The nuclear receptors LXRα and LXRβ have been implicated in the control of lipogenesis and cholesterol homeostasis. Ligand activation of these receptors in vivo induces expression of the LXR target gene SREBP-1c and increases plasma triglyceride levels. Expression of fatty acid synthase (FAS), a central enzyme in de novo lipogenesis and an established target of the SREBP-1 pathway, is also induced by LXR ligands. The effects of LXR ligands on FAS expression have been proposed to be entirely secondary to the induction of SREBP-1c. We demonstrate here that LXRs regulate FAS expression through direct interaction with the FAS promoter as well as through activation of SREBP-1c expression. Induction of FAS expression in HepG2 cells by LXR ligands is reduced, but not abolished, under conditions where SREBP processing is suppressed. Moreover, LXR ligands induce FAS expression in CHO-7 cells without altering expression of SREBP-1. We demonstrate that in addition to tandem SREBP sites, the FAS promoter contains a high affinity binding site for the LXR/RXR heterodimer that is conserved in diverse animal species including birds, rodents, and humans. The LXR and SREBP binding sites independently confer LXR responsiveness on the FAS promoter, and maximal induction requires both transcription factors. Transient elevation of plasma triglyceride levels in mice treated with a synthetic LXR agonist triggers induction of the FAS promoter at multiple sites (7, 15). Overexpression of nuclear SREBP-1 is sufficient to induce expression of the FAS gene in cultured cells as well as transgenic mice (5, 8).

Recent work has also implicated the nuclear receptors LXRα and LXRβ in the control of lipogenesis. Both LXRs bind to DNA and regulate transcription of target genes in a heterodimeric complex with RXR (16). Although early studies on LXRs focused on their role in cholesterol metabolism, mice carrying a targeted disruption in the LXRα gene were noted to be deficient in expression of FAS, SCD-1, ACC, and SREBP-1, consistent with a role in lipogenesis as well (17). Further support for this idea came with the observation that the synthetic LXR ligand T1317 to mice triggers induction of the lipogenic pathway and raises plasma triglyceride levels (18). The demonstration that the SREBP-1c promoter is a direct target for regulation by LXR/RXR heterodimers provided a straightforward explanation for the ability of LXR ligands to induce hepatic lipogenesis (19, 20). Until now, the effects of LXR activation on the expression of lipogenic genes, including FAS, have been presumed to be entirely indirect.

We demonstrate here that the FAS promoter is a direct target for regulation by the LXR/RXR heterodimer as well as SREBPs. This novel mechanism for the regulation of FAS expression and lipogenesis by LXRs has implications for the development of LXR agonists as modulators of human lipid metabolism.

**EXPERIMENTAL PROCEDURES**

Reagents and Plasmids—Expression plasmids for RRA and LXRs, and nuclear SREBP-1a, -1c, and -2 have been described (21, 22). GW3965 (23) and T0901317 (18) were provided by Timothy M. Willson (GlaxoSmithKline). Ligands were dissolved in Me2SO prior to use in cell culture. The –1594, –700, –150, and –135 rat FAS promoter luciferase reporter constructs were described previously (3). Mutations were

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introduced into the 700 FAS reporter using the QuikChange site-directed mutagenesis kit (Stratagene).

Cell Culture and Transfections—HepG2 cells were cultured in modified Eagle’s medium containing 10% fetal bovine serum or lipoprotein-deficient fetal bovine serum (LPDS). THP-1 cells were cultured in RPMI 1640 containing 10% fetal bovine serum and differentiated for 24 h with 40 ng/ml phorbol myristyl acetate (PMA). Transient transfections of HepG2 cells were performed in triplicate in 48-well plates. Cells were transfected with reporter plasmid (100 ng/well), receptor plasmids (5–50 ng/well), pCMV-β-galactosidase (50 ng/well), and pTKCIII (to a total of 205 ng/well) using the MBS mammalian transfection kit (Stratagene). Following transfection, cells were incubated in modified Eagle’s medium containing 10% LPDS and the indicated ligands or vehicle control for 24 h. Luciferase activity was normalized to β-galactosidase activity.

RNA Analysis—Total RNA was isolated using Trizol reagent (Invitrogen). S1 nuclease analysis was carried out as described (3). Real time quantitative PCR assays were performed using an Applied Biosystems 7700 sequence detector as in Ref. 23. Briefly, 1 μg of total RNA was reverse transcribed with random hexamers using Taqman reverse transcription reagents kit (Applied Biosystems). Each amplification mixture (50 μl) contained 50 ng of cDNA, 900 nM forward primer, 900 nM reverse primer, 100 nM dual-labeled fluorogenic probe (Applied Biosystems), and 25 μl of Universal PCR master mix. All samples were analyzed for 36 cycles or 18 S rRNA expression in parallel in the same run. Quantitative expression values were extrapolated from separate standard curves for controls and unknowns generated with 10-fold dilutions of cDNA. All assays were performed in duplicate. Primer and probe sequences are available upon request.

