Tissue-specific autoregulation of the LXRα gene facilitates induction of apoE in mouse adipose tissue

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Abstract The functions of the liver X receptors (LXRs) are not well documented in adipose tissue. We demonstrate here that expression of the LXRα gene is highly induced in vivo and in vitro in mouse and human adipocytes in the presence of the synthetic LXR agonist T0901317. This autoregulation is caused by an identified LXR-responsive element motif in the mouse LXRα promoter, which is conserved in the human LXRα promoter. Using different LXR-deficient mice, we demonstrate that the basal expression level of LXRα is increased in LXRβ$^{-/-}$ mice, whereas the basal expression level of LXRβ is unchanged in LXRα$^{-/-}$ mice. The two LXRs can compensate for each other in mediating ligand-activated regulation of LXR target genes involved in lipid homeostasis in adipose tissue. Sterol regulatory element binding protein-1 (SREBP-1), ATP binding cassette transporter A1 (ABCA1), ABCG1, as well as apolipoprotein E (apoE) are induced in vivo by T0901317 in wild-type, LXRα$^{-/-}$ or LXRβ$^{-/-}$ mice but not in LXRα$^{-/-}$β$^{-/-}$ mice. Although SREBP-1 and ABCG1 are induced in liver, muscle, and adipose tissue, the apoE, glucose transporter-4 (GLUT4), and LXRα genes are specifically induced only in adipose tissue. We suggest that an important aspect of LXRα autoregulation in adipose tissue may be to increase the level of LXRα over a threshold level necessary to induce the expression of certain target genes.—Ulven, S. M., K. T. Dalen, J-Å. Gustafsson, and H. I. Nebb. **Tissue-specific autoregulation of the LXRα gene facilitates induction of apoE in mouse adipose tissue. *J. Lipid Res.* 2004.** 45: **2052–2062.**

Supplementary key words liver X receptor • sterol regulatory element binding protein-1 • peroxisome proliferator-activated receptor γ • ATP binding cassette • lipid homeostasis • apolipoprotein E

The nuclear liver X receptors LXRα (NR1H3) and LXRβ (NR1H2) belong to the superfamily of ligand-activated transcription factors (1–3). They are activated by cholesterol derivatives and metabolites, including oxysterols such as 22(*R*)-hydroxycholesterol and 24(*S*),25-epoxycholesterol (4, 5), and bind as heterodimers with the retinoid X receptor (RXR) to direct repeat 4 (DR-4)-type sequence motifs, termed LXR-responsive elements (LXREs), in the regulatory regions of target genes. LXRβ is ubiquitously expressed in adults (1), whereas the expression of LXRα is predominantly restricted to tissues known to play important roles in lipid metabolism, such as liver, kidney, macrophages, small intestine, spleen, and adipose tissue (3, 6). Targeted disruption of the LXRα gene in mice uncovered roles for this receptor in the regulation of hepatic bile acid synthesis, hepatic lipogenesis, and intestinal cholesterol absorption (7–9). Recently, LXRs have also been shown to be involved in cholesterol efflux and reverse cholesterol transport (9–12). This indicates an important regulatory role of LXRs in several metabolic signaling pathways in the body.

Greater knowledge about adipose tissue has become increasingly more important in light of the increasing incidence of obesity and its associated disorders, such as type 2 diabetes and cardiovascular diseases. Despite the fact that both LXRs are highly expressed in adipose tissue, few studies have focused on the role of LXRs in this tissue (10, 13–16). The mechanisms controlling expression of the LXRα gene also are not well understood. We demonstrated previously that the peroxisome proliferator-activated receptor γ (PPARγ) directly regulates LXRα mRNA expression in 3T3-L1 mouse adipocytes (15). This cross-talk has also been demonstrated in macrophages (17, 18). In addition, LXRs regulate the human LXRα gene in macrophages through an identified LXRE in the human LXRα promoter (19–21). We have previously demonstrated that LXR activation in vitro in mature 3T3-L1 cells induces the accumulation of lipid droplets (15). In addition, we observed an increase in the expression of the LXRα gene and sterol regulatory element binding protein-1 (SREBP-1)

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Abbreviations: apoE, apolipoprotein E; CMC, carboxymethyl-cellulose; DR-4, direct repeat 4; GLUT4, glucose transporter-4; LXR, liver X receptor; LXRE, LXR-responsive element; PPARγ, peroxisome proliferator-activated receptor γ; RXR, retinoid X receptor; SREBP-1, sterol regulatory element binding protein-1; WAT, white adipose tissue.

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in response to LXR activation in vivo in mouse adipose tissue, which indicates that LXR activation may induce lipogenesis in adipose tissue (15). Recent reports have also linked LXR to metabolic disorders such as type 2 diabetes and insulin resistance (13, 16). It has been demonstrated that activation of LXRs in adipose tissue increases basal glucose uptake (16, 22). Recently, we demonstrated that the expression of glucose transporter-4 (GLUT4) in adipose tissue is directly regulated by LXRs (13) and that the basal expression of GLUT4 is selectively dependent on LXRα.

Despite the fact that adipose tissue contains the largest pool of nonesterified cholesterol in the body (23), little is known about the role of LXR in cholesterol efflux and trafficking in this tissue. Several studies have shown that efflux of free cholesterol from cells is accomplished through a pathway involving the cholesterol/phospholipid transporter ABCA1 and apolipoprotein E (apoE) (24–26), and the roles of LXRs in cholesterol efflux and reverse cholesterol transport in macrophages and intestine are well established (9–12). In addition, other members of the ATP binding cassette transporters, such as ABCG1, have been suggested to be involved in lipid efflux in macrophages, although their functions are less well understood (27, 28). Both ABCA1 and apoE genes are expressed in adipose tissue (10, 29), and apoE is induced by administration of a synthetic LXR activator in adipose tissue (10). Recently, apoD, a member of the lipocalin family of transporters (30) involved in lipid transport, has been demonstrated to be a direct LXR target gene in adipose tissue, linking LXR activation in adipose tissue to ligand transport and reverse cholesterol transport (14).

Here, we demonstrate that the expression of the murine LXRα gene is autoregulated via a conserved LXRE motif in the LXRα promoter. Studies in LXR-deficient mice treated with T0901317 demonstrate that both LXRs are able to mediate ligand-activated induction of target genes in lipid homeostasis in adipose tissue. We further demonstrate that the apoE and LXRα genes are specifically induced in adipose tissue and not in liver and muscle, suggesting that autoregulation of LXRα is important to increase the level of LXRα necessary to induce the expression of certain target genes in the presence of an activator.

