Skeletal Muscle Lipid Accumulation in Type 2 Diabetes May Involve the Liver X Receptor Pathway

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Liver X receptors (LXRs) are important regulators of cholesterol and lipid metabolism and are also involved in glucose metabolism. However, the functional role of LXRs in human skeletal muscle is at present unknown. This study demonstrates that chronic ligand activation of LXRs by a synthetic LXR agonist increases the uptake, distribution into complex cellular lipids, and oxidation of palmitate as well as the uptake and oxidation of glucose in cultured human skeletal muscle cells. Furthermore, the effect of the LXR agonist was additive to acute effects of insulin on palmitate uptake and metabolism. Consistently, activation of LXRs induced the expression of relevant genes: fatty acid translocase (CD36/FAT), glucose transporters (GLUT1 and -4), sterol regulatory element–binding protein-1c, peroxisome proliferator–activated receptor-γ, carnitine palmitoyltransferase-1, and uncoupling protein 2 and 3. Interestingly, in response to activation of LXRs, myotubes from patients with type 2 diabetes showed an elevated uptake and incorporation of palmitate into complex lipids but an absence of palmitate oxidation to CO_2. These results provide evidence for a functional role of LXRs in both lipid and glucose metabolism and energy uncoupling in human myotubes. Furthermore, these data suggest that increased intramyocellular lipid content in type 2 diabetic patients may involve an altered response to activation of components in the LXR pathway. ***Diabetes*** **54:1108–1115, 2005**

Liver X receptors (LXRs) are known as important actors in cholesterol and lipid metabolism (1–5) and have recently also been linked to glucose metabolism (6,7). Several studies have demonstrated that LXRs play an important role in fatty acid metabolism by directly or indirectly controlling the gene expressions of sterol regulatory element–binding protein-1c (SREBP-1c), fatty acid synthase (FAS), and stearoyl-CoA desaturase-1 (SCD-1) (8–11). Observations in liver suggest that the insulin-mediated activation of these genes is dependent on LXRs (10). In addition, LXRs have been proven to influence glucose metabolism and act as a regulator of GLUT4 gene expression in liver, adipose tissue, and skeletal muscle (6,7). Furthermore, accumulation of hepatic triacylglycerol has been reported as a consequence of chronic LXR agonist treatment in diabetic mice (12).

Recently, two studies demonstrated that treatment of skeletal muscle with the LXR receptor agonist T0901317 upregulated known LXR target genes such as FAS, SREBP-1c, apolipoprotein E, and ATP-binding cassette transporter A1 (3,13). However, no studies have fully addressed the functional role of LXRs in skeletal muscle, even though it accounts for ~40% of the total body weight and is known to be the major site of glucose utilization and fatty acid oxidation (14). Because previous studies describe LXRs to be important regulators of lipid and glucose metabolism, we initiated a study to determine whether LXRs play similar roles in human skeletal muscle and whether LXRs may play a role in the pathophysiology of insulin resistance and type 2 diabetes. In the present study, we used primary human skeletal muscle cells from both control and type 2 diabetic subjects to investigate the effects of chronic LXR agonist treatment (T0901317) on the uptake and metabolism of both palmitate and glucose and on the mRNA expression of relevant target genes.

RESEARCH DESIGN AND METHODS

Dulbecco's modified Eagle's medium (DMEM)-Glutamax, FCS, Ultroser G, penicillin-streptomycin-amphotericin B, and trypsin-EDTA were obtained from Life Technology (Paisley, U.K.). [1-^{14}C]palmitate (54 mCi/mmol), 2-[^{3}H(G)]deoxy-D-glucose (6.00 Ci/mmol), and D-[^{14}C(U)]glucose (12.10 mCi · mmol^{-1} · l^{-1}) were purchased from NEN Life Science Products (Boston, MA). D-[1-^{14}C]glucose (55 mCi/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). Palmitate, BSA (essentially fatty acid–free), cytochalasin B, and extracellular matrix gel were purchased from Sigma (St. Louis, MO). Insulin Actrapid was from Novo Nordisk (Bagsvaerd, Denmark). RNeasy minikit and RNase-free DNase were purchased from Qiagen Sciences (Oslo, Norway). The primers, SYBR Green, and TaqMan reverse-transcription reagents kit were obtained from Applied Biosystems (Warrington, U.K.). The protein assay kit was purchased from BioRad (Copenhagen, Denmark). T0901317 was obtained from Cayman (Ann Arbor, MI). All other chemicals used were of standard commercial high-purity quality.

Human study subjects. Eight obese type 2 diabetic patients (aged 50.1 ± 1.5 years) and eight lean control subjects (aged 51.3 ± 1.1 years) participated in the study. Only sedentary subjects were recruited. Diabetic patients were treated either with diet alone or in combination with either sulfonylurea, metformin, or insulin, which was withdrawn 1 week before biopsies were

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CD36/FAT, fatty acid translocase; CPT-1, carnitine palmitoyltransferase-1; DAG, diacylglycerol; DMEM, Dulbecco's modified Eagle's medium; FABP4, fatty acid–binding protein 4; FAS, fatty acid synthase; FFA, free fatty acid; LXR, liver X receptor; PDK4, pyruvate dehydrogenase kinase 4; PPAR, peroxisome proliferator–activated receptor; SCD-1, stearoyl-CoA desaturase-1; SREBP-1c, sterol regulatory element–binding protein-1c; UCP, uncoupling protein.

taken. The patients had no diabetic complications apart from simplex retinopathy. The control subjects had normal glucose tolerance and no family history of type 2 diabetes. The groups were matched with respect to age, but they differed by BMI (24.1 ± 0.6 vs. 33.2 ± 1.3 kg/m^2 for lean control vs. diabetic patients, $P < 0.01$), fasting plasma glucose concentrations (5.6 ± 0.1 vs. 10.3 ± 0.7 mmol/l, $P < 0.001$), fasting serum insulin levels (23.9 ± 6.5 vs. 91.9 ± 10.1 pmol/l, $P < 0.05$), hyperinsulinemic-euglycemic clamp (391.4 ± 20.2 vs. 119.1 ± 20.6 mg · min^{-1} · m^{-2}, $P < 0.001$), and HbA_{1c} (5.6 ± 0.1 vs. 8.0 ± 0.6, $P < 0.05$). Muscle biopsies were obtained from m. vastus lateralis in the fasted state by needle biopsy under local anesthesia. All subjects gave written informed consent, and the local ethics committee of Funen and Vejle County approved the study.

