Liver X receptor (LXR) ligands are currently being evaluated as potential therapeutic agents for the treatment of low HDL. The LXR ligand T0901317 elevates ATP binding cassette transporter A1 (ABCA1) and HDL levels in animal models and induces moderate lipogenesis through upregulation of sterol regulatory element binding protein 1c (SREBP1c). Because insulin may also regulate lipogenesis through SREBP1c and fatty acid synthase (FAS), we investigated the effect of an LXR ligand in hyperinsulinemic mice. Administration of T0901317 to male db/db mice for 12 days resulted in a more severe hypertriglyceridemia and hepatic triacylglycerol accumulation than observed in nondiabetic mice. The LXR target genes ABCA1, SREBP1c, FAS, and stearoyl-CoA desaturase 1 were upregulated by T0901317 treatment in both diabetic db/db and nondiabetic C57BLKS mice. Changes in lipogenic gene expression were independent of mouse strain, indicating that the severe lipogenesis observed in LXR ligand-treated db/db mice was not due to additive effects of insulin on lipogenic gene expression. Phosphoenolpyruvate carboxykinase expression was suppressed, suggesting that a shift from gluconeogenesis toward lipogenesis could partially explain our observations in db/db mice. Our data suggest that LXR ligands that have effects on both fatty acid and carbohydrate metabolism should be carefully evaluated in obesity, insulin, and leptin resistance.

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Liver X receptor (LXR) ligands are currently being investigated as potential therapeutic agents for the treatment of low HDL, a disorder common in both nondiabetic and diabetic humans. Active research in this area has resulted from discoveries identifying the LXR receptors, LXRα and LXRβ, as important members of a family of nuclear receptors, including the farnesoid X receptor, retinoic acid X receptors, and peroxisome proliferator-activated receptors, which regulate the transcription of genes involved in lipid and sterol metabolism and balance (1–5).

Although the importance of LXR receptors, in particular LXRα, in regulating sterol metabolism was recognized by the identification of cyp7A1 (7α-hydroxylase) as an LXR-regulated gene (6), the real potential of LXR as a therapeutic target only became evident from work identifying putative endogenous ligands for LXR (7, 8) and, subsequently, LXR as a potent regulator of the ATP binding cassette transporter A1 (ABCA1) (9, 10). Other genes found to be either regulated or partially regulated by LXR are sterol regulatory element binding protein 1c (SREBP1c), LXRα, ABCG1, ABCG3, ABCG8, cholesteryl ester transport protein, apolipoprotein E (apoE), phospholipid transport protein, lipoprotein lipase (LPL), and fatty acid synthase (FAS) (11–18). LXR appears to be a regulator of not only cholesterol but also triacylglycerol and glucose metabolism (19, 20).

ABCA1 is a membrane transporter that was identified as the defective protein in Tangier disease (21–24), a disease characterized by the absence of HDL. A number of mouse models confirmed that ABCA1 is obligatory for the formation of HDL, a determinant of HDL levels, and potentially important in the prevention of foam cell development and atherosclerosis (25–32). Based on these studies and clinical data identifying a strong inverse correlation between HDL levels and coronary heart disease (CHD) (33, 34), efforts have been undertaken to identify potent LXR receptor ligands as therapeutics.

Recent work by Joseph et al. (35) showed that the LXR ligand GW3965 can reduce the development of atherosclerosis in mouse models independent of changes in HDL. In this study, they suggested the effect might be at the level of the artery wall and, more recently, they have shown that this may occur partly through LXR-mediated repression of macrophage inflammatory pathways (36,
37). The most well characterized synthetic ligand to date is T0901317, a highly selective LXR agonist that has been shown both in vitro and in vivo to regulate LXR target genes such as ABCA1 (1, 2). Schultz et al. (1) showed that the feeding of this compound to mice and hamsters resulted in a dose-dependent increase in HDL levels and elevated plasma and hepatic triacylglycerols. This elevation in plasma and liver triacylglycerols was due to induction of lipogenic genes, including FAS, acetyl-CoA carboxylase, and stearoyl-CoA desaturase 1 (SCD1), via upregulation of the SREBP1c pathway by LXR (1) and, in the case of FAS, direct regulation by LXR (18). Clearly, therapeutic agents that induce lipogenesis resulting in elevation of plasma triacylglycerols and fatty livers are undesirable. T0901317 reportedly has minimal selectivity toward either LXRα or LXRβ receptor subtypes (1), raising the possibility that the lipogenic effects of LXR ligands might be ameliorated by ligands that exhibit receptor subtype selectivity, through either differential activation of LXR target genes or realization of tissue selectivity via differences in LXR subtype tissue distribution.

