Plasma triglycerides, phospholipids, free fatty acids, HDL cholesterol, free cholesterol, and total cholesterol were determined using commercially available kits (Roche Diagnostics and Wako Chemicals). Lipoproteins of C57BL/6J mice, apoE/H11002–/–, double knockout mice and APOE*3-Leiden transgenic mice were separated using fast protein liquid chromatography (FPLC) on a Superose 6B 10/30 column (Amersham Biosciences). The contents of apoB100, apoB48, and apoA-I in the fractions were visualized by Western blotting. Proteins in the fractions were separated by SDS-PAGE using 4–15% gradient gels (Ready Gels, Bio-Rad) and transferred to nitrocellulose membranes (Hyperbound, Amersham Biosciences, Roosendaal, The Netherlands). The membranes were incubated with sheep anti-human apoB (Roche Diagnostics) or rabbit anti-human apoA-I (Calbiochem, San Diego, CA), followed by incubation with donkey anti-sheep IgG horseradish peroxidase or donkey anti-rabbit IgG horseradish peroxidase and visualized by ECL detection (all three from Amersham Biosciences, Uppsala, Sweden) according to the instructions from the manufacturer.

**VLDL Isolation and Analyses**—Plasma VLDL/TDL (d < 1.019 g/ml) was isolated by density gradient ultracentrifugation. For this, 300 μl of plasma was adjusted to 800 μl with a NaCl/KBr solution with a density of 1.205 g/ml, containing 0.05% bovine serum albumin and 0.02% Triton X-100 at 120,000 rpm in a Optima TM LX tabletop ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA). VLDL was isolated by tube slicing, and the volume was recorded by weight. ApoB100 and apoB48 were determined by Western blotting, using antibodies against human apoB raised in sheep. Triglyceride and cholesterol content were determined as described for plasma. Phospholipids were determined using a commercial kit (Wako Chemicals). Fatty acid composition was determined as described previously (34).

**VLDL Size Determination**—VLDL size and volume distribution profiles were analyzed by dynamic scattering using a Nicomp model 370 submicron particle analyzer (Nicomp Particle Sizing Systems, Santa Barbara, CA). Particle diameters were calculated from the volume distribution patterns provided by the analyzer.

**Cell Culture Experiments**—MCA-RH7777 (rat hepatoma) cells were plated in 35-mm six-well plastic dishes (Costar Corp., Cambridge, MA) in 2 ml of Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, 10% natural horse serum, penicillin/streptomycin, and genetin. At a confluence of ~30%, the medium was removed and cells were washed with Hanks’ balanced salt solution (HBSS) and subsequently incubated with Dulbecco’s modified Eagle’s medium + 10% fetal calf serum, containing either 0 or 10 μM T0901317 and the above mentioned antibiotics. After 24 h, the medium was removed and half of the wells were incubated with the same medium for another 41 h. The other half of the wells were incubated with the same medium to which was added 25 μM [3H]glycerol (4.4 μCi/well) for 41 h. The medium was then collected, centrifuged to remove debris and, stored at 4°C until further analysis. Cells were washed with ice-cold HBSS and either used for RNA isolation or
LXR Activation Leads to Large VLDL Particles

Primer and probes used for real-time PCR analysis of hepatic gene expression

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TABLE I

Lipid Analyses of Cells and Medium—Lipids secreted by McA-RH7777 cells into medium and cellular lipids were extracted as described previously (35). [3H]TG was separated from the other lipids by thin layer chromatography with hexane/diethyl ether/acidic ether (80/20/1 v/v/v) as developing solvent. After iodine staining, the spots containing [3H]TG (as a measure for VLDL-TG secretion) were scraped into vials and assayed for radioactivity by scintillation counting.

Lipids secreted by McA-RH7777 cells were isolated by using the SV Total RNA Isolation System (mega RNA, Madison, WI) according to the instructions from the manufacturer. RNA was processed for real-time PCR as described under “Hepatic RNA Isolation and Measurement of mRNA Levels by Real-time PCR (Taqman).”

Statistics—All values represent mean ± standard deviation for the number of animals, wells, or experiments indicated. Statistical analysis of two groups was assessed by Mann-Whitney U test. Level of significance was set at p < 0.05. Analyses were performed using SPSS for Windows software (SPSS, Chicago, IL).