MATERIALS AND METHODS

Materials and expression vectors

Restriction enzymes were purchased from Promega (Madison, WI). Cell culture plastic ware was obtained from Corning, Inc. (Corning, NY). Cell culture media (#D6546 and #D6421), carboxymethyl-cellulose (CMC), oligonucleotides, and other chemicals were obtained from Sigma (St. Louis, MO). T0901317 was obtained from Alexis (Lausen, Switzerland).

The pCMX, pCMX-mRXRα, and pCMX-hLXRα expression vectors (31) were kindly provided by D. J. Mangelsdorf (Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX). A hLXRβ cDNA inserted into pcDNA3 kindly provided by Peter Akerblad (Aztra Zeneca) was cut out with *Kpn*I and *Bam*HI and ligated into the pCMX vector to generate the pCMX-hLXRβ expression vector.

Cells

COS-1 and 3T3-L1 cells (ATCC) were cultured in DMEM supplemented with 10% fetal calf serum (F#7524; Sigma), 2 mM L-glutamine, penicillin (50 U/ml), and streptomycin (50 μg/ml) at 37°C in 5% CO_2. Cells were not allowed to grow confluent before the experiments.

3T3-L1 cells and human SGBS cells were cultured and differentiated into adipocytes by the addition of adipogenic factors as previously described (13, 32). For gene expression studies in fully differentiated adipocytes, cells were treated for 24 h with 0.1 μM T0901317 (SGBS) or 1 μM T0901317 (3T3-L1). These concentrations were chosen based on initial dose-response experiments (data not shown).

Animal experiments

All animal use was approved and registered by the Norwegian Animal Research authority in Norway and the regional ethical committee for animal experiments in Sweden. Male C57BL/6J mice 10 weeks of age (25–30 g; B and K Universal Ltd., Sollentuna, Sweden), wild-type LXRα$^{+/+}$β$^{+/+}$ (controls), LXRα$^{-/-}$β$^{+/+}$, LXRα$^{+/+}$β$^{-/-}$, and LXRα$^{-/-}$β$^{-/-}$ mice (7–9 weeks old) were maintained in a temperature-controlled (22°C) facility with a strict 12 h light/dark cycle and given free access to food and water. The generation of the LXRα$^{-/-}$β$^{+/+}$, LXRα$^{+/+}$β$^{-/-}$, and LXRα$^{-/-}$β$^{-/-}$ mice has been described elsewhere (7, 33). All LXR transgenic mice and corresponding controls used in this study had mixed genetic backgrounds based on 129/Sv and C57BL/6J strains, backcrossed in C57BL/6J mice for six generations. Correct genotypes of the transgenic mice were confirmed by the altered LXRα and LXRβ transcript sizes in Northern blot analysis.

In the feeding experiment, mice were gavage-fed twice (24 and 8 h before the mice were killed) with vehicle [1% (w/v) CMC/H_2O; 10 μl/g mouse] or the synthetic LXR activator T0901317 (30 or 50 mg/kg in 1% CMC). Mice were killed by cervical dislocation. White adipose tissue (epididymal), liver, and muscle (gastrocnemius) were rapidly frozen in liquid nitrogen and stored at −70°C until isolation of total RNA.

Total RNA extraction and Northern blot analysis

Total RNA was extracted with TRIZOL® reagent (Invitrogen, Carlsbad, CA). Fifteen or 20 μg of total RNA was separated on a 1% agarose formamide/MOPS gel and wet blotted with 10× SSPE (1.8 M NaCl, 100 mM NaH_2PO_4, and 10 mM EDTA pH 7.4) onto Hybond-N membranes (Amersham Biosciences, Buckinghamshire, UK). Hybridization and stripping of membranes were performed as recommended (Clontech, Palo Alto, CA; #PT1200-1). Membranes were probed with [α-^{32}P]dCTP (Amersham)-radiolabeled cDNAs synthesized using the Multiple DNA Labeling System (Amersham). Results from cell culture experiments show averages ± SD of three independent experiments performed in duplicate normalized to acidic ribosomal phosphoprotein PO (36B4). Results from animal experiments show averages of five to six animals normalized to 36B4. The Northern analysis results shown in Fig. 4A, B were pieced together from three membranes. Each membrane contained samples isolated from wild-type mice, controls treated with CMC or treated with T0901317, and samples isolated from one of the three different modified LXR animal models fed by CMC or T0901317. Quantification of the results is based on wild-type control mice from each mem-

brane. Probes used were mouse (m), and rat (r): rLXRα, mLXRβ, mSREBP-1, mABCG1, mABCA1, mApoE, mPPARγ, maP2, and m36B4. Primer and probe sequences are available upon request. Radioactive signals were visualized by densitometry scanning and analyzed by ImageQuant™ software (Amersham Biosciences).

Electrophoretic mobility-shift assay

Proteins were synthesized from pCMX, pCMX-mRXRα, pCMX-hLXRα, or pCMX-hLXRβ expression vectors using a TNT T7-coupled in vitro transcription/translation system (Promega). For preparation of nuclear extract, COS-1 cells were transfected with RXR and/or LXR expression vector(s) by use of Lipofectamine (Invitrogen) for 48 h before nuclear extraction as described (13). Annealed double-stranded oligonucleotide probes [only one strand is shown; mouse LXRα promoter LXRE-wt, 5′-ATGGGATGAA**T-GGCCA**GCAG**TAACCT**CAACGCCTG-3′; mouse LXRα promoter LXRE-mut, 5′-ATGGGATGAA**TGG**GG**A**GCAG**TAA**GG**T**CAACGC-CTG-3′ (boldface nucleotides represent the LXRE sequence; the underlined nucleotides are mutated)] were labeled using T4 polynucleotide kinase (Promega) and [γ-^{32}P]ATP (Amersham). Binding reaction and separation of the protein-DNA complexes from free probe were performed as described (13).

Identification and mutagenesis of the mouse LXRα promoter, and transfection and luciferase assay

A previously described sequence of the mouse LXRα promoter [accession number AJ132599 (34)] was used as bait to identify a longer form of the mouse LXRα promoter by BLAST search (35) against *nr* and *htgs* sequences at NCBI (annealed to accession number AC016982). The obtained promoter sequence was extracted and analyzed by a consensus LXRE (DGGTYA HWHW MGKKCA) generated by the GCG program (Wisconsin Package Version 10.0; Genetics Computer Group, Madison, WI) to localize potential LXREs.

The mouse LXRα promoter was amplified by PCR using a fragment of the λ2-1 clone described by Alberti, Steffensen, and Gustafsson (34) as a template, and the obtained PCR product (promoter from −2164 to +1833) was subsequently inserted into the pGL3-basic vector to generate the full-length mouse LXRα reporter. Site-directed mutagenesis of the LXRE in the reporter was introduced by PCR with Pfu Turbo (Stratagene) as described (13), using the mutated oligonucleotides used in the electrophoretic mobility-shift assay (EMSA) assay as mutation-targeting primers. Positive clones were identified by restriction analysis.

