Skeletal muscle as a target of LXR agonist after long-term treatment: focus on lipid homeostasis

Amena Archer,1 Jurga Laurencikiene,2 Osman Ahmed,1,3 Knut R. Steffensen,1 Paolo Parini,1,3 Jan-Åke Gustafsson,1,4 and Marion Korach-André1

1Department of Biosciences and Nutrition and Center for Biosciences at NOVUM, Karolinska Institute, Huddinge, Sweden; 2Department of Medicine, Karolinska Institute, Lipid Laboratory, Huddinge, Sweden; 3Division of Clinical Chemistry, Department of Laboratory Medicine, Karolinska Institute at Karolinska University Hospital, Huddinge, Sweden; and 4Department of Biology and Biochemistry, Center for Nuclear Receptors and Cell Signaling, Houston, Texas

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The liver X receptors (LXR)α and LXRβ are ligand-dependent nuclear receptors activated by oxidized derivatives of cholesterol (oxysterols) and have been implicated in the control of cholesterol and fatty acid metabolism in multiple cell types (13). LXR function has been extensively explored in the liver, but much less is known about its function in the adipocyte, although both LXRα and LXRβ are highly expressed in murine and human white adipose tissue (WAT). In addition, LXR activation has recently been shown to have opposite effects in different tissues in vivo; acute LXR activation induces lipogenesis in liver (27, 29) and reduces lipogenesis in WAT (17). The effects of LXR agonists on plasma lipid homeostasis, especially triglyceride (TG) levels, are still controversial and depend on animal strain, genetic background, duration of the treatment, selectivity of LXR agonist, and/or dietary challenges (2, 3, 7–9, 14, 15, 17, 18, 20). A large number of studies have shown that LXR regulates expression of the main genes involved in lipid hydrolysis, such as Atgl, Hsl, and Lpl, but results remain controversial (2, 9, 17, 19, 27, 33). Previous studies have suggested that activation of LXR induces lipolysis in human adipocyte (34) and lipid oxidation in both human and murine adipocytes (33). Stenson et al. (34) have shown that LXR activation by GW3965 increases basal lipolysis in differentiated human adipocytes, while lipolysis and lipogenesis were both induced in isolated adipocytes from WAT of LXRAβ/−/− mice compared with WT mice (17). In addition, activation of LXR has been reported to regulate the switch between glucose and fatty acid oxidation in both human and murine adipocytes (33). Finally, we recently showed that chronic (5-wk) LXR activation in ob/ob mice leads to a redistribution of fat storage from the visceral region toward the subcutaneous region and modifies lipid composition toward less lipotoxic lipids (2). Collectively, to date the role of LXR in the regulation of lipid homeostasis in adipocytes remains unclear. Elucidating the LXR pathways that control lipid homeostasis is likely to identify novel opportunities for intervention in metabolic diseases. In addition, to our knowledge, the effect of chronic LXR activation on metabolism homeostasis in WT mice, which is of great interest from a therapeutic perspective, has not yet been explored.

Here, we demonstrate that the nonsteroidal LXR selective agonist GW3965 given for 5 wk has potent antilipolytic and antilipogenic activities in mouse adipocytes. In addition, we provide evidence that these regulations are mainly mediated via LXRβ. We also show that LXRA is required for the basal state of adipocyte metabolism. Furthermore, our data demonstrate that chronic LXR activation has opposite effects between...
WAT and muscle tissues (i.e., promotes lipogenesis and reverse cholesterol transport in muscle) through LXRβ and promotes lipolysis through activation of both LXR isoforms in skeletal muscles.

MATERIALS AND METHODS

Animals. Eight-week-old female WT C57Bl/6J mice and LXRα and LXRβ knockout (KO) mice as previously described (1) were housed under standard conditions with free access to water and chow diet throughout the study. Mice were fed for 5 wk a Chow diet alone (Control; TD 7001; Harlan-Teklad, WI) containing 4% (wt/wt) of total lipids (<12% of its calories as animal fat) or together with the synthetic LXR agonist GW3965 (Treated, 40 mg/kg body wt) directly incorporated in the pellet diet. At the end of the experiments, mice were euthanized under 4% isoflurane, and blood was collected by heart puncture. Liver, gonadal fat tissue (representing WAT) and tibialis anterior (TA) muscle were quickly collected, immediately frozen in liquid nitrogen, and stored at −80°C for further analysis. For metabolic experiments, fresh adipose tissue was used. The local Ethics Committee of the Swedish National Board of Animal Experiments approved all experiments.

