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Differential Effects of Activation of Liver X Receptor on Plasma Lipid Homeostasis in Wild Type and Lipoprotein Clearance-Deficient Mice

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Abstract

The effects of liver X receptor (LXR) agonists on plasma lipid homeostasis, especially triglyceride metabolism are controversial. Here we examined the effect of long-term activation of LXR on plasma lipid homeostasis in wild-type C57BL/6 and LDL receptor deficient (LDLR−/−) mice given the LXR agonist T0901317 for 4 weeks. LXR agonist treatment of wild-type mice decreased plasma total triglycerides by 35% due to a significant reduction of plasma VLDL triglycerides. In contrast, in LDLR−/− mice T0901317 treatment increased plasma total cholesterol and triglycerides. An increase in the level of smaller VLDL particles was also observed in T0901317-treated LDLR−/− mice. The changes in circulating lipoprotein profiles in response to T0901317 treatment in these two animal models reflect the balance between synthesis and secretion on the one hand and lipolysis and clearance on the other. In both models there was both an increase in VLDL production and secretion and in an increase in LPL production and activity in T0901317-treated animals. In wild-type mice lipolysis and clearance predominates, while in the absence of the LDLR, which plays a major role in the clearance of apoB-containing lipoproteins, the increased output predominates. The generation of elevated levels of small VLDL particles due to increased lipolysis may represent an additional risk factor for atherosclerosis.

Keywords

LXR; VLDL; LPL; lipoprotein clearance deficiency; triglycerides

1. Introduction

LXRs are members of the nuclear receptor superfamily and are involved in the regulation of lipid and glucose metabolism and inflammation [1]. The discovery that activation of LXR facilitates reverse cholesterol transport by upregulation of ABCA1, ABCG1 and apoE in macrophages has opened a window to a new therapeutic strategy in the prevention and

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treatment of atherosclerosis [2–4]. However, LXR activation in liver induces the expression of sterol regulatory element binding protein 1c (SREBP-1c), which consequently triggers the expression of fatty acid synthase (FAS) and acetyl-coenzyme A carboxylase (ACC), enzymes responsible for de novo lipogenesis and triglyceride accumulation in both liver and blood [5, 6]. In addition, multiple lipoprotein remodeling-related genes, such as lipoprotein lipase (LPL), phospholipid transfer protein (PLTP), and cholesterol ester transfer protein (CETP) are also under the regulation of the LXR signaling pathway [7–9]. Therefore, activation of LXR will influence the blood lipid profile depending upon the physiological context.

The effects of LXR agonists on plasma lipid homeostasis, especially triglyceride metabolism, are still controversial and very dependent on animal strain and genetic background, duration of treatment, selectivity of LXR agonist and diet used in the experiment [6,10–15]. In wild-type C57BL/6 mice 4 to 5 days treatment (short term) with the LXR agonist T0901317 increases hepatic production of VLDL triglycerides and leads to the secretion of large triglyceride-rich VLDL particles [6]. However treatment for more than 30 days (long term) with T0901317 reduces plasma triglycerides in wild-type BALB/c mice [10]. In mice with impaired lipoprotein particle clearance such as apoE−/− or LDLR−/− mice, both short-term and long-term treatments with T0901317 induce hypertriglyceridemia [6,11,12]. However, long-term treatment with T0901317 in LDLR−/− fed an atherogenic diet containing sodium cholate, a natural agonist of farnesoid X receptor, only transiently elevates plasma triglycerides [13]. Furthermore long-term treatment of LDLR−/− mice with organ-selective LXR agonists GW3965 or ATI-829 does not increase plasma triglyceride levels [14,15].

Evidence suggests that triglycerides are an independent risk factor for coronary heart disease [16]. Hypertriglyceridemia is prevalent in industrial countries, affecting 10–20% of the population in the United States [17]. In people over 50 years old, the prevalence increases to over 40% [18]. Therefore, information regarding the impact of activation of LXR on plasma lipid homeostasis, especially that involving triglycerides, is very critical for targeting LXR for pharmaceutical development. In this report we have examined the consequences of long-term (4 weeks) treatment with LXR agonist T0901317 in both wild-type C57BL/6 mice and lipoprotein clearance-deficient mice (LDLR−/− mice) on a C57BL/6 background. While treatment with LXR agonist T0901317 induced hepatic de novo lipogenesis in both strains of mice, the level of plasma VLDL triglycerides differed between these two models. In wild-type mice VLDL triglycerides were lowered after long-term LXR agonist treatment, while in LDL receptor-deficient mice, LXR agonist increased the level of VLDL remnants including small-sized VLDL and IDL/LDL. Increased plasma LPL activity may account for the decrease in plasma triglycerides in T0901317-treated wild-type mice. However, when lipoprotein clearance is impaired because of the absence of the LDLR, increased plasma LPL activity reduces the size of the accumulating triglyceride-rich VLDL particles with the potential of generating more atherogenic particles. Our work suggests that an organ-selective LXR agonist devoid of liver activity might be an ideal drug candidate for cardiovascular diseases, especially when the patients have either a defect in lipoprotein clearance or have hypertriglyceridemia.

