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Liver X Receptor α Interferes with SREBP1c-mediated *Abcd2* Expression

*NOVEL CROSS-TALK IN GENE REGULATION**

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The peroxisomal ATP binding cassette (ABC) transporter adrenoleukodystrophy-related protein, encoded by *ABCD2*, displays functional redundancy with the X-linked adrenoleukodystrophy-associated protein, making *ABCD2* up-regulation of therapeutic value. Cholesterol lowering activates human *ABCD2* in cultured cells. To investigate *in vivo* regulation by sterols, we first characterized a sterol regulatory element (SRE) in the murine *Abcd2* promoter that is directly bound by SRE-binding proteins (SREBPs). Intriguingly, this element overlaps with a direct repeat 4, which serves as binding site for liver X receptor (LXR)/retinoid X receptor heterodimers, suggesting novel cross-talk between SREBP and LXR/retinoid X receptor in gene regulation. Using fasting-refeeding and cholesterol loading, SREBP accessibility to the SRE/direct repeat 4 was tested. Results suggest that adipose *Abcd2* is induced by SREBP1c, whereas hepatic *Abcd2* expression is down-regulated by concurrent activation of LXRα and SREBP1c. In cell culture, SREBP1c-mediated *Abcd2* induction is counteracted by ligand-activated LXRα. Finally, hepatic *Abcd2* expression in LXRα,β-deficient mice is inducible to levels vastly exceeding wild type. Together, we identify LXRα as negative modulator of *Abcd2*, acting through a novel regulatory mechanism involving overlapping SREBP and LXRα binding sites.

X-linked adrenoleukodystrophy (X-ALD[2]; OMIM 300100) is a neurodegenerative disorder caused by mutations in the *ABCD1* (*ALD*) gene, encoding the peroxisomal ATP binding cassette (ABC) half-transporter adrenoleukodystrophy protein (ALDP) (1). Currently, no satisfying therapy is available for X-ALD patients.

Next to ALDP, three additional mammalian peroxisomal ABC half-transporters have been identified: adrenoleukodystrophy-related protein (ALDRP), 70-kDa peroxisomal membrane protein (PMP70), and PMP70-related protein (P70R), sharing 66, 39, and 25% amino acid identity with ALDP, respectively. Thus, the *ABCD2*-encoded ALDRP is the protein most closely related to ALDP and upon overexpression can functionally compensate for ALDP deficiency in X-ALD fibroblasts and *Abcd1*-deficient mice (2, 3). Therefore, pharmacological stimulation of *ABCD2* expression has been targeted as an alternative therapeutic strategy for X-ALD (4), requiring detailed knowledge about how the *ABCD2* gene is transcriptionally regulated.

We recently showed that human *ABCD2* is induced upon cholesterol depletion in cultured cells via a mechanism requiring the activation and binding of sterol regulatory element (SRE)-binding proteins (SREBPs) (5). SREBPs, which are synthesized as membrane-bound precursors and cleaved to generate the active nuclear form, are a class of transcription factors known to play a major role in regulating genes involved in fatty acid and cholesterol synthesis (reviewed in Ref. 6). To date, three SREBPs have been identified: SREBP1a and SREBP1c, which are produced from a single gene and preferentially regulate fatty acid synthesis, and SREBP2, encoded by a separate gene and controlling expression of cholesterogenic genes (7). Actively growing cultured cells predominantly produce SREBP1a, whereas *in vivo*, SREBP1c is much more abundant (8).

Abcd1-deficient mice mimic the biochemical defect found in X-ALD patients, *i.e.* accumulation of very long-chain fatty acids in tissues (9–11). Thus, a model is available to evaluate the efficacy of cholesterol-lowering drugs on SREBP maturation and *Abcd2* induction as a future therapeutic agent for X-ALD. This requires investigation of *in vivo* accessibility and inducibility of *Abcd2* by SREBPs. Intriguingly, the *Abcd2* SRE overlaps with a direct repeat separated by 4 nucleotides (DR-4) that fits the consensus sequence of the 3,5,3′-triiodothyronine thyroid hormone receptor (TR) or the liver X receptor (LXR) response element. Thus, in this particular case, TR or LXR could possibly interfere with binding and activation of the *Abcd2* promoter by SREBP.

Recently, direct binding of TRβ homodimer or TRβ/RXRα heterodimer complexes to the rat *Abcd2* SRE/DR-4 and inducibility by triiodothyronine has been demonstrated (12). In contrast, no study has addressed a possible role of LXRα in *Abcd2* regulation. The LXR subfamily of nuclear receptors, consisting of LXRα and LXRβ, emerged as key mediator of cholesterol homeostasis (13). LXRs function as cholesterol sensors that respond to elevated sterol concentrations by initiating expression of genes involved in cholesterol efflux, bile acid production, and lipid transport (14). LXRα has a dual role in gene expression: the ligand-receptor complex up-regulates many target genes, whereas the unliganded receptor suppresses gene expression by recruiting corepressor proteins (15). In addition, activated LXRs down-regulate transcription of a subset of genes, as in the case of matrix metalloproteinase-9 or 11β-hydroxysteroid dehydrogenase type 1 (16, 17). However, so far no investigation has demonstrated that the underlying mechanism

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[2] The abbreviations used are: X-ALD, X-linked adrenoleukodystrophy; *ABCD2/Abcd2*, ATP binding cassette transporter subfamily D member 2 gene; ALDRP, adrenoleukodystrophy-related protein; DR-4, direct repeat spaced by 4 nucleotides; LXRα, liver X receptor α; LXRE, LXR response element; RXRα, retinoid X receptor α; SRE, sterol regulatory element; SREBP, SRE-binding protein; TRβ, thyroid hormone receptor β; 22-(*R*)-hydroxycholesterol, 22-(*R*)-HC; EMSA, enzyme-linked immunosorbent assay; QRT, quantitative reverse transcriptase; PPAR, peroxisome proliferator-activated receptor; nt, nucleotide.

involves a direct interaction of LXR with LXREs in the repressed target genes.

The aim of the present study was to (*a*) evaluate whether murine *Abcd2* is inducible by SREBP, (*b*) investigate the relevance of the SRE motif for *in vivo* transcriptional regulation of *Abcd2* by either fasting-refeeding or dietary "cholesterol loading" or "cholesterol depletion" of mice, (*c*) characterize the physiological relevance of the overlapping SRE and DR-4 motifs for *Abcd2* expression, and (*d*) evaluate a putative modulatory effect of LXR on Abcd2 mRNA levels in cell culture and in mice.

EXPERIMENTAL PROCEDURES

Animals and Treatment—Male 4–6-month-old wild type C57BL/6 mice were kept at a temperature of 22 °C with equal periods of darkness and light. For high cholesterol or lovastatin/colestipol treatment, mice were fed *ad libitum* either a standard diet, a standard diet enriched with 2% cholesterol, or a standard diet supplemented with 0.1% lovastatin and 2% colestipol (ssniff). Groups consisting of three animals were treated for either 1 or 6 weeks and sacrificed in the middle of the light phase.

For fasting-refeeding, 3-month-old male wild type mice were divided into three groups, each consisting of three mice: nonfasted, fasted, and fasted/refed. The nonfasted group was fed *ad libitum* a standard chow (ssniff). The fasted group was starved for 24 h (sacrificed at the end of the light phase), and the fasted/refed group was fasted for 24 h and then refed a high carbohydrate/low fat diet (ssniff) for 12 h prior to study (sacrificed at the beginning of the light phase).

For studies involving treatment of wild type and LXRα,β-deficient mice with the synthetic RXRα and LXR ligands, LG268 and T0901317, respectively, 3–4-month-old mixed C57BL/6:129/SvEv mice were used (6 animals/group). Mice were fed *ad libitum* a standard chow diet (number 7001; Teklad) supplemented with either T0901317 (50 mg/kg body weight) or LG268 (30 mg/kg body weight) or both. Control animals were dosed with vehicle. Animals were treated for 12 h and sacrificed at the beginning of the light phase.

Cell Culture—COS-7 and THP-1 cells (ATCC) were cultivated in Dulbecco's modified Eagle's medium with 10% fetal calf serum, 2 mM L-glutamine, 50 units/ml penicillin, and 100 μg/ml streptomycin (Biowhittaker). For cholesterol depletion, standard fetal calf serum was substituted with 10% lipoprotein-deficient fetal calf serum (Sigma). 22-(*R*)-hydroxycholesterol (22-(*R*)-HC; Sigma) was used at 10 μM for 24 h.