* Gel Shift Assays—In vitro translated RXRs and LXRα were generated from pCMX-RXRα and pCMX-hLXRα using the TNT Quick Coupled transcription/translation system (Promega). Gel shift assays were performed as described (24) using in vitro translated proteins. The sequences of the oligonucleotides used were (only one strand shown): rFAS, 5’-gatcgctgcc-3’; ME LXRE, 5’-gatcgctgcc-3’; ME LXRE, 5’-gatcgctgcc-3’; ME LXRE, 5’-gatcgctgcc-3’; ME LXRE, 5’-gatcgctgcc-3’; ME LXRE, 5’-gatcgctgcc-3’; ME LXRE, 5’-gatcgctgcc-3’; ME LXRE, 5’-gatcgctgcc-3’.

Animals—C57BL/6 mice (5 animals/group) were maintained on standard mouse chow and dosed with 50 mg/kg of T0901317 or vehicle alone (0.5% methylcellulose) by oral gavage once a day for 3 or 7 days. Animals were sacrificed 4 h after the last treatment on days 3 and 7. Plasma triglyceride levels were determined on an Instrumentation Laboratories Llab600 Clinical Chemistry Analyzer.

**RESULTS**

Previous work has shown that expression of FAS and SREBP-1c in rodent liver is induced in response to the synthetic LXR agonist T1317 (18). Induction of FAS expression by LXR has been presumed to be secondary to the induction of SREBP-1c; however, this hypothesis has not been tested. We identified FAS as an LXR-responsive gene in macrophages using Affymetrix cDNA microarrays (data not shown). As shown in Fig. 1A, real time quantitative PCR (Taqman) analysis confirmed that FAS expression was induced in 12-O-tetradecanoylphorbol-13-acetate (TPA)-differentiated THP-1 macrophages in response to treatment with either of the non-steroidal LXR agonists, T1317 (18) or GW3965 (23, 25). The RXR agonist LG268 also induced FAS expression, and the combination of LG268 and T1317 had an additive effect. Consistent with previous results in liver (19, 20), treatment of THP-1 macrophages with LXR ligands also led to a significant induction of SREBP-1 RNA (data not shown).

To investigate whether LXR activation induced FAS expression in other peripheral cells we examined CHO-7 cells. S1 nuclease analysis demonstrated that T1317 strongly induced expression of FAS (Fig. 1B). However, unlike THP-1 cells and HepG2 cells, this induction occurred in the absence of significant induction of either SREBP-1 or the SREBP target gene HMG-CoA reductase. This observation suggests that the ability of LXR to regulate FAS expression is not entirely dependent on the ability to induce SREBP-1 expression. We further explored the regulation of the FAS promoter in HepG2 cells under different cellular sterol conditions. As shown in Fig. 1C, basal FAS expression and induction by T1317 were highest in sterol-depleted cells (cultured in LPDS, 5 μM simvastatin, 100 μM mevalonic acid) in which levels of nuclear SREBPs are expected to be high. However, a significant induction of FAS expression was also observed in the presence of 2.5 μM/ml 25-hydroxycholesterol (25-HC) and 5 μM/ml cholesterol, conditions under...
which SREBP cleavage is suppressed. Again, these observations suggest that LXR can regulate FAS expression, at least in part, by a mechanism that is independent of SREBP-1c.

The regulatory regions that mediate induction of the FAS gene by LXR ligands have not been defined. In an effort to map these sequences, we analyzed the ability of LXRα and synthetic LXR ligands to regulate the FAS promoter in transient transfection assays. HepG2 cells were cotransfected with a luciferase reporter containing sequences from −1594 to +67 bp of the rat FAS promoter or PGL-2 control vector along with expression vectors for LXRα and LXRβ, or LXRβ and RXRα. Cells were treated with 1 μM T1317 3-h post-transfection and harvested after 24 h of ligand treatment. Data are presented as luciferase activity normalized to β-galactosidase activity and represent the average of triplicate experiments.

mulation of SREBP, cells were cultured in the presence of high levels of cholesterol and 25-HC, conditions known to suppress SREBP cleavage and reduce the concentration of nuclear SREBPs to undetectable levels (26). Under these conditions the vast majority of the LXR response is conferred by sequences between −700 and −150 bp (Fig. 3C). The −150 and −135 FAS constructs were not significantly induced by LXR or LXR ligand in the presence of cholesterol and 25-HC. These results are consistent with previous work localizing the sterol response sequences of the FAS promoter to two tandem SREBP binding sites between −71 and −54 bp (Fig. 3A and Ref. 15). Thus, LXR can regulate FAS expression through an SREBP-dependent pathway involving sequences between −135 and +67 bp as well as an SREBP-independent pathway involving sequences present between −700 and −150 bp.