Cell culture. Cell cultures were established from satellite cells as previously described (15). Human myoblasts from control and type 2 diabetic subjects were allowed to differentiate at a physiological concentration of insulin (25 pmol/l) and glucose (5.5 mmol/l) for 4 days. On day 4, myotubes were exposed to different T0901317 concentrations (0.1, 0.5, and 1.0 μmol/l) for another 4 days before experiments were performed. Myotubes were rinsed twice and incubated for 4 h with either labeled palmitate or glucose. Corresponding values for palmitate uptake, lipid profile and oxidation, glucose uptake, glucose oxidation, and glycogen synthesis were determined at a basal insulin concentration (25 pmol/l) and under acute insulin stimulation (1 μmol/l) for 4 h. The protein content of each sample was determined (16). Also, corresponding RNA extractions were obtained.

Palmitate uptake and lipid distribution. Myotubes were exposed to DMEM supplemented with 0.24 mmol/l BSA, 0.5 mmol/l L-carnitine, 20 mmol/l HEPES, [1-^{14}C]palmitate (0.5 μCi/ml, 0.6 mmol/l), and 25 pmol/l or 1 μmol/l insulin for 4 h to study basal and insulin-mediated palmitate uptake and lipid distribution. Myotubes were placed on ice, washed three times with PBS (1 ml), harvested into a tube in two additions of 250 μl distilled water, and homogenized. The radioactivity in the cell fraction (50 μl) was quantified by liquid scintillation (Packard Tri-Carb 1900 TR) (17). The cells were later assayed for protein (16), and cellular lipids were extracted (17). Briefly, the homogenized cell fraction (300 μl) was extracted, lipids were separated by thin-layer chromatography, and the radioactivity was quantified by liquid scintillation.

Acid-soluble metabolites. Myotubes were exposed to DMEM supplemented with 0.24 mmol/l BSA, 0.5 mmol/l L-carnitine, [1-^{14}C]palmitate (0.5 μCi/ml, 0.6 mmol/l), and 25 pmol/l or 1 μmol/l insulin for 4 h to study basal and insulin-mediated palmitate oxidation. The incubation media were transferred to new tubes and assayed for labeled acid-soluble metabolites as previously described (18). Acid-soluble metabolites give an estimation of fatty acid β-oxidation.

CO_2. Myotubes were exposed to DMEM supplemented with 0.24 mmol/l BSA, 0.5 mmol/l L-carnitine, 20 mmol/l HEPES, [1-^{14}C]palmitate (0.5 μCi/ml, 0.6 mmol/l), and 25 pmol/l or 1 μmol/l insulin for 4 h to study basal and insulin-mediated palmitate oxidation as previously described (17).

Glucose uptake. Cultures were exposed to DMEM supplemented with 0.24 mmol/l BSA, 2-[^{3}H]deoxy-D-glucose (0.2 μCi/well), and 25 pmol/l or 1 μmol/l insulin for 4 h to study basal and insulin-mediated glucose uptake. Cells were solubilized by the addition of 500 μl of 0.1 mol/l NaOH. An aliquot (50 μl) was removed for protein determination (16). The remaining fluid was counted by liquid scintillation (19).

Glycogen synthesis. Cells were grown and differentiated in 12-well plates as described above. Cultures were exposed to DMEM supplemented with 0.24 mmol/l BSA, D-[^{14}C(U)]glucose (1.0 μCi/well), and 25 pmol/l or 1 μmol/l insulin for 4 h to study basal and insulin-mediated glycogen synthesis. The reaction was stopped after 4 h by aspirating the reaction mixture and rapidly rinsing each well four times with PBS at 4°C. Cells were solubilized by the addition of 500 μl of 1.0 mol/l KOH and heated at 70°C for 20 min. The samples were added 100 μl saturated Na_2SO_4, 100 μl 25 mg/ml glycogen in distilled water (freshly made), and 9 ml ice-cold absolute ethanol and then left at −70°C for 48 h to precipitate glycogen. The tubes were centrifuged (2000*g* for 20 min at 4°C), and the supernatant was immediately removed and discarded. The glycogen precipitate was redissolved in 500 μl distilled water by heating at 70°C for 10 min, 9 ml ice-cold absolute ethanol was added, and samples were left at −20°C for a minimum of 24 h. Again, the tubes were centrifuged, the supernatants were removed, 500 μl distilled water was added, and glycogen was dissolved and counted by liquid scintillation (19).

Glucose oxidation. Cultures were incubated with DMEM supplemented with 0.24 mmol/l BSA, [1-^{14}C]D-glucose (2.0 μCi/ml), and 25 pmol/l or 1 μmol/l insulin for 4 h to study basal and insulin-mediated glucose oxidation. Flasks were made airtight by using a rubber stopper. After 4 h, 300 μl phenyl ethylamine-methanol (1:1, vol/vol) were added with a syringe to a center well containing a folded filter paper. Perchloric acid (300 μl, 1.0 mol/l) was subsequently added to the cells through the stopper tops using a syringe. The flasks were placed for a minimum of 1 h at room temperature to trap labeled CO_2. Cell-free flasks (no-cell controls) were included to correct for unspecific CO_2 trapping (19).