Plasmid Construction and in Vitro Mutagenesis—*Abcd2* promoter fragments were generated by PCR using the antisense primer containing a XhoI site (−96, 5′-GCCTCCCATGCTCTCGAGCTTGTCTCC-3′) and various sense primers containing a MluI site (−3623, 5′-ATGCTTCAAGACGCGTGGGCAAGCAC-3′, 3515 bp; −1594, 5′-CACACAAGTGACGCGTTGTGATGTGAC-3′, 1486 bp; −607, 5′-CTGACGCGTTTCCCTGGGCCGGTTCCG-3′, 499 bp). PCR fragments were cloned into the XhoI/MluI-digested vector pGL3basic (Promega) upstream from the luciferase gene. The expression vector pGLUC (18), containing the minimal β-globin promoter upstream from a luciferase gene, was used for studies of the isolated response element. The annealed double-stranded SRE sequence containing oligonucleotides with HindIII and BamHI overhangs (mAbcd2-SRE: 5′-AGCTTATCGATAGCAGCTGACCTCATTCGACCG-3′, 5′-GATCCGGTCGAATGAGGTCAGCTGCTATCGATA-3′) were ligated into BamHI/HindIII-digested pGLUC to generate pGl-mAbcd2-SREluc. The point mutations M1 and M2 were introduced using the following pairs of annealed oligonucleotides: pGl-mAbcd2-SRE-M1, 5′-AGCTTATCGATCGCCGCTGACCTCATTCGACCG-3′, 5′-GATCCGGTCGAATGAGGTCAGCGGCGATCGATA-3′; pGl-mAbcd2-SRE-M2, 5′-AGCTTATCGATCGCGGCTGACCTCATTCGACCG-3′, 5′-GATCCGGTCGAATGAGGTCAGCCGCGATCGATA-3′. Mutations in the *Abcd2* promoter constructs were introduced using the QuikChange site-directed mutagenesis kit (Stratagene) and complementary degenerate primers (5′-AGCAGCTGACCTCATTCGgCCgCTCCAGAAATAG-3′ and 5′-CTATTTCTGGAGcGGcCGAATGAGGTCAGCTGCT-3′).

Transient Transfection Experiments—COS-7 cells were transfected using Lipofectamine 2000 (Invitrogen) with 0.5 μg of pGLUC or pGL3basic construct, 0.1 μg of SREBP1a/1c/2-expression vector (kindly provided by Dr. Timothy Osborne, University of California, Irvine, CA), 0.1 μg of LXRα expression vector (19), and 0.05 μg of pCMV-β-Gal (Clontech). Transfected cells were incubated with or without 22-(*R*)-HC for 24 h and assayed for luciferase and β-galactosidase activity (5).

Electrophoretic Mobility Shift Assay (EMSA)—SREBP1a, LXRα, and RXRα were synthesized *in vitro* using the TNT T7 Quick for PCR *in vitro* transcription/translation system (Promega) and the primers SREBP1a 5′-GGATCCTAATACGACTCACTATAGGGAACAGCCACCATGGACGAGCCACCCTTCAG-3′ (forward), 5′-T $_{29}$ACTATGTCAGGCTCCGAGTCACTGCCA-3′ (reverse); LXRα 5′-GGATCCTAATACGACTCACTATAGGGAACAGCCACCATGTCCTTGTGGCTGGGGGCC-3′ (forward), 5′-T $_{29}$TCATTCGTGCACATCCCAGATCTCAG-3′ (reverse); RXRα 5′-GGATCCTAATACGACTCACTATAGGGAACAGCCACCATGGACACCAAACATTTCCTG-3′ (forward), 5′-T $_{29}$ACTAGGTGGTTTGATGTGGGGCCTCCAG-3′ (reverse) and the SREBP1a, LXRα, and RXRα expression plasmids as templates, respectively. Complementary oligonucleotides were 5′-GTTCTCCAGCAGCTGACCTCATTCGACCT-3′ and 5′-TGGAGAGGTCGAATGAGGTCAGCTGCTGG-3′, mAbcd2-SRE/DR-4; 5′-AGGCAGAGGGAACAGCAGATTGTG-3′ and 5′-TCTGCACAATCTGCTGTTCCCTC-3′, mScd1-SRE; 5′-AGGTGGTTGACCCGAGGTAACCCCT-3′ and 5′-CGAGGGGTTACCTCGGGTCAAC-3′, chicken acetyl-CoA carboxylase-LXRE.

Oligonucleotides for competition experiments were murine growth hormone receptor 3 (*Ghr3*) 5′-CTGGTTAGTTCATATTGTTGTTCCACCAATAGGGTTG-3′ and 5′-GTCTGCAACCCTATTGGTGGAACAACAATATGAACTAA-3′. Labeling of probes and binding experiments were performed as described (5). For EMSA experiments involving LXRα, incubation occurred with or without 22-(*R*)-hydroxycholesterol (100 μM). The gel migration was documented by exposure to x-ray films.

RNA Isolation and Northern Blot—Total RNA was isolated from murine liver using TriPure isolation reagent (Roche Applied Science) and a Polytron PT3100 homogenizer (Kinematica). Membranes containing 10 μg of total RNA/lane were hybridized with a [α-^{32}P]dCTP-labeled cDNA probe as described (5). Probes were: acidic ribosomal phosphoprotein P0 (36B4, nucleotides 9–969, GenBank™ accession code BC011106), Abcd2 (nucleotides 92–405, GenBank™ accession code BC019187), cytochrome p450 7α1 (Cyp7A1, nucleotides 1033–1435, GenBank™ accession code L23754), and SREBP1c (GenBank™ accession code NM004176).

Real-time Quantitative RT-PCR (QRT-PCR)—From total RNA, cDNA was generated by reverse transcription (GeneAmp RNA PCR kit; Applied Biosystems), and 12 ng per assay was analyzed using the iCycler iQ real-time PCR detection system (Bio-Rad). For quantification of murine Abcd2 mRNA the forward primer nt1959, 5′-CACAGCGTGCACCTCTAC-3′, reverse primer nt2032, 5′-AGGACATCTTTCCAGTCCA-3′, and the TaqMan fluorescent probe nt1986, 5′-HEX-CAA-

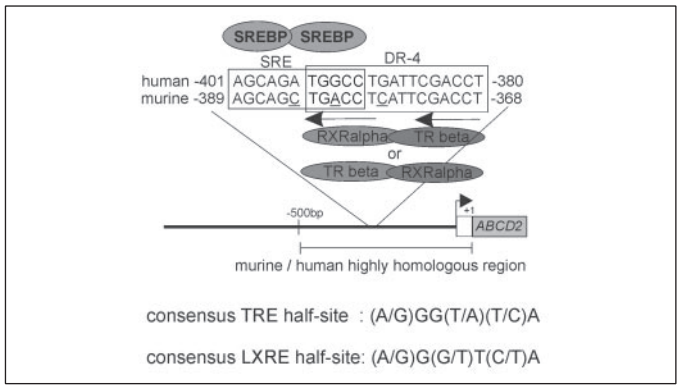

FIGURE 1. **The SRE and DR-4 motifs of the human and murine *ABCD2* promoter.** An SRE was identified at nucleotide position −401 to −391 and −389 to −379 with respect to the translation start site of the human and murine *ABCD2* genes, respectively. Nucleotides that differ between species are *underlined*. The SRE sequence overlaps 5 bp with a direct repeat hexameric sequence of the DR-4 type that fits the TR element or LXRE consensus sequence. The DR-4 half-sites are indicated by *arrows* below the sequence.

AGAGAAGGAGGATGGGATGC-TAMRA-3′ were used. For human ABCD2, primers were nt1883 5′-TCCTACACAATGTCCATCTC-T-3′ (forward), nt1961 5′-AGGACATCTTTCCAGTCCA-3′ (reverse), nt1915 5′-HEX-CAAAGAGAAGGAGGATGGGATCG-TAMRA-3′ (TaqMan probe). As a control, hydroxy-phosphoribosyl transferase mRNA levels were quantified: murine, nt 430, 5′-AAAGTTATTGGT-GGAGATGA-3′ (forward), nt518, 5′-TGCATTGTTTTACCAGTGT-C-3′ (reverse), nt454, 5′-FAM-TCAACTTTAACTGGAAAGAATG-TCTTGA-DABCYL-3′ (TaqMan probe); human, nt504 5′-GCAAAA-CAATGCAGACTT-3′ (forward), nt613 5′-TGGCTTATATCCAAC-ACTTC-3′ (reverse), nt567 5′-CAAGCTTGCTGGTGAAAAGGA-3′ (TaqMan probe). The standard curves for quantification were generated by serial dilution of plasmids containing ABCD2 (20) or hydroxy-phosphoribosyl transferase cDNA (21), kindly provided by Dr. D. W. Melton (University of Edinburgh, UK). The thermocycler was programmed: 95 °C for 10 min followed by 50 cycles at 95 °C for 20 s and 58 °C for 50 s. For quantification of ATP binding cassette transporter 1 (Abca1), QRT-PCR was performed as described (22).