2. Materials and methods

2.1. Animals and diets

All work with mice followed National Institutes of Health guidelines for care and use of animals in experimentation. Animal work was reviewed and approved by the University of Chicago Institutional Animal Care and Use Committee. Male C57BL/6 and LDLR−/− mice on a C57BL/6 background were obtained from The Jackson Laboratory. C57BL/6 mice were fed a standard chow diet (2918, Harlan TEKLAD, Madison, WI) and LDLR−/− mice were fed a standard chow diet or a Western diet (TD88137, Harlan TEKLAD, Madison, WI). Starting at 8 to 10 weeks of age, mice were administered the LXR agonist T0901317 (Cayman Chemical

Company, Ann Arbor, MI) daily at a dose of 1 mg/kg by gavage in a 20% microemulsion [19] or vehicle (control, microemlusion only) for 4 weeks. T0901317 delivery using a 20% microemulsion increases T0901317 bioavailability in mice and lowers the dose required for physiologic responses [12,15]. At the end of the treatment period, mice were fasted for 4 hours, anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), and bled from the retro-orbital plexus. The mice were then perfused transcardially with PBS. Blood samples were used for lipid and lipoprotein analysis. Liver samples were stored in RNAlater (Ambion, Austin, TX) at −70°C for RNA isolation. Liver samples for lipid analysis were stored frozen at −70° C.

2.2. Lipid and lipoprotein analysis

Two hundred μl of plasma was fractionated on tandem Superose 6 fast protein liquid chromatography (FPLC) columns [20]. Cholesterol and triglycerides in the even-numbered FPLC fractions and in plasma samples were measured by using commercial kits (Stanbio Laboratory, Boerne, TX). VLDL, IDL/LDL, and HDL lipids were analyzed from fractions 6–14, 15–35, and 36–52, respectively. Liver samples were homogenized, and the lipids were extracted as described [21] and measured as described above.

2.3. Electron microscopy (EM) of lipoproteins

VLDL and IDL/LDL particles from pooled FPLC fractions were placed on a carbon-coated EM grid and negatively stained with 1% uranyl acetate. Particles were examined with a FEI Tecnai F30 electron microscope (FEI Company, Hillsboro, OR). OpenLab software version 3.1.5 (Improvision Inc., Lexington, MA) was used in the measurement of particle size.

2.4. Nascent lipoprotein analysis

C57BL/6 mice fed a standard chow diet or LDLR−/− mice fed a Western diet were gavaged daily with vehicle or 1 mg/kg T0901317 for 4 weeks. After a 16 h fast, mice were anesthetized as described above and injected with 500 mg/kg body weight of Triton WR1339 (Tyloxapol, Sigma-Aldrich, St. Louis, MO) (15% w/v Triton WR1339 in 0.9% NaCl) or 0.9% NaCl via the retro-orbital sinus. Three hours after injection, mice were bled from the contra-lateral retro-orbital plexus. The plasma samples were then fractionated by FPLC for lipoprotein lipid analysis.

2.5. RNA isolation and gene expression analysis

Total RNA from mouse liver was isolated by using the RNeasy Mini System (QIAGEN, Valencia, CA). First strand cDNA was synthesized by utilizing SuperScript III System (Invitrogen Life Technologies, Carlsbad, CA). Sequences of gene-specific primers and probes are shown in supplemental data and were purchased from IDT (Coralville, IA). Quantitative real time PCR was performed by using “TaqMan Universal PCR Master Mix” from Applied Biosystems. The results were normalized to 36B4 mRNA.

2.6. Lipase analysis

Mice were fasted for 4 hours and then injected with 500 U/kg body weight of heparin via the retro-orbital sinus. After 5 minutes, mice were bled from the contralateral retro-orbital plexus and post-heparin plasma prepared. Total and hepatic lipase activity was measured by using a CONFLUOLIP™ total or hepatic lipase kit (RDI division of Fitzgerald Industries Intl., Concord, MA) according to the manufacturer’s instructions by using a Wallac Victor 1420 (Perkin-Elmer, Boston, MA) fluorescent plate reader.

2.7. Statistical analysis

Values are presented as means ± standard error of the mean (SEM) and differences between the means of different treatment groups were analyzed by using the unpaired Student *t*-test. A statistically significant difference is set at $p<0.05$.

3. Results

3.1. T0901317 reduces plasma triglycerides in C57BL/6 mice

Male C57BL/6 mice were fed a standard chow diet and gavaged daily with vehicle or 1 mg/kg of T0901317 for 4 weeks. Plasma samples were prepared and subjected to FPLC for lipoprotein profile analysis. Total plasma cholesterol increased after T0901317 treatment, due primarily to an increase in cholesterol in the IDL/LDL and HDL particles (Fig. 1A). Interestingly, plasma total triglycerides decreased by 35% after 4 weeks treatment with T0901317 (Fig. 1B). The decrease in plasma total triglycerides was due to a marked reduction in plasma VLDL triglycerides (Fig. 1B). Plasma IDL/LDL triglycerides from T0901317-treated mice were not different from control mice (Fig. 1B). Furthermore, treatment with T0901317 significantly decreased the ratio of triglyceride to cholesterol in VLDL particles from 6.17 ± 0.71 in the control group to 3.7 ± 0.22 ($p<0.05$) and in IDL/LDL particles from 0.7 ± 0.06 in the control group to 0.45 ± 0.03 ($p<0.05$).

The decrease of plasma triglycerides after T0901317 treatment may be due to increased post-secretion remodeling of plasma VLDL, such as the increased hydrolysis of VLDL triglycerides. This latter possibility is suggested by the T0901317-induced elevation of LPL expression and activity (Fig. 1 E and G.). But it is also possible that there is a decrease in lipid secretion by the liver. To address this question, standard chow diet-fed C57BL/6 mice after 4 weeks of vehicle or T0901317 treatment were injected with Triton WR1339 to prevent lipolysis and clearance of triglyceride-rich lipoprotein. Mice were fasted for 16 hours to exclude exogenous sources (dietary fat) of plasma triglycerides before the Triton WR1339 injection. Under these conditions the main source of plasma triglycerides would be from endogenous hepatic synthesis. Three hours after injection of Triton, mice were bled and the plasma samples were fractionated by FPLC for lipoprotein lipid analysis. Total plasma cholesterol levels were more than 40% higher in the T0901317-treated mice, due primarily to a significant increase in the VLDL cholesterol (Fig. 1C). Plasma total triglycerides were 3-fold higher in the T0901317-treated mice with large increases in triglyceride levels observed in all classes of lipoproteins (Fig. 1D). However, the major contributor to increased plasma triglycerides was a large increase in VLDL triglycerides (Fig. 1D). Furthermore, treatment with T0901317 significantly increased the ratio of triglyceride to cholesterol in VLDL particles from 8.03 ± 1.66 in the control group to 13.45 ± 1.27 ($p<0.05$) in the T0901317 treated group and raised the ratio in IDL/LDL particles from 1.3 ± 0.19 in the control group to 4.25 ± 1.48, though this difference was not statistically different.