Isolation of adipocytes from adipose tissue and measurement of lipogenesis and lipolysis. Fat cells were isolated from gonadal WAT according to Rodbell (28). Lipogenesis (basal and insulin-stimulated glucose incorporation into lipids) in isolated fat cells was assayed as described in detail previously (22). In brief, 3.6% (vol/vol) adipocyte solution in KRP buffer supplemented with 2% albumin and 5 μM glucose was prepared. Under constant stirring, 100 μl of the cell suspension was transferred to vials, and 5 million DPM/ml labeled [3-3H]glucose (37 MBq/ml; PerkinElmer-Cetus, Norwalk, CT) was added. Insulin was added in concentrations of 10−16 to 10−8 mol/l and incubated at 37°C for 2 h in a shaking water bath, upon which the reaction was stopped by cooling on ice and addition of 50 μl of 6 mol/l H2SO4. Scintillation fluid extracting lipid fraction [toluene with 5 g/l 2,5-diphenyloxazol and 0.3 g/l 1,4-Bis (4-methyl-5-phenyl-2-oxazolyl)-benzene; all from Sigma-Aldrich] was added, and glucose incorporation into lipids was quantified in a beta-counter. In blank samples, lipogenesis was stopped with H2SO4 prior to incubation. Mean adipocyte volume was determined as described previously (4, 28). Lipolysis experiments were done on isolated fat cells as described (21). In brief, a diluted suspension (3.6%) of isolated fat cells was incubated for 2 h in duplicate samples with air as the gas phase at 37°C in KRP buffer supplemented with glucose (8.6 mmol/l). Ascorbic acid (0.1 mg/ml), and BSA (20 mg/ml) in the absence (basal) or presence of increasing concentrations of norepinephrine (a nonselective β- and α-adrenergic receptor agonist). After the incubation, an aliquot of the medium was removed, and glycerol (lipolysis index) was analyzed using a bioultramimicence method (11).

Energy expenditure. Mice were individually housed in calorimeter cages and acclimatized to the climber for 2 days before gas exchange measurements. Indirect calorimetry was performed using a computer-controlled SOMEDIC Metabolic System-INCA (SOMEDIC Sales) for 2 days with a 12:12-h light-dark cycle at 23°C with full access to food and water. Oxygen consumption and carbon dioxide production were measured for each mouse over a 48-h period. EE and glucose and lipid oxidation were calculated as previously described (16).

Biochemical analysis of serum and tissue. After blood collection, serum was prepared by centrifugation and aliquoted to avoid repeated thawing/freezing. Blood glucose concentration was measured in tail blood using the OneTouch Ultra glucometer (AccuChek Sensor, Roche Diagnostics). Serum insulin level was determined by ELISA kit (LINCO Research, St. Charles, MO). Homeostasis model assessment (HOMA) was calculated with the formula: [(fasting plasma glucose) × (fasting plasma insulin)/22.5] × 100. Nonesterified fatty acid (NEFA) levels and LPL activity were quantified in serum samples by a colorimetric enzymatic kit (Wako Chemicals, Richmond, VA) and Abcam (ab65331; Cambridge, MA) and a fluorescence assay (Roar Biomedical, New York, NY) respectively. Liver and TA lipids were extracted according to Folch et al. (6). Liver, skeletal muscles, and serum TG were measured by enzymatic assay using commercially available kits (Roche Diagnostics, Mannheim, Germany). Liver and TA lipid levels were normalized to the tissue protein content.

Oral glucose and intraperitoneal insulin and pyruvate tolerance tests. Mice were all fasted in the morning from 6:00 AM. Mice were deprived of food for 4 h before an oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) and 6 h prior to a pyruvate tolerance test (PTT). Mice were administered 1 g glucose (1.0 g/kg by gavage), 2 insulin (0.8 U/kg Actrapid; Penfill, Novo Nordisk), and 3 pyruvate (2 g/kg) intraperitoneally. Blood samples were obtained via tail nick at 0, 15, 30, 60, and 120 min in each test, and glucose was measured with the OneTouch Ultra glucometer (AccuChek Sensor). The tolerance tests were conducted at least 3 days apart. The area under the curve (AUC) was calculated as follows: [Glc(T1) + Glc(T2) × (T2 − T1)]/2 for each consecutive point and summed.