In reporter transfection experiments, performed in triplicate in three independent experiments, SGBS cells were transfected in 12-well dishes with reporter (3.7 μg), pTK Renilla luciferase (Promega; 0.3 μg as an internal control), and expression vectors (0.5 μg each). DNA was first dissolved in 100 μl of OptiMEM®I (Invitrogen) and then mixed with 12.5 μl of Lipofectamine2000 (Invitrogen)/100 μl of OptiMEM®I and incubated at room temperature for 20 min. Cells were incubated in medium without antibiotics for 24 h before transfection, and the culture medium was renewed (1 ml) containing ligand (0.2 μM) before addition of the transfection mixture (200 μl). After 24 h of incubation, cells were harvested in 100 μl of 1× Passive Lysis Buffer and luciferase activity was measured with the Dual-Luciferase Reporter™ assay system (Promega).

COS-1 cells were transiently transfected in six-well dishes with mouse LXRα promoter reporters (5 μg) and cotransfected with pCMX-mRXRα, pCMX-hLXRα (1 μg each), and pSV-β-galactosidase (3 μg) expression vectors with calcium phosphate precipitation (36). Relative luciferase activity was normalized against β-galactosidase activity.

RESULTS

LXR activation induces LXRα expression in white adipose tissue in mice

The mechanisms controlling expression of the LXRα gene are not well understood. We and others have demonstrated that PPARγ directly regulates LXRα in adipocytes (15) and macrophages (17). In addition, in vitro studies suggest that the human LXRα gene is induced via an identified LXRE in the LXRα promoter (19–21). It has been claimed that autoregulation of the LXRα gene is tissue- and species-dependent and only occurs in humans (19–21). However, we have observed an increased level of LXRα in 3T3-L1 cells and in mouse adipose tissue after treatment with a potent LXR agonist (15), suggesting that autoregulation of the LXRα gene also occurs in mouse. To further explore whether LXRα is autoregulated in vivo, we gavage-fed C57BL/6J male mice (10 weeks of age) with a potent synthetic LXR agonist, T0901317 (37). In three independent feeding experiments, mice were given vehicle alone (1% CMC) or T0901317 (2 × 50 mg/kg) during a 24 h period (three to six animals in each group). LXRα expression was significantly upregulated by T0901317 in epididymal white adipose tissue (WAT; 2.2- ± 0.6-fold induction) but not in liver (0.9- ± 0.2-fold change) (**Table 1**). We did not observe any change in LXRβ mRNA expression after T0901317 feeding in either of the two tissues examined (1- ± 0.3-fold and 0.8- ± 0.1-fold change in epididymal WAT and liver, respectively). As a positive control for LXR activation in both tissues, we found an increased expression of SREBP-1 in both tissues, in agreement with previous results (15, 37).

Induction of endogenous LXRα expression in response to LXR agonist in differentiated human and mouse adipocytes in culture

To test whether activation of LXRs affected the expression of LXRα in cultured adipocytes, mouse 3T3-L1 cells were differentiated for 13 days and stimulated with T0901317 (1 μM) for 24 h, and samples were examined by Northern blot analysis. Exposure of differentiated 3T3-L1 cells to the LXR agonist induced the LXRα mRNA level significantly (1.7-fold; **Fig. 1**). To examine whether a similar reg-

TABLE 1. Male C57BL/6J mice were treated twice with vehicle (1% carboxymethyl-cellulose) or a synthetic LXR activator, T0901317 (50 mg/kg), during a 24 h period

Tissue	LXRα	LXRβ	SREBP-1
WAT: vehicle	1 ± 0.4	1 ± 0.2	1 ± 0.6
WAT: T0901317	2.2 ± 0.6[a]	1 ± 0.3	5.2 ± 3.0[a]
Liver: vehicle	1 ± 0.2	1 ± 0.2	1 ± 1.1
Liver: T0901317	0.9 ± 0.2	0.8 ± 0.1	7.6 ± 5.5[a]

Mice were killed 8 h after the last treatment. The table shows means ± SD of three independent experiments with three to six animals in each group. Epididymal white adipose tissue (WAT) and liver were rapidly taken out, and total RNA was extracted before Northern analysis. LXR, liver X receptor; SREBP-1, sterol regulatory element binding protein-1.

[a] $P < 0.01$ using Student's *t*-test.

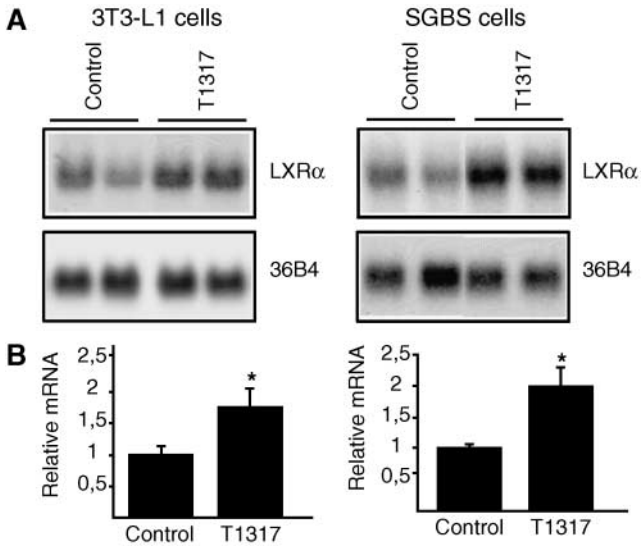

Fig. 1. Liver X receptor α (LXRα) expression is induced by LXR agonist in fully differentiated mouse and human adipocytes. A: Expression of LXRα mRNA in adipocytes cultured in vitro after LXR agonist treatment for 24 h. 3T3-L1 cells (differentiated until day 13) and SGBS cells (differentiated until day 17) were stimulated with 1 and 0.1 μM T0901317, respectively. B: Expression of LXRα in 3T3-L1 and SGBS cells relative to the expression of 36B4. Control = 1. The results are given as means ± SD of three independent experiments performed in duplicate. *P* values were determined by Student's *t*-test (* $P < 0.01$).

ulation also occurs in humans, differentiated human adipocytes (SGBS cells) were stimulated with T0901317 (0.1 μM) for 24 h. A significant 2-fold induction of the LXRα mRNA transcript was observed (Fig. 1).