LXR target gene expression profiles were analyzed in livers of mice treated for 4 weeks with vehicle or T0901317 by using quantitative real-time PCR. The LXR target genes ABCA1, FAS and LPL mRNA were significantly increased in livers from T0901317-treated mice (Fig. 1E). SREBP-1c and PLTP mRNA, also LXR targets, were not significantly changed (data not shown). Consistent with the increase in FAS mRNA after T0901317 treatment, liver triglycerides were significantly increased after 4 weeks of treatment with T0901317 (Fig. 1F). Activity of plasma post-heparin total lipase significantly increased up to 20% after 4 weeks of T0901317 treatment (Fig. 1G). However, activity of plasma post-heparin hepatic lipase (HL) did not change (Fig. 1G). Because the activity of plasma total lipase is due to both LPL and HL, the actual increase in plasma post-heparin lipase activity after T0901317 treatment is

probably attributable to increased LPL activity. Thus the LXR agonist reduces plasma triglyceride and VLDL by enhancing VLDL lipolysis and not attenuating hepatic output.

3.2. T0901317 treatment exacerbates hyperlipidemia in mice with lipoprotein clearance deficiency

Male LDLR−/− mice fed a Western diet are hyperlipidemic due to the accumulation of VLDL and LDL particles. To determine the effect of LXR agonist on plasma lipids in the context of this hyperlipidemia, we treated LDLR −/− mice for 4 weeks with vehicle or T0901317 and analyzed plasma lipoprotein profiles by FPLC. A large increase in plasma total, VLDL and IDL/LDL cholesterol was observed in T0901317-treated hyperlipidemic mice (Fig. 2A). Plasma total, VLDL and IDL/LDL triglycerides were also significantly increased after T0901317 treatment (Fig. 2B). Treatment with T0901317 significantly raised the ratio of triglyceride to cholesterol in VLDL particles from 1.03 ± 0.09 in the control group to 2.14 ± 0.18 ($p<0.05$) in the T0901317-treated group and in IDL/LDL particles from 0.22 ± 0.01 in the control group to 0.75 ± 0.07 ($p<0.05$).

To further examine the influence of T0901317 on the size of plasma VLDL particles, pooled VLDL particles (FPLC fractions 6 to 14) were analyzed by EM. While very large VLDL particles (>100 nm in diameter) were observed in hypertriglyceridemic mice treated with vehicle (Fig. 2C), very few very large VLDL particles (>100 nm in diameter) were found in T0901317-treated mice (Fig. 2D). The average diameter of the VLDL particles in the plasma of hypertriglyceridemic mice after T0901317 treatment was significantly reduced compared to vehicle-treated mice (Fig. 2E). The percent of total VLDL particles with diameters less than 40 nm was dramatically increased (Fig. 2F), while the number of larger (> 50 nm in diameter) VLDL particles was significantly decreased in T0901317-treated hypertriglyceridemic mice (Figure 2F). IDL/LDL size was similar in both vehicle and T0901317-treated mice (Fig. 2G).

The accumulation of small VLDL in the plasma of the T0901317-treated mice could be due to the secretion of smaller VLDL particles from the liver or to increased lipolysis of newly secreted triglyceride-rich lipoprotein particles in the plasma. To further explore this, Western diet-fed LDLR−/− mice with and without T0901317 treatment for 4 weeks were injected with Triton WR1339 or saline after a 16 hour fast. The size of nascent plasma VLDL from Triton-injected mice was increased markedly compared to the plasma VLDL from non-Triton-treated mice (compare Fig. 3C with Fig. 3A and B). The average size of nascent plasma VLDL from mice treated with T0901317 was significantly increased compared to the nascent plasma VLDL from mice treated with vehicle (Fig. 3D). This increase in nascent VLDL average size in T0901317-treated mice was due to a significant decrease in the number of small VLDL (diameter < 40 nm) and marked increase in the number of giant VLDL (diameter > 150 nm) (Fig. 3E). In this Triton protection experiment, T0901317 treatment significantly raised plasma total cholesterol up to 50% and triglycerides up to 55% compared to vehicle treatment (data not shown).

The effect of LXR agonist on liver gene expression in LDLR −/− mice was analyzed. In addition to the lipid transport-related gene ABCA1 and de novo lipogenesis-related gene FAS, the gene LPL associated with lipoprotein remodeling was also induced by T0901317 (Fig. 3F). Again, in agreement with the upregulation of de novo lipogenesis-related gene expression, liver triglycerides were increased after T0901317 treatment (Fig. 3G). The activity of plasma post-heparin total lipase was significantly increased by 60% following T0901317 treatment (Fig. 3H). Since the activity of plasma post-heparin HL was not altered (Fig. 3H), increased LPL activity is probably responsible for the increase in total lipase activity.