Quantitative PCR. Total RNA extraction was performed using TRIzol according to the manufacturer’s instructions (Invitrogen). Expression levels of mRNA were quantified using an ABI 7500 instrument and the FAST SYBR green technology (Applied Biosystems). Relative gene expression levels were calculated by the comparative Ct method using TFIIB as the internal reference gene for liver and muscle tissues and using 36B4 as the internal reference gene for WAT.

Statistical analysis. Data are expressed as means ± SE. Statistical analyses were performed by two-tailed Student’s t-test (control vs. treated) or one-way ANOVA (WT vs. KO mice) followed by a Newman-Keuls post hoc test for multiple comparisons when appropriate. The level of significance was set at $P < 0.05$.

RESULTS

Five-week GW3965 treatment induces liver TG level but drastically reduces serum TG concentration. To investigate the effect of chronic LXR activation on lipid homeostasis in vivo, we treated 8-wk-old female WT mice with the synthetic dual LXR agonist GW3965 included directly into the chow diet for 5 wk. No differences in body weight (BW) were observed between groups before treatment (Table 1), but addition of GW3965 in the diet led to a significantly lower BW gain with no changes in food intake (Fig. 1) which indicated a lower food efficiency (calculated as total weight gain divided by total food intake during the 5-wk dietary challenge) upon treatment in WT mice (Table 1). LXRs are regulators of liver TG content through the regulation of Srebp1c expression and downstream lipogenic genes (9). Liver TG level was induced three times by

| Table 1. Expression level of selective LXR target genes in liver of WT mice in response to GW3965 for 5 wk |
|-----------------|-----------------|-----------------|----------------|
| Genes          | Control         | Treated         | P    |
| Lxra           | 1.0 ± 0.1       | 0.9 ± 0.1       | NS   |
| Lcrf           | 1.0 ± 0.1       | 0.9 ± 0.1       | NS   |
| Abca1          | 1.0 ± 0.1       | 1.3 ± 0.3       | NS   |
| Abcg5          | 1.0 ± 0.1       | 1.1 ± 0.2       | NS   |
| Abcg8          | 1.0 ± 0.1       | 0.9 ± 0.1       | NS   |
| Cyp7a1         | 1.0 ± 0.1       | 0.6 ± 0.1       | NS   |
| Me1            | 1.0 ± 0.1       | 0.7 ± 0.1       | NS   |
| Fas            | 1.0 ± 0.9       | 0.9 ± 0.2       | NS   |

Data are means ± SE; n = 7 animals per group. WT, wild type; LXR, liver X receptor; NS, not significant.
LXRs CONTROL LIPID BALANCE

Fig. 1. Chronic GW3965 (a synthetic dual LXR agonist) treatment induces liver triglyceride (TG) in wild type (WT) mice only but reduces serum TG in all 3 genotypes. A: mean body weight (BW) gain and food intake during 5-wk period in control (filled bars) and treated (open bars) WT mice. B: liver TG content in control (filled bars) and treated (open bars) WT mice and liver relative mRNA expression level of liver X receptor (LXR) target genes involved in lipid metabolism in control (filled bars) and treated (open bars) WT mice. C: serum level of TG, free glycerol, and NEFA and total lipase activity in serum in control (filled bars) and treated (open bars) WT mice. Liver (D) and serum TG (E) levels in control (filled bars) and treated (open bars) WT, LXRα−/−, and LXRβ−/− mice. F: total lipase activity in serum in control (filled bars) and treated (open bars) WT, LXRα−/−, and LXRβ−/− mice (data from WT mice are the same as those presented in B and C). Values are means ± SE; n = 7. *P < 0.05 WT vs. LXR knockout mice; #P < 0.05 control vs. treated mice.