RNA isolation and analysis of gene expression by real-time RT-PCR. Myotubes were washed and pelleted before total RNA was isolated by an RNeasy minikit (Qiagen Sciences) according to the supplier's total RNA isolation protocol. RNA samples were incubated with RNase-free DNase (Qiagen Sciences) for a minimum of 15 min in an additional step during the RNA isolation procedure. Total RNA (0.1 μg/μl) was reverse-transcribed with a TaqMan reverse-transcription reagents kit (Applied Biosystems), using a Perkin-Elmer Thermal Cycler 9600 (25°C for 10 min, 37°C for 1 h, and 99°C for 5 min). Real-time quantitative PCR was performed using an ABI Prism 7000 Detection System. DNA expression was determined by SYBR Green (Applied Biosystems), and the following primers were designed using Primer Express (Applied Biosystems): GLUT1 (accession no. K03195), GLUT4 (accession no. M20747), SREBP-1c (accession no. U00968), uncoupling protein 2 (UCP2; accession no. AF019409.1), UCP3 (accession no. AF050113), pyruvate dehydrogenase kinase 4 (PDK4; accession no. BC040239), peroxisome proliferator–activated receptor-γ (PPAR-γ; accession no. L40904), LXR-α (accession no. U22662), LXR-β (accession no. U07132), fatty acid translocase (CD36/FAT; accession no. L06850), carnitine palmitoyltransferase-1 (CPT-1; accession no. L39211), fatty acid–binding protein 4 (FABP4; accession no. J02874), myogenin (accession no. X17651), and β-actin (accession no. NM 001101). Each target gene was quantified in quadruplicate and carried out in a 25-μl reaction volume according to the supplier protocol. All assays were run for 40 cycles (95°C for 12 s followed by 60°C for 60 s). The transcription levels were normalized to the housekeeping control gene β-actin, and there were no differences in expression between control and type 2 diabetic subjects ($P = 0.4$). Another house-keeping control gene tested, 36B4, gave similar results as β-actin.

Statistical analysis. Data in text and figures are given as means ± SE, and all experiments were run in triplicate or quadruplicate. Comparisons of different treatments and groups were evaluated by ANOVA and Fisher's protected least-significant difference (StatView; SAS Institute, Cary, NC). Dose-response effects were evaluated by paired Students' *t* test, and $P \leq 0.05$ was considered significant.

RESULTS

Cell culture. T0901317 treatment showed no difference in appearance during phase contrast microscopy (not shown). To confirm the purity of the cell culture, the expression of myogenin and the adipocyte marker FABP4 were measured. Basal expression of myogenin for control and type 2 diabetic myotubes was found to be equal ($P = 0.85$). FABP4 expression was very low, but no differences could be detected between basal and T0901317-treated myotubes (not shown). LXR agonist treatment proved not to be toxic to the cells, as verified by measurements of cellular protein content for control myotubes (0.22 ± 0.03 vs. 0.23 ± 0.03 mg protein/well for basal vs. 1 μmol/l T0901317, $P = 0.3$) and type 2 diabetic myotubes (0.19 ± 0.02 vs. 0.19 ± 0.02 mg protein/well, $P = 0.7$).

Palmitate uptake. Palmitate uptake increased in a dose-dependent manner after LXR agonist treatment and additional insulin further increased palmitate uptake for both control and type 2 diabetic myotubes (Fig. 1). Control myotubes showed a 45% increased palmitate uptake after T0901317 treatment (1 μmol/l) and a 31% increased uptake after insulin treatment, and treatment with both agents showed an additive effect with an 86% increase in palmitate uptake (Fig. 1). Most interestingly, the LXR agonist–induced (1 μmol/l) palmitate uptake was further increased in myotubes established from type 2 diabetic subjects compared with control subjects (Figs. 1 and 2*F*).

Lipid distribution. Basal incorporation of labeled palmitate into various lipid classes showed that cellular levels of free fatty acids (FFAs), diacylglycerol (DAG), triacylglycerol, phospholipids, and cholesteryl ester were increased after exposure to T0901317 (1μmol/l) (Fig. 2, ■). Levels of

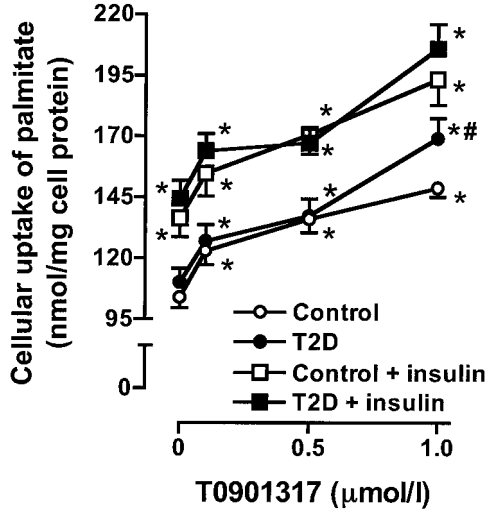

FIG. 1. Total palmitate uptake after 4 days of pretreatment with T0901317 and 4 h of incubation with insulin. Differentiated myotubes were pretreated with 0, 0.1, 0.5, and 1 μmol/l T0901317 for 4 days and incubated with [1-^{14}C]palmitate (0.6 mmol/l) for 4 h ± 1 μmol/l insulin. Results are the means ± SE (n = 7–8). *$P \leq 0.05$ vs. basal; #$P \leq 0.05$ vs. control myotubes. T2D, type 2 diabetes.