RESULTS

Effects of T0901317 on Hepatic and Plasma Lipids in C57BL/6J Mice—Livers from mice that had received T0901317 were clearly heavier than those in the control group (Table II). The hepatic TG content in treated mice was −10 times higher than in controls. There were no differences in protein, phospholipid, and free cholesterol contents, whereas the cholesteryl ester content was slightly decreased after treatment (Table II). Oil-Red-O staining for neutral lipids on frozen liver sections showed massive lipid accumulation in periporal (zone 1) as well as in perivenous (zone 3) hepatocytes in mice receiving T0901317 (Fig. 1). The hepatic fatty acid composition was changed upon treatment: the relative amount of oleate (C18:1) was sharply increased (Fig. 2), whereas the relative...
TABLE II
Comparison of hepatic lipid parameters of mice treated with T0901317 or its solvent

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>T0901317</th>
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<tbody>
<tr>
<td>Liver weight (% of body weight)</td>
<td>4.6 ± 0.6</td>
<td>6.6 ± 0.3*</td>
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<tr>
<td>Protein (mg/g liver)</td>
<td>225 ± 17</td>
<td>225 ± 17</td>
</tr>
<tr>
<td>Triglycerides (nmol/mg liver)</td>
<td>6.92 ± 2.65</td>
<td>57.74 ± 16.61*</td>
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<tr>
<td>Free cholesterol (nmol/mg liver)</td>
<td>4.21 ± 0.50</td>
<td>3.83 ± 0.71*</td>
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<tr>
<td>Cholesterylester (nmol/mg liver)</td>
<td>1.44 ± 0.52</td>
<td>0.98 ± 0.40*</td>
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<tr>
<td>Phospholipids (nmol/mg liver)</td>
<td>35.74 ± 3.48</td>
<td>36.43 ± 3.63</td>
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</tbody>
</table>

Fig. 1. Uniform Oil-Red-O staining for neutral fat in livers of T0901317-treated mice. No staining was observed in liver sections from male C57BL/6J mice receiving the solvent (A). Massive staining was found in liver sections from mice treated 4 days with 10 mg/kg LXR agonist T0901317 (B). V, central vein; P, portal vein. Original magnification, ×50.

Fig. 2. Changes in relative hepatic concentrations of major fatty acid species upon LXR activation. Fatty acid composition was determined in ~15 mg of liver tissue by gas chromatography after methylation, with heptadecanoic acid (C17:0) as internal standard. *p < 0.01 versus control.

Fig. 3. Changes in relative hepatic gene expression upon LXR activation, determined by real-time PCR. mRNA levels of genes encoding proteins involved in de novo lipogenesis were 3.5-4.5 times higher than in controls. No induction of mRNA levels of Srebp-1a was observed.

The plasma concentrations of free cholesterol, cholesterol esters, phospholipids, and free fatty acids (FFA) were significantly elevated in the treated group as compared to controls (Table III). As expected, HDL cholesterol was increased by ~60%, but the TG concentration was not affected by LXR activation in these experiments. Upon FPLC separation of plasma lipoproteins, distribution of TG appeared to be unaffected after LXR activation (Fig. 4A). Cholesterol contents of fractions 25–29 were higher in the group treated with T0901317 (Fig. 4B). Fig. 4C shows that apoA-I was present in these earlier fractions after LXR activation, indicating that the “shoulder” in the cholesterol profile could be attributed to the presence of large HDL particles.