Treatment. Surprisingly, this was associated with a small induction of liver Srebp1c expression level only, with no effect on well-known downstream lipogenic genes (Acc1 and Scd1) or Lxrα and Lxrβ (Fig. 1B and Table 1). Even more surprisingly, serum TG levels were reduced by 74% upon treatment as opposed to NEFA and free glycerol levels that were induced four- and twofold, respectively (Fig. 1C). To investigate the pathway by which LXR activation could increase the liver TG level in mice without significant stimulation of the lipogenic pathway, we measured the expression level of the main genes involved in TG hydrolysis, such as hormone sensitive lipase (Hsl), adipocyte TG lipase (Atgl) and hepatic lipase (Hl) in the liver of WT control and WT-treated mice. A 40% reduction of Atgl and Hsl expression levels was observed in liver of treated mice compared with control but no effect on Hl expression level (Fig. 1B), in line with the increased TG storage in liver upon chronic GW3965 treatment. In addition, lipolytic activity, measured as lipase activity in serum (sum of LPL and HL activities) was increased by 47% in serum of WT mice treated with GW3965 compared with control (Fig. 1C). These results give new insight on the role of LXR in the regulation of serum lipid levels and agree with the increased NEFA and free glycerol levels and the decreased TG level observed in treated compared with control WT mice. Both LXRα and LXRβ are expressed in WT mice; to address the question of the specific role of each isoform in the response to chronic LXR activation, we fed LXRα−/− and LXRβ−/− female mice the chow diet supplemented or not with GW3965 for 5 wk. Liver TG level was twice higher in LXRβ−/− compared with WT mice in the control group. Treatment had no effect on liver TG levels either in LXRα−/− or in LXRβ−/− mice (Fig. 1D). Of note, liver TG level was already high in LXRβ−/− mice before treatment and stayed high after treatment. This suggests that in LXRβ−/− mice the liver was already steatotic before treatment and this was not worsened by treatment. Conversely, in LXRα−/− mouse liver, TG level was low and remained low after treatment, indicating that LXRα is required for liver TG storage upon LXR activation during chronic GW3965 treatment, as already shown after acute LXR activation treatment (27, 29). Surprisingly, serum TG levels dropped with treatment in both genotypes as observed in WT mice (Fig. 1E). We thus measured lipase activity in serum of LXRα−/− and LXRβ−/− mice. Interestingly, a different mechanism than when both isoforms were present seemed to operate in single-KO mice. Lipase activity was significantly higher (74%) in LXRα−/− than in WT mice in the control group, with no effect of treatment (Fig. 1F). LXRβ−/− mice showed similar lipase activity, as WT mice both in control and treated groups with no effect of treatment. All together we can conclude that chronic LXR activation by GW3965 induces TG level in liver through...
activation of LXRα. Surprisingly, this induction was associated with a strong reduction of serum TG level in WT mice due to a significant induction of serum lipase activity after chronic GW3965 treatment.

Chronic GW3965 treatment suppresses lipolysis and lipogenesis in mouse adipocytes. NEFA released into the circulation are believed to come mostly from TG lipolysis in adipocytes and skeletal muscle (25) while the liver releases circulating TG. We observed an increase of NEFA level and a sixfold drop of TG level in serum after treatment (Fig. 1C).

Therefore, we harvested gonadal WAT from control and treated WT mice, isolated adipocytes, and measured basal and stimulated lipolysis. Visceral fat mass as well as adipocyte volume were not changed upon treatment (Table 2). In agreement with previous findings, after acute LXR activation, expressions of the main lipolysis-regulating genes Hsl and Atgl and lipid droplet coating protein perilipin 1 (Plin1) were strongly repressed by chronic LXR agonist treatment of WT mice. Likewise, catecholamine-stimulated lipolysis was reduced by the treatment (Fig. 2A). Even basal adipocyte lipolysis was attenuated (Table 3), which is in contrast with previous in vitro studies on differentiated human and murine adipocytes using shorter treatments with GW3965 LXR agonist (34). These data may indicate that after chronic LXR activation the increased NEFA level in the circulation of WT mice might not come from adipocyte release.

Cluster of differentiation (CD)36 protein, first discovered in platelets 30 years ago, is now characterized as a membrane glycoprotein that is widely expressed on the surface of a variety of cell types, including adipocytes and hepatocytes (30). CD36 has been shown to promote fatty acid uptake into the adipocyte and thereby to regulate serum lipid profile. Here, we found that Cd36 expression level in WAT was drastically reduced by treatment of WT mice (Fig. 2B), in line with the observed elevated NEFA serum level after treatment compared with control WT mice.