FFAs, DAG, and triacylglycerol were increased twofold after treatment with T0901317. In the presence of acute insulin, FFAs and DAG increased by 30%, and triacylglycerol was elevated twofold (Fig. 2, *light-hatched columns*). The combined effect of T0901317 and insulin further increased incorporation of palmitate into FFAs, DAG, and triacylglycerol (Fig. 2). Phospholipid levels were also elevated after exposure to insulin and insulin + T0901317, although not to the same extent as FFAs, DAG, and triacylglycerol, whereas cholesteryl ester only responded to T0901317 (Fig. 2*E*). Myotubes from type 2 diabetic subjects showed an elevated level of free palmitate and an increased incorporation of palmitate into complex lipids compared with control cells after LXR agonist treatment (Fig. 2*B*, *C*, *E*, and *F*). In addition, it should be noted that in type 2 diabetic cells, basal incorporation of palmitate into triacylglycerol was also further increased by 57%, and incorporation into cholesteryl ester was increased by 66% (Figs. 2*C* and *E*).

Lipid oxidation. Fatty acid oxidation was evaluated by measurements of acid-soluble metabolites representing fatty acid β-oxidation (Fig. 3*A*) and CO_2 representing complete oxidation of palmitate (Fig. 3*B*). After T0901317 treatment, acid-soluble metabolite formation was increased by 20% in both control and type 2 diabetic myotubes (Fig. 3*A*). Moreover, CO_2 formation was elevated by 20% (Fig. 3*B*) after exposure to T0901317 in control but not in diabetic myotubes (Figs. 3*B* and 2*F*). Insulin had no detectable effect on palmitate oxidation. Partitioning of labeled palmitate between fatty acid oxidation (acid-soluble metabolites) and complex lipids (Fig. 3*C*) demonstrated that treatment with T0901317, insulin, and the combination of insulin and T0901317 changed palmitate distribution in favor of lipid accumulation compared with palmitate oxidation (acid-soluble metabolites). No statistical differences between control and type 2 diabetic cells were observed, although there was a ten-

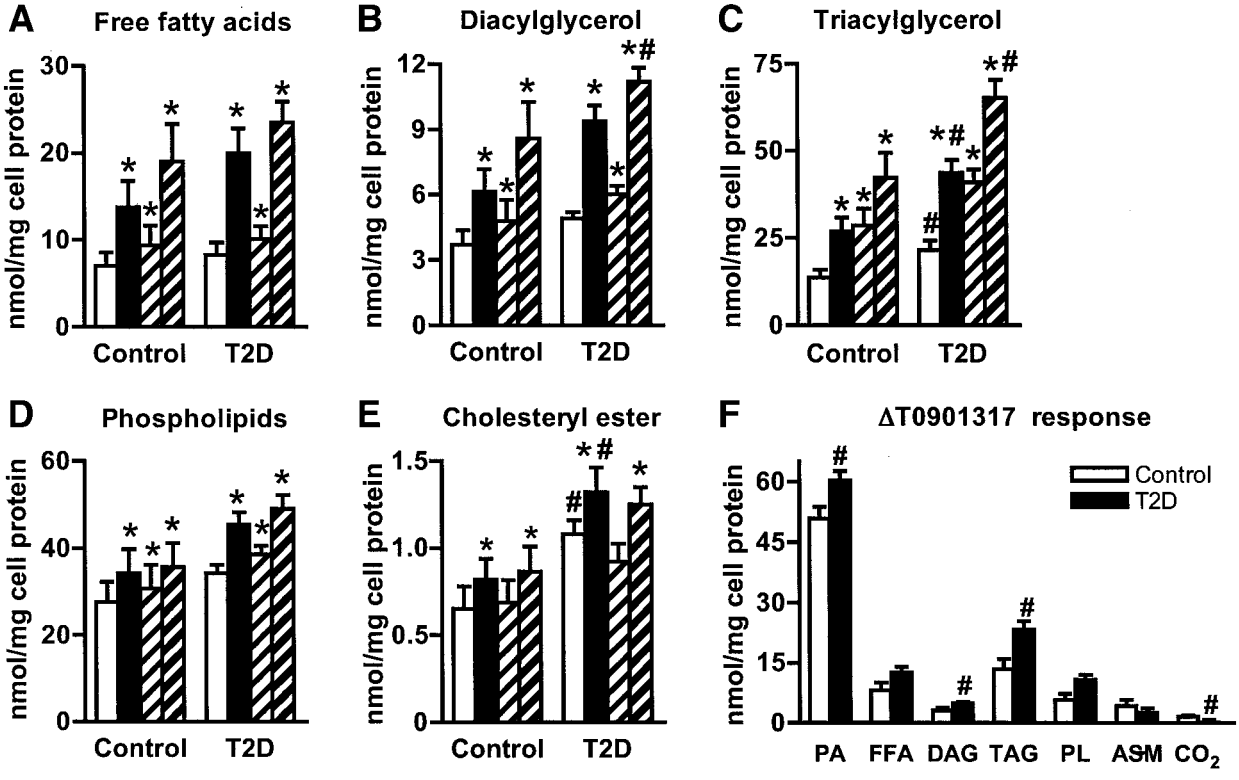

FIG. 2. Palmitate distribution into cellular lipids after pretreatment with T0901317 and insulin. Differentiated myotubes were pretreated with 1 μmol/l T0901317 as described in Fig. 1. The figure shows cellular free palmitate (*A*), palmitate incorporated into various lipid classes (*B–E*), and ΔT0901317 response (*F*). *A*: FFAs. *B*: DAG. *C*: Triacylglycerol. *D*: Phospholipids. *E*: Cholesteryl ester. Results are the means ± SE (n = 8). The different scaling of y-axes should be noted. *$P \leq 0.05$ vs. basal; #$P \leq 0.05$ vs. control myotubes. □, basal; ■, T0901317; light-hatched bars, insulin; dark-hatched bars, T0901317 + insulin. ASM, acid soluble metabolites; PA, palmitate; PL, phospholipids; T2D, type 2 diabetes; TAG, triacylglycerol.

