LXR-SREBP-1c-Phospholipid Transfer Protein Axis Controls Very Low Density Lipoprotein (VLDL) Particle Size*

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Liver X receptors (LXRs) activate triglyceride synthesis in liver directly and indirectly by inducing sterol regulatory element–binding protein-1c (SREBP-1c). When administered to wild-type mice, the LXR activator T0901317 produces a mild and transient hypertriglyceridemia. Here, we show that T0901317 produces massive hypertriglyceridemia when given to mice lacking low density lipoprotein (LDL) receptors (Ldlr<sup>−/−</sup> mice). Triglycerides ranged from 4000 to 6000 mg/dl, and the plasma turned milky. The median diameter of VLDL particles, measured by electron microscopy, increased from 43 to 112 nm, 87% exceeding 80 nm, the size of chylomicrons. Hypertriglyceridemia was prevented in Ldlr<sup>−/−</sup>;Srebp-1c<sup>−/−</sup> double knock-out mice. In Ldlr<sup>−/−</sup> mice, T0901317 increased mRNAs not only for enzymes of fatty acid and triglyceride synthesis, but also for phospholipid transfer protein (PLTP), which transfers phospholipids into nascent VLDL, allowing particle expansion. The PLTP increase was blunted in Ldlr<sup>−/−</sup>;Srebp-1c<sup>−/−</sup> animals. When Ldlr<sup>−/−</sup>;Srebp-1c<sup>−/−</sup> mice received an adenovirus encoding Pltp, the hypertriglyceridemic response to T0901317 was partially restored and the VLDL size increased. We conclude that LXR agonists activate triglyceride synthesis and PLTP transcription by activating Srebp-1c. In concert with the increase in TG synthesis, the increased PLTP permits triglyceride incorporation into abnormally large VLDL, which are removed from plasma by LDL receptors. In the absence of LDL receptors, the large VLDLs accumulate and produce massive hypertriglyceridemia.

Synergy between liver X receptors (LXRs) and sterol regulatory element–binding protein-1c (SREBP-1c) increases the synthesis of triglycerides in liver and their secretion into plasma in very low density lipoproteins (VLDL) (1, 2). As a result, the administration of T0901317, a synthetic LXR activator, produces prominent fatty liver and mild hypertriglyceridemia in mice (2). LXR agonists enhance fatty acid synthesis largely by activating the transcription of the gene encoding SREBP-1c, which in turn activates transcription of lipogenic genes (3, 4). LXRs also directly stimulate the transcription of certain lipogenic genes, including acetyl-CoA carboxylase (Acc) (5) and fatty acid synthase (FAS) (6). The synergistic action of LXRs and SREBP-1c was confirmed by experiments in mice with a deletion of the gene encoding SREBP-1c (3). When these animals were treated with T0901317, the increase in mRNAs encoding ACC and fatty acid synthase was reduced by 50–60%. Moreover, the increase in mRNA encoding glycerol-3-phosphate acyltransferase (GPAT), the initiating enzyme in triglyceride synthesis, was eliminated. As a result, hepatic accumulation of triglycerides was reduced by two-thirds.

When administered for 1 to 7 days to normal mice, T0901317 causes a mild 2-fold increase in plasma triglycerides (2, 7). When administered for 4 days to double knock-out mice lacking both the LDL receptor and apolipoprotein E, the hypertriglyceridemia was much more pronounced, increasing as much as 12-fold (7). Under these conditions the plasma contained abnormally large triglyceride-rich VLDL particles. These findings led to the suggestion that LXRs enhance the production of large VLDL particles and that these are normally cleared from the circulation by LDL receptors (7). When LDL receptors are absent, the large VLDL particles accumulate. The mechanism by which LXRs enhance the production of large VLDL particles has not been addressed.

In the current studies, we administered T0901317 to Ldlr<sup>−/−</sup> mice and observed that the plasma turned milky and the triglyceride level rose as high as 6,000 mg/dl (~70 mm) after 9 days, a time when triglyceride levels were normal in wild-type mice treated with the same compound. The plasma of the T0901317-treated Ldlr<sup>−/−</sup> mice contained abnormally large VLDL particles ~80 nm in diameter, accounting for the milky appearance. To find a mechanism for this response, we administered T0901317 to mice with a combined deficiency of LDL receptors and SREBP-1c (Ldlr<sup>−/−</sup>;Srebp-1c<sup>−/−</sup> mice). Remarkably, these double knock-out mice were completely protected from hypertriglyceridemia when treated with T0901317, indicating that at least one SREBP-1c target gene is essential. We provide evidence that an essential SREBP-1c target gene encodes phospholipid transfer protein (PLTP), a protein that has been shown previously to play a role in the secretion of VLDL (8–10). Together with the increase in mRNAs encoding enzymes of fatty acid and triglyceride synthesis, the elevation of PLTP allows production of abnormally large VLDL. These results establish a functional pathway proceeding from LXR to...
SREBP-1c to PLTP, which governs the size of VLDL particles secreted by the liver.