3.3 T0901317 treatment exacerbates plasma lipid profile in lipoprotein clearance-deficient mice without severe hyperlipidemia

Western diet-fed LDLR−/− mice have much higher lipid levels than standard chow diet-fed wild-type mice (compare Fig. 1A, B with Fig. 2A, B). As the results just presented could represent an altered balance between substrate (triglyceride rich lipoproteins) and lipoprotein lipase, we undertook a similar experiment on LDLR−/− fed a standard chow diet. To determine whether diet-induced hypertriglyceridemia is important for the accumulation of small VLDL, male LDLR−/− mice fed a standard chow diet were treated with T0901317 or vehicle for 4 weeks. Plasma lipoprotein profiles were analyzed by FPLC. Relative to the vehicle-treated control mice, a remarkable increase in plasma total, VLDL, IDL/LDL and HDL cholesterol was observed in T0901317-treated mice (Fig. 4A). Plasma total, VLDL, IDL/LDL and HDL triglycerides were also significantly increased after T0901317 treatment (Fig. 4B). Furthermore, treatment with T0901317 non-significantly decreased the ratio of triglyceride to cholesterol in VLDL particles from 2.41 ± 0.75 in the control group to 1.54 ± 0.06 and did not change the ratio in IDL/LDL particles (0.7 ± 0.07 in the control group vs. 0.68 ± 0.02 in treatment group).

To further examine the influence of T0901317 on the size of plasma VLDL particles, pooled VLDL particles (FPLC fractions 6 to 14) were analyzed by EM. Very large VLDL particles (>100 nm in diameter) were observed in mice treated with vehicle (Fig. 4C). The average diameter of the VLDL particles in T0901317-treated mice was significantly reduced compared to vehicle-treated mice (Fig. 4E). The percent of total VLDL particles with diameters less than 40 nm or between 40 to 70 nm was dramatically increased (Fig. 4F), while the number of larger (between 70 to 100 nm or > 100 nm in diameter) VLDL particles was significantly decreased in T0901317-treated mice (Fig. 4F). Again, almost no very large VLDL particles (>100 nm in diameter) were found in T0901317-treated mice (Fig. 4D and F).

4. Discussion

In this work we show that the activation of LXR by the agonist T0901317 results in multiple influences on lipoprotein metabolism. To some extent, the results depend on the animal model employed and the duration of treatment. We have studied wild-type animals and animals lacking the LDL receptor, which accounts for a substantial proportion of the clearance of apoB-containing lipoproteins, namely VLDL and IDL/LDL. In both models, the LXR agonist induces an increase in lipid synthesis and triglyceride-rich lipoprotein output. Also both show an accumulation of hepatic triglyceride. Triglyceride-rich lipoprotein secretion is assessed after acute administration of Triton WR-1339 to fasting animals. With the extent of fasting employed (16 hours), we believe we are largely measuring hepatic secretion of triglyceride-rich lipoprotein.

The steady-state levels of triglyceride-rich lipoproteins are different in the two models. In wild-type mice, despite the increase in hepatic triglyceride output, the level of plasma triglycerides and VLDL is reduced in T0901317-treated mice. We attribute this to the induction of lipoprotein lipase in the liver and increased lipolysis of VLDL in the plasma following extended treatment with the LXR agonist. An increase in total lipase activity, without any change in hepatic lipase activity, was noted in animals receiving the LXR agonist and indicated an increase in plasma LPL activity in LXR agonist-treated mice. The increase in lipoprotein lipase expression in the liver, possibly reflecting activity of non-hepatocytes especially Kupffer cells [22] and/or hepatocytes [23], is consistent with a report in the literature [7]. In addition, multiple LXR-target genes are involved in the regulation of LPL activity. Activation of the apoE/C-I/CIV/C-II gene cluster [24] by LXR agonist is polytrophic upon LPL activity. apoC-II is generally believed to be an activator of LPL [25], but overexpression of apoC-II increases the apoC-II/apoE ratio in VLDL and decreases its accessibility to cell surface lipases or receptors

within their glycosaminoglycan matrices [26]. Usually, apoC-I is considered an inhibitor of LPL [27,28]. The role of apoE as an activator or inhibitor of LPL is still controversial [29, 30]. Angiopoietin-like protein 3, another LXR-target gene, has a suppressive effect on LPL activity [31]. Therefore, regulation of LPL activity by LXR pathway is complex and a particular outcome may depend on the exact circumstances. In the presence of a fully functional LDL receptor, the VLDL acted upon in the plasma by lipase is readily cleared from the plasma accounting for the reduction in plasma triglyceride and VLDL despite increased hepatic production. In addition the increased lipoprotein lipase could function as ligand for VLDL uptake by the other hepatocyte receptor of the LDLR family, LRP [32].

On the other hand, in the LDLR−/− mice fed a Western-type diet, the plasma triglyceride and VLDL was notably increased, even in the face of an increase in plasma lipase activity. The fact that plasma triglyceride levels are sustained in this circumstance is probably attributable to impaired clearance of triglyceride-containing lipoproteins due to the absence of the LDL receptor. However a careful examination of the VLDL composition and size by electron microscopy indicates a significant VLDL remodeling takes place in the plasma under the influence of the increased lipase activity. This results in the accumulation of small-sized VLDL in the plasma of T0901317-treated LDLR−/− animals when fed Western-type or chow diet. Obviously the level of these lipoproteins is much higher in the Western diet-fed animals. These small-sized VLDL particles are not primary products of the output by the liver, as shown in the Triton experiment when very large VLDL particles accumulate in the plasma of T0901317-treated animals (Fig. 3). Rather these small VLDL particles are generated by the remodeling of nascent hepatic lipoproteins in the plasma under the influence of active lipoprotein lipase. This explanation is supported by a previous in vitro study in which exogenous lipoprotein lipase added to whole plasma hydrolyzed lipids in chylomicrons and VLDL leading to increases in IDL, LDL and HDL2 [33].