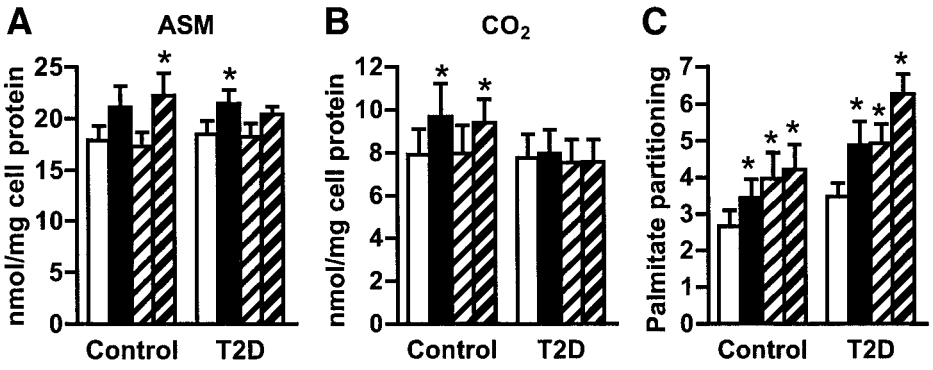

FIG. 3. Palmitate oxidation after pretreatment with T0901317 and insulin. Differentiated myotubes were pretreated with 1 μmol/l T0901317 as described in Fig. 1. The diagrams show palmitate oxidation to acid-soluble metabolites (*A*), CO_2 (*B*), and partitioning of labeled palmitate between the sum of complex lipids divided by acid-soluble metabolites (*C*). Results are the means ± SE (n = 7–8). *$P \leq 0.05$ from basal. □, basal; ■, T0901317; light-hatched bars, insulin; dark-hatched bars, T0901317 + insulin. T2D, type 2 diabetes.

dency toward a more profound lipid accumulation in type 2 diabetic myotubes (Fig. 3*C*).

Expression of genes involved in lipid metabolism. The expression of certain genes important for fatty acid transport and accumulation were measured after T0901317 treatment, and mRNA levels of LXR-α, LXR-β, and SREBP-1c were measured to confirm the expected gene responses to the LXR agonist and to detect any possible differences between control and type 2 diabetic myotubes. The expression of LXR-α (Fig. 4*A*) was increased 7.5-fold, SREBP-1c (Fig. 4*C*) 6- to 8-fold, and PPAR-γ (Fig. 4*D*) 2.5-fold, and the increases were similar in control and type 2 diabetic cells. LXR-β, on the other hand, was increased significantly for control myotubes by 60% (Fig. 4*B*). The mRNA expression of CD36/FAT and CPT-1 (Fig. 4*E* and *F*) showed a 2.6-fold and a 30–60% increase, respectively.

Glucose uptake. T0901317 has been reported to improve glucose tolerance in vivo in mice (7). This observation may result from increased glucose uptake into skeletal muscle. Insulin-stimulated glucose uptake in control and type 2 diabetic myotubes increased from basal by 30% on average for all T0901317 concentrations examined (Fig. 5*A*). No differences were observed in insulin-stimulated glucose uptake between the groups. On the other hand, basal glucose transport in control myotubes was significantly increased by 15% after chronic exposure to T0901317 (1 μmol/l), whereas no response in type 2 diabetic cells was observed (data not shown).

Glycogen synthesis and glucose oxidation. To further evaluate intracellular glucose turnover after T0901317 treatment, glycogen synthesis and glucose oxidation were studied. As previously shown (20), glycogen synthesis was decreased on average by 30% in type 2 diabetic cells compared with control myotubes in both the absence and presence of insulin, and LXR agonist treatment did not change glycogen synthesis (data not shown). In contrast, glucose oxidation assessed by CO_2 trapping was markedly increased by the LXR agonist (Fig. 5*B*). Oxidation of glucose increased by 65% in control myotubes and 45% in type 2 diabetic myotubes, and no additive effect of insulin was seen.

Expression of genes involved in glucose metabolism. Pretreatment of myotubes with the LXR agonist increased both basal and insulin-stimulated glucose transport and especially glucose oxidation. In support of these findings, levels of GLUT1 mRNA showed a 60–80% increase in both control and type 2 diabetic myotubes after T0901317 treatment (Fig. 6*A*), whereas GLUT4 mRNA levels were increased 5.5- to 6.5-fold (Fig. 6*B*). No differences in mRNA expression were observed for GLUT1 or -4 between type 2 diabetic and control myotubes. PDK4, a negative regulator of glycolysis (21,22), was increased by 50% after exposure to T0901317 in both control and type 2 diabetic cells, but it reached statistical significance only in diabetic myotubes (data not shown).

Expression of UCPs. Overexpression of UCP3 in human myotubes has recently been shown to increase glucose uptake, glucose oxidation, and lipid oxidation (23). To possibly explain some of our resembling observations, we investigated whether mRNA expression for UCP2 and -3 were increased by T0901317 treatment of myotubes. T0901317 increased mRNA expression twofold for both UCP2 and -3 in both control and diabetic myotubes (Figs. 7*A* and *B*).