Even with LXR ligands such as T0901317 that induce lipogenesis, there are indications that the plasma effect may be temporary. Joseph et al. (18) found that the elevation in plasma triacylglycerols with T0901317 was normalized by day 7 of treatment and concluded that the effect was transient and reversible. However, less is known about whether triacylglycerol accumulation in the liver is transient or more persistent.

In the present study, we provide data suggesting that the lipogenic effects of LXR ligands mediated through SREBP1c and FAS may be drastically exacerbated under certain physiological conditions, and that hepatic lipid accumulation is persistent. Using the db/db mouse strain as a model of obesity and type II diabetes caused by a leptin receptor mutation (Leprdb) that leads to obesity, hyperinsulinemia, hyperleptinemia, and hyperglycemia (38, 39), we have shown that treatment with T0901317 results in a much more severe lipogenic pathology than observed in nonpathological strains. Given the potential overlap of LXR and insulin regulation on SREBP1c- and FAS-mediated lipogenesis, we suggest that even mild LXR-induced increases in SREBP1c-mediated lipogenesis may be severely aggravated under pathophysiological states often associated with the human metabolic syndrome.

MATERIALS AND METHODS

Synthetic LXR ligand

T0901317 was synthesized at CV Therapeutics, Inc., by the Department of Bio-Organic Chemistry. This compound can also be obtained commercially from Sigma (St. Louis, MO).

Animals

Male C57BLKS/J (BKS) and BKS-Cg-m +/+ Leprdb (db/db) mice from 5 to 6 weeks of age were obtained from Jackson Labs (Bar Harbor, ME) and fed mouse chow ad libitum. At between 7 and 8 weeks of age, mice were administered T0901317, 50 mg/kg/day in vehicle [10% Cremaphore (BASF/saline), or vehicle alone by intraperitoneal injection daily for 12 days. In one study, male C57BL/6j mice between 7 and 11 weeks old were administered T0901317, 50 mg/kg/day in vehicle (0.75% carboxymethylcellulose) or vehicle alone by gavage daily for the indicated period. At study completion, blood and tissue samples were collected from 5–6 h-fasted animals and processed as described. The CV Therapeutics, Inc., Institutional Animal Use and Care Committee approved all animal experiments.

Assays

Lipid analysis was performed on EDTA-treated plasma using enzymatic kits (cholesterol and triacylglycerols, Roche Diagnostics, Indianapolis, IN; free cholesterol and phospholipids, Wako Chemicals). Liver lipids were determined enzymatically using kits after extraction as previously described (40). Plasma glucose (Sigma), aspartate aminotransferase (AST) (Wako Chemicals), and alanine aminotransferase (Wako Chemicals) were measured on fresh plasma using kits. Insulin and leptin levels were measured by immunoassay using mouse-specific kits (Crystal Chem, Downers Grove, IL). Lecithin-cholesterol (v/v) acyltransferase activity was measured as described (41) using recombinant HDL. LPL triacylglycerol lipase activity in postheparin plasma was measured by the method of Jackson and McLean (42) using heat-inactivated mouse plasma as a source of apoC-II [as described in reference (43)].

Glucose tolerance

Glucose tolerance tests were performed on db/db mice following 12 days of treatment with T0901317. On the morning of day 12, mice were treated and fasted for 5 h prior to an intraperitoneal glucose challenge (2 g/kg). Mice were bled from the retro-orbital sinus at 0, 15, 30, 60, and 120 min, and glucose and insulin levels during the study were measured by enzymatic kit and ELISA, respectively. The total change in glucose and insulin levels was determined by measuring the area under the curve (AUC), and the insulin sensitivity index was calculated as the product of glucose AUC and insulin AUC.

Western blots

Plasma (0.1 μl) from individual mice, three per group, was separated by SDS-PAGE using 4–20% ready gels (Biorad, Hercules, CA) and transferred to Nitrocellulose (Schleicher and Schuell). apoB, apoA-I, and apoE were probed with mouse-specific apolipoprotein antiserum (Biodesign, Saco, ME) and detected with horseradish peroxidase-labeled secondary antibodies (Pierce, Rockford, IL) and Super Signal chemiluminescent substrate (Pierce).