Western Blot Analysis—Murine liver was homogenized in five volumes of buffer A (10 mM HEPES, pH 8.0; 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM EDTA, 5 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, protease inhibitor mix complete (Roche Applied Science)) and centrifuged at 100,000 × *g* for 60 min at 4 °C. Pellets were resuspended in buffer A containing 0.5% Nonidet P-40, separated by SDS-PAGE (8%), and transferred to a nitrocellulose membrane (Schleicher & Schnell). Proteins were detected using the antibodies anti-SREBP1: 2A4 (1:2500), anti-SREBP2: H164 (1:1000) (both Santa Cruz Biotechnology, Santa Cruz, CA), anti-β-actin: Mab1501 (1:10,000, Chemikon) and horseradish peroxidase-coupled anti-mouse (Dako) or anti-rabbit (Bio-Rad, both 1:50,000). Visualization was performed using Supersignal West Pico chemiluminescence substrate (Pierce). For reprobing, blots were washed three times with buffer B (0.2 M glycine, 0.5 M NaCl, pH 2.5) and neutralized with Tris-buffered saline-T.

RESULTS

A Functional SRE Is Present in the Murine Abcd2 Promoter That Is Preferentially Activated by SREBP1—A computer-based search of 3.5 kb of murine *Abcd2* promoter sequence (GenBank™ accession code AF302500) led to the identification of an SRE-like motif located at a similar position as in the human *ABCD2* gene (5) and differing by only two bp (Fig. 1). To test the functionality of the putative SRE, various extents between 0.5 and 3.5 kb (see "Experimental Procedures") of the murine *Abcd2* promoter were fused to a luciferase gene. These reporter constructs were used to transiently transfect COS-7 cells, together with a plasmid encoding either the mature forms of SREBP1a, SREBP1c, or SREBP2 or the empty vector pCMV5 (Fig. 2*a*). The luciferase activity increased in an SREBP-dependent manner with all three *Abcd2* promoter constructs. The highest levels of induction were observed after cotransfection with cDNAs encoding the SREBP1 isoforms. In contrast, cotransfection with SREBP2 resulted only in a low and, with the exception of the intermediate promoter construct (Abcd2–1486luc), barely statistically significant increase in reporter activity (Fig. 2*a*).

The fact that the shortest *Abcd2* promoter construct (Abcd2–499luc) was still activated by SREBP strongly suggested a proximal location of the SRE. To test whether the candidate SRE sequence (5′-AG-CAGCTGACC-3′, nucleotide −389 to −379) is sufficient to confer SREBP-dependent activation, it was cloned into a luciferase reporter construct upstream from a minimal β-globin promoter to generate pGl-mAbcd2-SREluc. Cotransfection with this construct and SREBP expression vectors clearly identified this element as a functional SRE (Fig. 2*b*). Again, induction was best conferred by SREBP1, reflecting the situation seen with the reporter constructs harboring the SRE in its natural context of the *Abcd2* promoter (Fig. 2*a*).

To confirm the requirement of the identified regulatory element in SREBP-mediated induction of the *Abcd2* promoter, we replaced the putative SRE in the minimal β-globin promoter context with two different mutated versions (pGl-mAbcd2-SRE-M1luc and pGl-mAbcd2-SRE-M2luc in Fig. 2*c*). For both constructs, the activation in response to SREBP1c cotransfection was significantly impaired, whereas only mutation M2 abrogated the induction by SREBP1a (Fig. 2*c*).

SREBP Directly Interacts with the SRE from the Abcd2 Gene—To establish whether the *Abcd2*-SRE can be directly bound by SREBP, EMSAs were carried out (Fig. 2*d*). The incubation of a ^{32}P-labeled Abcd2 SRE oligonucleotide probe with *in vitro* produced mature SREBP1a resulted in the formation of a protein-DNA complex (Fig. 2*d*, *lane 4*). SREBP1c was not incorporated in this study because its DNA-binding domain is identical to that of SREBP1a. To assess the specificity of this protein-DNA interaction, cross-competition using a 50-fold molar excess of unlabeled oligonucleotides containing the SRE from the murine stearyl-CoA desaturase 1 (*Scd1*) promoter was performed, resulting in reduced amounts of complex formation (Fig. 2*d*, *lane 5*). In contrast, the unrelated competitor mGh3 (murine growth hormone receptor 3) DNA was unable to inhibit SREBP1a binding (Fig. 2*d*, *lane 6*). As a positive control, complex formation between labeled oligonucleotide containing the *Scd1* SRE and SREBP1a is shown (Fig. 2*d*, *lane 3*). Thus, the results indicate that a direct interaction is possible between the SRE from the *Abcd2* gene and SREBP1.

In Fasted-Refed Mice, Abcd2 Expression Correlates with SREBP1c-activated Genes in Adipose and PPARα-regulated Genes in the Liver—To determine whether SREBP1c also regulates *Abcd2* expression *in vivo*, a fasting-refeeding protocol (see "Experimental Procedures") was employed. In this paradigm, typical SREBP1c-regulated genes like fatty acid synthase *(Fas)* are induced after fasting when mice are refed a high carbohydrate/low fat diet (23). When mice were fasted, Abcd2 mRNA levels markedly declined in white adipose tissue and were re-induced when mice were refed a high carbohydrate/low fat diet (Fig. 3*a*). These results show that during a fasting-refeeding protocol, adipose *Abcd2* expression correlates with the level of mature SREBP1c and thus with genes induced under conditions of fatty acid and triglyceride synthesis.

In the liver, fasting led to increased Abcd2 mRNA levels that returned to pre-fasted levels upon refeeding (Fig. 3, *b* and *c*). As expected, hepatic *Fas* expression was found to be strongly induced in the refed state,