DISCUSSION

The present study shows that chronic treatment of human myotubes with the LXR agonist T0901317 increases cellu-

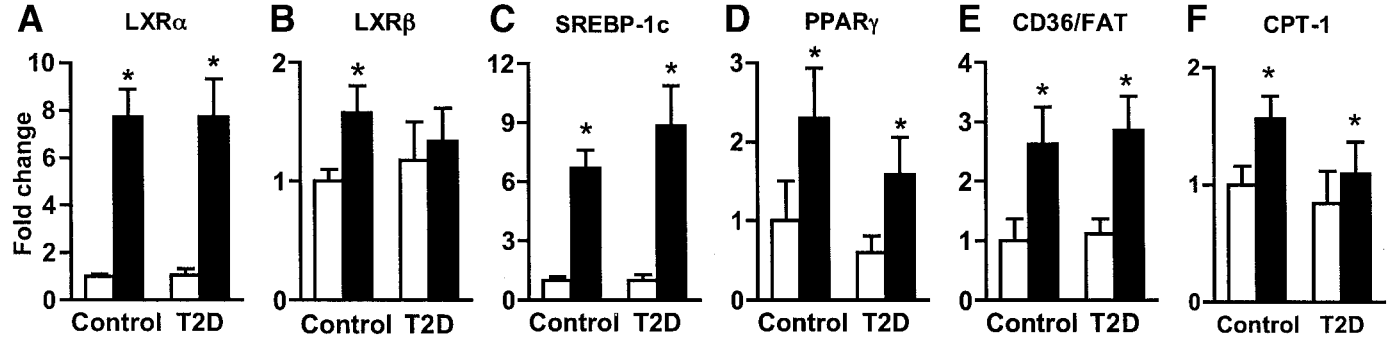

FIG. 4. mRNA expression of LXR-α (*A*), LXR-β (*B*), SREBP-1c (*C*), PPAR-γ (*D*), CD36/FAT (*E*), and CPT-1 (*F*) after pretreatment with T0901317. Differentiated myotubes were pretreated with 1 μmol/l T0901317 (■) for 4 days. Total RNA was extracted, reverse-transcribed, and analyzed by real-time RT-PCR. Results are normalized to levels of β-actin and presents means ± SE (n = 6–8). *$P \leq 0.05$ vs. basal. □, basal. T2D, type 2 diabetes.

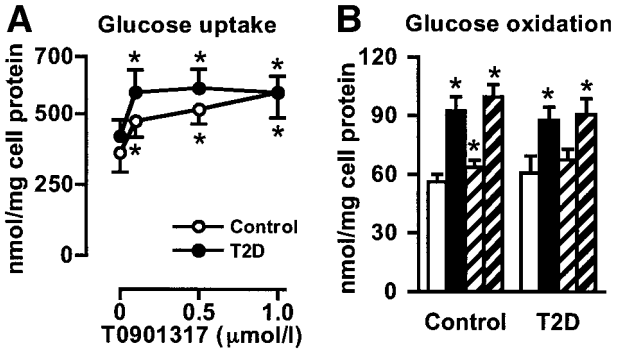

FIG. 5. Glucose uptake (*A*) and glucose oxidation (*B*) after pretreatment with T0901317. *A*: Differentiated myotubes were pretreated with 0, 0.1, 0.5, and 1 μmol/l T0901317 for 4 days and incubated with [^{3}H]2-deoxy-D-glucose ± 1 μmol/l insulin for 4 h. The curves represent insulin-stimulated glucose uptake as the means ± SE (n = 8). *B*: Differentiated myotubes were pretreated with 1 μmol/l T0901317 for 4 days before incubation for 4 h with [1-^{14}C]glucose ± 1 μmol/l insulin. CO_2 formation is the means ± SE (n = 6–7). ★$P \leq 0.05$ vs. basal. □, basal; ■, T0901317; light-hatched bars, insulin; dark-hatched bars, T0901317 + insulin. T2D, type 2 diabetes.

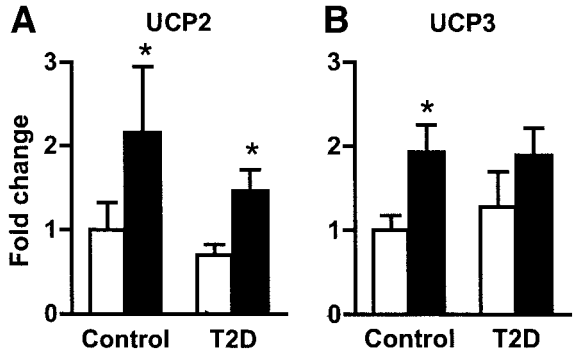

FIG. 7. mRNA expression of UCP2 (*A*) and UCP3 (*B*) after pretreatment with T0901317. Differentiated myotubes were pretreated as described in Fig. 4. Results are normalized to levels of β-actin and are the means ± SE (n = 7–8). ★$P \leq 0.05$ vs. basal. □, basal; ■, 1 μmol/l T0901317. T2D, type 2 diabetes.

lar uptake of palmitate, its incorporation into complex lipids (despite elevated palmitate oxidation), and cellular uncoupling. Glucose oxidation and glucose uptake were also increased, whereas glycogen synthesis remained unaffected. Moreover, LXR activation increased palmitate uptake and accumulation into lipids to a higher extent in type 2 diabetic myotubes and did not increase palmitate oxidation to CO_2. These findings imply that the increased intramyocellular lipid levels found in muscles from type 2 diabetic subjects may be related to altered response of the LXR pathway.