Lipoprotein analysis

HDL was measured by precipitation from plasma diluted 3:2 (v/v) with saline using an HDL precipitation reagent (Sigma), and nonprecipitable total cholesterol was determined using a kit (Roche Diagnostics). Complete lipoprotein profiles were determined after separation of 100 μl of plasma using two Superose 6/30 columns (Amersham Biosciences) linked in a series, and integration of the HDL peak was used to confirm HDL cholesterol levels. Fractions were eluted in phosphate-buffered saline, pH 7.4, containing 0.02% NaN3 at a flow rate of 0.5 ml/min. Cholesterol and triacylglycerols in each fraction were determined using kits (Roche Diagnostics).

RNA preparation and gene expression analysis

Tissues were crushed in liquid N2 using a mortar and pestle, and the RNA from 5–20 mg of tissue was isolated using an RNA Easy kit (Qiagen) and DNase treatment (Qiagen) according to the manufacturer’s instructions. One microgram of total RNA was
transcribed using a TaqMan Reverse Transcription Reagents kit (Applied Biosystems, Foster City, CA) in a 50 µl reaction using random hexamers. cDNAs were diluted 1:5 (v/v) and real-time quantitative PCR was performed in an ABI 5700 instrument using the SYBR green kit in a 25 µl reaction (Applied Biosystems). Gene expression was calculated by interpolation of the threshold cycle number values on standard curves generated from cDNA dilutions. All data are normalized to the amount of cyclophilin in the cDNA sample. Primer sequences are available by request.

RESULTS
The LXR ligand T0901317 was administered to male db/db mice and mice of the background strain BKS for 12 days at 50 mg/kg/day in an intraperitoneal dosage formulation. A dose of 50 mg/kg/day was chosen for these studies based on dose-finding studies in C57BL/6J mice, where a dose in the 5–50 mg/kg/day range was required to see reproducible ABCA1 upregulation and increased HDL cholesterol. Interestingly, at 5 h post-dose, the in vivo EC_{50} for ABCA1 was 2.4 µM, corresponding to a dose of 5 mg/kg/day and was approximately 100 times greater than the in vitro EC_{50} (1).

Lipid composition analysis of fasted plasma (Fig. 1) demonstrated that the compound had profound effects on db/db mice, with less-pronounced effects on the background strain. Treatment of db/db mice with the LXR ligand resulted in dramatically increased plasma unesterified cholesterol (Fig. 1A) and phospholipids (Fig. 1B) and an approximately 30-fold increase in plasma triacylglycerols (Fig. 1C). Esterified cholesterol was not changed by treatment (Fig. 1D), and HDL cholesterol (Fig. 1E) was significantly reduced in this experiment. In repeat experiments, the overall HDL cholesterol reduction varied from 10–80%, likely due to differences in liver function and degree of HDL triacylglycerol enrichment. There were no significant changes in blood lipids of the control strain in this experiment, although we have observed moderately increased triacylglycerols in some experiments and a reproducible trend toward increased HDL cholesterol. In similar experiments with C57BL/6J mice, we observed increased HDL and triacylglycerols as reported by others (1), although the degree of plasma triacylglycerol elevation was highly variable beyond the first few days of treatment. In a 4-week daily dosing study, the extent of the LXR ligand-mediated elevation in plasma triacylglycerols was time dependent and tended toward normalization after the first week (Fig. 2A); however, liver triacylglycerol accumulation was persistent out to 28 days treatment (Fig. 2B). Based on these observations, liver triacylglycerol levels appear to be a more robust measure of enhanced lipogenesis than plasma triacylglycerols. All animals in the study maintained their body weight (Fig. 3), although LXR ligand-treated db/db mice showed less weight gain than mice in other study groups.