Identification of an LXRE motif within the mouse LXRα promoter

The above experiments suggest that autoregulation of LXRα occurs in both humans and mice, in disagreement with previous results (19–21). To investigate the molecular basis for the regulation of the mouse LXRα gene by the potent LXR activator, we analyzed the 5′-flanking region of the mouse LXRα gene. We identified one potential mouse LXRE (**Fig. 2A, B**), which is located in an enhancer region that is conserved between mouse and human (19). Although three putative DR-4 elements in the promoter of the human LXRα gene have been reported, only one of them plays a critical role in the induction of the human LXRα gene and seems to function as an LXRE (19–21). Interestingly, our identified mouse DR-4 element differed only in one nucleotide from the human DR-4 sequence, and it has never been characterized. We therefore tested whether this mouse LXRE is functional and mediates LXR-responsive gene transcription in mouse adipose tissue and cells. To demonstrate in vitro binding of LXRα to the potential mouse LXRE, we performed EMSA with double-stranded oligonucleotides containing the human and mouse LXREs with in vitro translated protein extracts. A protein/DNA complex was only observed when both RXRα and LXRα proteins were incubated together with the mouse and human LXRα LXRE (Fig. 2C and data not shown). A similar protein/DNA complex was also observed in the presence of RXRα and LXRβ proteins, demonstrating that the binding to the LXRE is not receptor-specific (Fig. 2D). These protein/DNA complexes were specific, because the binding was eliminated by excess unlabeled wild-type but not by mutated oligonucleotides. Similarly, a strong specific binding was observed when nuclear extracts from COS-1 cells transfected with RXRα and LXRα expression vectors were used (Fig. 2E).

To further demonstrate the functionality of the LXRE, a full-length construct containing the murine LXRα 5′-flanking reporter (sequence spanning from −2164 to +1833) inserted into the pGL3-basic reporter was used in transient transfection assays. The wild-type reporter and a reporter containing the targeted mutation of the LXRE were transfected into SGBS cells in the presence of RXRα and LXRα expression vectors and stimulated with the LXR ligand (T0901317; 0.2 μM for 24 h). As expected, only the wild-type reporter construct responded to the LXR agonist (Fig. 2F). The same results were obtained in COS-1 cells (data not shown). These results are similar to what was observed for the human LXRα promoter (19–21), suggesting that autoregulation of the murine LXRα gene is mediated through this particular LXRE. These analyses suggest that the ability for autoregulation of the LXRα gene is conserved among humans and mice.

Basal expression of LXRα is increased in LXRβ$^{-/-}$ mice

To determine whether LXRα is able to autoregulate the LXRα gene by T0901317 treatment in vivo, wild-type control, LXRα$^{-/-}$, and LXRβ$^{-/-}$ mice (7−9 weeks old; five to six animals per group) were fed orally with vehicle (1% CMC) or with T0901317 (2 × 30 mg/kg in CMC). As expected, in wild-type mice the LXRα transcript was induced after LXR agonist treatment (2.3-fold) (**Fig. 3A**). Interestingly, in LXRβ$^{-/-}$ mice, the basal expression of LXRα was increased (2.4-fold) with an even higher expression after T0901317 treatment (3.4-fold) compared with wild-type control mice (Fig. 3A). It is likely that the lower relative induction of the LXRα gene by T0901317 treatment observed in LXRβ$^{-/-}$ mice (from 2.4- to 3.4-fold) might be attributable to the increased basal LXRα expression in these mice. Thus, it seems appropriate to conclude that the LXRα transcript is autoregulated in both wild-type and LXRβ$^{-/-}$ mice, demonstrating that autoregulation may be mediated through LXRα. As expected from our previous analysis (Table 1), the LXRβ gene was not induced in wild-type or LXRα$^{-/-}$ mice after stimulation with T0901317 (Fig. 3B). Furthermore, neither was the basal expression level of this gene altered in LXRα$^{-/-}$ mice.

Both LXRs enhance the expression of genes involved in lipid homeostasis in adipose tissue after T0901317 treatment

We have previously suggested that basal expression of GLUT4 in adipose tissue is selectively dependent on LXRα