Lipid storage is a balance between lipid synthesis and lipid breakdown; we thus measured basal and insulin-stimulated lipolysis

Table 2. Physiological parameters in control and treated mice

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<tr>
<th></th>
<th>Wild Type</th>
<th>LXRα−/−</th>
<th>LXRβ−/−</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>24.2 ± 0.5</td>
<td>20.5 ± 0.6#</td>
<td>24.7 ± 0.5</td>
</tr>
<tr>
<td>Food efficiency, a.u.</td>
<td>2.7 ± 0.2</td>
<td>1.4 ± 0.1#</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>Visceral fat, mg</td>
<td>577 ± 57</td>
<td>664</td>
<td>623 ± 105</td>
</tr>
<tr>
<td>Adipocyte volume, ml³</td>
<td>125 ± 12</td>
<td>132 ± 11</td>
<td>120 ± 7</td>
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</table>

Data are means ± SE; n = 5–8 animals per group. *P < 0.05 wild-type vs. LXR knockout (KO) mice; #P < 0.05 control vs. treated.
glucose incorporation into lipids (as a measure of lipogenesis) in WT adipocytes. Basal lipogenesis in adipocytes was not affected by LXR agonist treatment in WT mice (Table 3). However, insulin-stimulated lipogenesis was suppressed, suggesting that overall metabolic activity in adipocytes was reduced by chronic administration of LXR agonist. This effect was associated with a significant reduction of a well-known target gene of LXR, the main player of lipogenesis Srebp1c (Fig. 2B). Glyceroneogenesis, synthesis of glycerol for TG production, has been shown to be the major pathway of glycerol production in adipocytes, liver, and skeletal muscle in rat (24). Interestingly, the expression level of phosphoenolpyruvate carboxykinase (Pepek) gene, the rate-limiting step of glyceroneogenesis in liver and WAT, was strongly repressed by LXR activation in the WAT of WT mice (Fig. 2B).

To determine which of the LXR isoforms might mediate the observed repressing effect of LXR activation on lipolysis and lipogenesis in isolated adipocytes, we performed lipolysis and insulin-stimulated lipogenesis experiments with adipocytes obtained from gonadal fat of LXRα−/− and LXRβ−/− mice treated or not with GW3965. Interestingly, LXRα−/− mice showed lower basal lipolysis than LXRβ−/− and WT mice. In addition, basal lipolysis in LXRα−/− mice was not affected by treatment (Table 3). In contrast, LXRβ−/− mice showed higher rate of basal lipolysis after treatment.

LXRα−/− adipocytes had lower and LXRβ−/− adipocytes had higher capacity to respond to norepinephrine compared with WT mice in the nontreated group. This could imply that LXR isoforms have different impacts on the regulation of lipolysis and might affect it in different directions. Chronic treatment by LXR agonist reduced stimulated lipolysis in LXRα−/− adipocytes but had only a very mild effect (significant only at 10 nM of norepinephrine) in LXRβ−/− mice.

Evaluation of basal and insulin-stimulated lipogenesis confirmed that LXRα and LXRβ might have very different roles in regulation of adipocyte metabolism. Basal relative lipogenesis was lower in LXRα−/− mice and higher in LXRβ−/− mice compared with WT control animals. The treatment increased basal relative lipogenesis in LXRα−/− but repressed it in LXRβ−/− mice. LXRβ−/− mice had much higher induction of insulin-stimulated lipogenesis than WT or LXRα−/− mice, which was suppressed by treatment. LXRα−/− adipocytes had low insulin-stimulated lipogenesis, and no effect of treatment was observed. These observations were in line with the expression level of the master gene of lipogenesis, Srebp1c, as shown in Fig. 2D. These data indicate that LXRα is required for basal adipocyte metabolism whereas LXRβ acts as a suppressor. Most likely it is a balance between the two isoforms that defines metabolic effects of LXR agonist.