**EXPERIMENTAL PROCEDURES**

*Animals—*C57BL/6J* and *Ldlr<sup>−/−</sup> mice (B6.129S7-Ldlb<sup>tm1Herf</sup>J) were obtained from The Jackson Laboratory. *Srebp-1c<sup>−/−</sup>/* mice (3) were originally generated on a mixed C57BL/6J × 129Sv background and then backcrossed to C57BL/6J background for 10 generations (N10). The *Ldlr<sup>−/−</sup>*/*Srebp-1c<sup>−/−</sup> double knockout mice were generated by breeding *Ldlr<sup>−/−</sup>/* (11) mice with *Srebp-1c<sup>−/−</sup>/−* mice. All mice were housed in colony cages with a 12-h light/12-h dark cycle and maintained on a standard chow diet containing 6% fat (Teklad Mouse/Rat Diet 7002 from Harlan Teklad Premier Laboratory Diets, Madison, WI). For the T0901317 treatment study, mice were fed a powder chow diet (Teklad Mouse/Rat Diet 7001 from Harlan Teklad Premier Laboratory Diets) or a chow diet supplemented with the indicated amount of T0901317 (J-Star Research, South Plainfield, NJ). For the experiments in which mice were treated with T0901317 for different durations, the starting times were staggered so that all mice were sacrificed at the same time, which was at the end of the dark cycle. For all the animal studies, blood was drawn from the retro-orbital sinus and plasma was separated immediately for analysis. All animal experiments were performed with the approval of IACUC at the University of Texas Southwestern Medical Center at Dallas.

**Fast Performance Liquid Chromatography Analysis**—Pooled plasma (1.7 ml) from 4 male mice of C57BL/6J and *Ldlr<sup>−/−</sup>* fed a chow diet or a chow diet containing 0.025% (w/w) T0901317 for 3 days prior to the study. Primary hepatocytes were isolated and allowed to attach for 3 h (2.5 × 10<sup>6</sup> cells per 10-cm dish in 5 ml of medium A) as described above. After washing by phosphate-buffered saline, cells were incubated at 37 °C with 5 ml of fresh medium A for 6 h. VLDL fractions were then isolated from the medium by ultracentrifugation, negatively stained, and visualized by electron microscopy. The diameters of VLDL particles were measured by ImageJ 1.39u software (NIH).

**DNA Microarray Analysis**—Two microarray experiments were analyzed. Experiment A used a previously described data set in which the mRNA expression profiles were compared in livers from chow-fed wild-type (WT) mice, transgenic mice that overexpress the nuclear forms of SREBP-1a, and knockout mice lacking Scap in the liver (L-Scap<sup>−/−</sup>) (15). We identified a total of 98 putative SREBP-1 target genes whose mRNA expression profile showed a combination of having a >2-fold increase in the livers of the transgenic SREBP-1a mice and a >20% decrease in livers of L-Scap<sup>−/−</sup> mice. In Experiment B, the mRNA expression profiles of the 98 putative SREBP-1 target genes identified in Experiment A were examined in the livers from WT, *Ldlr<sup>−/−</sup>*/*Srebp-1c<sup>−/−</sup> mice fed a chow diet with or without 0.015% T0901317 for 3 days prior to the study. Primary hepatocytes were isolated and allowed to attach for 3 h (2.5 × 10<sup>6</sup> cells per 10-cm dish in 5 ml of medium A) as described above. After washing by phosphate-buffered saline, cells were incubated at 37 °C with 5 ml of fresh medium A for 6 h. VLDL fractions were then isolated from the medium by ultracentrifugation, negatively stained, and visualized by electron microscopy. The diameters of VLDL particles were measured by ImageJ 1.39u software (NIH).