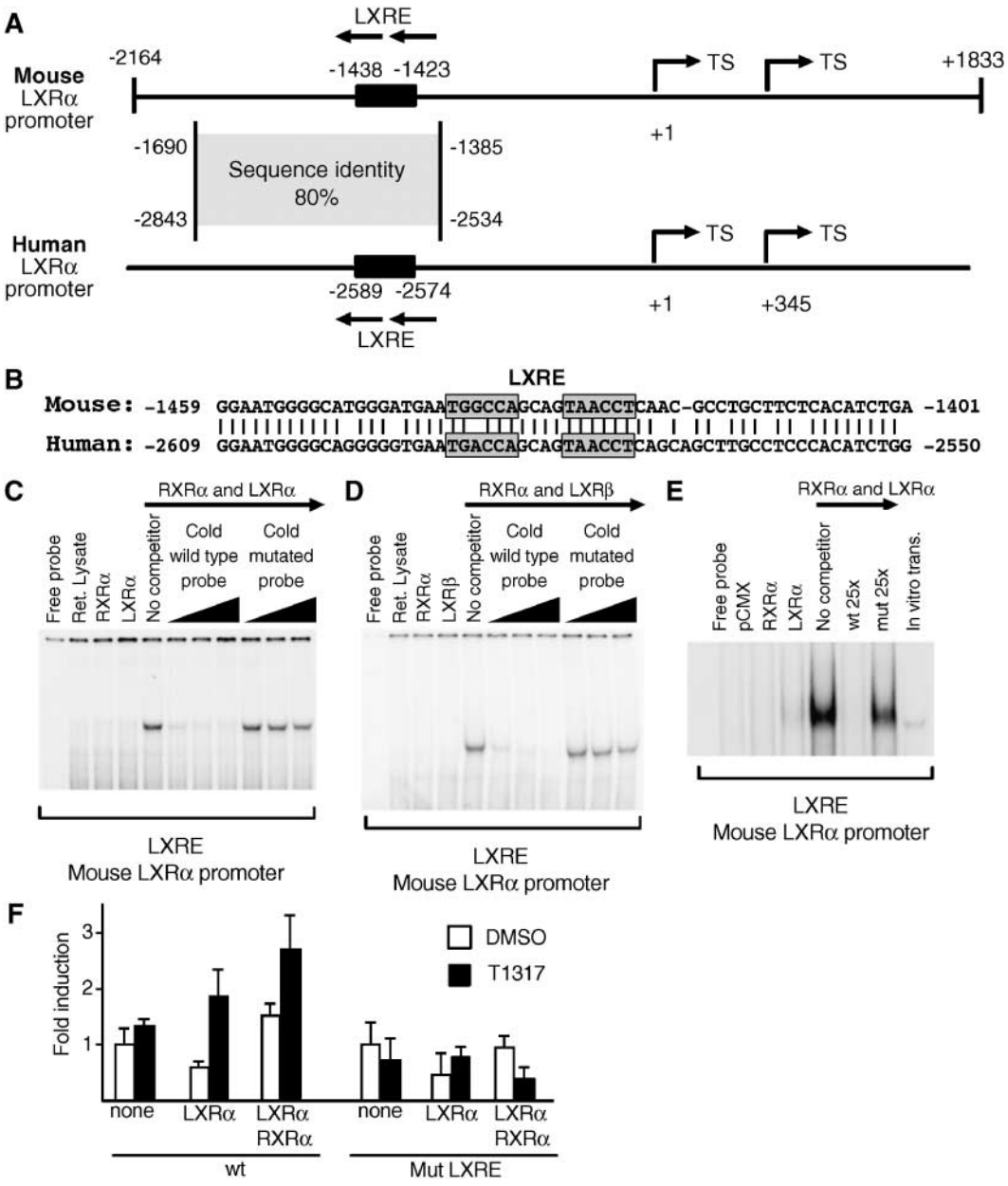

Fig. 2. Characterization of the human and mouse LXRα promoters. A: Schematic presentation of the mouse LXRα promoter (nucleotides −2164 to +1833) and the corresponding human LXRα promoter. The position and nucleotide sequence identity of the distal conserved enhancer are emphasized. The two identified transcription start sites are marked with bent arrows. The nucleotide positions of the black boxes point out the location of the LXR-responsive element (LXRE). B: Sequence alignment and relative sequence identity between the nucleotides surrounding the mouse and human LXRα LXRE. The two half-sites in the conserved LXRE are enclosed with boxes. Conserved nucleotides are indicated by vertical lines. C: Direct and specific binding of the LXRα/RXRα protein complex to the LXRE in the mouse LXRα promoter. Electrophoretic mobility-shift assay (EMSA) was performed with annealed and ^{32}P-labeled mLXRα-LXRE oligonucleotide and incubated in the presence of in vitro translated RXRα and/or LXRα proteins as indicated. D: Direct and specific binding of the LXRβ/RXRα protein complex to the LXRE in the mouse LXRα promoter. EMSA was performed with annealed and ^{32}P-labeled mLXRα-LXRE oligonucleotide and incubated in the presence of in vitro translated RXRα and/or LXRβ proteins as indicated. E: Nuclear extracts (0.5 μg) isolated from COS-1 cells transfected with pCMX, pCMX-RXRα, or pCMX-LXRα expression vectors form a strong specific complex with the LXRα LXRE nucleotide only when extracts from RXRα- and LXRα-transfected cells were combined. F: Transient transfection of full-length wild-type- (wt) and mutant (Mut) LXRα LXRE reporters into SGBS cells. Cells were cotransfected with pCMX, pCMX-LXRα, or pCMX-RXRα and pCMX-LXRα expression plasmids and stimulated with vehicle (Me_2SO; white bars) or T0901317 (0.2 μM; black bars). Results from one representative experiment performed in triplicate are shown.

but that both LXRs compensate for each other in regulating GLUT4 after LXR agonist treatment (13). We therefore investigated whether there were differences in the expression of other LXR target genes among the different LXR-deficient mice. First we explored whether SREBP-1, which is believed to be an important LXR target gene in adipose tissue (15), was preferentially regulated by any of the LXRs. Wild-type control, LXRα$^{-/-}$, LXRβ$^{-/-}$, and

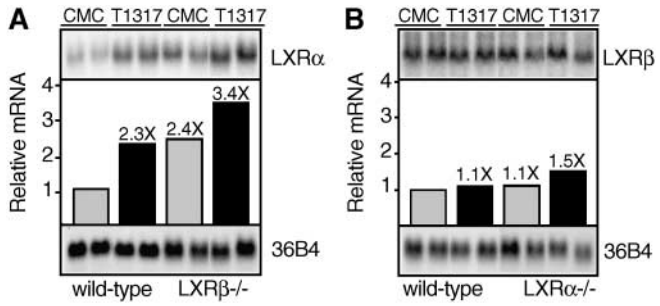

Fig. 3. Basal expression of LXRα is increased in LXRβ$^{-/-}$ mice. Mice were gavage-fed with vehicle [1% carboxymethyl-cellulose (CMC)] or T0901317 (2 × 30 mg/kg) and killed 8 h after the last administration of T0901317. Adipose tissue [epididymal white adipose tissue (WAT)] was rapidly taken out, and total RNA was isolated before being subjected to Northern blot analysis [two or three animals per lane (n = 5 or 6), 15 μg total RNA/lane]. A: Expression of LXRα in wild-type and LXRβ$^{-/-}$ mice. B: Expression of LXRβ in wild-type and LXRα$^{-/-}$ mice. Relative levels of the LXRα and LXRβ mRNA transcripts are given as averages of the two lanes. Control = 1 for wild-type vehicle-treated animals.

LXRα$^{-/-}$β$^{-/-}$ mice (7–9 weeks old; five to six animals per group) were fed orally with vehicle (1% CMC) or with the synthetic LXR agonist, T0901317 (2 × 30 mg/kg in CMC). In wild-type and LXRα$^{-/-}$ mice, the SREBP-1 gene was induced ~6-fold compared with wild-type control mice after treatment with T0901317 (**Fig. 4A**). Interestingly, the basal expression of SREBP-1 was found to be 4-fold higher in LXRβ$^{-/-}$ mice and induced by as much as 14-fold compared with wild-type control mice after T0901317 treatment. As expected, no induction of SREBP-1 was observed in LXRα$^{-/-}$β$^{-/-}$ mice. These data suggest that the induced SREBP-1 expression in WAT after T091317 treatment might be mediated through both LXRs. However, the observed increased basal and T0901317-stimulated SREBP-1 expression levels in LXRβ$^{-/-}$ mice are interesting in view of the increased basal LXRα expression in these mice.

Because it has been demonstrated that SREBP-1 is able to stimulate the transcription of PPARγ (38) and that PPARγ directly regulates the LXRα gene in 3T3-L1 mouse adipocytes (15), SREBP-1c-PPARγ-LXRα cross-talk could be involved in the observed induction of LXRα after T0901317 feeding. To rule out this possibility, we examined whether the expression level and activation of PPARγ was changed by T0901317 treatment. We did not observe any change in PPARγ mRNA expression in wild-type mice or any of the LXR-deficient mice. Furthermore, the expression level of the aP2/aFABP gene, which is a well-characterized PPARγ target gene (39), also was not regulated after T0901317 feeding, except perhaps for a small induction in the LXRα$^{-/-}$ mice. This result strongly suggests that the observed induction of LXRα by short-term T0901317 treatment is the result of LXR autoregulation and not of SREBP-1c-PPARγ-LXRα cross-talk.