The analysis of the composition of VLDL in chow-fed wild-type or LDL receptor-deficient mice showed a reduction in the triglyceride to cholesterol ratio in T090137-treated mice, particularly in the wild-type animals. These are circumstances in which intravascular lipolysis is permissible and indicates an ongoing remodeling of VLDL in the plasma. On the other hand with Triton WR1337 treatment, the triglyceride to cholesterol ratio is dramatically increased in the T0901317-treated wild-type animals. This indicates a very dynamic homeostasis between production, lipolysis and clearance when all elements of the homeostatic mechanism are intact. On the other hand, the reduction in triglyceride to cholesterol ratio is not seen in T090137-treated Western-type diet-fed LDLR−/− mice because in this circumstance increased production slightly dominates over intraplasma remodeling and clearance. It is notable that the triglyceride to cholesterol ratio is monitoring the total VLDL pool. The lack of a reduction in the ratio of triglyceride to cholesterol in VLDL does not, however, mean an absence of remodeling in this murine atherosclerosis model, given the increased generation of small VLDL. These small VLDL may perhaps contribute to increased atherogenesis in this model. Our prior work has reported on two aspects of atherosclerosis in LDLR−/− mice that are relevant in this context. First, treatment of Western-type diet-fed animals with T0901317 results in a notable decline in atherosclerosis in innominate artery but not in the aortic root [12]. We have also reported that VLDL cholesterol and triglyceride represents an important risk factor for aortic root atherosclerosis but not innominate artery atherosclerosis in LDLR−/− mice fed the Western-type diet [34]. Considering these two observations together with the lipoprotein changes associated with T0901317 treatment in this study, one possibility to account for the site-selective influence of T0901317 treatment on atherosclerosis might be that the remodeled VLDL of these animals represents a powerful pro-atherogenic influence for the aortic root, but not the innominate artery. Thus the potential atheroprotective effect of targeting LXR may be substantially attenuated in aortic root, associated with the increment in pro-atherogenic small VLDL.

One of the major side effects of LXR activation in mice is the genesis of triglyceride-rich particles in hyperlipidemic animals due to impaired lipoprotein clearance. Whether or not a similar effect would be observed in hyperlipidemic patients exhibiting some impairment of lipoprotein homeostasis is not clear. Plasma triglycerides have been reported to be a risk factor for cardiovascular disease [16]. This study suggests that any hepatotrophic LXR agonist will aggravate this risk by two mechanisms: by promoting hepatic triglyceride synthesis and by modulating plasma VLDL catabolism to generate a higher concentration of small VLDL particles that are capable of entering the vascular wall and promoting atherogenesis. Some of these concerns have led us to explore LXR agonists that are targeted less to the liver and do not lead to the induction of hypertriglyceridemia [15]. In the light of the fact that LXR activation selectively increases lipoprotein lipase production in the liver and not in many other tissues [7], an agonist that does not target the liver will likely not result in an increase in VLDL production and also will limit the occurrence of this more atherogenic small VLDL under the circumstance of lipoprotein clearance deficiency.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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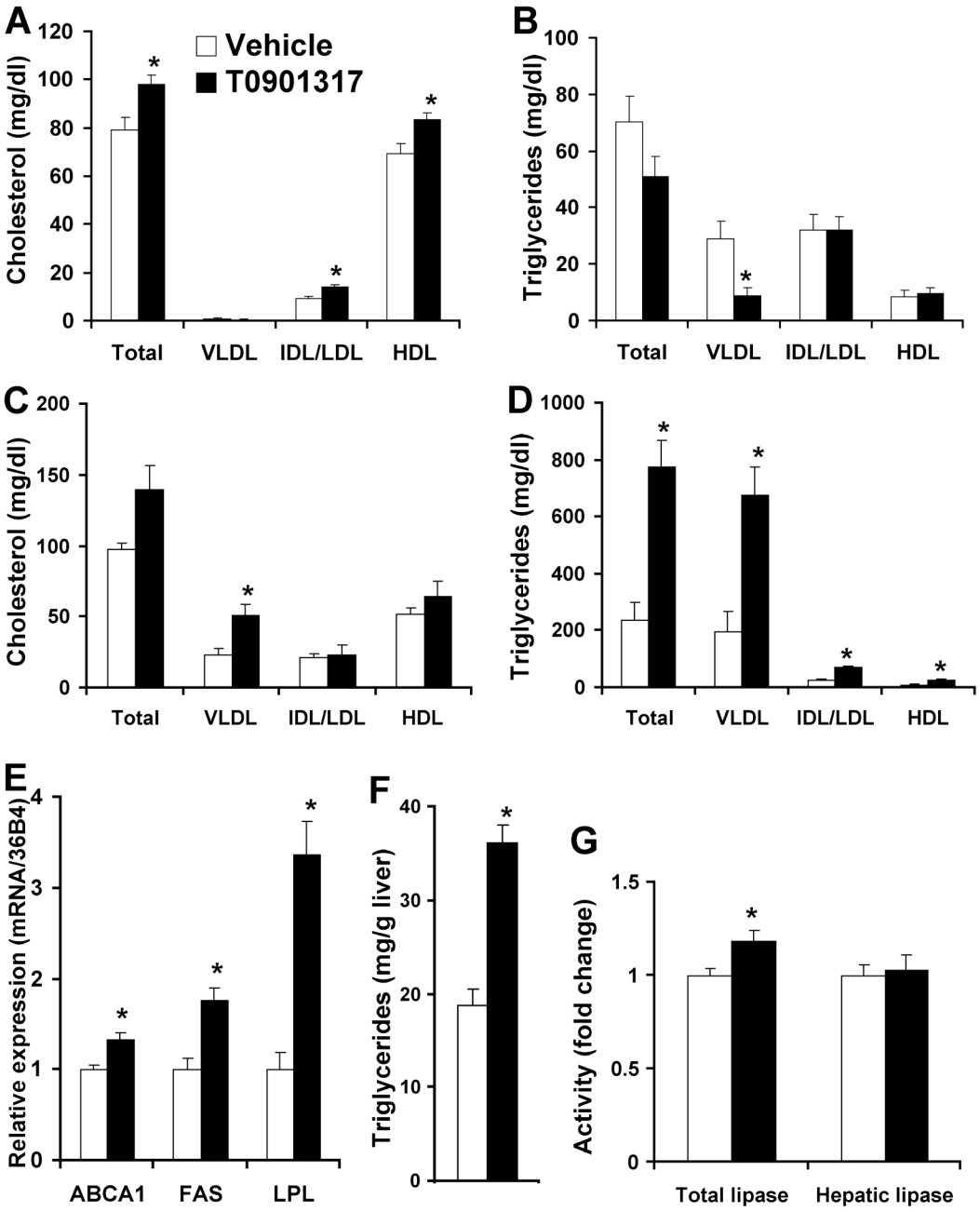