**Quantitative Real Time PCR Analysis**—Total RNA was prepared from mouse livers using an RNA STAT-60 kit (TEL-TEST “B”) (Friendswood, TX) and subjected to quantitative real time PCR as previously described (3). All reactions were done in triplicate, and the relative amounts of mRNAs were calculated using the comparative C<sub>T</sub>-method. Mouse cyclophilin mRNA was used as the invariant control. Most of the primers for real time PCR analysis were described previously (3, 16). The new primers are as follows: Acc-2 (AF290178),...
Hypertriglyceridemia in Ldlr<sup>−/−</sup> Mice

A and B, plasma lipid levels in wild-type (WT) and Ldlr<sup>−/−</sup> mice (9–14 weeks old, male) fed an ad libitum chow diet supplemented with 0.025% T0901317 for the indicated time. The starting times for the feeding regimens were staggered so that all mice were sacrificed at the same time, which was at the end of the dark cycle on day 9. Each value represents the mean ± S.E. of data from 4 mice. Inset shows the plasma of Ldlr<sup>−/−</sup> mice treated with (+) or without (−) T0901317 for 9 days. C and D, FPLC profiles of plasma lipoproteins from Ldlr<sup>−/−</sup> mice (13–14 weeks old, male) fed a chow diet with (+) or without (−) 0.025% T0901317 for 10 days. Plasma from 4 mice was pooled and subjected to ultracentrifugation at d = 1.215 g/ml. The lipoprotein fractions (d < 1.215 g/ml) were subjected to gel filtration by FPLC, and the content of cholesterol (C) and triglycerides (D) in each fraction were measured. HDL, high density lipoprotein.

### TABLE 1
Composition of plasma VLDL from wild-type, Ldlr<sup>−/−</sup>, and Ldlr<sup>−/−</sup>;Srebp-1c<sup>−/−</sup> mice fed a chow diet or chow diet containing LXR agonist T0901317

| Component of VLDL | wild-type | +T0901317 | −T0901317 | −T0901317 | +T0901317 |
|-------------------|-----------|-----------|-----------|-----------|-----------|
| Triglycerides     | 69        | 287       | 78        | 3742      | 26        |
| Total cholesterol | 4.1       | 14        | 15        | 575       | 5.4       |
| Free cholesterol  | 2.9       | 12        | 9.1       | 385       | 1.8       |
| Cholesteryl ester | 1.2       | 2.2       | 5.7       | 190       | 3.6       |
| Phospholipids     | 15        | 46        | 24        | 730       | 7.6       |
| Protein           | 7.2       | 18        | 13        | 278       | 4.6       |

Mice of the indicated genotype (3–6 months old) were fed a chow diet with or without 0.0075% T0901317 for 6 days. Plasma samples from 3 mice (2 male, 1 female) per group were pooled and subjected to ultracentrifugation at d < 1.006 to isolate the VLDL fraction. Lipids and protein concentrations in the VLDL were measured as described under “Experimental Procedures.”

RESULTS

When wild-type mice were fed T0901317 for 9 days, they exhibited a mild increase in plasma cholesterol (Fig. 1A) and a mild and transient increase in plasma triglycerides that soon returned to baseline (Fig. 1B). In marked contrast to wild-type mice, Ldlr<sup>−/−</sup> mice treated with T0901317 exhibited a massive increase in plasma cholesterol, reaching over 2000 mg/dl at day 9 (Fig. 1A), and an even more massive and sustained increase in plasma triglycerides, reaching over 4,000 mg/dl (Fig. 1B). The inset to Fig. 1B shows the milky appearance of plasma in the Ldlr<sup>−/−</sup> mice treated with the T0901317 for 9 days. Fractionation of the lipoproteins by fast performance liquid chromatography revealed that the elevated cholesterol and triglycerides were contained in large VLDL particles (Fig. 1, C and D; Table 1).

As a first step in exploring the mechanism for this massive hyperlipidemia, we bred the Ldlr<sup>−/−</sup> mice with mice lacking the...
gene encoding SREBP-1c to produce \(Ldlr^{-/-};sreb\beta{-1c}{-/-}\) mice. Previous studies have shown that activators of LXR increase fatty acid and triglyceride synthesis in liver in part by direct actions and in part by increasing SREBP-1c levels (1, 2).

Fig. 2 shows an experiment comparing the responses of the \(Ldlr^{-/-}\) mice and the \(Ldlr^{-/-};sreb\beta{-1c}{-/-}\) mice to T0901317 over a 6-day period. Again, we observed massive hypercholesterolemia and hypertriglyceridemia in the \(Ldlr^{-/-}\) mice fed T0901317 (Fig. 2, B and E). These responses were nearly abolished in the \(Ldlr^{-/-};sreb\beta{-1c}{-/-}\) mice (Fig. 2, C and F; Table 1).