Treatment increases EE and induces a switch from glucose toward lipid oxidation in WT mice. Mice were placed in an individual calorimetry chamber for 2 days to measure gas exchanges and EE prior to and after the treatment. Total EE over a 24-h period was higher after treatment compared with baseline (Fig. 3A). In line with this, treatment induced lipid oxidation in WT mice (Fig. 3B). GW3965-treated LXRα−/− and LXRβ−/− mice were placed in a calorimetry chamber for 2 days to establish the role of each isoform in this regulation. Treated LXRα−/− and LXRβ−/− mice showed higher EE than control WT mice (Fig. 3B). Interestingly, this increased EE was mainly the consequence of increased fatty acid oxidation in LXRα−/− mice and increased glucose oxidation in LXRβ−/− mice compared with WT mice (Fig. 3B). SM metabolism (glucose and lipid oxidation) is a major determinant of EE both at rest and during exercise. Interestingly, expression levels of LXR target genes involved in lipid synthesis [Srebplc and fatty acid synthase (Fas)] and in reverse cholesterol transport [ATP-binding cassette (Abca1 and Abcb1)] were markedly induced in TA SM (Fig. 3C) of WT mice after treatment compared with control. This may indicate that after a chronic LXR agonist treatment SM is the target site for regulation of lipid and cholesterol homeostasis and may be a major contributor to the control of lipid balance. In addition, this induction was also largely observed in LXRα−/− mice but to a much lower extent in LXRβ−/− mice (Fig. 3E), which indicates that LXRβ might be the main LXR isoform regulating lipid homeostasis in SM tissue.

To evaluate the contribution of SM in the regulation of lipid flux, we measured cholesterol and TG contents in TA muscle, as shown in Fig. 3D. Cholesterol content in TA was significantly reduced in all genotypes after treatment compared with control groups, indicating that chronic LXR activation facilitates cholesterol excretion from SM through activation of both LXR isoforms. TG level in TA was significantly reduced in treated LXRβ−/− mice only (Fig. 3D), and the expression levels of the main genes of the lipolytic pathway in SM (Hsl, Lpl, and Atg1) were upregulated by treatment in single-KO mice only. Chronic LXR activation had no effect on the expression levels of Lxra and Lxrβ in SM (Fig. 3D).

Chronic GW3965 treatment improves insulin sensitivity and represses gluconeogenesis in mice. Serum glucose level was not different between control and treated WT mice, but treated WT mice showed reduced serum insulin level compared with control WT mice. As a consequence, HOMA was significantly reduced in treated WT mice compared with control WT mice (Fig. 4A). These data may indicate an overall improved response to insulin after treatment. Glucose and insulin tolerance tests revealed no major changes in the metabolic responses.

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**Table 3. Effect of chronic GW3965 treatment on basal lipolysis and basal lipogenesis in isolated adipocytes from gonadal fat depot of wild-type and LXR KO mice**

<table>
<thead>
<tr>
<th>LXR isoform</th>
<th>Control</th>
<th>Treated</th>
<th>Control</th>
<th>Treated</th>
<th>Control</th>
<th>Treated</th>
</tr>
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<tbody>
<tr>
<td>Lipogenesis</td>
<td>1.2 ± 0.1</td>
<td>0.9 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>5.0 ± 0.3</td>
<td>2.0 ± 0.0</td>
</tr>
<tr>
<td>Lipolysis</td>
<td>1.8 ± 0.3</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>2.7 ± 0.1</td>
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Data are means ± SE; n = 6–8 animals per group. GW3965, synthetic dual LXR antagonist. *P < 0.05 wild type vs. LXR KO mice; #P < 0.05 control vs. treated.
following glucose absorption or insulin injection after treatment compared with control; both WT control and WT treated mice were glucose tolerant and insulin sensitive (Fig. 4, B and C). Nevertheless, fasting glucose levels prior to all tests were significantly lower after GW3965 treatment, probably due to a reduced hepatic glucose production following treatment, as observed after pyruvate injection (Fig. 4D). The observed repression of gluconeogenesis was supported by a reduction of the expression level of the rate-limiting enzyme of gluconeogenesis (Pepck) in liver (Fig. 4E). Finally, expression levels of the key genes of glycolysis and glucose uptake, such as glucokinase (Gk), the first enzyme of the glycolysis pathway and, the insulin-dependent glucose transporter Glut4 (Fig. 4C) were significantly induced by treatment in TA muscle (Fig. 4E). Taken together, these data may suggest an induced glucose uptake/oxidation and an improved insulin sensitivity upon chronic GW3965 LXR activation in SM.