Fig. 1.

Effect of T0901317 on plasma lipid levels, hepatic gene expression and triglyceride levels and plasma lipase activity in male C57BL/6 mice fed a standard chow diet. Plasma total cholesterol (A) and triglycerides (B) were measured after treatment with vehicle (n=9) or 1 mg/kg of T0901317 (n=9) daily for 4 weeks. Plasma total levels of cholesterol (C) and triglycerides (D) in vehicle (n=4) or T0901317-treated (n=4) mice were measured after injection of Triton WR-1339. The effect of T0901317 on gene expression in liver (E), hepatic triglycerides (F), and plasma lipase activity (G) was determined. Specific mRNA levels were measured by quantitative RT-PCR, normalized with 36B4, and presented relative to controls. For gene expression analysis and hepatic triglyceride measurement, 9 mice were in both vehicle and

treatment groups. For plasma lipase activity, 6 and 5 mice were in vehicle and treatment groups, respectively. Values are the mean ± SEM. Significant differences between vehicle and treatment groups are indicated as * $P<0.05$.

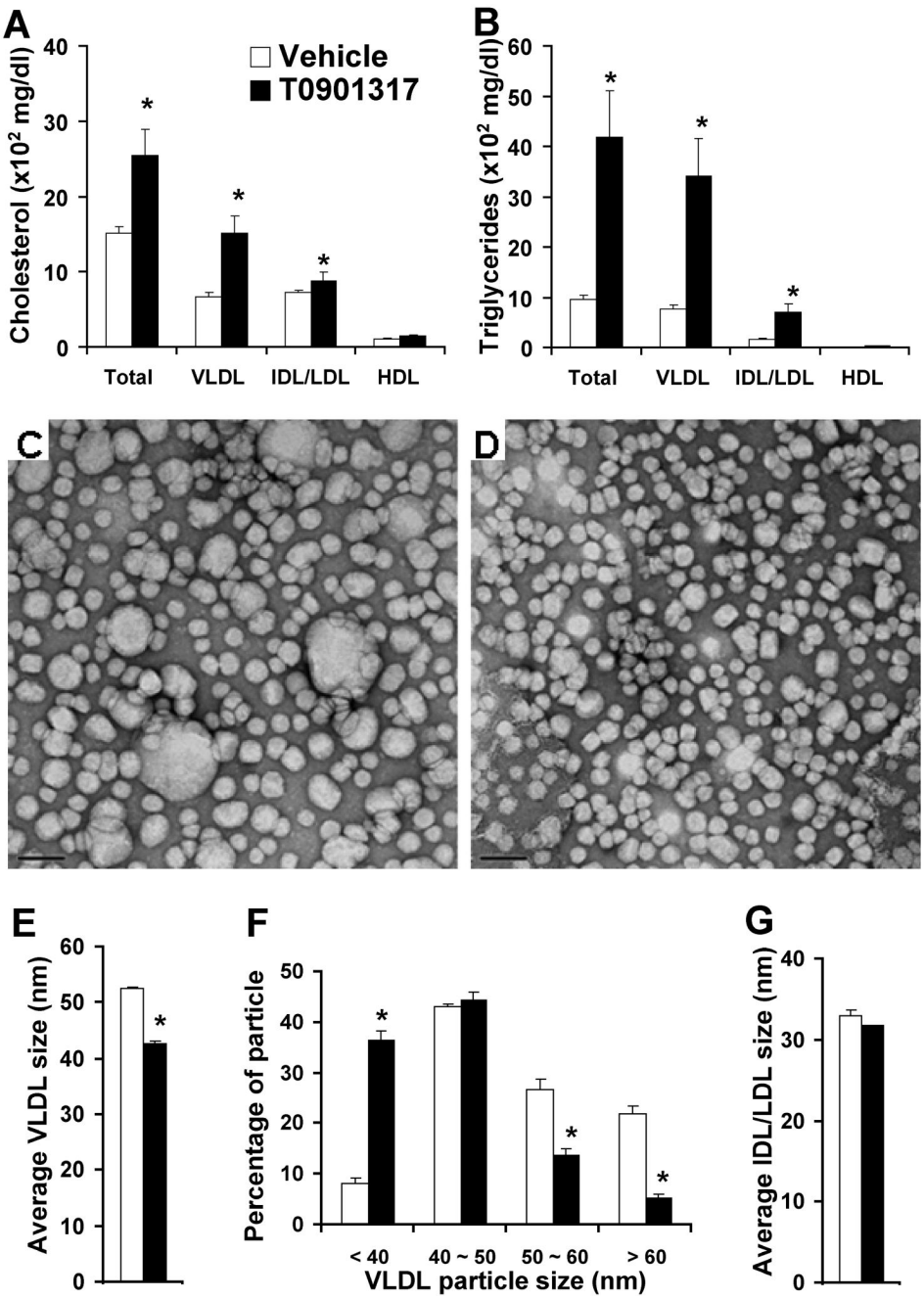

Fig. 2.