Fig. 3A shows the size of the lipoproteins in the \(d < 1.006\) fraction as determined by negative stain electron microscopy. To quantify these data, we measured the diameters of more than 300 randomly selected particles in each sample (Fig. 3B). In wild-type mice fed ad libitum with T0901317 for 6 days, we observed a distinct shift to larger particles (red bar in Fig. 3B, upper panel). The median diameter increased from 60 to 85 nm, and there was a skewed distribution to even larger particles with diameters up to 170 nm. Particles greater than 80 nm are generally considered to be in the range of chylomicrons (18). The shift to larger particles was even more pronounced in the \(Ldlr^{-/-}\) mice (Fig. 3B, middle panel). Here, T0901317 treatment increased the median particle size.

**FIGURE 2.** Comparison of blood lipid levels in \(Ldlr^{-/-}\) mice and \(Ldlr^{-/-};sreb\beta{-1c}{-/-}\) mice treated with T0901317 for 6 days. Male mice with the indicated genotype (10–18 weeks old) were fed a chow diet with (●) or without (○) 0.0075% T0901317. Aliquots of blood were obtained by retro-orbital bleeding at the indicated time after the start of the diet experiment and used for measurement of the plasma content of triglycerides (A–C) and cholesterol (D–F). Each value represents the mean ± S.E. of data from 3 mice.

**FIGURE 3.** Electron microscopy of negatively stained plasma VLDL from wild-type, \(Ldlr^{-/-}\), and \(Ldlr^{-/-};sreb\beta{-1c}{-/-}\) mice fed a chow diet with or without 0.0075% T0901317 for 6 days. A, plasma samples from 3 male mice of each group (10–18 weeks old) were pooled and VLDL (\(d < 1.006\) g/ml) was isolated for viewing by electron microscopy as described under “Experimental Procedures.” Magnification, \(\times 100,000\). B, size distribution of VLDL particles. The diameters of more than 300 VLDL particles from each group were measured, and the percentages of VLDL particles of different size are shown.
diameter to 112 nm. The chylomycin-like particles (>80 nm) represented 59% of the particles in T0901317-treated wild-type mice and 87% in the Ldlr<sup>−/−</sup> mice. In stark contrast, T0901317 produced no increase in particle diameter in the Ldlr<sup>−/−</sup>;Srebp-1c<sup>−/−</sup> mice, and only 0.3% of the particles were greater than 80 nm (Fig. 3B, lower panel). To minimize the possible contribution of intestinally derived chylomicrons to the VLDL fractions, we carried out a separate study identical in design to that in Fig. 3 except that the mice were fasted for 4 h prior to obtaining the blood for electron microscopy. The VLDL particle sizes did not differ significantly from those in Fig. 3B.

To confirm that the liver was capable of producing very large VLDL particles after T0901317 treatment, we fed mice with T0901317 for 3 days, isolated hepatocytes, and incubated them in vitro for 6 h. The medium was subjected to ultracentrifugation and the d < 1.006 fraction was examined by electron microscopy (Fig. 4). After T0901317 treatment, hepatocytes from wild-type and Ldlr<sup>−/−</sup> mice produced a population of large VLDL particles (Fig. 4A). In hepatocytes from wild-type mice, the proportion of particles >80 nm in diameter increased from 0.8 to 13% after T0901317 treatment (Fig. 4B, top panel). Similarly, in Ldlr<sup>−/−</sup> mice the proportion of very large VLDL rose from 1.3 to 21% (middle panel). No particles greater than 80 nm were secreted by hepatocytes from Ldlr<sup>−/−</sup>;Srebp-1c<sup>−/−</sup> hepatocytes with or without T0901317 treatment (lower panel).

The production of large VLDL particles implies that triglyceride synthesis is enhanced in hepatocytes of wild-type and Ldlr<sup>−/−</sup> mice after T0901317 feeding. To test this hypothesis, we fed mice with T0901317 for 3 days, isolated hepatocytes, incubated them with [14C]glycerol, and measured the incorporation of radioactivity into triglycerides (Fig. 5). Indeed, the rate of triglyceride synthesis was markedly elevated in the hepatocytes from T0901317-treated wild-type mice (Fig. 5A) as well as Ldlr<sup>−/−</sup> mice (Fig. 5B). The increase was much smaller in hepatocytes from Ldlr<sup>−/−</sup>;Srebp-1c<sup>−/−</sup> mice (Fig. 5C).

Considered together, the data so far indicate that T0901317 treatment increases triglyceride synthesis in wild-type and Ldlr<sup>−/−</sup> mice, and this increase is associated with the secretion of abnormally large VLDL particles. Both of these processes are severely blunted when the Srebp-1c gene is deleted.