**DISCUSSION**

The regulation of lipid and glucose metabolism is closely linked. Impairment of one of these can lead to several disorders including obesity, insulin resistance, and T2D. In this study, we present robust data to infer the nuclear receptors LXRα and LXRβ as pivotal actors in lipid and cholesterol homeostasis in the gonadal WAT and the muscle tissues. After 5 wk of treatment with the synthetic LXR ligand GW3965 (40 mg/kg), serum TG level drastically dropped as opposed to acute treatment, whereas liver TG level stayed high. We show evidence that this drop is, at least in part, due to an increase of serum lipase activity in WT mice upon chronic GW3965 treatment (Fig. 1C). Interestingly, lipogenesis in adipose tissue was blunted in LXRβ−/− mice, with no effect of GW3965, highlighting the critical role of LXRα in the regulation of lipid metabolism in mouse adipocytes. In addition, we show that stimulated lipolysis is reduced by treatment, and this repression requires LXRβ. Importantly, we demonstrate that SM are major players of lipid homeostasis after chronic LXR activation. Indeed, both lipogenic and reverse cholesterol transport pathways were highly induced (50- to 20-times increases of Srebp1c and Abcg1 expression levels) in WT and LXRα−/− mice after 5-wk treatment and to a much lower extent in LXRβ−/− mice, suggesting a crucial role of LXRβ in lipid synthesis and cholesterol homeostasis in SM. Interestingly, both LXRα and LXRβ are important regulators of lipolysis in SM and are thereby important contributors to lipid trafficking in the body, as shown in Fig. 3C. Finally, we show that chronic treatment with GW3965 induces EE in mice and provokes a switch from glucose toward lipid oxidation as a major fuel for energy production in mice. This is supported by the observation of lower body weight gain in treated mice compared with control.
control mice with a similar food intake (Fig. 1A). These new data would be of high interest in the long-term treatment of metabolic diseases and are summarized in Fig. 5A.

Whereas the effects of acute treatment (3–5 days) with LXR agonists has been largely explored in liver metabolism (26, 29, 35), the effect of long-term treatment is poorly documented (2). While acute LXR activation is well known to induce target genes of TG synthesis and cholesterol transport/excretion in liver such as $\text{Srebp1c}$, $\text{Abca1}$, $\text{Abcg5}$, or (cytochrome P-450) $\text{Cyp7a1}$, we found in the current study that chronic treatment had no effect on the expression of these genes, suggesting that liver is no longer the target organ at the transcriptional level of LXR activation after 5-wk treatment (Fig. 1B and Table 1).

Interestingly, Muscat et al. (23) suggest that SM could be a crucial site for reverse cholesterol transport and may contribute to the control of HDL-cholesterol levels after 7 days’ treatment with the synthetic LXR agonist T0901317 in mice. In line with this, expression level of LXR target genes controlling the reverse cholesterol transport ($\text{Abcg1}$ and $\text{Abca1}$) was highly induced in TA muscle after treatment (Fig. 3E). Gene expression was sustained by a 50% reduction of cholesterol content in this muscle compared with control groups (Fig. 3F) in all genotypes. Interestingly, the effect of GW3965 treatment on the induction of reverse cholesterol target genes was lower in both LXR$^+/+$ and LXR$^-/-$ mice compared with WT mice, suggesting a role of both LXR isoforms in the regulation of cholesterol homeostasis in the SM.

LXR is known to regulate lipid homeostasis by controlling expression levels of genes of the lipogenic pathway such as $\text{Srebp1c}$ and downstream genes in liver and in adipocytes (12), but the control of SM TG metabolism is of high interest in the treatment of metabolic diseases. Here, we provide data showing that LXR is an important contributor to TG homeostasis in the SM. In our study, the $\text{Srebp1c}$ expression level was increased 45-fold in TA after 5-wk treatment with GW3965 and $\text{Scd1}$ and 2.2-fold, respectively (Fig. 3C and data not shown), compared with control WT mice. Interestingly, this induction was restrained in LXR$^-/-$ mice compared with control mice (Fig. 3C), and TG level measured in TA muscle was significantly reduced in LXR$^-/-$ mice compared with control group. These data suggest a crucial role for LXR$^-$ as a regulator of TG homeostasis in the SM and is potentially important information for LXR-mediated drug development.

The capacity of LXRs to regulate fatty acid metabolism in SM has crucial consequences for both glucose and lipid homeostasis and is closely linked to insulin action. Indeed, insulin is a master player of glucose uptake in SM and induces $\text{Srebp1c}$ expression in this tissue; these actions are flawed in T2D patients (5). LXR signaling is thus closely linked to insulin action in muscle tissue. In our study, both $\text{Glut4}$ and $\text{Srebp1c}$ expression levels were highly induced after chronic LXR activation, whereas insulin in serum was significantly reduced (Figs. 3, A and C, and 4E). These data indicate that
LXRα/β signaling pathway controls lipid metabolism.