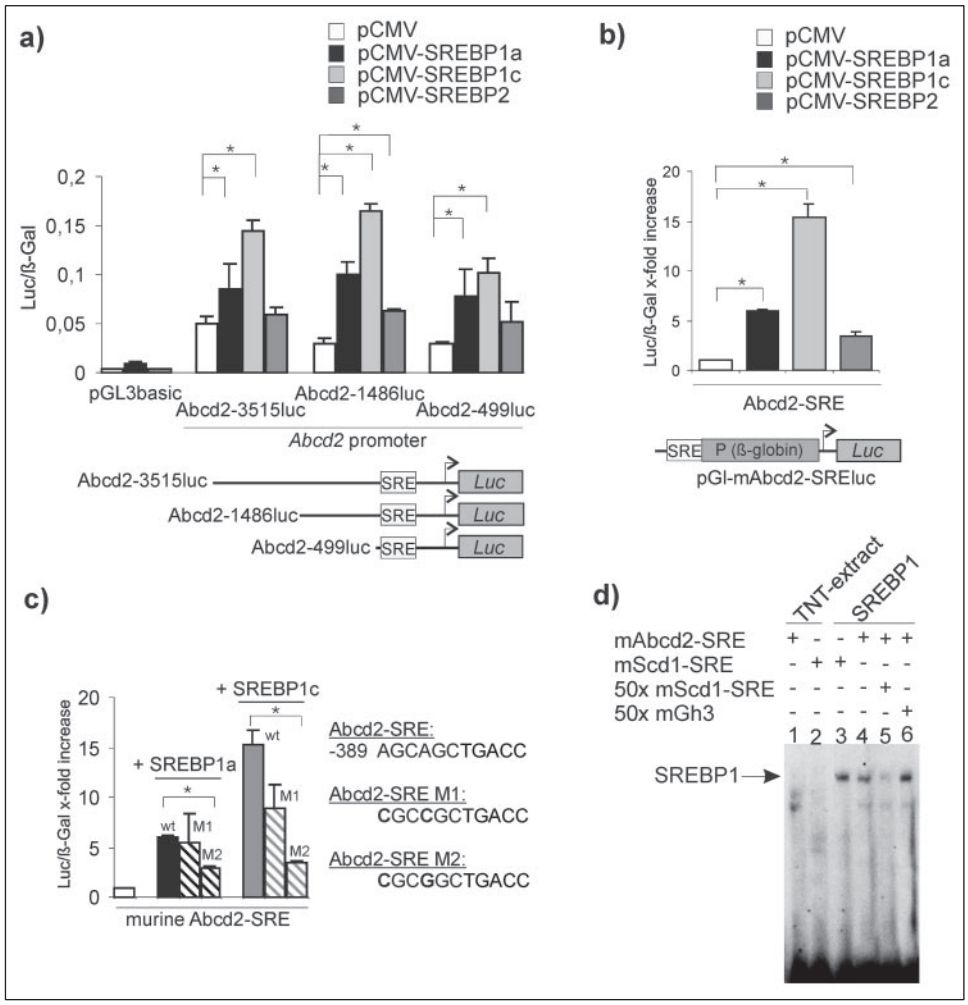

FIGURE 2. **Murine *Abcd2* expression is directly stimulated by SREBP.** COS-7 cells were transiently cotransfected with luciferase reporter constructs driven by murine *Abcd2* promoter regions of various length (3,515, 1,486, or 460 bp) (*a*) or the isolated wild type SRE from the murine *Abcd2* gene (*b*) or the SRE sequence with introduced point mutations M1 and M2 (pGl-mAbcd2-SRE-M1luc and pGl-mAbcd2-SRE-M2luc) cloned into the β-globin minimal promoter context (*c*), together with expression plasmids encoding the mature form of either human SREBP1a, SREBP1c, or SREBP2 or the empty vector pCMV5 as a negative control. Cotransfection with pCMV-βGal allowed for correction of the transfection efficiency in all experiments. The data are shown as luciferase activity (*Luc*) normalized to β-galactosidase activity (*β-Gal*) (*a*) and as -fold induction of Luc normalized to β-galactosidase (*b*, *c*) after SREBP cotransfection. Data represent the mean ± S.E. of four samples; for pGl-mAbcd2-SRE-M2luc, $n = 2$. Statistically significant differences by Student's *t* test are indicated by *asterisks* ($p < 0.05$). *d*, EMSA using *in vitro* synthesized SREBP1a and a ^{32}P-labeled oligonucleotide probe corresponding to the murine *Abcd2* SRE show a band shift (*lane 4*). Specificity of the interaction was tested by cross-competition with excess of unlabeled oligonucleotides containing the *Scd1* SRE (*lane 5*) and with the unrelated competitor mGh3 (*lane 6*). Radioactively labeled *Scd1* SRE oligonucleotide was used as a positive control (*lane 3*). Incubation of the labeled probe with unprimed reticulocyte lysate did not result in complex formation (*lanes 1* and *2*). Specific bands are marked by an *arrow*.

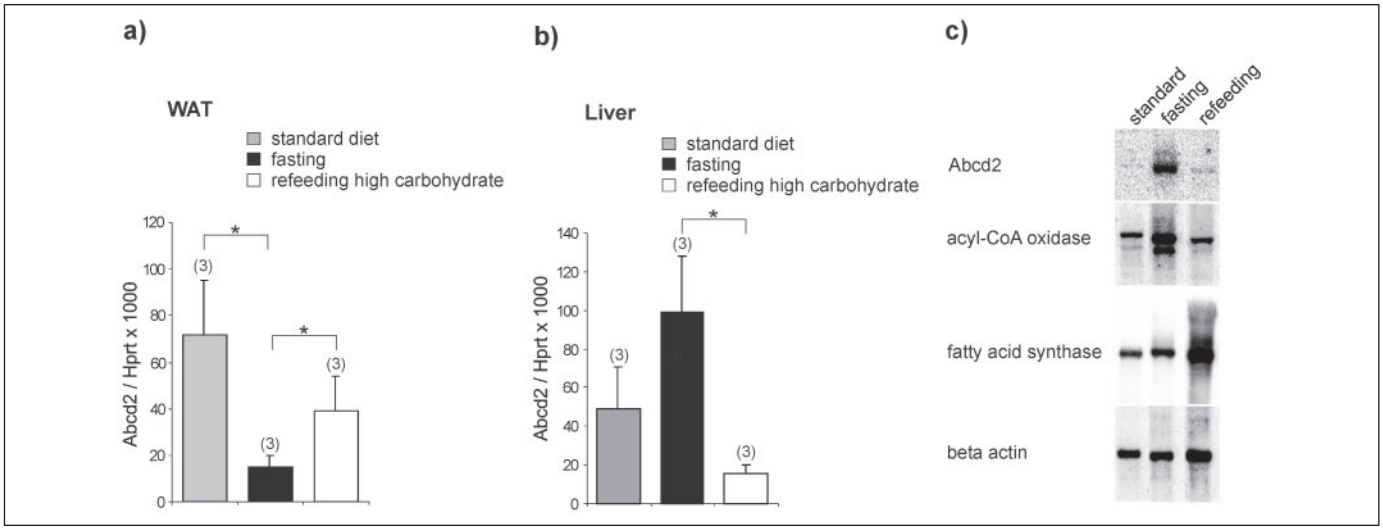

FIGURE 3. **Fasting-refeeding of mice reveals that *Abcd2* expression in the liver is regulated by PPARα, whereas Abcd2 mRNA levels in white adipose tissue correlate with SREBP1c-stimulated genes.** Wild type C57BL/6 mice were fasted for 24 h and then refed a high carbohydrate/low fat diet for 12 h. Total RNA was isolated from adipose tissue and liver and Abcd2 mRNA levels were evaluated by (*a*, *b*) QRT-PCR performed in triplicate wells and normalized to the number of hydroxy-phosphoribosyl transferase mRNA copies (results are shown as mean ± S.E. with the number of treated mice in parentheses and statistically significant differences by Student's *t* test are indicated by *asterisks* ($p < 0.05$)) and (*c*) Northern blot analysis, with detection of acyl-CoA oxidase and fatty acid synthase mRNA levels as a control for fasting and refeeding, respectively. β-Actin was used as a control for equal loading and transfer.

whereas fasting induced acyl-CoA oxidase, encoding the key enzyme of the fatty acid degradation pathway, which is controlled by the nuclear peroxisome proliferator-activated receptor (PPAR) α (Fig. 3*c*). Thus, in accordance with our previous studies involving synthetic PPARα agonists (20, 24), hepatic *Abcd2* regulation correlated with a PPARα and not with a SREBP1c target gene, although several investigations failed to

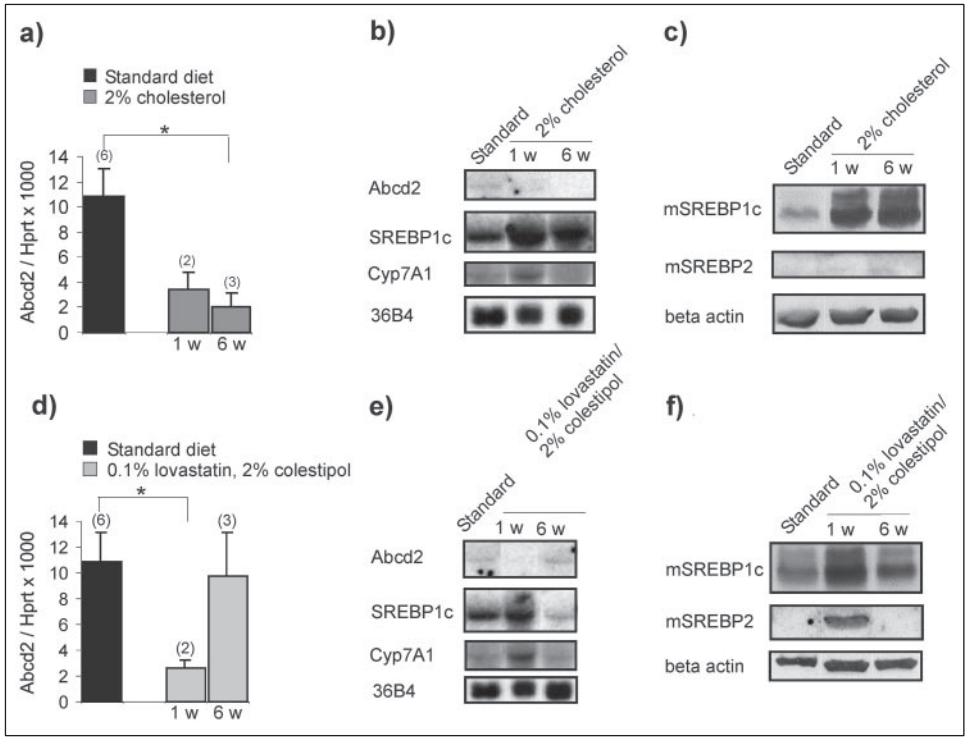

FIGURE 4. **Hepatic *Abcd2* expression in cholesterol- or lovastatin/colestipol-treated mice inversely correlates with LXRα induction.** Wild type C57BL/6 mice were fed a diet supplemented with either 2% cholesterol (*a–c*) or 0.1% lovastatin and 2% colestipol (*d–f*) for 1 or 6 weeks. Total RNA was isolated from the liver to evaluate Abcd2 mRNA levels using quantitative real-time RT-PCR (*a*, *d*) or Northern blot analysis (*b*, *e*), with detection of SREBP1c and Cyp7A1 mRNAs as controls for the feeding paradigm. 36B4 was used as a control for equal loading and transfer. *c*, Western blot analysis of equal amounts of homogenized liver samples (100,000 × *g* pellet) using antibodies recognizing mature SREBP1c (*mSREBP*) or mature SREBP2 (*mSREBP2*). As a loading control for the Western blot analysis, detection of β-actin is shown. *a* and *d*, results were normalized to the amount of hydroxy-phosphoribosyl transferase mRNA in each sample and are shown as mean ± S.E. with the number of analyzed mice in *parentheses*. Each sample was analyzed in triplicate.

localize a PPARα binding site in the human or rodent *ABCD2* promoter (18, 24, 25).