Fast-protein liquid chromatography analysis of pooled plasma lipoproteins further confirmed the whole-plasma lipid measurements, indicating increased HDL cholesterol in plasma from BKS mice treated with T0901317.
without increased VLDL cholesterol (Figs. 4A, 5A). In db/db mice (Fig. 4B), the elevated plasma cholesterol was localized to VLDL- and LDL-sized particles, and this cholesterol colocalized with triacylglycerol (Fig. 5B), indicating that the majority of the triacylglycerol was in VLDL-sized particles with some enrichment of all lipoprotein fractions at the expense of cholesterol esters. The previously observed decrease in HDL cholesterol (Fig. 1E) was confirmed (Fig. 4B). Chemical composition analysis of pooled fractions (Table 1) revealed that the elevated plasma triacylglycerols following db/db mouse treatment with T0901317 were localized in VLDL of similar composition and surface:core ratio as nontreated animals, suggesting that the increased triacylglycerol was due to overproduction and secretion of hepatic VLDL rather than dramatically increased VLDL size. A moderate increase in VLDL size has been reported for T0901317-treated C57BL/6J mice (44). We attempted to measure plasma triacylglycerol accumulation as a measure of VLDL production using the Triton WR-1339 method (45); however, T0901317-treated db/db mice did not tolerate detergent administration. We suspect this may be related to the extremely high plasma triacylglycerol levels.

To further confirm that the increased VLDL triacylglycerol was due mainly to increased VLDL production and not simply increased VLDL size, Western blots for plasma apoB$_{100}$ and apoB$_{48}$ were performed. Consistent with increased VLDL particle number, there was a large increase in VLDL apoB$_{100}$ and apoB$_{48}$ content in T0901317-treated db/db mice.
in the abundance of apoB\textsubscript{100} and apoB\textsubscript{48} in the plasma of db/db-treated mice (Fig. 6). Consistent with this, apoE levels were also elevated. ApoA-I levels were near normal, supporting the conclusion that decreased HDL cholesterol was the result of HDL triacylglycerol enrichment at the expense of cholesteryl ester, rather than a decrease in HDL particle number.

At necropsy, the livers from T0901317-treated BKS and db/db animals were observed to be severely enlarged and engorged with lipid (Fig. 7). Livers from the db/db strain had a 3-fold increase in liver-to-bodyweight mass (Fig. 8A) and a 6-fold increase in triacylglycerol (Fig. 8B). Contrary to the normal plasma triacylglycerol levels, accumulation of hepatic triacylglycerol was observed in LXR ligand-treated BKS mice, indicating enhanced lipogenesis. This further supports the concept that liver triacylglycerol is a more reliable marker of LXR-induced lipogenesis. Further analysis of liver lipid extracts demonstrated that the neutral lipid enrichment following treatment was due solely to increased triacylglycerol mass and not to cholesteryl ester accumulation (Table 2), as cholesteryl ester levels were very low and the changes not significant.

Further characterization of parameters of plasma lipid metabolism, liver function, and glucose control were carried out and are reported in Table 3. Plasma nonesterified fatty acid (NEFA) levels were moderately increased in BKS mice and decreased in db/db mice following T0901317 treatment. This may reflect either decreased plasma and/or tissue lipolysis or increased tissue uptake. In contrast to data reported by Cao et al. (19), we did not observe a statistically significant decrease in plasma glucose in plasma from LXR ligand-treated db/db mice, although we have always observed a lowering trend. Potentially, this may be due to the site of blood sampling. When db/db mice treated with T0901317 were tested for glucose tolerance, glucose levels in plasma from peripheral blood drawn from the retro-orbital sinus prior to administration of glucose were found to be significantly reduced by 70% (data not shown). Glucose tolerance was also modestly improved (Fig. 9A), as has been previously reported for LXR ligand-treated diet-induced obesity mice; however, there was no significant improvement in insulin sensitivity (Fig. 9B). Insulin and leptin measurements confirmed that the db/db mice used were hyperinsulinemic and hyperleptinemic, and, with the exception of decreased leptin in treated db/db mice, LXR ligand treatment did not significantly alter these parameters.

The presence of increased amounts of hepatic enzymes in the plasma is indicative of liver dysfunction. Consistent with this, we observed modestly increased alkaline phosphatase activity in both strains following LXR ligand treatment and increased AST in the db/db mice. Because a hallmark of liver dysfunction is often decreased HDL (46), variable degrees of liver damage, in addition to differences in triacylglycerol enrichment between experiments, might explain the variable changes in HDL cholesterol levels we observed between experiments. Decreased HDL is also often related to decreased lecithin: cholesteryl acyltransferase (LCAT)-mediated plasma esterification of HDL (46). However, in two separate experiments