LXR Activation Leads to Formation of Larger Particles—Fig. 5 shows the plasma TG concentration versus time curve after injection of Triton WR-1339 in control and T0901317-treated mice. The VLDL-TG production rates were calculated from these curves. Upon LXR activation, the production rate was 2.56 times higher than control values: 201.9 ± 36.4 versus 78.8 ± 18.7 μmol/kg/h (Fig. 5, inset). The concentrations of free cholesterol, cholesterol esters, phospholipids, and TG in nascent VLDL particles isolated from plasma 4 h after Triton WR-1339 injection are summarized in Figure IV. The mol% of surface lipids (free cholesterol and phospholipids) was reduced relative to that of core lipids (TG and cholesterol esters), leading to an increase of the core:surface ratio by 14.4%. Accordingly, direct measurement of particle sizes revealed that after LXR activation the mean diameter of the VLDL particles was increased by 48% (129 ± 9 versus 94 ± 12 nm, p < 0.001). The diameter was used to calculate the particle volume. The in-
Male C57BL/6J mice were treated with 10 mg/kg synthetic LXR agonist T0901317 or its solvent (control) during 4 days. Blood was collected by cardiac puncture, and plasma obtained by centrifugation. Concentrations of lipids were measured as described under "Experimental Procedures." Each value represents the mean ± S.D.; n = 10, *p < 0.05.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>T0901317</th>
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<tbody>
<tr>
<td>Free cholesterol (mM)</td>
<td>0.60 ± 0.07</td>
<td>0.87 ± 0.05*</td>
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<tr>
<td>Cholesterylester (mM)</td>
<td>1.36 ± 0.31</td>
<td>2.27 ± 0.21*</td>
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<tr>
<td>HDL cholesterol (mM)</td>
<td>1.35 ± 0.27</td>
<td>2.15 ± 0.14*</td>
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<tr>
<td>Phospholipids (mM)</td>
<td>2.55 ± 0.41</td>
<td>3.94 ± 0.21*</td>
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<tr>
<td>Triglycerides (mM)</td>
<td>0.46 ± 0.16</td>
<td>0.49 ± 0.21</td>
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<tr>
<td>Free fatty acids (mM)</td>
<td>0.34 ± 0.07</td>
<td>0.47 ± 0.08*</td>
</tr>
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Fig. 4. Effect of LXR activation on distribution of triglycerides and cholesterol in plasma lipoprotein fractions. Lipoproteins were separated using FPLC on a Superose 6B 10/30 column (Amersham Biosciences). Plasma of 10 male C57BL/6J mice per group was pooled, and 0.2 ml was applied to the column and eluted with phosphate-buffered saline at a flow of 0.5 ml/min. Triglycerides and cholesterol were measured as described under "Experimental Procedures." A, profiles of triglycerides. B, profiles of cholesterol. C, mice receiving the solvent (control); ●, mice treated 4 days with 10 mg/kg LXR agonist T0901317; ○, mice receiving the solvent (control).

Fig. 5. Plasma triglyceride concentrations in T0901317-treated and control mice after injection of Triton WR-1339. Male C57BL/6J mice received at t = 0 an injection of 12.5 mg of Triton WR-1339 in 100 μl of phosphate-buffered saline via the penile vein. Triglyceride concentration was measured in the plasma samples collected at indicated time points as described under "Experimental Procedures." Open bar, mice receiving the solvent (control). Closed bar, mice treated 4 days with 10 mg/kg LXR agonist T0901317. Values represent the mean ± S.D.; n = 6 (T0901317), n = 7 (control); *, p < 0.05.

Table III
Comparison of plasma lipid parameters of mice treated with T0901317 or its solvent

Table IV
Comparison of composition and size of nascent VLDL particles of mice treated with T0901317 or its solvent

Male C57BL/6J mice were treated with 10 mg/kg synthetic LXR agonist T0901317 or its solvent (control) during 4 days. Mice were injected with Triton WR-1339 on the fifth day. At 4 h after injection, blood was collected by cardiac puncture and VLDL was isolated from plasma by density gradient ultracentrifugation. Concentrations of lipids were measured as described under "Experimental Procedures" and particle diameter was measured using a particle analyzer. Each value represents the mean ± S.D.; n = 6 (T0901317); *, p < 0.05.
of palmitate (C16:0), linoleate (C18:2), and arachidonate (C20:4) were decreased upon LXR activation.

To gain further insight into the molecular background of LXR-induced stimulation of VLDL-TG secretion, the expression of genes involved in the control of this process was investigated. Fig. 8A shows that expression of the Apob gene was slightly but significantly reduced upon LXR activation. No changes in expression of Mttp and Apoe were noted. Likewise, expression of Apobec-1, encoding the apoB mRNA-editing protein, was not affected. In contrast, a marked induction of Lpl and Pltp mRNA levels was found (Fig. 8B), the latter encoding the phospholipid transfer protein. Expression of Ldlr, encoding the low density lipoprotein receptor involved in the clearance of TG-rich lipoproteins, was slightly induced.