Hepatic Abcd2 Expression Is Down-regulated by Dietary Cholesterol—To investigate whether SREBP1c plays a role in regulating hepatic *Abcd2* expression under conditions when PPARα is not activated, we studied the effect of a dietary modulation of cholesterol levels in mice fed a diet containing 2% cholesterol (for comparison, the standard mouse chow contains only 0.01% cholesterol). In this feeding paradigm, excess cholesterol results in LXRα activation in the murine liver, which induces *SREBP1c* transcription but also cholesterol storage and efflux through conversion to bile acids (26). Hepatic *Abcd2* expression was evaluated after 1 and 6 weeks by QRT-PCR and Northern blot analysis (Fig. 4, *a* and *b*). Surprisingly, a high cholesterol diet led to significantly reduced Abcd2 mRNA levels in the liver after 1 week and a further down-regulation after 6 weeks of treatment (Fig. 4, *a* and *b*). In contrast, *SREBP1c* was consistently induced after 1 and 6 weeks of cholesterol feeding. In addition, cholesterol 7α-hydroxylase (Cyp7A1) mRNA, encoding the rate-limiting enzyme of bile acid synthesis, was up-regulated after 1 week (Fig. 4*b*). As both genes are directly controlled by LXRα, their induction serves as a positive control for the treatment paradigm, indicating that LXRα was activated by cholesterol feeding. At the protein level, high cholesterol feeding resulted in increased levels of cleaved SREBP1c, whereas SREBP2 was barely detectable by Western blot analysis before and after feeding a cholesterol-enriched diet (Fig. 4*c*), thus confirming previous studies. From these results, it appears that although a functional SRE is present in the *Abcd2* promoter and SREBP1c is amply available, SREBP recruitment is not possible under conditions of concomitant LXRα activation.

Abcd2 Expression Correlates Inversely with LXRα-mediated SREBP1c Induction in the Liver of Cholesterol-depleted Mice—To clarify whether depletion of sterols also affects hepatic *Abcd2* expression, mice were treated with a diet supplemented with 0.1% lovastatin and 2% colestipol for 1 and 6 weeks. This feeding paradigm is known to decrease the hepatic cholesterol content by (*a*) lovastatin inhibiting HMG-CoA reductase, the rate-controlling enzyme of cholesterol biosynthesis, and (*b*) colestipol binding bile acids in the intestine, thus decreasing their reabsorption and stimulating the liver to convert more cholesterol to bile acids. Abcd2 mRNA levels were quantified using QRT-PCR and Northern blotting (Fig. 4, *d* and *e*). After 1 week of treatment, Abcd2 mRNA levels were reduced in the liver, comparable with the down-regulation seen after feeding a cholesterol-enriched diet. After 6 weeks of treatment, however, Abcd2 mRNA returned to pretreatment levels (Fig. 4, *d* and *e*). Northern blot analysis verified the results obtained by QRT-PCR and revealed that both SREBP1c and Cyp7A1 mRNA levels were moderately induced after 1 week of cholesterol depletion but returned to pretreatment or lower levels after 6 weeks (Fig. 4*e*). The moderate and transient increase in *SREBP1c* expression observed after 1 week was also reflected by an increase in mature SREBP1c (Fig. 4*f*). As expected, cleaved SREBP2 was barely detectable by Western blot analysis in mice fed normal chow but strongly increased when the diet was supplemented with lovastatin/colestipol for 1 week, thus reflecting a response aimed at inducing hepatic *de novo* cholesterol synthesis (Fig. 4*f*). After 6 weeks, the amounts of mature SREBP2 returned to pretreatment levels, indicating a transient effect of lovastatin/colestipol on hepatic cholesterol metabolism in mice (Fig. 4*f*). In summary, these results suggest that a combinatorial treatment of lovastatin/colestipol for 1 week stimulates cholesterol and bile acid synthesis through activation of SREBP2 and LXRα, respectively, as shown by maturation of SREBP2 and induction of *SREBP1c* and *Cyp7A1* expression, which correlates with a down-regulation of Abcd2 mRNA levels.

LXRα Interferes with SREBP-mediated Stimulation of Abcd2 Promoter Reporter Constructs—Because *Abcd2* expression inversely correlates with induction of SREBP1c *in vivo*, we considered LXRα as a potential candidate that could interfere with the access of SREBP1c to the *Abcd2* SRE by binding to the overlapping DR-4 element (Fig. 1). To test this hypothesis, we next examined whether LXRα directly affects SREBP1c-dependent stimulation of the *Abcd2* promoter. COS-7 cells were cotransfected with the reporter construct containing 3.5 kb of the

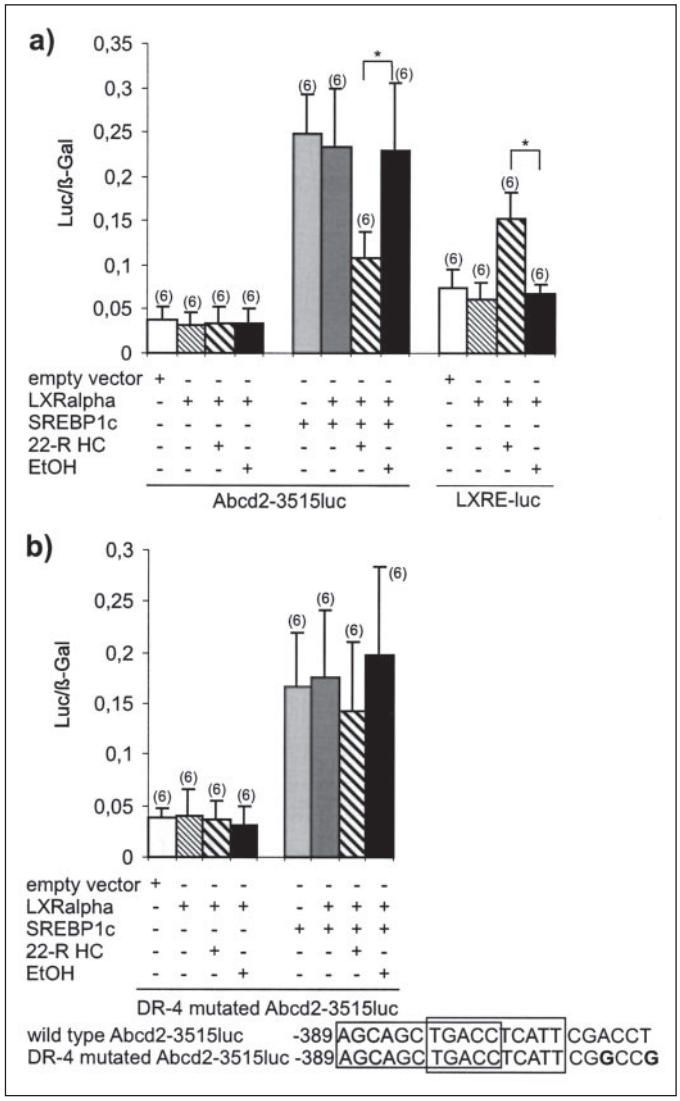

FIGURE 5. **LXRα interferes with SREBP1c-mediated stimulation of *Abcd2*.** COS-7 cells were transiently cotransfected with a luciferase reporter construct driven by 3.5 kb of the murine *Abcd2* promoter (*Abcd2–3515luc*) (*a*) or a 3.5-kb *Abcd2* promoter reporter construct with a mutated DR-4 motif and expression plasmids encoding human LXRα, the mature form of human SREBP1c, or the empty vector pCMV5 as a negative control (*b*), as indicated. To allow correction of transfection efficiency, pCMV-βGal was cotransfected in all experiments. The mutations that were introduced in the DR-4 of the *Abcd2* gene are indicated in bold at the bottom of *panel b*. For ligand activation of LXRα, 24-h post-transfection cells were treated for 24 h with 10 μM 22-(*R*)-hydroxycholesterol (*22-R-HC*). The LXRE of the human *ABCA1* gene was cloned into the β-globin minimal promoter context of the reporter construct (*LXRE-luc*) and used as a positive control for LXRα stimulation. Data represent the mean ± S.E. of six samples. Statistically significant differences by Student's *t* test are indicated by *asterisks* ($p < 0.05$).