The established LXR target gene, ABCA1, involved in cholesterol release from peripheral cells is regulated during adipose differentiation of 3T3-L1 cells, and an induced expression of ABCA1 mRNA is observed after LXR agonist treatment in vitro (40). To further explore the function of LXR in adipose tissue, we tested whether apoE and members of the ATP binding cassette transporter family were induced in adipose tissue after T091317 treatment in wild-type and LXR-deficient mice. Treatment of wild-type mice with the synthetic LXR agonist induced the expression of apoE (2.3-fold), ABCA1 (3.0-fold), and ABCG1 (12-fold) in adipose tissue (Fig. 4B). As expected, the induction of these genes by T0901317 treatment was abolished in LXRα$^{-/-}$β$^{-/-}$ mice. However, interesting differences in the expression pattern were observed in the single knockout mice (LXRα$^{-/-}$ and LXRβ$^{-/-}$ mice). The induction of apoE after LXR agonist treatment was lower in the LXRα$^{-/-}$ mice (1.7-fold) compared with wild-type control mice (2.3-fold). This was not observed in LXRβ$^{-/-}$ mice, in which the induction of apoE (2.5-fold) was similar to that in wild-type mice.

In liver, no regulation of apoE was observed after T0901317 treatment in either of the mice (Fig. 4C), but the basal expression level tended to be lower in the LXRα$^{-/-}$ mice. In fully differentiated adipocytes cultured in vitro, treatment with T0901317 for 24 h significantly induced the expression level of apoE by 1.9-fold (SGBS cells) and 2.7-fold (3T3-L1 cells) (Fig. 4D).

The ABC transporters were on the other hand almost oppositely regulated compared with apoE in WAT of LXR-deficient mice. In the LXRα$^{-/-}$β$^{-/-}$ mice, the basal expression level of ABCG1 was increased by 4.5-fold. The ABCG1 was also induced to a higher level in LXRα$^{-/-}$ mice (19-fold) compared with wild-type control mice after T0901317 feeding. Furthermore, in LXRβ$^{-/-}$ mice, the basal expression level was similar to that in wild-type controls, and the expression level was less induced (6.7-fold) after T0901317 treatment compared with that in control mice. Interestingly, the ABCA1 gene seems to be regulated specifically by LXRβ, because there is no induction in LXRβ$^{-/-}$ mice after T0901317 feeding.

In conclusion, these observations suggest that both LXRs are able to enhance the expression of SREBP-1c, apoE, and ABCG1 in adipose tissue when activated with the potent LXR agonist (T0901317). However, whereas apoE and SREBP-1 seem to be preferentially induced by LXRα, ABCG1 and ABCA1 seem to be more selectively induced by LXRβ. This tendency is also reflected by the altered basal expression level in the absence of the potent activator, where a lack of LXRα increases the basal expression of ABCG1 but an increased expression of LXRα in the absence of LXRβ increases the basal expression of SREBP-1 in adipose tissue.

LXRα autoregulation is tissue-specific and facilitates the tissue-specific induction of the apoE gene in adipose tissue

LXR specificity, with regard to the regulation of LXR target genes in adipose tissue, led us to examine whether characterized LXR target genes were differently expressed and regulated in three metabolic tissues such as liver, muscle (gastrocnemius), and WAT (epididymal). Male C57BL/6J mice were fed vehicle (1% CMC) or T0901317 (2 × 30