Previous in vivo animal and cell culture experiments suggest that LXRs play an important role in lipid accumulation. It has been shown in rodents that LXR activates the coordinate expression of major hepatic fatty acid biosynthetic genes (lipogenesis) (5). LXR agonist treatment has also been reported to increase lipid accumulation in differentiating adipocytes, and fat deposits in LXR-α/-β–deficient mice have been shown to be smaller than in age-matched wild-type littermates (1,24). In this study T0901317 treatment was linked to increased palmitate uptake and incorporation into cellular lipids. An important factor involved in fatty acid uptake and intracellular transport is CD36/FAT. This protein is ubiquitously expressed, its expression correlates with long-chain fatty acid uptake into both heart and muscle, and it is induced by PPAR-γ activation (25). It has recently been found that CD36/FAT can be translocated to the plasma membrane within minutes in response to muscle contraction and insulin to increase long-chain fatty acid uptake (26). The upregulation of CD36/FAT mRNA levels seen after T0901317 treatment in our study may explain the increased palmitate uptake. In support of this finding, we observed that chronic LXR agonist treatment increased the mRNA level of both PPAR-γ and SREBP-1c, which suggests that LXR could influence CD36/FAT expression, either by a direct or indirect effect through PPAR-γ (25). However, direct regulation of CD36/FAT by LXRs has not previously been described. An indirect effect through SREBP-1c is more likely because SREBP-1c has previously been described to upregulate PPAR-γ (27), and 4 days of treatment is sufficient time for this to occur.

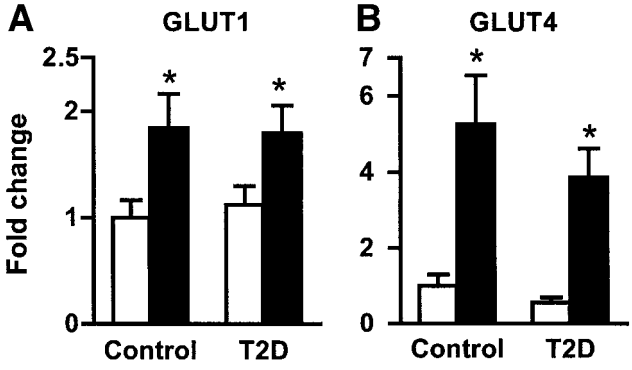

FIG. 6. mRNA expression of GLUT1 (*A*) and GLUT4 (*B*) after pretreatment with T0901317. Differentiated myotubes were pretreated as described in Fig. 4. Results are normalized to levels of β-actin and are the means ± SE (n = 7–8). ★$P \leq 0.05$ vs. basal. □, basal; ■, 1 μmol/l T0901317. T2D, type 2 diabetes.

Interestingly, we observed a significant increase in LXR-β mRNA levels for control myotubes after LXR agonist treatment, although this was not observed for type 2 diabetic myotubes. The two LXR subtypes, LXR-α and -β, probably do not play identical roles in vivo, as judged by the phenotypes of knockout mice. LXR-α knockout mice massively accumulate cholesterol on cholesterol feeding and seem to exert a more noticeable effect on lipid storage (9,28). No observable phenotype has been ascribed to LXR-β knockout mice, but it is reported that LXR-β activation promotes less lipogenesis than LXR-α. It is also possible that different gene selectivities for LXR-α and -β can be explained by different ratios in different tissues (29) where muscle express much more LXR-β than -α. However, studies to support this hypothesis have yet to be carried out.

T0901317 increased not only palmitate uptake but also the accumulation of labeled palmitate into cellular lipids. LXR agonist treatment was reported to increase lipid accumulation in differentiating adipocytes (1,24) and cellular triacylglycerol content in macrophage-forming foam cells (30). However, the effect of LXR agonists on lipid composition has not previously been investigated. We observed that all palmitate-derived lipid fractions increased in the myotubes and that the triacylglycerol and DAG pools showed the highest fold change.

The current study also shows that T0901317 increases

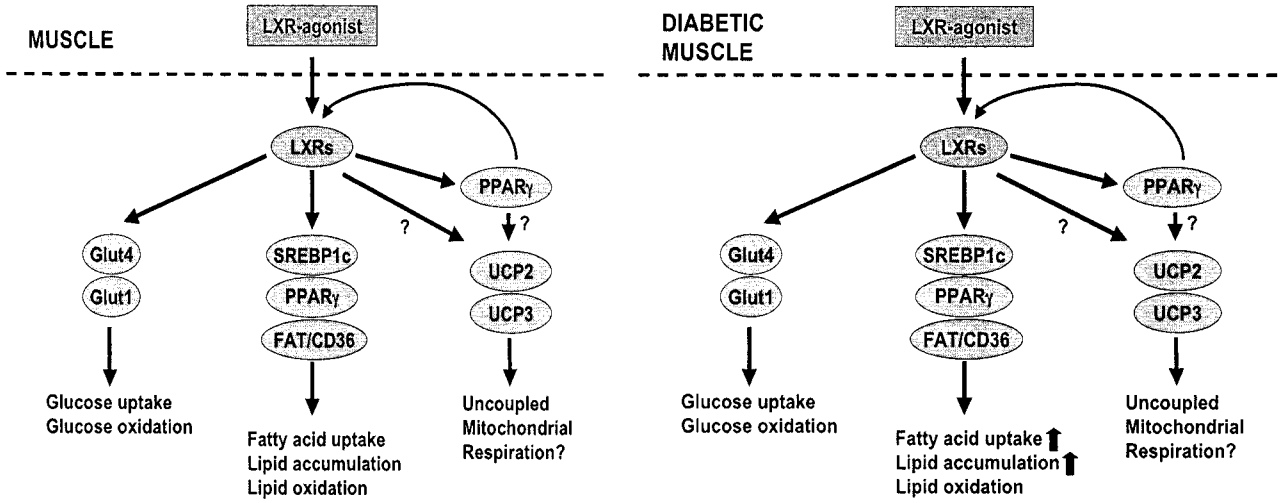

FIG. 8. A role for LXRs in lipid and glucose metabolism in human skeletal muscle. Chronic LXR activation led to increased glucose uptake and oxidation and increased uptake and accumulation of palmitate into lipids in myotubes. Type 2 diabetic myotubes showed enhanced uptake and accumulation of palmitate, which implies that increased triacylglycerol found in type 2 diabetic subjects muscle could be related to an altered response of the LXR pathway.