We next sought to identify SREBP-1c target genes that are required for hypertriglyceridemia in the T0901317-treated Ldlr<sup>−/−</sup> mice. As a first step, we quantified the mRNA levels of various lipogenic genes in livers of wild-type, Ldlr<sup>−/−</sup>, and Ldlr<sup>−/−</sup>;Srebp-1c<sup>−/−</sup> mice.

FIGURE 4. Electron microscopy of negatively stained VLDL secreted by primary hepatocytes from wild-type, Ldlr<sup>−/−</sup>, and Ldlr<sup>−/−</sup>;Srebp-1c<sup>−/−</sup> mice. A, primary hepatocytes from male mice (4–6 months old) with the indicated genotype fed a chow diet with or without 0.015% T0901317 for 3 days were isolated and cultured in 10-cm dishes containing 5 ml of medium A. After incubation for 6 h, the VLDL fractions (d < 1.006 g/ml) were isolated from the medium by ultracentrifugation, negatively stained, and visualized by electron microscopy as described under “Experimental Procedures.” Magnification ×100,000. B, size distribution of VLDL particles. The diameters of more than 300 VLDL particles from each group were measured, and the percentages of VLDL particles of different size are shown.
Hypertriglyceridemia in Ldlr\(^{-/-}\) Mice

Ldlr\(^{-/-}\);Srebpb-1c\(^{-/-}\) mice. As shown in Table 2, in wild-type and Ldlr\(^{-/-}\) mice, T0901317 induced the mRNAs for all fatty acid and triglyceride biosynthetic enzymes. These increases were blunted in Ldlr\(^{-/-}\);Srebpb-1c\(^{-/-}\) mice, consistent with our previous observations that LXR-induced lipogenic response is partially mediated by the LXR-stimulated increase of SREBP-1c (3). Genes that are known to be direct targets of LXR were induced by T0901317 to similar levels in wild-type, Ldlr\(^{-/-}\), and Ldlr\(^{-/-}\); Srebpb-1c\(^{-/-}\) mice. These genes included Abc-a1, Abc-g5, Abc-g8, cholesterol 7α-hydroxylase, and lipoprotein lipase.

Because expression of lipogenic genes in T0901317-treated double knock-out mice is not completely abolished, these SREBP-1c target genes alone cannot fully account for the dramatic difference in triglyceride levels in Ldlr\(^{-/-}\) mice versus Ldlr\(^{-/-}\);Srebpb-1c\(^{-/-}\) mice. Therefore, we carried out the following analysis to identify other SREBP-1c target genes that might be required for T0901317-induced hypertriglyceridemia in Ldlr\(^{-/-}\) mice. We compared mRNA expression in livers of transgenic mice that overexpress SREBP-1a in liver and in L-scap\(^{-/-}\) mice that lack Scap in the liver. The L-scap\(^{-/-}\) mice are unable to process any SREBPs to their active nuclear forms. These microarray data were obtained from experiments that were published previously (15). As criteria for SREBP-1 specificity, we selected all mRNAs whose expression was increased by at least 2-fold in the SREBP-1a transgenic mice and decreased by at least 20% in the L-scap\(^{-/-}\) mice. A total of 98 mRNAs met these criteria. We then examined the expression of these mRNAs by microarray analysis in livers of wild-type, Ldlr\(^{-/-}\), and Ldlr\(^{-/-}\);Srebpb-1c\(^{-/-}\) mice that were fed a chow diet with or without T0901317 for 6 days. We asked whether any of these 98 mRNAs increased by more than 1.5-fold in livers of T0901317-treated wild-type and Ldlr\(^{-/-}\) mice, but showed no increase in livers of T0901317-treated Ldlr\(^{-/-}\);Srebpb-1c\(^{-/-}\) mice. Four of the 98 mRNAs met these criteria. These four mRNAs encoded ACC-2, GPAT, PLTP, and a protein of unknown function designated S14 (19). To confirm the microarray data, we measured the amounts of these four mRNAs by quantitative reverse transcription-PCR (Fig. 6A). These mRNAs all rose after T0901317 treatment of wild-type and Ldlr\(^{-/-}\) mice.