We hypothesized that the FAS promoter might be a target for direct regulation by LXR/RXR heterodimers as well as SREBPs. The preferred binding site for LXR/RXR is a DR-4 (direct repeat with a 4-nucleotide spacing) hormone response element (16). An alignment of the FAS promoter (−699 to +1 bp relative to the transcriptional start site) from human, rat, and chicken is shown in Fig. 4. Previous work has demonstrated that the actions of SREBPs on the FAS promoter are mediated primarily by a pair of non-canonical binding sites located between −71 and −54 bp (rat) (15). These regulatory elements, as well as binding sites for SP-1 and NF-Y, are highly conserved. However, the SREBP site at −150, which is a perfect match to the human LDL receptor SRE in the human and rat FAS promoters, is not completely conserved in the chicken promoter. Surprisingly, although the sequence upstream of −150 bp is quite divergent in these three species, a DR-4 element present between −669 and −655 bp in the rat promoter is highly conserved. The striking conservation of this element suggested that it was likely to be an important regulatory sequence. Interestingly, the sequence of this DR-4 element in rodents and humans is identical to that of the LXR response element (LXRE) identified previously in the murine SREBP-1c promoter (Fig. 5A) (19, 20). Gel mobility shift analysis using in vitro translated LXRα and RXRα proteins and radiolabeled oligonucleotides confirmed that FAS LXRE binds LXRα/RXR heterodimers (Fig. 5B). Competition assays using unlabeled oligonucleotides revealed that the affinity of this site for LXR/RXR was slightly greater than that of the previously identified LXRE from the human apoE macrophage enhancer (27) (Fig. 5C).

The presence of binding sites for both LXRα/RXRα and SREBP in the FAS promoter suggests that these two classes of transcription factors both contribute to the regulation of FAS expression. In support of this idea, we found that cotransfection of expression vectors for LXRα and RXRα along with expression vectors for the nuclear forms of SREBPs had a dramatic effect on the induction of the −700 FAS promoter in transient transfection assays. At the highest amount of expression vector tested, the combination of nuclear SREBP-1a expression vector and LXR activation led to a greater than 50-fold increase in FAS promoter activity (Fig. 6A). Expression of SREBP-1c also had an additive effect (Fig. 6B), although ~10-fold more expression vector was needed to achieve the same level of promoter activity as with SREBP-1a. This is consistent with the known difference in activity between the SREBP-1a and -1c isoforms (14, 22). We further analyzed the dose response of the =700 FAS promoter to the synthetic LXR ligand T1317 in the presence and absence of SREBP. Fig. 6C illustrates that the maximal response of FAS promoter to the LXR ligand is observed in the presence of expression vectors for both LXRα/RXRα and nuclear SREBP-1c.

To definitively demonstrate that the effects of LXR on the
FAS promoter are mediated by the combined action of the LXRE and tandem SREBP sites we analyzed FAS promoter constructs carrying specific mutations in these elements (Fig. 7A). Consistent with the results of the FAS promoter deletion analysis (Fig. 3), mutation of either the LXRE or the tandem SREBP sites between −71 and +54 reduced the activity of the promoter in the presence of cotransfected LXR expression vector and synthetic LXR ligand (Fig. 7B). Simultaneous mutation of both sites virtually abolished promoter activity. We further examined the effect of these mutations on activation of the FAS promoter by SREBP-1a. In agreement with previous work (15), mutation of the tandem SREBP sites resulted in a complete loss of induction by the SREBP-1a expression vector (Fig. 7C), despite the presence of the SRE element at −150 bp. The activity of the SREBP mutant FAS promoter in the presence of both LXR and SREBP was not different from that with LXR alone. As expected, mutation of the LXRE had no effect on the ability of SREBP to activate the FAS promoter, but eliminated the additive effect of LXR and SREBP. Taken together, these results indicate that LXR/RXR heterodimers and SREBPs additively regulate the FAS promoter and that this regulation requires the combined action of both the LXR and SREBP binding sites.