palmitate oxidation to acid-soluble metabolites for both groups, whereas CO_2 was only increased in control myotubes. In line with this, Campbell et al. (31) suggested a role for CD36/FAT in mitochondrial fatty acid uptake, and they suggested that CD36/FAT mitochondrial content matched the increased capacity for fatty acid oxidation, possibly involving translocation of CD36/FAT to the mitochondria. Moreover, we observed a similar increase in CPT-1 expression for control as well as type 2 diabetic myotubes, which mainly regulates mitochondrial fatty acid oxidation (32). This did not explain the lack of increased CO_2 formation in type 2 diabetic myotubes after LXR activation, the mechanisms of which need further characterization. Finally, data showing partitioning of palmitate between fatty acid oxidation and formation of complex lipids clearly demonstrate a shunting of palmitate toward lipids after exposure to the LXR agonist. Thus, the observed increase in palmitate oxidation was not sufficient to handle the increased palmitate uptake.

Interestingly, the increase in intramyotubular lipids did not induce insulin resistance. Quite the contrary, the acute effects of insulin was additive to the chronic effect of the LXR agonist on palmitate uptake and metabolism. In the presence of insulin, palmitate partitioning was also further changed toward increased lipid synthesis. Similar acute effects of insulin on palmitate metabolism have previously been reported in human myotubes (17), and LXR has been suggested to function as a regulatory factor in insulin-mediated fatty acid metabolism in hepatocytes and LXR-α/-β knockout mice (10).

Chronic treatment of human myotubes with the LXR agonist resulted in increased glucose transport in the presence of insulin and markedly elevated glucose oxidation to CO_2. The increase observed in glucose transport correlated with the elevated mRNA levels detected for GLUT1 in control cells and for GLUT4 in both groups, although the increase in mRNA levels for these glucose transporters suggested a more profound effect than that shown by the functional data. Previously, Tortorella et al. (33) showed that C2C12 treated with dexametasone caused a 10-fold increase in GLUT4 expression in myoblasts, without altering insulin-stimulated glucose transport, despite normal expression of vesicle-associated membrane proteins. LXRs have also been shown to upregulate GLUT1 mRNA and protein in 3T3-L1 adipocytes (34), suggesting that the increase in basal glucose uptake may be mediated through GLUT1. Moreover, Dalen et al. (6) reported that GLUT4 contains a conserved LXR-responsive element directly upregulated by LXR in both human and mouse adipocytes. They also showed a slight increase in mRNA levels for GLUT4 in mouse skeletal muscle (6). Laffitte et al. (7) demonstrated that LXR activation by T0901317 in mice improves glucose tolerance in vivo and that GLUT4 is upregulated in adipose tissue.

To our knowledge, it has not previously been described that LXR stimulation influences glucose oxidation, as demonstrated in our current study. We measured the mRNA expression of PDK4 in control myotubes and were not able to verify an increased PDK4 expression, thus suggesting that the PDK4 expression level may not be central to regulating glucose oxidation in our cells.

Furthermore, UCP2 and -3 mRNA expression was significantly increased during chronic T0901317 stimulation. Overexpression of UCP3 in human myotubes has recently been shown to increase glucose uptake, glucose oxidation, and lipid oxidation (23), thus questioning whether a part of our findings may be secondary to increased UCP2 and -3 expression. There is no literature supporting a direct regulation of UCPs by LXRs. However, evidence that increasing levels of cellular lipids could induce expression of UCPs by acting as ligands for PPARs (35) supports our hypothesis that upregulation of LXRs can indirectly influence UCP expression. Despite elevated UCP3 expression, possibly indicating increased energy expenditure, treatment of cells with T0901317 appears to cause a relatively higher increase in energy intake rather than energy expenditure, thereby allowing healthy muscle to accumulate lipids.

In this study, we also compared myotubes established from control subjects with cells from obese type 2 diabetic

subjects. Type 2 diabetes is associated with an increase in intramuscular fatty acid metabolites, such as long-chain acyl-CoA, DAG, and triacylglycerol (36–40). We observed significantly higher palmitate uptake in type 2 diabetic myotubes and further elevated levels of intracellular complex lipids, whereas basal glucose uptake was not increased. Thus, type 2 diabetic myotubes seem to be more responsive to T0901317 treatment with respect to palmitate uptake and lipid accumulation compared with control myotubes. In addition, T0901317 failed to increase CO_2 formation from palmitate in type 2 diabetic cells, as was seen in control myotubes, suggesting that the diabetic myotubes have lower energy turnover, conserving more energy for storage as lipids than control myotubes. Moreover, it has been observed that lipid oxidation is reduced in muscle fibers from obese and type 2 diabetic subjects (41,42) and that complete palmitate oxidation (CO_2 production) is decreased in type 2 diabetic myotubes (17).

A recent study in *db/db* mice showed that 12 days of treatment with the LXR agonist T0901317 resulted in more severe hypertriglyceridemia and hepatic triacylglycerol accumulation in diabetic mice than that observed in nondiabetic control animals (12). In the current study, the exaggerated increase in lipid accumulation seen in type 2 diabetic myotubes was not supported by a more profound increase in mRNA levels of CD36/FAT, SREBP-1c, PPAR-γ, or LXR-α compared with control myotubes, so the molecular basis for the discrepancy between control and diabetic myotubes remains unclear at present.

Altogether, our study shows that chronic LXR activation by T0901317 results in increased uptake and accumulation of palmitate into lipids in myotubes, which is, however, enhanced in type 2 diabetic myotubes partly because there is no increase in palmitate oxidation to CO_2. These findings imply that activation of LXR may in part be responsible for the accumulation of intracellular lipids in skeletal muscle and furthermore that the increased intramyocellular triacylglycerol found in muscle from type 2 diabetic subjects could be related to an altered response of the LXR pathway (Fig. 8).

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