Table 2: Relative amounts of mRNAs in livers of wild-type, Ldlr\(^{-/-}\), and Ldlr\(^{-/-}\);Srebpb-1c\(^{-/-}\) mice fed with ad libitum chow diet or chow diet containing LXR agonist T0901317.

| mRNA          | Wild-type | Ldlr\(^{-/-}\) | Ldlr\(^{-/-}\);Srebpb-1c\(^{-/-}\) |
|---------------|-----------|--------------|-------------------------------|
| SREBP pathway |           |              |                               |
| LDL receptor  | 1.0       | 2.2          | 0.0                           |
| SREBP-1c      | 1.0       | 7.3          | 0.7                           |
| SREBP-1a      | 1.0       | 2.5          | 0.7                           |
| SREBP-2       | 1.0       | 1.3          | 0.6                           |
| Scap          | 1.0       | 1.3          | 0.6                           |

Fatty acid and triglyceride synthesis

| mRNA                  | Chow diet | Chow + T0901317 |
|-----------------------|-----------|-----------------|
| ATP citrate lyase     | 1.0       | 2.1             |
| Acetyl-CoA carboxylase-1 | 1.0   | 4.3             |
| Fatty acid synthase   | 1.0       | 5.0             |
| Long chain fatty acyl elongase-6 | 1.0  | 7.2             |
| Stearoyl-CoA desaturase-1 | 1.0  | 3.4             |
| Glycerol-3-phosphate acyltransferase | 1.0  | 2.8             |
| Malic enzyme          | 1.0       | 4.2             |
| Glucose-6-phosphate dehydrogenase | 1.0  | 14.2            |

Other LXR targets

| mRNA | Chow diet | Chow + T0901317 |
|------|-----------|-----------------|
| ABC-A1 | 1.0      | 2.6             |
| ABC-G5 | 1.0      | 6.9             |
| ABC-G8 | 1.0      | 7.3             |
| Cholesterol 7α-hydroxylase | 1.0 | 6.0             |
| Lipoprotein lipase | 1.0 | 8.6             |

Figure 5: Triglyceride synthesis in primary hepatocytes from wild-type, Ldlr\(^{-/-}\), and Ldlr\(^{-/-}\);Srebpb-1c\(^{-/-}\) mice. Male mice with the indicated genotype (4–6 months old) were fed a chow diet with or without 0.0075% T0901317 for 6 days. On day 3, primary hepatocytes were isolated from the mice and used for measurement of triglyceride synthesis by determining the incorporation of [14C]glycerol into [14C]triglycerides for the indicated time as described under "Experimental Procedures." The rate of triglyceride synthesis was expressed as nanomole of [14C]glycerol incorporated into triglycerides/mg of protein in the cell lysates. Each value represents the average of duplicate incubations.
mice, but not significantly in $\text{Srebp-1c}^{-/-}$ mice (Fig. 6A). In contrast, the mRNAs for ACC-1 and fatty acid synthase showed a definite, but blunted, increase in the $\text{Ldlr}^{-/-}$ mice. As expected, the mRNA for Scap showed no change in the double knock-out mice.

Inasmuch as PLTP has been reported to influence hepatic VLDL secretion (9, 20) and inasmuch as the $\text{Ldlr}^{-/-}$ mice fail to show an increase in PLTP after treatment with T0901317, we decided to test the hypothesis that the lack of PLTP activation is responsible for failure of the VLDL size to increase in the double knock-out animals. For this purpose, we prepared a recombinant adenovirus encoding PLTP under control of the cytomegalovirus promoter. For controls, we prepared similar viruses encoding S14 and GAP, two other proteins whose mRNA failed to rise when T0901317 was administered to the SREBP-1c-deficient mice (Fig. 6A). The adenoviruses were injected into $\text{Ldlr}^{-/-}$ mice. After 3 days the triglyceride level averaged 1812 mg/dl in the six mice injected with the $\text{Pltp}$ adenovirus (Fig. 6B). In contrast, the mean value was 351, 335, or 365 mg/dl in mice injected with an adenovirus encoding β-galactosidase ($\text{lacZ}$), S14, or GAP, respectively. Plasmid triglyceride levels in mice injected with $\text{Pltp}$ plus S14 or GAP viruses did not differ significantly from that in mice injected with the $\text{Pltp}$ virus alone (Fig. 6B). Negative stain electron microscopy revealed the size of plasma VLDL particles in the $\text{Ldlr}^{-/-}$ mice injected with the $\text{Pltp}$ adenovirus (Fig. 6C). The median diameter increased from 31 to 39 nm, and 3% of the particles exceeded 80 nm in diameter (Fig. 6C). In $\text{lacZ}$-injected mice, only 0.7% of particles exceeded 80 nm in diameter.