Effect of T0901317 on plasma lipid levels and VLDL size in male LDLR−/− mice fed a Western diet. Plasma total levels of cholesterol (A) and triglycerides (B) were measured in mice after treatment with vehicle (n=8) or 1 mg/kg of T0901317 (n=8) daily for 4 weeks. The effect of T0901317 on plasma VLDL size was determined in mice after treatment with vehicle (C) or 1 mg/kg/day of T0901317 (D) daily for 4 weeks. Pooled FPLC fractions were used for VLDL (C, D) and IDL/LDL EM analysis (G). Size bar is 100 nm. The average size (E) and size distributions (F) of VLDL particles from mice treated with vehicle or T0901317 were assessed. Six mice were in both vehicle and treatment groups and 250 particles from each mouse were

measured. Values are the mean ± SEM. Significant differences between vehicle and treatment groups are indicated as * $P<0.05$.

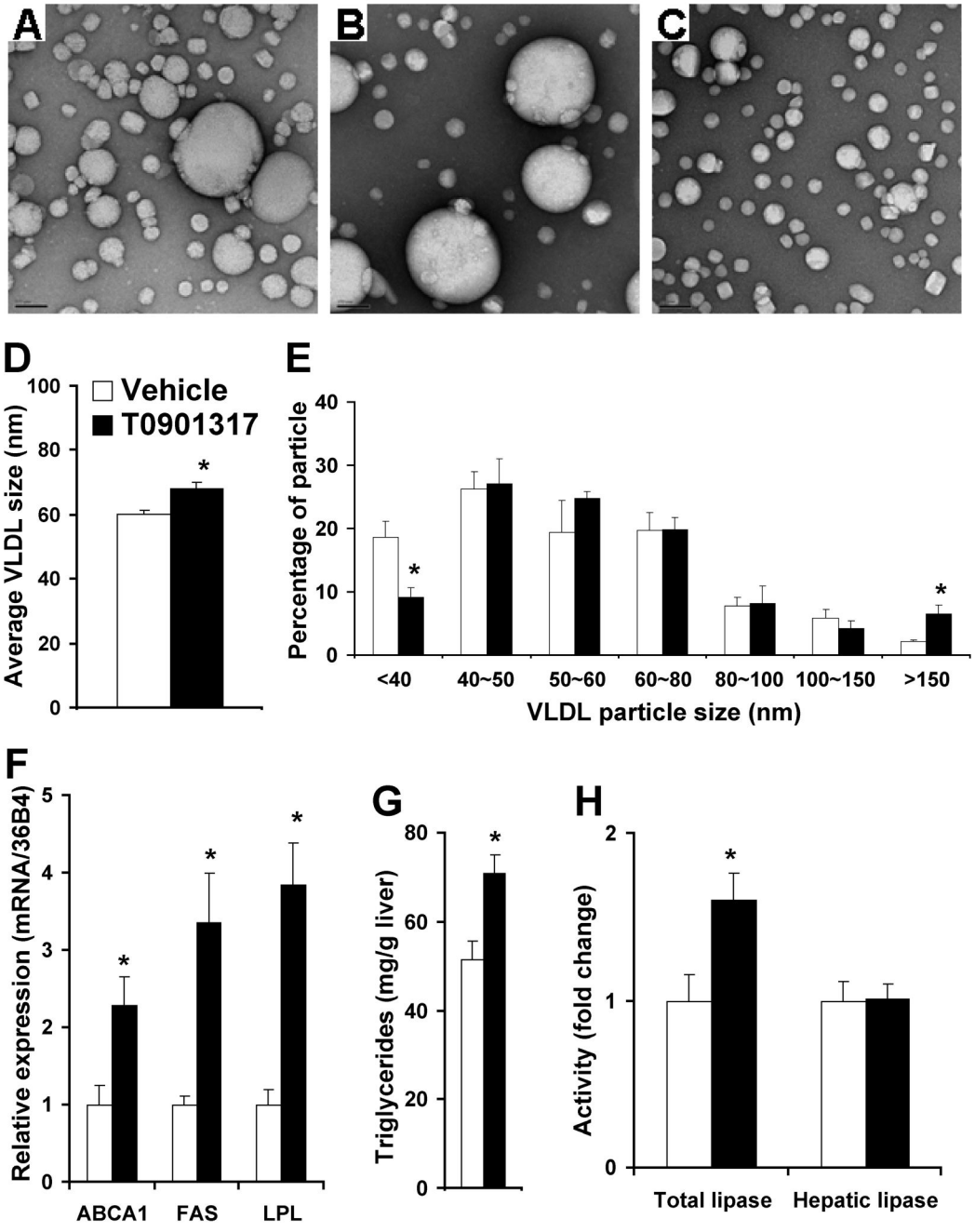

Fig. 3.