| Group       | Unesterified Cholesterol | Esterified Cholesterol | Triacylglycerols | Phospholipid | Surface:Core |
|-------------|--------------------------|------------------------|------------------|--------------|--------------|
| **VLDL**    |                          |                        |                  |              |              |
| BKS         | 8                        | 11                     | 64               | 17           | 0.33         |
| BKS - T0901317 | 6                      | 10                     | 65               | 18           | 0.33         |
| db/db       | 5                        | 6                      | 80               | 9            | 0.16         |
| db/db - T0901317 | 6                   | 2                      | 80               | 13           | 0.25         |
| **LDL**     |                          |                        |                  |              |              |
| BKS         | 2                        | 41                     | 30               | 28           | 0.41         |
| BKS - T0901317 | 2                    | 36                     | 37               | 25           | 0.37         |
| db/db       | 6                        | 34                     | 37               | 22           | 0.41         |
| db/db - T0901317 | 7                  | 8                      | 60               | 25           | 0.47         |
| **HDL**     |                          |                        |                  |              |              |
| BKS         | 6                        | 28                     | 16               | 50           | 1.27         |
| BKS - T0901317 | 2                    | 48                     | 28               | 22           | 0.32         |
| db/db       | 5                        | 48                     | 20               | 26           | 0.47         |
| db/db - T0901317 | 7                  | 12                     | 52               | 30           | 0.56         |

BKS, C57BLKS/J; db/db, BKS-Cg-m +/- + Lepr\textsuperscript{db}. Mouse plasma from BKS and db/db mice treated with vehicle or T0901317 for 12 days was pooled, and lipoprotein fractions were separated by fast-protein liquid chromatography. Column fractions corresponding to VLDL, LDL, and HDL were isolated and the lipid composition determined as described in Materials and Methods.
LCAT activity was found to be unaffected by LXR treatment in either the BKS or db/db strains.

Hepatic lipase activity was ~50% of normal following treatment, and there was a strong but statistically insignificant trend toward increased plasma LPL. Both of these changes are consistent with measured changes in hepatic gene expression (Table 4), where hepatic lipase mRNA was strongly decreased in db/db-treated animals (30%), and LPL was increased almost 3-fold. The known LXR target genes ABCA1, SREBP1c, FAS, SCD1, LPL, and apoC-II were significantly upregulated in both strains by drug treatment. ABCG5 and ABCG8 were robustly upregulated in the T0901317-treated BKS mice but were unchanged in LXR-treated db/db animals. LXRα, which was previously reported by Tobin et al. (47) to be basally upregulated in db/db mice in response to high plasma insulin levels, tended to be elevated when compared with normoinsulinemic BKS mice. LXR ligand treatment did not affect LXRα expression in BKS mice, and LXRα expression was significantly reduced in T0901317-treated db/db mice. Surprisingly, the magnitude of the drug-induced elevation of lipogenic gene expression was not enhanced in treated db/db mice. This suggests that the massive overproduction of triacylglycerol in treated db/db mice cannot be explained simply by an additive effect of insulin and an LXR ligand on SREBP1c and target lipogenic gene expression or enhanced activation of the lipogenic pathways due to increased basal expression of LXRα.

In contrast to the increased expression of lipogenic genes, phosphoenolpyruvate carboxykinase (PEPCK) expression in both mouse strains treated with the LXR ligand was dramatically downregulated, confirming results of previous work that showed T0901317 decreased hepatic gluconeogenesis in db/db mice (19). No changes in the expression of apoA-II or apoC-III were observed.

|               | Unesterified Cholesterol | Esterified Cholesterol | Triacylglycerols | Phospholipid | µg/mg protein |
|---------------|--------------------------|------------------------|-----------------|--------------|---------------|
| BKS           | 10.4 ± 0.4               | 1.5 ± 0.2              | 19 ± 11         | 104 ± 4      |
| BKS-T0901317  | 8.7 ± 0.6                | ND                     | 178 ± 22        | 137 ± 9      |
| db/db         | 10.0 ± 0.6               | 0.3 ± 0.2              | 114 ± 17        | 104 ± 6      |
| db/db-T0901317| 9 ± 3                    | 1.5 ± 0.7              | 662 ± 60        | 188 ± 10     |

ND, none detected. Liver lipid compositions from vehicle and T0901317-treated (50 mg/kg/day, 12 days) mice were determined enzymatically after solvent extraction and solubilization in 1% Triton X-100. Protein was determined from liver homogenates. Data are means ± SEM of n = 7.