Abcd2 promoter (Abcd2–3515luc; compare Fig. 2*a*) and expression plasmids encoding either LXRα or SREBP1c or both. Because COS-7 cells endogenously express RXRα, the heterodimer partner of LXRα, cotransfection with a RXRα expression plasmid was not necessary. Neither unactivated LXRα nor 22-(*R*)-HC-activated LXRα influenced expression from the *Abcd2* promoter reporter construct (Fig. 5*a*). When the cells were cotransfected with LXRα and mature SREBP1c, the reporter gene was activated to a level comparable with the induction mediated by SREBP1c alone. However, cotransfection with SREBP1c and LXRα in the presence of the LXRα ligand 22-(*R*)-HC resulted in repression of SREBP1c-mediated stimulation of the *Abcd2* promoter (Fig. 5*a*). To confirm that activated LXRα directly interferes with SREBP1c stimulation via the DR-4 element, two point mutations were introduced into the DR-4 half-site that does not overlap with the SRE of the *Abcd2* promoter reporter construct. When this mutated construct, designated DR-4 mutated Abcd2–3515luc, was cotransfected with SREBP1c and LXRα expression plasmids, 22-(*R*)-HC-activated LXRα was no longer capable of repressing SREBP1c stimulation of the *Abcd2* promoter (Fig. 5*b*). These results strongly suggest that ligand-activated LXRα has a negative effect on *Abcd2* gene activation by SREBP and that the intact DR-4 element is necessary to mediate this repression.

LXRα/RXRα Interacts Directly with the DR-4 from the Abcd2 Gene—The ability of LXRα/RXRα to specifically bind to the SRE/DR-4 sequence from the *Abcd2* gene was tested by EMSA (Fig. 6). The wild type oligonucleotides were radiolabeled with [α-^{32}P]dCTP and incubated with *in vitro* synthesized LXRα and RXRα. In the presence of both receptors, the formation of a protein-DNA complex was observed (Fig. 6*a*, *lane 3*) that was not dependent, as assessed by the incorporation of the LXRα agonist 22-(*R*)-HC (Fig. 6*a*, *lane 4*). The specificity of binding was shown by the ability of a 50-fold molar excess of unlabeled LXRE oligonucleotides from the chicken acetyl-CoA carboxylase gene to reduce the binding of the LXRα/RXRα heterodimer (Fig. 6*a*, *lane 6*), whereas the unrelated competitor DNA mGhr3 was unable to inhibit the formation of the complex (Fig. 6*a*, *lane 5*). As a positive control, complex formation between LXRα/RXRα and labeled oligonucleotides containing the acetyl-CoA carboxylase-LXRE is shown (Fig. 6*a*, *lane 2*). To test whether LXRα interferes with binding of SREBP1a to the SRE/DR-4 from the *Abcd2* promoter, *in vitro* synthesized SREBP1a was incorporated in the assay system (Fig. 6*b*). As expected, incubation of the mAbcd2-SRE/DR-4-containing oligonucleotides with SREBP1a alone resulted in formation of a DNA-protein complex (Fig. 6*b*, *lane 1*). In the presence of both SREBP1a and LXRα/RXRα, two specific bands corresponding to SREBP1a and LXRα/RXRα protein-DNA complexes were formed (Fig. 6*b*, *lane 2*) that were not affected by the addition of 22-(*R*)-HC (Fig. 6*b*, *lane 3*). However, no supershifted bands appeared that would indicate a direct interaction between SREBP and LXRα. In summary, these results show that the LXRα/RXRα heterodimer directly binds the Abcd2 SRE/DR-4 element and furthermore suggest that a concurrent binding of SREBP1 and LXRα/RXRα to this sequence is not possible.

In LXRα,β-deficient Mice Hepatic Abcd2 Expression Can Be Induced to Levels Vastly Exceeding Those in Wild Type—The effect of ligand-activated LXRα on hepatic *Abcd2* expression was further examined by comparing the response to LXR agonist treatment in wild type and LXRα,β-deficient mice (Fig. 7*a*). By QRT-PCR, basal Abcd2 mRNA levels were found to be slightly but not significantly elevated in the liver of LXRα,β-deficient mice compared with wild type controls. In addition, when animals were treated with the synthetic LXR ligand T0901317 for 12 h, Abcd2 mRNA levels did not significantly differ from untreated controls in both genotypes. In contrast, treatment with the synthetic RXRα ligand LG268 resulted in an almost 6-fold increase of hepatic *Abcd2* expression in both wild type and LXRα,β-deficient mice. As this induction was also observed in LXRα,β-deficient mice, the underlying mechanism seems not to involve LXRs as heterodimeric partner for RXRα. Strong hepatic stimulation of the LXRα target gene *Abca1* requires activation by both LXRα and RXRα ligands, and thus a combinatorial treatment with T0901317 and LG268 was performed. As expected, Abca1 mRNA levels were significantly induced in the liver of wild type but not in LXRα,β-deficient mice. Surprisingly, in wild type mice administration of both LXRα and RXRα ligands abolished the induction of *Abcd2* observed with the RXRα agonist alone, indicating a negative effect of activated LXR on the stimulation of *Abcd2* expression. In contrast, LXRα,β-deficient mice treated with both LXRα and RXRα

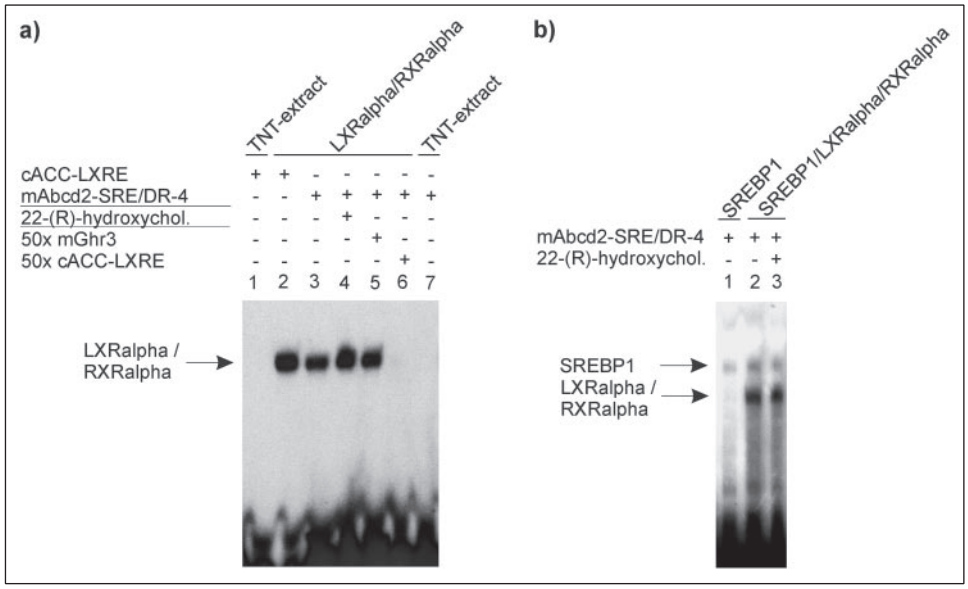

FIGURE 6. **LXRα/RXRα specifically binds the SRE/DR-4 sequence of the murine *Abcd2* promoter.** EMSA using *in vitro* transcribed and translated LXRα/RXRα (*a*) and SREBP1 and LXRα/RXRα/SREBP1 and a ^{32}P-labeled oligonucleotide probe corresponding to the mAbcd2 SRE/DR-4 sequence (*b*), as indicated. Specificity of the interaction was tested by cross-competition with excess of unlabeled acetyl-CoA carboxylase-LXRE oligonucleotide (*panel a*, *lane 6*) or unspecific mGhr3 oligonucleotides (*panel a*, *lane 5*). Radioactively labeled acetyl-CoA carboxylase-LXRE oligonucleotide was used as a positive control (*panel a*, *lane 2*). Incubation of the labeled probe with unprimed reticulocyte lysate did not result in complex formation (*panel a*, *lane 1*). Specific bands are marked by an *arrow*.