Effect of T0901317 on nascent plasma VLDL size, hepatic gene expression and triglycerides and plasma lipase in male LDLR−/− mice fed a Western diet. Mice were gavaged with vehicle (A) or 1 mg/kg/day of T0901317 (B, C) daily for 4 weeks and then fasted for 16 hours before receiving an injection of Triton WR-1339 (A, B) or 0.9% NaCl (C) as described in "Materials and methods". Three hours after the injections, mice were bled and plasma fractionated by FPLC. Pooled FPLC fractions were used for VLDL EM analysis. Size bar is 100 nm. The average size (D) and size distributions (E) of VLDL particles from vehicle or T0901317-treated mice injected with Triton WR-1339 were determined using 3 mice per group. A total of 100–150 particles from each mouse were measured. The effect of T0901317 on gene expression in

liver (F), hepatic triglycerides (G) and plasma lipase activity (H) was measured in LDLR−/− mice fed a Western diet and gavaged daily for 4 weeks with vehicle or 1 mg/kg of T0901317. Specific mRNA levels were measured by quantitative RT-PCR, normalized with 36B4, and presented relative to controls with 8 mice in both vehicle and treatment groups for gene expression analysis and hepatic triglyceride measurement. For plasma lipase activity 5 mice were in both vehicle and treatment groups, respectively. Values are the mean ± SEM. Significant differences between vehicle and treatment groups are indicated as * $P<0.05$.

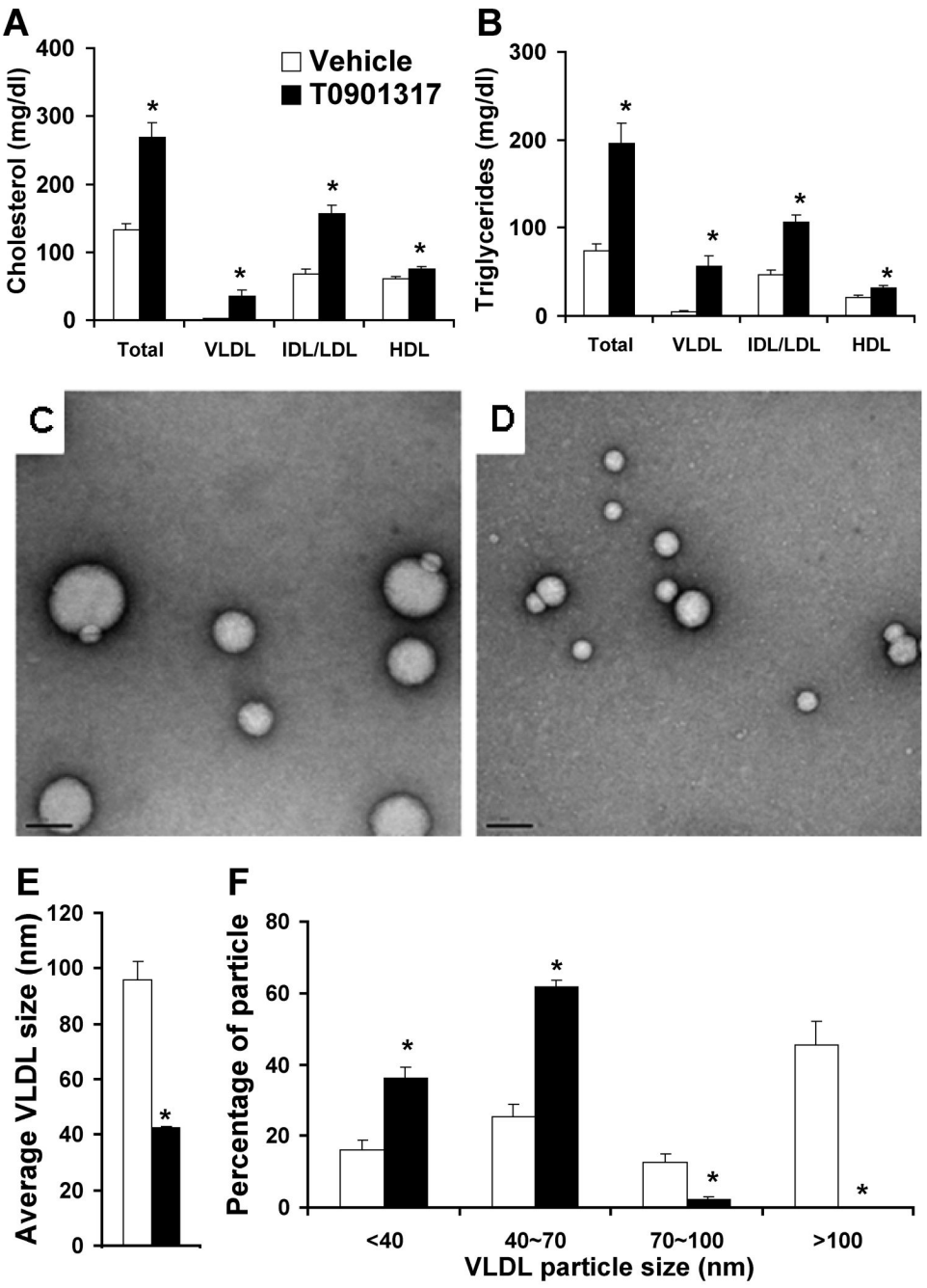

Fig. 4.

Effect of T0901317 on plasma total levels of cholesterol, triglycerides and VLDL size in male LDLR−/− mice fed a standard chow diet. Mice were gavaged with vehicle (n=6) or 1 mg/kg of T0901317 (n=7) for 4 weeks and then plasma total levels of cholesterol (A) and triglycerides (B) were measured. Plasma from vehicle (C) or T0901317-treated (D) mice was then fractionated by FPLC and pooled for VLDL EM analysis. Size bar is 100 nm. The average size (E) and size distributions (F) of VLDL particles from mice treated with vehicle (n=4) or 1mg/kg/day of T0901317 (n=5) were determined. Fifty particles from each mouse were measured. Values are the mean ± SEM. Significant differences between vehicle and treatment groups are indicated as * $P<0.05$.