* Results are significant versus vehicle controls at P < 0.01.
type II diabetes and CHD. Two of the key metabolic modulators that have emerged are the nuclear receptor LXR, which has been found to be a regulator of sterol, lipid, and carbohydrate metabolism, and SREBP1c, which through regulation by both LXR and insulin appears to play a key role in modulating lipogenesis. Not surprisingly, both of these molecules are under investigation as potential therapeutic targets. A number of synthetic LXR ligands have recently been identified and are currently being investigated in animal models (1, 35).

While LXR activation clearly results in desirable changes in cholesterol metabolism, including increased ABCA1 expression, increased cholesterol efflux, increased HDL in hamsters and mice, and reduced atherosclerosis in animal models, the concurrent and undesirable induction of lipogenesis through direct activation of SREBP1c and FAS remains a serious concern (1, 11, 18, 35, 48). Accumulated data for T0901317 indicate that in vitro, the compound robustly induces SREBP1c and other LXR targets and leads to triacylglycerol accumulation in cells (49). When administered to animals, the compound increased hepatic triacylglycerol accumulation and, depending on the dose used and the length of the study, resulted in variable elevation of plasma triacylglycerols (1, 18). In daily dosing studies of up to 4 weeks in C57BL/6J mice, we observed that increased plasma triacylglycerols were mostly normalized after 1 week (Fig. 2A). However, liver triacylglycerol accumulation persisted (Fig. 2B). Given the variability in plasma triacylglycerols, hepatic lipid accumulation appears to be a more reliable marker of increased lipogenesis. Published data on GW3965 suggest that this LXR ligand does not appreciably induce SREBP1c or result in significantly elevated triacylglycerols at doses suffi-

**TABLE 3.** Plasma analytes and lipolytic enzyme activities

|                      | BKS       | Vehicle | T0901317 | Vehicle | T0901307 |
|----------------------|-----------|---------|----------|---------|----------|
| NEFA, meq/l          | 0.4 ± 0.0 | 0.6 ± 0.1^a | 1.2 ± 0.2 | 0.7 ± 0.1^b |         |
| Glucose, mmol/l      | 20 ± 2    | 19 ± 1  | 41 ± 6   | 30 ± 5   |         |
| Insulin, pmol/l      | 20 ± 10   | 49 ± 7  | 354 ± 63 | 377 ± 49 |         |
| Leptin, pmol/l       | 0.7 ± 0.2 | 0.5 ± 0.2 | 76 ± 3   | 40 ± 3^b |         |
| Alk Phos, U/l        | 409 ± 39  | 530 ± 27^a | 795 ± 128 | 1530 ± 149^a |         |
| ALT, U/l             | 285 ± 88  | 193 ± 81 | 365 ± 71 | 896 ± 151^a |         |
| ALT, U/l             | 110 ± 41  | 109 ± 74 | 294 ± 106 | 448 ± 220 |         |
| LCAT, µmol cholesteryl oleate formed/ml/h | 30 ± 3 | 34 ± 3 | 30 ± 3   | 34 ± 3   |         |
| HTGL, µmol oleic acid released/ml/h | ND | ND | 4.5 ± 0.4 | 2.1 ± 0.5^c |         |
| LPL, µmol oleic acid released/ml/h | ND | ND | 4.4 ± 0.4 | 7.9 ± 1.8 |         |

NEFA, nonesterified fatty acids; Alk Phos, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LCAT, lecithin: cholesterol acyltransferase; HTGL, hepatic triacylglycerol lipase; ND, not determined; LPL, lipoprotein lipase. Data are means ± SEM of n = 5–7.

^a Significant versus the same strain vehicle control at P < 0.01.

^b Significant versus the same strain vehicle control at P < 0.05.

**DISCUSSION**

Over the last few years, considerable progress has been made in understanding the complex interplay between sterol, lipid, and carbohydrate metabolism and how this relates to the development of metabolic diseases such as

![Fig. 9](image.png)

Fig. 9. Glucose tolerance in db/db mice. Glucose area under the curve (AUC) (A) and insulin sensitivity index (B). db/db mice were treated with T0901317 (T) (50 mg/kg/day ip, 12 days) and were fasted for 5 h after the last injection prior to the intraperitoneal administration of 2 g/kg glucose. Plasma glucose and insulin were measured as described in Materials and Methods. glucose and insulin AUC were calculated on individual animals using Graphpad Prism. The insulin sensitivity index was calculated as glucose AUC × insulin AUC. Data are means ± SEM of n = 5–6, and significant differences were determined for each strain by a t-test using Sigmapstat (SPSS).