We next sought to determine whether restoration of PLTP expression could restore the ability of T0901317 to increase the size of VLDL particles in mice of mice lacking SREBP-1c. For this purpose, we injected the $\text{Pltp}$ adenovirus into $\text{Ldlr}^{-/-}$;Srebp-1c$^{-/-}$ mice that were treated with T0901317 and compared these animals with $\text{Ldlr}^{-/-}$;Srebp-1c$^{-/-}$ mice injected with a control $\text{lacZ}$ adenovirus and treated with T0901317. As shown in Fig. 7A, the $\text{Pltp}$ adenovirus increased the plasma triglyceride level to 1344 mg/dl as compared with a value of 400 mg/dl in the $\text{Ldlr}^{-/-}$;Srebp-1c$^{-/-}$ mice injected with the control adenovirus. The triglyceride-to-protein ratio in the isolated VLDL fraction increased from 5.9 to 9.6. The median size of plasma VLDL particles increased from 29 to 42 nm, and the percentage of particles $>$80 nm increased from 0 to 9% (Fig. 7, B and C). A major increase in VLDL particle size was also seen when we incubated isolated hepatocytes from the PLTP injected mice (Fig. 7, B and C). The median size of hepatocyte-derived VLDL increased from 39 to 52 nm, and the percentage of particles $>$80 nm increased from 0 to 14%. These data indicate that overexpression of PLTP can partially restore the increase of

![FIGURE 6. Identification of PLTP as a SREBP-1c target gene required for hypertriglyceridemia in the T0901317-treated Ldlr$^{-/-}$ mice. A, relative amount of mRNAs in livers from WT, Ldlr$^{-/-}$, and Ldlr$^{-/-}$;Srebp-1c$^{-/-}$ male mice (10–18 weeks old) fed a chow diet with or without 0.0075% T0901317 for 6 days. Total RNA from 3 mouse livers per group was pooled and quantified by real-time PCR. Cyclophilin was used as the invariant control. Values represent the amount of mRNA relative to that in chow-fed WT mice, which is arbitrarily defined as 1. B, plasma content of triglycerides in Ldlr$^{-/-}$ mice injected with different adenoviruses. For mice that received adenovirus encoding lacZ, S14, GAP, or PLTP, a total of 1.5 × 10$^{11}$ particles of lacZ virus or 0.75 × 10$^{11}$ particles of lacZ plus 0.75 × 10$^{11}$ particles of the indicated virus was injected into the jugular vein of each Ldlr$^{-/-}$ mouse (male, 8–14 weeks old) fed an ad libitum chow diet. For mice that received the PLTP+S14 or PLTP+GAP viruses, each mouse received 0.75 × 10$^{11}$ particles of Pltp plus 0.75 × 10$^{11}$ particles of S14 or Gap viruses. Three days after injection, aliquots of blood were obtained by retro-orbital bleeding and used for measuring the plasma content of triglycerides. The results represent data obtained from two separate studies. Each value represents the value from an individual mouse. C, size distribution of VLDL particles from Ldlr$^{-/-}$ mice injected with different adenoviruses. The mice used here are the same as those described in B. Plasma samples from 3–4 mice of each group were pooled, and VLDL fractions were isolated, negatively stained, and visualized by electron microscopy. The diameters of more than 300 VLDL particles from each group were measured, and the percentages of VLDL particles of different size are shown. Magnification, ×100,000.](image-url)
plasma triglycerides in \(Ldlr^{-/-}\);\(Srebp-1c^{-/-}\) mice treated with T0901317.

**DISCUSSION**

The current studies reveal a delicate relationship between LXRs, SREBP-1c, PLTP, and LDL receptors in governing plasma triglycerides in mice. In wild-type mice administration of T0901317, an LXR activator increased triglyceride synthesis in hepatocytes (Fig. 5), but produced only a mild and transient increase of triglycerides in plasma (Figs. 1 and 2). The delicate nature of this balance was unmasked when T0901317 was given to mice treated with T0901317. Therefore, other SREBP-1c-activated genes are also important. The likely candidate genes are those involved in fatty acid and triglyceride synthesis such as Acc-1, Fas, and Gpat (see Table 2 and Fig. 6). The T0901317-mediated increases of these lipogenic genes were blunted or abolished in the absence of SREBP-1c. Among the lipogenic genes, Gpat was the one most dependent on SREBP-1c for expression (Fig. 6A). However, restoration of GPAT expression with a \(Gpat\) adenovirus failed to raise plasma triglycerides in the \(Ldlr^{-/-}\) mouse (Fig. 6B).