**LXR Activation Leads to Hypertriglyceridemia in ApoE−/−; LDLr−/− Double Knockout and APOE*3-Leiden Transgenic Mice**—Increased VLDL-TG production in C57BL/6J mice was not accompanied by elevated fasting TG levels (Table III), possibly as a result of efficient particle metabolism and clearance. To test this option, effects of LXR activation on plasma TG levels were also studied in mouse models with defective particle clearance, i.e. apoE−/−; LDLr−/− double knockout and APOE*3-Leiden transgenic mice. Fig. 9 shows that in both apoE−/−; LDLr−/− mice and in APOE*3-Leiden transgenic mice, treatment with T0901317 resulted in a very strong, progressive increase in plasma TG concentrations. As expected, TG was almost exclusively present in VLDL-sized lipoprotein fractions in T0901317-treated apoE−/−; LDLr−/− double knockouts and in APOE*3-Leiden transgenics upon FPLC separation of plasma lipoproteins (data not shown).

**Effects of LXR Activation on VLDL-TG Production in Cultured Liver Cells**—To determine whether LXR effects on VLDL production can be achieved via direct actions on liver cells, the rat hepatoma cell line McA-RH7777 was exposed to the LXR agonist T0901317 for 65 h. Fig. 10 shows the effects of LXR activation on mRNA expression of genes involved in VLDL assembly and fatty acid synthesis. After incubation with T0901317, a 7-fold increase in Srebp-1c mRNA levels was observed as well as a 2-fold increase in both Acc1 and Fas mRNA levels. Similar to the in vivo situation, a 20% decrease in Apob and a slight decrease in Lxra mRNA levels were observed after exposure of the cells to the LXR agonist.

After 24 h of incubation with T0901317 alone, the medium was replaced by medium also containing $[^{3}H]$glycerol. After another 41-h incubation, lipids were isolated from cells and media to determine the amount and secretion of newly synthesized VLDL-TG. Incubation with T0901317 resulted in a 2.6-fold induction of VLDL-TG secretion and a 3.4-fold induction of TG synthesis (Fig. 11A). Intracellular total TG mass was also increased upon incubation with T0901317 (1.4-fold compared with control situation). The amount of apoB isolated from medium after incubation with T0901317 was compared with that in the control medium. No significant differences in apoB100 or apoB48 were detected (Fig. 11B).

**DISCUSSION**

Pharmacological activation of LXR leading to accelerated reverse cholesterol transport has been advocated as a potential novel treatment or prevention of atherosclerosis (17). This study shows that the beneficial raise of HDL levels is accompanied by potentially adverse effects on triglyceride metabolism. In mice, an increased VLDL-TG production was seen upon LXR activation by the LXR agonist T0901317, caused by the formation of large, TG-rich particles. Large VLDL particles, also known as VLDL1 particles, are considered pro-atherogenic and are metabolized to small dense low density lipoprotein. Increased production of VLDL1 is a hallmark of diabetes type 2-related hyperlipidemia (37).

The development of a severe hepatic steatosis, also reported previously (18), is likely related to increased de novo lipogenesis in combination with an increased FFA flux toward the liver. Slight quantitative differences in T0901317-induced gene expression patterns between our and other studies (18, 22, 25) are probably the result of differences in treatment protocols. For instance, Liang et al. (25) supplied T0901317 via the food during 12 h, which may have resulted in a more constant exposure of the liver to the agonist. Livers were removed immediately after this 12-h period. In our study mice received T0901317 daily for 4 days, and livers were taken out 20 h after the last administration. Second, effects of the agonist may be transient because Joseph et al. (22) showed that hepatic expression of Fas and Scd-1 was higher after 3 days of LXR activation than after 7 days.

The accumulated neutral lipids were distributed uniformly
across the liver lobule, in contrast to most models of steatosis, e.g. the ob/ob mice (38), in which fat accumulates mainly in perivenous (zone 3) hepatocytes. After LXR activation, TG accumulated both in perivenous (zone 3) hepatocytes, which may reflect increased de novo lipogenesis (39), and in periportal (zone 1) hepatocytes. The fatty acid profile showed a specific increase in 18:1 fatty acids, in accordance with increased expression of Scd-1 after LXR activation (25). The fatty acid composition of VLDL was comparable with that of liver homogenates, indicating that TG containing specific fatty acids are not preferentially recruited for VLDL assembly.

The increase of HDL cholesterol was mainly the result of the presence of larger particles in the circulation. Elevated HDL levels are probably caused by increased cholesterol efflux from peripheral and/or liver cells caused by induction of ABCA1 and ABCG1. The HDL elevating action of the LXR agonist may be, in part, counteracted by the LXR-induced expression of Pltp, because overexpression of Pltp is shown to reduce HDL in mice (40). However, induction of Pltp expression might also contribute to the presence of large HDL particles, as Pltp is a main determinant of the size and composition of HDL. These latter particles are efficient acceptors of cholesterol from peripheral cells.