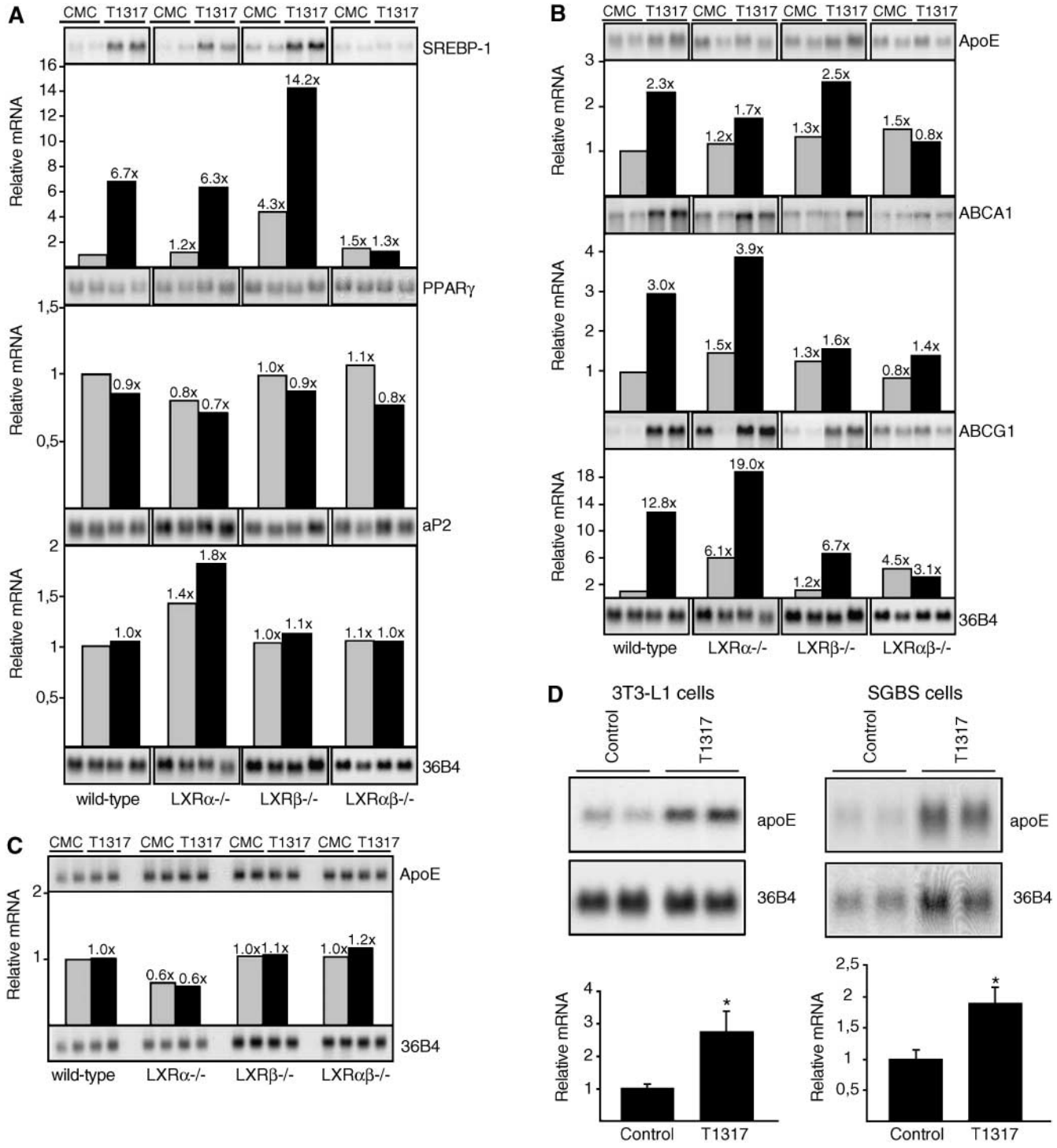

Fig. 4. LXR target genes in adipose tissue are differently regulated by the two LXRs. Mice were gavage-fed as described for Fig. 3, WAT and liver were rapidly taken out, and total RNA was isolated before being subjected to Northern blot analysis. A: Expression of sterol regulatory element binding protein-1 (SREBP-1), peroxisome proliferator-activated receptor γ (PPARγ), and aP2/aFABP in WAT from wild-type, LXRα$^{-/-}$, LXRβ$^{-/-}$, and LXRα$^{-/-}$β$^{-/-}$ mice correlated against the ribosomal probe m36B4. Relative levels of the SREBP-1 mRNA transcripts are given as averages of the two lanes [two or three animals per lane (n = 5 or 6), 15 μg total RNA/lane]. Control = 1 for wild-type vehicle-treated animals. B: Relative expression of the apolipoprotein E (apoE), ABCA1, and ABCG1 genes in WAT quantified as described in A. C: Relative expression of apoE in liver isolated from wild-type, LXRα$^{-/-}$, LXRβ$^{-/-}$, and LXRα$^{-/-}$β$^{-/-}$ mice correlated against the ribosomal probe m36B4 (RNA from six animals was pooled and loaded in two lanes, 20 μg total RNA/lane). D: Expression of apoE mRNA in adipocytes cultured in vitro after LXR agonist treatment for 24 h. 3T3-L1 cells (differentiated until day 13) and SGBS cells (differentiated until day 17) were stimulated with 1 and 0.1 μM T0901317, respectively, relative to the expression of 36B4. Control = 1. The results are given as means ± SD of three independent experiments performed in duplicate. *P* values were determined by Student's *t*-test (* $P < 0.01$).

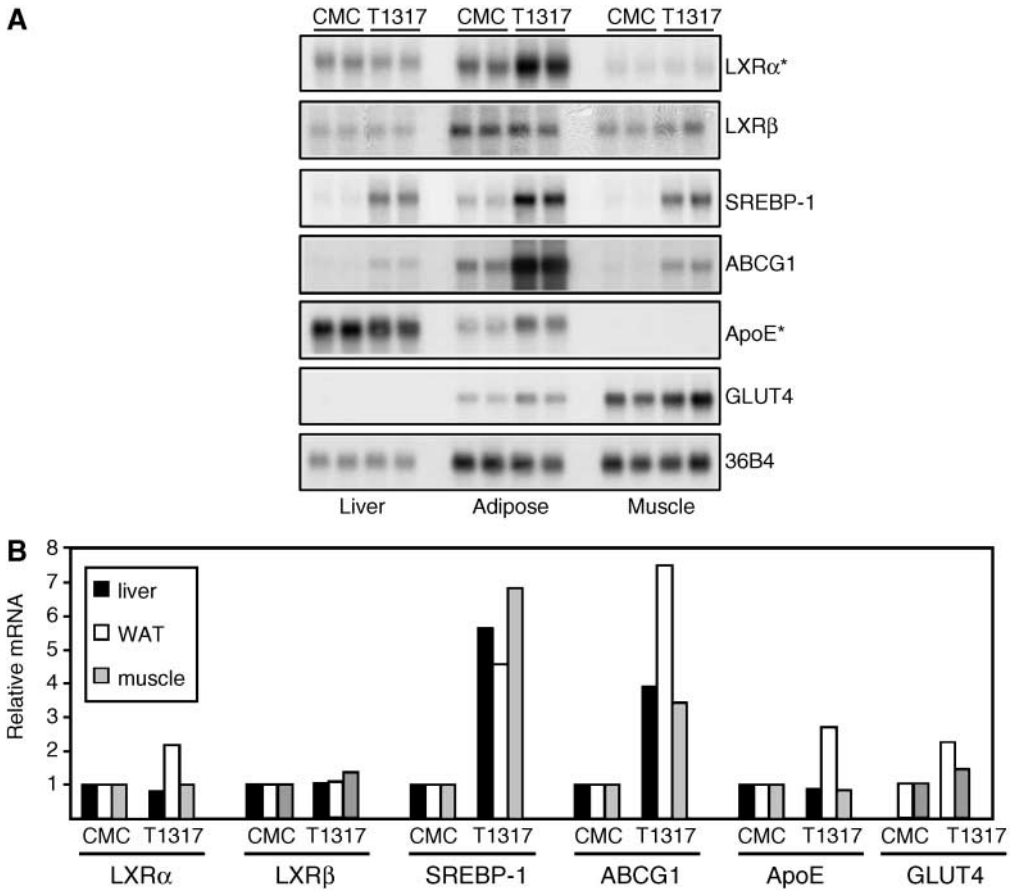

Fig. 5. LXRα autoregulation and target gene induction are tissue-specific and facilitate induction of the apoE gene in adipose tissue. Mice (C57BL/6J male mice) were gavage-fed with vehicle alone (1% CMC) or T0901317 (2 × 30 mg/kg) and killed 8 h after the last administration of T0901317. Adipose tissue (epididymal fat), liver, and muscle (gastrocnemius) were rapidly taken out, and total RNA was isolated before being subjected to Northern blot analysis. A: Expression of LXRα, LXRβ, SREBP-1, ABCG1, apoE, GLUT4, and 36B4 in liver, adipose tissue, and muscle (RNA from six animals was pooled and loaded in two lanes, 20 μg total RNA/lane). * Quantification of the muscle expression of these genes was performed after longer exposure. B: Relative expression of LXRα, LXRβ, SREBP-1, ABCG1, apoE, and GLUT4 in liver, adipose tissue, and muscle correlated against the ribosomal probe m36B4 signal. Results are given as averages of the two lanes. CMC-treated mice = 1.

mg/kg) for 24 h, and total RNA was extracted from the three tissues and applied on the same Northern blot. This enabled us to detect differences in the basal and LXR-activated induction of the selected LXR target genes in these three tissues. Interestingly, the examined target genes SREBP-1 and ABCG1 were induced by treatment with the LXR agonist in all three tissues examined, whereas LXRα, apoE, and GLUT4 were only induced in adipose tissue (**Fig. 5**). This suggests that autoregulation of the LXRα gene is associated with induction of apoE and GLUT4, and with additional support from other results (Fig. 4), we suggest that the level of LXRα expression is crucial for the LXR agonist induction of the apoE gene.