PLTP is a secreted protein whose mRNA is expressed in multiple tissues, including liver, lung, and adipose tissue (21). Previous studies have demonstrated an important action of PLTP in plasma where it transfers phospholipids from VLDL to high density lipoprotein as the VLDL particles shrink during lipolysis (8, 10). A role for PLTP in VLDL secretion was first sug-

![Figure 7. Effect of PLTP adenovirus injection on the level of plasma triglycerides (A) and the size of plasma VLDL particles (B and C) in \(Ldlr^{-/-}\);Srebp-1c^{-/-} mice treated with T0901317. Male \(Ldlr^{-/-}\);\(Srebp-1c^{-/-}\) mice (5–8 months old) were fed with an ad libitum chow diet supplemented with 0.015% T0901317 for 7 days and then injected via the jugular vein with \(lacZ\) or \(Pltp\) recombinant adenoviruses (0.8 × 10¹¹ particles per mouse). A, 3 days after the injection, aliquots of blood were obtained by retro-orbital bleeding and used for determining the plasma content of triglycerides. Each value represents the measurement from an individual mouse. B, 3 days after the injection, plasma samples from 4 mice of each group were pooled, and VLDL fractions were isolated, negatively stained, and visualized by electron microscopy. Magnification, ×100,000. C, size distribution of VLDL particles. The diameters of more than 300 VLDL particles from plasma (top panel) or more than 100 VLDL particles secreted by hepatocytes (bottom panel) from each group were measured, and the percentages of VLDL particles of different size are shown.

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gested by Jiang et al. (20), who found that VLDL secretion was reduced in mice with targeted disruption of the Pltp gene. In the current study, we also appear to be observing an intracellular action of PLTP, in this case by inserting phospholipids into the outer layer of nascent VLDL particles, allowing them to grow larger. The intracellular role of PLTP is suggested by the observation that hepatocytes from mice that overexpress PLTP secreted abnormally large VLDL particles into the culture medium (Fig. 7C).

Considered together, our data suggest that SREBP-1c coordinates regulates two processes essential for the LXR-stimulated increase of large VLDL particles. One is the activation of the fatty acid and triglyceride biosynthetic genes to increase the production of triglycerides. The other is the increase of PLTP to insert additional phospholipids into VLDL particles, thereby enabling them to expand to accommodate the increased triglycerides. Without PLTP, the larger VLDL particles cannot be produced.

If T0901317 increases the synthesis of large VLDLs in both wild-type and Ldlr<sup>−/−</sup> mice, why do the particles accumulate to a much greater degree in the Ldlr<sup>−/−</sup> animals? We hypothesized that wild-type mice clear these large particles through a process that requires the LDL receptor and that this clearance is blunted in Ldlr<sup>−/−</sup> mice. In experiments to be described in a subsequent report, we show that the large VLDL particles produced by T0901317 activation of the LXR-SREBP-1c-PLTP axis are deficient in apoA-V. This apolipoprotein deficiency rendered these large VLDL particles dependent on the LDL receptor for their clearance into the liver.

Our current data in the Ldlr<sup>−/−</sup>Srebpd<sup>−/−</sup>-Srebpc<sup>−/−</sup> mice suggest that activation of Pltp transcription by LXRs requires SREBP-1c, and hence activation is severely blunted when T0901317 is fed to mice lacking SREBP-1c (Fig. 6A). LXRs are known to activate transcription of the Srebpc<sup>−/−</sup> gene (1, 2, 4). The data of Fig. 6A indicate that SREBP-1c is also required for LXR-mediated activation of Pltp gene transcription. In addition to its ability to activate the Pltp promoter indirectly through SREBP-1c induction, LXRs can activate the gene directly by binding to LXR elements (22–24). It seems likely that full activation of the Pltp gene requires the synergistic actions of LXRs and SREBP-1c. Elimination of Srebpd<sup>−/−</sup> disrupts this synergy and severely reduces the induction of Pltp expression by T0901317 (Fig. 6A).

The current studies have relevance to the hypertriglyceridemia that is common in insulin-resistant individuals with type 2 diabetes. Extensive studies in mice and rats have shown that insulin stimulates the production of SREBP-1c and its processing to the active nuclear form (25–28). Studies of mouse models have shown that insulin resistance does not extend the ability of insulin to activate production of SREBP-1c (26, 29). Indeed, in these models insulin resistance is associated with major increases in hepatic SREBP-1c mRNA and protein and consequently an increase in triglyceride synthesis. The current studies suggest that Pltp is an important target of SREBP-1c in these circumstances. In this context, it is worth noting that plasma PLTP levels were shown to be elevated by 2-fold in human subjects with type 2 diabetes (30). Inhibitors of PLTP or SREBP-1c might be useful in treating extreme cases of diabetic hyperlipemia.

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