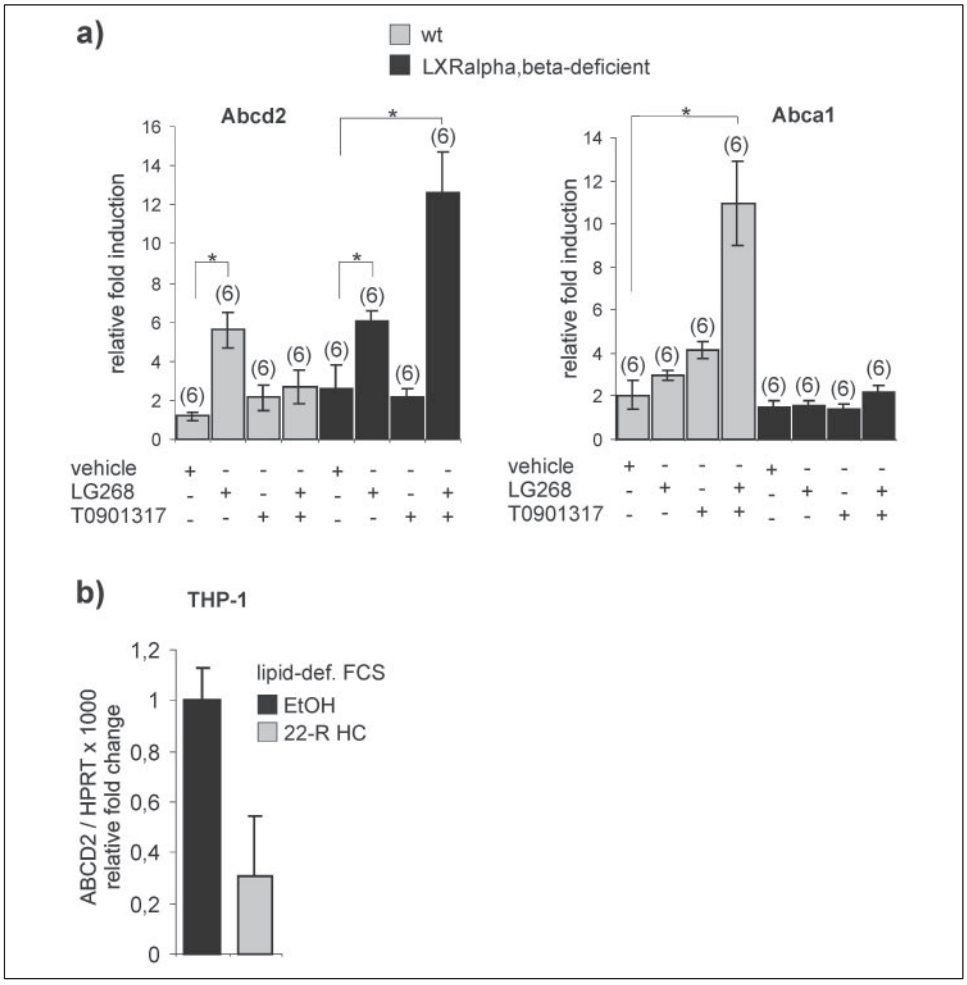

FIGURE 7. **Investigation of the negatively modulatory effect of LXRα,β-deficient mice and human monocytic cells.** *a*, wild type mixed C57BL/6:129/SvEv and LXRα,β-deficient mice were treated with either the synthetic RXRα ligand LG268, the LXR ligand T0901317, or both for 12 h. Total RNA was isolated from the liver to evaluate Abcd2 and Abca1 mRNA levels using QRT-PCR. The results are shown as mean ± S.E. with the number of analyzed mice in *parentheses*. *b*, human monocytic THP-1 cells were cultured in medium containing lipid-deficient fetal calf serum and treated with 10 μM 22-(*R*)-hydroxycholesterol for 24 h. The number of Abcd2 mRNA copies was evaluated by QRT-PCR. Data represent the mean ± S.E. of two samples. Statistically significant differences by Student's *t* test are indicated by *asterisks* ($p < 0.05$).

ligands demonstrated hepatic Abcd2 mRNA levels vastly exceeding those observed in LG268-treated animals, thus reflecting the loss of negative LXR action on *Abcd2* expression.

Activation of LXRα under Conditions of Induced SREBP Maturation Results in Reduced ABCD2 mRNA Levels in Human Monocytic Cells—We have previously shown that SREBP activation by cholesterol depletion in human and murine monocyte or microglial cell lines results in substantially increased ABCD2 mRNA levels (5). To test whether activation of LXRα under cholesterol depletion influences endogenous *ABCD2* expression, human monocytic THP-1

cells were incubated with 10 μM 22-(*R*)-HC in sterol-deficient medium for 24 h, and ABCD2 mRNA levels were quantified by QRT-PCR (Fig. 7*b*). Treatment with 22-(*R*)-HC under concurrent SREBP activation results in a considerable down-regulation of *ABCD2* expression, indicating that also in cultured human cells, activated LXRα is a negative modulator of *ABCD2*.

DISCUSSION

Pharmacological stimulation of the *ABCD2* gene has been suggested as an alternative therapeutic strategy for X-ALD (4), necessitating a careful characterization of endogenous *ABCD2* expression and function of the encoded protein. Our recent work has uncovered a link between human *ABCD2* expression and cholesterol metabolism in cultured cells (5). Thus, the goal of the present study was to investigate the *in vivo* effect of cholesterol and SREBP maturation on murine *Abcd2* expression.

Our cell culture experiments show that murine *Abcd2* expression is also regulated by SREBPs and revealed the presence of a functional SRE in the murine *Abcd2* promoter, which was preferentially stimulated by SREBP1. Interestingly, no significant stimulation of reporter gene expression was observed with SREBP2, indicating that in cell culture this SREBP isoform stimulates the human, but not the murine, *ABCD2* gene.

To investigate whether *in vivo* SREBP1 also regulates *Abcd2* expression, different feeding paradigms were applied. In adipose, fasting, reduced, and refeeding a high carbohydrate/low fat diet induced Abcd2 mRNA levels. Together with the presence of a functional SRE in the *Abcd2* promoter region, this suggests that Aldrp in white adipose tissue is connected to fatty acid and triglyceride synthesis and lipid storage. This proposition is further supported by the finding that the SRE motifs present in both the murine and the human *ABCD2* promoter region show striking similarity to the SRE of the SREBP1c-regulated *Scd1* promoter (5, 27). Scd1 is involved in fatty acid synthesis by catalyzing the Δ^9-cis desaturation of acyl-CoAs with palmitoyl-CoA and stearyl-CoA as preferred substrates.

In the liver, conditions of increased fatty acid oxidation through PPARα activation during fasting led to induced *Abcd2* expression. When mice were refed a high carbohydrate/low fat diet resulting in reduced need for degradation of fatty acids as energy source, hepatic Abcd2 mRNA levels were down-regulated. Thus, regulation of hepatic *Abcd2* expression correlated with a PPARα, and not with a SREBP1c, target gene. Combined with a peroxisomal localization, these results suggest that in the liver, Aldrp is somehow involved in degradation of fatty acids. Interestingly, this dual regulation of expression by the two factors SREBP1c and PPARα, which possess opposing activation properties depending on nutritional state, has also been demonstrated for several desaturase genes like *Scd1* (28) and seems to place Aldrp and Scd1 in a position distinct from most other proteins involved in lipid metabolism.

To better understand the role of Aldrp in hepatic cholesterol and lipid metabolism, mice were treated with either high cholesterol diets or lovastatin and colestipol. Unexpectedly, these feeding paradigms demonstrated that although SREBP1c activation occurred through LXRα-mediated stimulation of transcription, *Abcd2* expression was repressed in the liver of these mice. We thus hypothesized that hepatic *Abcd2* expression is repressed under conditions of concomitant SREBP1c and LXRα activation because the SRE might not be accessible for SREBP binding. In support of this notion, both the murine and the human *ABCD2* promoters show an overlap between the SRE and one half-site of a DR-4 element that matches the consensus sequence for LXR/RXRα binding. In an effort to explore whether the repression of SREBP1c-induced *Abcd2* expression seen under conditions of concurrent SREBP1c and LXRα activation could be explained by competitive binding of LXRα/RXRα to the DR-4 sequence, thus obstructing the SRE, reporter gene studies, *in vitro* mutagenesis, and gel shift assays were carried out. The results obtained indeed show that LXRα is able to directly bind the DR-4 element and that LXRα, upon ligand-binding, interferes with SREBP1c-mediated activation of the *Abcd2* promoter.

In addition to LXRα/RXRα heterodimers, previous studies demonstrated that TRβ homodimers or TRβ/RXRα heterodimers also can bind the *Abcd2* SRE/DR-4 sequence (12). The ability of different nuclear steroid receptors to heterodimerize with RXRα and to bind the same DR-4 response element adds an additional level to regulation of gene expression (29). In whole body metabolism SREBPs, TRβ, and LXRα are directly functionally linked through a complex transcriptional regulatory network. When sterols are in excess, LXRα mediates transcriptional activation of *SREBP1c*, thus providing a mechanism for inducing fatty acid synthesis necessary for storage of cholesterol (30). For the *SREBP2* gene, a direct regulation by TRβ binding to a TR-response element in the 5′-flanking region could be demonstrated (31). Thus, a clear link exists between TRβ, LXRα, and SREBP, strongly suggesting a physiological relevance of the overlapping SRE and DR-4 elements for *ABCD2* expression.