**Figure 1A** shows the proposed role of LXRα and LXRβ in lipid metabolism. Chronic LXR activation by GW3965 treatment for 5 wk induced a significant reduction in TG levels. This reduction was accompanied by an increased serum level of lipase activity, indicating that the reduced TG level was significantly induced by treatment in WT mice. In contrast, in single-KO mouse groups, lipolytic gene expression levels were reduced and the serum NEFA level was induced. Consequently, lipid oxidation was induced by treatment (Fig. 1B). In conclusion, we suggest that LXR could regulate lipid trafficking by controlling glyceroneogenesis and/or LPL activity. Clearly, additional studies are required to further explore this hypothesis.

**Figure 1C** illustrates the complex role of LXRα and LXRβ in lipid metabolism. Acute LXR activation leads to reduced hepatic glucose production and increased cholesterol excretion through induction of Cyp7a1 and bile acid synthesis. This well-known side effect of LXR activation is an increase in hepatic and serum lipoprotein metabolism via the induction of Srebp1c and all downstream genes of the lipogenic pathway. This secondary effect of LXR activation has reduced interest in targeting LXR in metabolic diseases. Interestingly, after 5-wk treatment with GW3965, we observed an increase in TG content in liver (Fig. 1D) but a drop in TG concentration (Fig. 1C) in serum as opposed to short-term treatment (10, 15). This drop in serum TG content was accompanied by an increased serum level of lipase activity, indicating that the reduced TG level was significantly induced by treatment in WT mice. Only. We can thus conclude that the reduced TG level and induced NEFA and glycerol levels in serum is due to the increase of lipase activity upon treatment when both LXRα and LXRβ are present. This new mechanism by which LXR controls serum lipid metabolism during chronic treatment is of high interest, especially because the observed secondary effect of acute LXR treatment was a drastic increase of serum TG content, which has decreased the interest in using LXR as a target in the control of lipid homeostasis. In addition, it is interesting to note that LXRα−/− mice showed a 73% higher serum lipase activity than the WT mice on a chow diet (Fig. 1F). This could be the major mechanism by which LXRα−/− mice have a lower TG level, as already demonstrated by several groups (27, 29). Both LXRα−/− and LXRβ−/− mice showed increased serum NEFA and glycerol levels upon GW3965 treatment with no significant increase of serum lipase activity. SM is likely to be the main contributor to this effect in single-KO mice. Indeed, in both single-KO mouse groups, lipolytic gene expression levels were increased by GW3965 (Fig. 3D). This new finding is summarized in Fig. 5B.

We also provide evidence that LXRα plays a crucial role in the glyceroneogenesis pathway in liver and adipose tissue; an important pathway in lipid homeostasis. Lipids are released from the WAT and SM as NEFA and from the liver as TG. Thus, glyceroneogenesis, the synthesis of glycerol for TG production, affects lipid metabolism in opposite ways in the two tissues; it restrains NEFA release from the WAT and the SM and enhances it in the form of TG from the liver. LXR could be the link between the three tissues to ensure appropriate control of the flux of fuel. Indeed, in the current study we show that LXR activation reduces Ppck expression level in liver and WAT, the key enzyme of glycerol synthesis. Additional experiments are required to further explore this new pathway of LXR regulation of glyceroneogenesis. Accordingly, the serum TG (mainly released by the liver) level was reduced and the serum NEFA level was induced. Consequently, lipid oxidation was induced by treatment (Fig. 3B). In conclusion, we suggest that LXR could regulate lipid trafficking by controlling glyceroneogenesis and/or LPL activity. Clearly, additional studies are required to further explore this hypothesis.

Taken together, our observations highlight the complexity of LXR action in vivo in the regulation of metabolism. Since LXR pathways are involved in many different organs and could have opposite roles between organs, a better understanding of LXR action in vivo during chronic treatment is of interest. Here, we identify the LXR signaling pathway as a potential target for pharmacological modulation of lipid metabolism in skeletal muscle in long-term treatment. Thus, the development of tissue-selective and/or LXRα- and LXRβ-selective LXR modulators may allow differential regulation among liver, WAT, and skeletal muscle and thereby may be beneficial in the treatment of metabolic disorders. These findings give new insights into the role of LXR in the regulation of lipid metabolism and contribute to the understanding of LXR action between organs.

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**DISCLOSURES**

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