DISCUSSION

Despite the fact that LXRα and LXRβ are oxysterol-regulated receptors that control lipid and cholesterol homeostasis and are expressed abundantly in adipose tissue, little is known about the function of LXR as a cholesterol sensor in adipose tissue. Here, we demonstrate that a synthetic LXR agonist, T0910317, induces the mouse LXRα gene in adipocytes in vitro and in vivo. In addition, in a human adipocyte cell line, LXR activation leads to an induction of the LXRα gene. The induction is gene specific, because only the LXRα gene and not the LXRβ gene is regulated via an autoregulatory loop. Several reports have shown that LXR agonists induce expression of the LXRα gene in human cell lines, such as macrophages, skin fibroblasts, preadipocytes, and liver HepG2 cells (19–21), and it has been claimed that LXRα autoregulation only occurs in various human cell lines (19, 20). Our findings demonstrate that autoregulation of LXRα also occurs in mouse adipose tissue both in vitro and in vivo mediated through the direct binding of an LXR/RXR heterodimer complex to a DR-4-type LXRE in the mouse LXRα promoter. This element is located in the highly conserved enhancer re-

gion (with 80% sequence identity between human and mouse) positioned −2843 to −2534 bp from the transcription start site in the human LXRα promoter (19–21) and at positions −1690 to −1385 in the mouse promoter. With our analysis, we demonstrate that the DR-4 is functional as an LXRE in mouse as well as in human adipose tissue. Because the three transcription factors SREBP-1, PPARγ, and LXRα have the potential to form a cross-regulatory loop (15, 38, 41, 42), we investigated whether PPARγ-LXR cross-talk is involved in the observed adipose regulation of the LXRα gene. We did not observe any induction of PPARγ mRNA level or the well-characterized PPARγ target gene aP2/aFABP (39) in our T0901317 feeding experiments; hence, we find it unlikely that the T0901317-induced regulation of LXRα is attributable to altered adipose PPARγ activity.

It has been demonstrated by others that both LXRα and LXRβ are able to regulate the human LXRα promoter in transfected human macrophages (19–21). In our studies, we cannot conclude which of the LXRs mediates the induction of the LXRα gene in vivo. Because the LXRα gene is autoregulated and the basal expression level of the LXRα gene is increased in LXRβ$^{-/-}$ mice, we speculate that the LXRβ isoform might inhibit the basal expression of LXRα in wild-type mice, perhaps by competing with LXRα binding to the LXRE in the LXRα promoter. This hypothesis will be difficult to prove experimentally, because we would have to measure the LXRα transcript in LXRα$^{-/-}$ mice. Unfortunately, it is uncertain whether the disruption of the LXRα transcript in LXRα$^{-/-}$ and LXRα$^{-/-}$β$^{-/-}$ mice alters the stability of the mutated transcript, making mRNA measurement of the LXRα gene in LXRα$^{-/-}$ and LXRα$^{-/-}$β$^{-/-}$ mice highly unreliable.

The synthetic LXR activator T0901317 is able to bind and activate both LXR isoforms (37). In accordance with a previous in vivo study in liver (41) and transient transfection studies in HEK293 cells (42), we show that the regulation of the LXR target gene SREBP-1 is mediated by both receptors in vivo in adipose tissue. ApoE has been demonstrated to be induced by LXR activation in adipose tissue (10), although little is known about other target genes involved in cholesterol efflux in vivo in this tissue. ABCA1 has been demonstrated to be induced by LXR activation in vitro in 3T3-L1 cells (40), but to our knowledge, there are no reports of ABCG1 expression in adipose tissue. We demonstrate that ABCA1 and ABCG1 are induced by LXR activation in adipose tissue in vivo. Our results support the notion that both LXR isoforms may mediate the ligand-activated induction of SREBP-1 and a number of other characterized LXR target genes in vivo. However, we observed differences in the basal and T0901317-stimulated expression of a number of characterized LXR target genes in adipose tissue between mice with targeted disruption of only one of the LXR genes. The basal and T091317-stimulated expression levels of both SREBP-1 and LXRα are higher in LXRβ$^{-/-}$ mice than in wild-type mice. The basal expression of apoE in adipose tissue is preserved in LXRα$^{-/-}$, LXRβ$^{-/-}$, and LXRα$^{-/-}$β$^{-/-}$ mice, and the principal role of LXRs seems to be to mediate the lipid inducibility of apoE. Interestingly, T0901317-mediated induction is lower in LXRα$^{-/-}$ mice, suggesting that LXRα is more important for LXR agonist-induced expression of apoE in adipose tissue.

The ability of the mouse LXRα gene to undergo autoregulation is therefore likely to have implications for LXR target gene expression and cellular function. To understand the role of autoregulation of the LXRα gene, we compared the expression of selected target genes in the three important cholesterol- and fatty acid-metabolizing tissues: liver, muscle, and WAT. Although SREBP-1 and ABCG1 were induced in all three tissues, the apoE, GLUT4, and LXRα genes were specifically induced only in adipose tissue by T0901317 treatment. This observation is in agreement with our previous report describing GLUT4 as an LXRα-specific target gene in adipose tissue (13). We suggest that an important aspect of LXRα autoregulation may be to increase the level of LXRα over a threshold level necessary for the induction of expression of certain target genes, notably apoE and GLUT4.

To date, the major focus on LXR research has been relatively limited to macrophages and liver. Recent experiments suggest that induced expression of the LXRα gene in macrophages increases cholesterol efflux and reverse cholesterol transport (9–12, 18). Adipose tissue contains the largest pool of nonesterified cholesterol in the body (23); it is therefore interesting to observe that the adipose tissue and lipid-loaded macrophages seem to regulate the level and activity of LXRα in a similar way (19, 20). In both cell types, PPARγ and LXR seem able to regulate the level of LXRα (15, 17). In addition, target genes such as ABCA1, ABCG1, and apoE are induced upon LXR agonist treatment in macrophages (10, 11, 19) as well as in adipose tissue. It has recently become clear that macrophages are able to infiltrate adipose tissue (43), where they are responsible for the secretion of cytokines and other factors from this tissue, thus we cannot in our in vivo studies fully exclude the possibility that the effects observed on apoE regulation by LXR activation are from adipose tissue macrophages. However, because we also observed induction of LXRα and apoE in mouse and human adipocytes cultured in vitro, we conclude that the effects are not attributable to macrophage infiltration but rather to regulation within the adipose cell. Our data therefore suggest that LXRs may have similar functions in macrophages and adipose tissue.

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