To determine whether *Abcd2* is also *in vivo* a direct target of LXRα, studies involving the synthetic LXR and RXRα ligands T0901317 and LG268, respectively, were carried out. Treatment of either wild type or LXRα,β-deficient mice with T0901317 for 12 h did not change Abcd2 mRNA levels. A possible explanation could be that the strong synthetic LXR ligand T0901317 is expected to be more potent in inducing *SREBP1c* expression than dietary cholesterol treatment and thus might result in a titration of the positive and negative effect of SREBP1c and LXRα, respectively, on the *Abcd2* promoter. When wild type mice and LXRα,β-deficient mice were exposed to the RXRα ligand LG268, a strong induction of hepatic *Abcd2* expression was observed, thus confirming previous studies demonstrating the inducibility of *ABCD2* by 9-cis retinoic acid-activated RXRα in cultured cells (25). As activation of RXRα also results in stimulation of *Abcd2* expression in the liver of LXR-deficient mice, a role for LXRs in this induction can be excluded. Possibly, the RXRα heterodimeric partner TRβ, which binds and activates the *Abcd2* promoter (12), is involved in this induction. An intriguing observation of this feeding experiment is the finding that co-administration of both LXRα and RXRα ligands, T0901317 and LG268, abrogated the induction of Abcd2 mRNA levels seen after RXRα activation. This suggests that with activation of both LXRα and RXRα, the repressive effect of LXRα on the *Abcd2* promoter seems to overwhelm the stimulation observed with the RXRα ligand alone, possibly by competing for binding to the *Abcd2* DR-4 element. This hypothesis is further supported by the finding that in LXR-deficient mice, co-administration of both T0901317 and LG268 does not influence the induction observed after treatment with LG268 alone. Indeed, a hepatic stimulation of *Abcd2* expression to levels vastly exceeding those in wild type is observed. This might be explained by involvement of other DR-4 binding nuclear receptors cross-activated by T0901317, *e.g.* farnesoid X receptor and pregnane X receptor (32, 33), that could possibly bind the *Abcd2* SRE/DR-4 in the absence of LXRα.

The aim of our work is to pharmacologically induce *ABCD2* in X-ALD patients. Thus, we also investigated whether the observation that LXRα interferes with SREBP1c-mediated *Abcd2* expression is conserved between man and mouse. When human monocytic cells were cultured under conditions of induced SREBP maturation, LXR ligand

treatment resulted in reduced ABCD2 mRNA levels, indicating that also in human cells, activated LXRα is a negative modulator of *ABCD2* expression.

In conclusion, our results have exposed a novel mode of cross-talk between LXRα and SREBP1c in gene regulation. It is tempting to speculate that an overlap between SRE and DR-4 elements might provide a mechanism by which a subset of SREBP1c-inducible genes are down-regulated under conditions of sustained LXRα activation. In combination with cell type or tissue-specific variation in activation or availability of LXRα, SREBP1c could stimulate *ABCD2* expression in some tissues, whereas LXRα-mediated repression is obtained in others. Moreover, transcriptional regulation by LXRα is dependent on different tissue-specific cofactors, further accounting for additional effects on target genes (34). To sum up, the observation that hepatic *Abcd2* is actively down-regulated under conditions of LXRα activation opens up a novel therapeutic strategy for X-ALD, targeted at bypassing repressive effects on *Abcd2* expression.

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REFERENCES

1. Moser, H. W., Smith, K. D., Watkins, P. A., Powers, J., and Moser, A. B. (2001) in *The Metabolic and Molecular Bases of Inherited Disease* (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds), 8th Ed., pp. 3257–3301, McGraw-Hill, New York
2. Netik, A., Forss-Petter, S., Holzinger, A., Molzer, B., Unterrainer, G., and Berger, J. (1999) *Hum. Mol. Genet.* **8,** 907–913
3. Pujol, A., Ferrer, I., Camps, C., Metzger, E., Hindelang, C., Callizot, N., Ruiz, M., Pampols, T., Giros, M., and Mandel, J. L. (2004) *Hum. Mol. Genet* **13,** 2997–3006
4. Kemp, S., Wei, H. M., Lu, J. F., Braiterman, L. T., McGuinness, M. C., Moser, A. B., Watkins, P. A., and Smith, K. D. (1998) *Nat. Med.* **4,** 1261–1268
5. Weinhofer, I., Forss-Petter, S., Zigman, M., and Berger, J. (2002) *Hum. Mol. Genet.* **11,** 2701–2708
6. Horton, J. D., Goldstein, J. L., and Brown, M. S. (2002) *J. Clin. Investig.* **109,** 1125–1131
7. Brown, M. S., and Goldstein, J. L. (1997) *Cell* **89,** 331–340
8. Shimomura, I., Shimano, H., Horton, J. D., Goldstein, J. L., and Brown, M. S. (1997) *J. Clin. Investig.* **99,** 838–845
9. Forss-Petter, S., Werner, H., Berger, J., Lassmann, H., Molzer, B., Schwab, M. H., Bernheimer, H., Zimmermann, F., and Nave, K. A. (1997) *J. Neurosci. Res.* **50,** 829–843
10. Kobayashi, T., Shinnoh, N., Kondo, A., and Yamada, T. (1997) *Biochem. Biophys. Res. Commun.* **232,** 631–636
11. Lu, J. F., Lawler, A. M., Watkins, P. A., Powers, J. M., Moser, A. B., Moser, H. W., and Smith, K. D. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 9366–9371
12. Fourcade, S., Savary, S., Gondcaille, C., Berger, J., Netik, A., Cadepond, F., El Etr, M., Molzer, B., and Bugaut, M. (2003) *Mol. Pharmacol.* **63,** 1296–1303
13. Peet, D. J., Turley, S. D., Ma, W., Janowski, B. A., Lobaccaro, J. M., Hammer, R. E., and Mangelsdorf, D. J. (1998) *Cell* **93,** 693–704
14. Zhang, Y., and Mangelsdorf, D. J. (2002) *Mol. Interv.* **2,** 78–87
15. Hu, X., Li, S., Wu, J., Xia, C., and Lala, D. S. (2003) *Mol. Endocrinol.* **17,** 1019–1026
16. Castrillo, A., Joseph, S. B., Marathe, C., Mangelsdorf, D. J., and Tontonoz, P. (2003) *J. Biol. Chem.* **278,** 10443–10449
17. Stulnig, T. M., Oppermann, U., Steffensen, K. R., Schuster, G. U., and Gustafsson, J. A. (2002) *Diabetes* **51,** 2426–2433
18. Fourcade, S., Savary, S., Albet, S., Gauthe, D., Gondcaille, C., Pineau, T., Bellenger, J., Bentejac, M., Holzinger, A., Berger, J., and Bugaut, M. (2001) *Eur. J. Biochem.* **268,** 3490–3500
19. Repa, J. J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J. M., Shimomura, I., Shan, B., Brown, M. S., Goldstein, J. L., and Mangelsdorf, D. J. (2000) *Genes Dev.* **14,** 2819–2830
20. Berger, J., Albet, S., Bentejac, M., Netik, A., Holzinger, A., Roscher, A. A., Bugaut, M., and Forss-Petter, S. (1999) *Eur. J. Biochem.* **265,** 719–727
21. Konecki, D. S., Brennand, J., Fuscoe, J. C., Caskey, C. T., and Chinault, A. C. (1982) *Nucleic Acids Res.* **10,** 6763–6775
22. Bookout, A. L., and Mangelsdorf, D. J. (2003) *NURSA e-Journal* **1**
23. Horton, J. D., Bashmakov, Y., Shimomura, I., and Shimano, H. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 5987–5992
24. Rampler, H., Weinhofer, I., Netik, A., Forss-Petter, S., Brown, P. J., Oplinger, J. A., Bugaut, M., and Berger, J. (2003) *Mol. Genet Metab.* **80,** 398–407
25. Pujol, A., Troffer-Charlier, N., Metzger, E., Chimini, G., and Mandel, J. L. (2000) *Genomics* **70,** 131–139
26. Tontonoz, P., and Mangelsdorf, D. J. (2003) *Mol. Endocrinol.* **17,** 985–993
27. Tabor, D. E., Kim, J. B., Spiegelman, B. M., and Edwards, P. A. (1999) *J. Biol. Chem.* **274,** 20603–20610
28. Nakamura, M. T., and Nara, T. Y. (2002) *Biochem. Soc. Trans.* **30,** 1076–1079
29. Quack, M., Frank, C., and Carlberg, C. (2002) *J. Cell. Biochem.* **86,** 601–612
30. Yoshikawa, T., Shimano, H., Amemiya-Kudo, M., Yahagi, N., Hasty, A. H., Matsuzaka, T., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Osuga, J., Harada, K., Gotoda, T., Kimura, S., Ishibashi, S., and Yamada, N. (2001) *Mol. Cell. Biol.* **21,** 2991–3000
31. Shin, D. J., and Osborne, T. F. (2003) *J. Biol. Chem.* **278,** 34114–34118
32. Houck, K. A., Borchert, K. M., Hepler, C. D., Thomas, J. S., Bramlett, K. S., Michael, L. F., and Burris, T. P. (2004) *Mol. Genet. Metab.* **83,** 184–187
33. Shenoy, S. D., Spencer, T. A., Mercer-Haines, N. A., Alipour, M., Gargano, M. D., Runge-Morris, M., and Kocarek, T. A. (2004) *Drug Metab. Dispos.* **32,** 66–71
34. Miao, B., Zondlo, S., Gibbs, S., Cromley, D., Hosagrahara, V. P., Kirchgessner, T. G., Billheimer, J., and Mukherjee, R. (2004) *J. Lipid Res.*