**TABLE 4.** Liver gene expression

| Gene  | BKS | Vehicle | T0901317 | Vehicle | T0901307 |
|-------|-----|---------|----------|---------|----------|
| ABCA1 | 1.0 ± 0.1 | 4.1 ± 0.3^a | 1.7 ± 0.3 | 3.3 ± 0.1^a |         |
| ABCG5 | 1.0 ± 0.2 | 4.1 ± 0.4^a | 2.7 ± 0.3 | 3.2 ± 0.2 |         |
| ABCG8 | 1.0 ± 0.3 | 4.8 ± 0.6^a | 1.2 ± 0.2 | 1.3 ± 0.2 |         |
| SREBP1c | 1.0 ± 0.2 | 7 ± 1^a | 1.7 ± 0.1 | 3.3 ± 0.2^a |         |
| FAS   | 1.0 ± 0.1 | 9 ± 1^a | 4 ± 1 | 10 ± 1^a |         |
| LXRα  | 1.0 ± 0.1 | 1.4 ± 0.5 | 2.0 ± 0.4 | 0.7 ± 0.2^b |         |
| SCD1  | 1.0 ± 0.3 | 12 ± 1^a | 8 ± 1 | 15 ± 1^a |         |
| PEPCK | 1.0 ± 0.1 | 0.3 ± 0.1^a | 2.0 ± 0.2 | 0.2 ± 0.04^a |         |
| HTGL  | 1.0 ± 0.1 | 1.2 ± 0.2 | 1.6 ± 0.1 | 0.5 ± 0.1^a |         |
| LPL   | 1.0 ± 0.1 | 9 ± 1^a | 2.3 ± 0.6 | 6.1 ± 0.6^b |         |
| Apo A-I | 1.0 ± 1.0 | 1.0 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 |         |
| Apo A-II | 1.00 ± 0.04 | 1.2 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.2 |         |
| Apo C-I | 1.00 ± 0.02 | 1.6 ± 0.1^a | 1.1 ± 0.1 | 2.2 ± 0.1^a |         |
| Apo C-III | 1.0 ± 1.0 | 1.2 ± 0.1 | 0.9 ± 0.1 | 0.83 ± 0.02 |         |

ABCA1, ATP binding cassette transporter A1; ABCG5, ABCG8, ATP binding cassette transporter G5; ABCG8, ATP binding cassette transporter G8; SREBP1c, sterol regulatory element binding protein 1c; LXRα, liver X receptor α; SCD-1, stearoyl-CoA desaturase 1; PEPCK, phosphoenolpyruvate carboxykinase; HTGL, human triacylglycerol lipase; apo, apolipoprotein; FAS, fatty acid synthase. Liver gene expression normalized to cyclophilin and BKS vehicle. Data are means ± SEM of n = 4.

^a Values are significant versus the same strain vehicle control at P < 0.01.

^b Values are significant versus the same strain vehicle control at P < 0.05.
cient to cause a reduction in atherosclerosis (35). However, it is unclear as to whether this LXR ligand leads to hepatic triacylglycerol accumulation or has tissue or LXR target gene specificity that would avoid induction of SREBP1c and FAS in lipogenic tissues.

Our current data indicates that the lipogenic effects of T0901317, and potentially of other LXR agonists, may be severely exacerbated in db/db mice. We believe that this is an additive effect of both LXR activation and the lipogenic potential of an insulin- and leptin-resistant state. In the present study, this was manifested by the accumulation of massive amounts of triacylglycerol in the plasma (Fig. 1C) and liver (Fig. 8B) of T0901317-treated db/db mice. The increase in plasma VLDL (Fig. 5) and plasma apoB and apoE (Fig. 6), without significant changes in VLDL composition, surface:core ratio (Table 1), or negative changes in LPL activity (Table 3) along with liver triacylglycerol engorgement, clearly points to hepatic overproduction of triacylglycerol. Surprisingly, HDL cholesterol levels were decreased to varying amounts over a series of studies even though plasma apoA-I levels were near normal (Fig. 6). This contrasts with the increased HDL observed in C57Bl/6J mice and hamsters treated with T0901317 (1), and it appears to reflect an exchange of triacylglycerol for cholesterol ester in db/db-treated mouse HDL (Table 1) and modest hepatic dysfunction without any change in plasma LCAT activity (Table 3). The lack of a statistically significant increase in HDL cholesterol in the BKS strain (Fig. 1E) following LXR treatment may be indicative of a reduced response in this strain.