The induced VLDL-TG production rate upon LXR activation was found to be the result exclusively of formation of larger particles. The increase in VLDL-TG production exactly matched the increased mean VLDL particle volume. The apoB blot is representative for the results from three independent experiments.
to formation of larger instead of more VLDL particles. The increased TG production by the liver did not lead to a raise in plasma TG levels in CL57BL/6J mice, but defective clearance in apoE−/− LDLr−/− double knockout and APOE3-Leiden transgenic mice clearly unmasked the hypertriglyceridemic actions of LXR activation. Plasma TG levels markedly and progressively increased upon treatment with the agonist in these mice. Interestingly, Schultz et al. (18) showed that treatment of wild type C57BL/6 mice with T0901317 during 7 days did lead to elevated plasma TG levels. The discrepancy with our results may be caused by a difference in duration of treatment. Possibly, net effects of T0901317-treatment on plasma lipid levels are determined by time-dependent effects on gene expression levels and their metabolic consequences.

It is tempting to speculate that LXR-stimulated de novo lipogenesis is the primary cause of the increased VLDL-TG secretion. The increased VLDL-TG production together with the induced transcription of hepatic Lpl might account for the elevated plasma FFA levels observed upon treatment (Table II). Lipoprotein lipase hydrolyzes TG and thereby catalyzes the conversion of VLDL into LDL, and subsequently of LDL into low density lipoprotein. Both conversions result in the release of FFAs into the bloodstream (43) and these FFAs will to large extent be taken up again by the liver. Increased supply of FFAs to liver cells stimulates VLDL-TG production (44); part of the observed stimulation of VLDL-TG production may therefore occur via this indirect mechanism. To investigate whether LXR activation stimulates the VLDL-TG secretion by liver cells directly, we performed a limited series of experiments with Mca-RH7777 cells kept under fixed FA concentrations. In these in vitro studies, we also found a gene expression profile consistent with increased de novo lipogenesis and stimulated cellular TG secretion, again without changes in apoB production. Administration of T0901317 to C57BL/6 mice also results in induction of Srebp-1 and Sdc-1 mRNA levels in intestine and kidney (18). A recent study from Mak et al. (45) showed that incubation of murine macrophages with T0901317 resulted in increased Srebp-1 and Fas mRNA levels. Nevertheless, actions of the LXR agonist in hepatocytes may be responsible for the observed increased VLDL-TG production rate in vivo because these cells are primarily involved in this process. It should be stressed that increased hepatic de novo lipogenesis is not always associated with increased VLDL-TG secretion. For instance, the strong induction of de novo lipogenesis upon inhibition of glucose-6-phosphatase activity in rats is not associated with increased VLDL production (46). Apparently, different regulatory factors dominate under different metabolic conditions.

Genes encoding proteins involved in assembly and secretion of VLDL, i.e. Apoe and Mttp, were not affected upon treatment. The production of larger VLDL particles might be accounted for by an intracellular action of PLTP. Jiang et al. (47) reported that PLTP-deficient mice show a decreased secretion of apoB-containing particles. Because this decreased apoB production rate was observed both in vivo and in hepatocytes in vitro, it was concluded that PLTP must also be active within the cells. This suggestion is supported by the fact that mice overexpressing the human PLTP show increased VLDL-TG production (48). However, neither of these studies provided information about the size of the particles produced in the presence or absence of PLTP. Alternatively, induction of Cyp7a1 expression and activity may contribute to increased VLDL-TG production, as mice overexpressing the human Cyp7a1 gene also show this feature (49). Thus, various mechanisms may be responsible for the production of larger VLDL particles after LXR activation, but the exact mode of action is currently not known. In conclusion, pharmacological activation of LXR is associated with development of hepatic steatosis and strongly affects the VLDL production process in mice, leading to formation of large, TG-rich particles. This combination of hepatic fat accumulation and overproduction of large VLDL strikingly resembles that observed in human diabetic states.

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LXR Activation Leads to Large VLDL Particles

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Stimulation of Lipogenesis by Pharmacological Activation of the Liver X Receptor Leads to Production of Large, Triglyceride-rich Very Low Density Lipoprotein Particles

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