Initially, we expected to find the expression of hepatic lipogenic genes further upregulated in treated db/db mice when compared with treated normoinsulinemic BKS mice as a result of both higher basal LXRα expression in db/db mice and as a result of direct insulin effects on lipogenic genes (18, 47). However, no additive or enhancing effect was observed between LXR ligand and the type II diabetic phenotype of db/db mice on the expression of lipogenic genes, including SREBP1c and FAS (Table 4).

We suspect the mechanism underlying our observations is much more complex than simple changes in hepatic gene expression, especially given that insulin resistance, obesity, and diabetes often result in defective energy metabolism, which favors lipid production and storage (50). Perhaps the additional upregulation of lipogenic genes by LXR ligand treatment reduces the enzymatic barriers that might be limiting the conversion of acetyl-CoA into fatty acids. Should more acetyl-CoA be available for fatty acid synthesis and the lipogenic pathway have increased capacity, then significant triacylglycerol overproduction would be favored.

Cao and colleagues (19) showed in two hyperinsulinemic models of type II diabetes, db/db mice and Zucker (fa/fa) rats, that T0901317 inhibited hepatic gluconeogenesis through inhibition of PEPCK and glucose-6-phosphate dehydrogenase. They concluded that this could be a beneficial antidiabetic action of LXR compounds. We have confirmed in our studies that PEPCK expression is also strongly downregulated by T0901317 (Table 4). However, we have been unable to detect a significant reduction in plasma glucose from cardiac blood and have only observed significant differences in glucose levels in peripheral blood samples. Because Cao et al. (19) used peripheral blood for their studies, and it has been recently shown that LXR may upregulate tissue glucose uptake, perhaps the major plasma glucose-lowering effect of LXR ligands results from increased peripheral tissue glucose uptake. Similar to studies with GW3965 in diet-induced obesity mice (20), we also observed a modest improvement in glucose tolerance with LXR ligand treatment (Fig. 9A); however, there was no significant improvement in the insulin sensitivity index (Fig. 9B).

Improvements in glucose uptake and inhibition of hepatic glucose production would normally be viewed as beneficial to the management of diabetes. However, it appears that in the setting of enhanced lipogenic gene expression, inhibition of gluconeogenesis may be negatively affecting metabolic balance. We suggest that a shift of metabolism away from glucose production without a concomitant increase in energy utilization would result in excess acetyl-CoA available for fatty acid synthesis and subsequently increased production of fatty acids and triacylglycerols.

The db/db mice have a mutation in the leptin receptor resulting in defective leptin signaling and leptin resistance. Defective leptin signaling (leptin resistance) has been shown in rodents to decrease the synthesis of malonyl-CoA from acetyl-CoA, inhibiting fatty acid oxidation and causing a metabolic shift toward fatty acid and triacylglycerol synthesis in some tissues (51). A further increase in acetyl-CoA under these conditions would likely further contribute to the overproduction of triacylglycerol in LXR ligand-treated diabetic mice.

Recent work has also suggested that the LXR ligands may play a key role in regulating fatty acid and triacylglycerol metabolism in adipocytes and can promote glucose uptake through upregulation of GLUT4, enhanced lipogenesis, and the release of NEFA (20, 49, 52). We observed a modest increase in plasma NEFA in T0901317-treated BKS mice but reduced NEFA in treated db/db mice (Table 3). This could be related to either the diabetic or hyperleptinemic background and may indicate decreased adipocyte or VLDL lipolysis and/or increased fatty acid uptake by adipocytes, the liver, or peripheral tissues.

Our observations strongly suggest that the induction of pathways regulated by LXR ligands may result in very different outcomes, depending on the existing metabolic balance. In particular, we suggest that SREBP1c- and FAS-mediated lipogenic effects of LXR ligands will result in substantially worsened lipogenesis under metabolic conditions favoring fatty acid synthesis and storage. Our data suggest that LXR ligands that have effects on both fatty acid and carbohydrate metabolism should be carefully evaluated in models of obesity, insulin, and leptin resistance. The antidiabetic potential of LXR ligands will likely be limited unless their effects on glucose metabolism can be pharmacologically or synthetically separated from lipogenic effects.
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