Finally, to investigate the potential contribution of direct LXR activation of the FAS promoter to the control of lipogenesis in vivo, we analyzed the ability of the synthetic LXR ligand T1317 to regulate FAS expression and influence plasma triglyceride and HDL levels in mice. C57Bl/6 mice (5 animals per group) were treated for 3 or 7 days with either vehicle or 50 mg/kg T1317. After 3 days of treatment, plasma triglycerides increased ~200% in response to T1317 (Fig. 8A), consistent with previous work (18). After 7 days, however, triglyceride levels in these mice had nearly normalized. Treatment with T1317 also led to a significant elevation of plasma HDL cholesterol (Fig. 8B, HDL-C). Unlike the effect on triglycerides, the effect on HDL persisted after 7 days. Next, we endeavored to
correlate changes in plasma lipid levels induced by T1317 with changes in hepatic gene expression. After 3 days of treatment, expression of FAS was induced ~15-fold by T1317 but had largely normalized by day 7, an effect that mirrored the normalization of triglyceride levels (Fig. 8C). In contrast, expression of SREBP-1c, SCD-1, and ABCG1 was induced at day 3 and remained elevated after 7 days. Thus, alterations in plasma triglyceride levels correlated closely with temporal changes in FAS expression. These data are consistent with the hypothesis that direct action of LXR on the FAS promoter as well as induction of SREBP-1c expression are likely to contribute to regulation of lipogenesis by LXR ligands in vivo.

**DISCUSSION**

The nuclear receptors LXRα and LXRβ are emerging as key regulators of lipid homeostasis. The physiologic ligands for these receptors are likely to be specific intermediates in the cholesterol biosynthetic pathway such as (24S,25)-epoxycholesterol. LXRα is expressed primarily in liver, intestine, adipose tissue, and macrophages, whereas LXRβ is ubiquitously expressed (16, 28). In peripheral cells such as macrophages LXRs play an important role in the regulation of reverse cholesterol transport and the induction of genes in response to cellular lipid loading. Multiple genes involved in the cholesterol efflux pathway, including those encoding the putative cholesterol/
phospholipid transporter ABCA1 (21, 29–31), ABCG1 (32), and apoE (27), have been identified as transcriptional targets of LXR. In the intestine, induction of ABCA1 expression by LXR or RXR ligands dramatically reduces dietary cholesterol absorption, presumably by actively transporting cholesterol from the enterocyte into the lumen of the gut (30). These results establish that both dietary cholesterol absorption and the rate of cholesterol efflux in peripheral cells are controlled by LXR signaling pathways.

In the liver, LXRs appear to regulate both cholesterol and fatty acid metabolism. Mice carrying a targeted disruption of the Lxrα gene fail to induce transcription of the gene encoding cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid synthesis, in response to dietary cholesterol (17). In addition, mice lacking LXRα are deficient in the expression of several genes involved in lipogenesis, including FAS, SCD-1, ACC, and SREBP-1. Further evidence for the involvement of LXRs in lipogenesis came with the observation that treatment of mice with the synthetic LXR ligand T1317 induces expression of lipogenic genes and raises plasma triglyceride levels (18). The recent demonstration that the SREBP-1c promoter is a direct target for regulation by LXR/RXR heterodimers provided a potential explanation for the ability of LXR ligands to induce hepatic lipogenesis (19, 20). Until now, the effects of LXR activation on the expression of lipogenic genes, including FAS, have been presumed to be entirely indirect.

We have shown here that LXRs regulate FAS expression through direct interaction with the FAS promoter as well as through indirect effects on SREBP-1c. The observation that synthetic LXR ligands induce FAS expression in certain cell

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**Fig. 7.** Activation of the FAS promoter by LXR requires binding sites for both LXR/RXR and SREBP. A. Sequences of wild type and mutant LXR and SREBP elements from the FAS promoter. B and C, HepG2 cells were transiently transfected with the wild type −700 FAS reporter or constructs containing specific mutations in the LXRRE and/or the tandem SREBP binding sites. Expression vectors for LXRα, RXRα, and SREBP-1a were included as indicated. Following transfection, cells were treated with vehicle or 1 μM T1317 for 24 h. Data are presented as luciferase activity normalized to β-galactosidase activity and represent the average of triplicate experiments.

**Fig. 8.** Temporal induction of hepatic FAS expression by LXR ligands in vivo correlates with plasma triglyceride levels. C57BL/6 mice (5 animals/group) were dosed with 50 mg/kg T0901317 or vehicle alone (0.5% methyl-cellulose) by oral gavage once a day for 3 or 7 days. Animals were sacrificed 4 h after the last treatment on days 3 and 7 and plasma triglyceride and HDL cholesterol levels were determined. Hepatic gene expression was analyzed by real time quantitative PCR assays.

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| Triglycerides | HDL-C | Hepatic gene expression |
|--------------|-------|-------------------------|
| Day 3        |       |                         |
| vehicle      |       |                         |
| T1317        |       |                         |
| Day 7        |       |                         |
| vehicle      |       |                         |
| T1317        |       |                         |
types in the absence of changes in SREBP-1 expression led us
to map the promoter sequences involved in LXR induction. We
found that in addition to binding sites for SREBPs, the FAS
promoter contains a high affinity binding site for the LXR/RXR
heterodimer that is conserved in birds, rodents, and humans.
The LXR and SREBP binding sites independently confer LXR-
responsiveness on the FAS promoter, and maximal induction
requires the binding of both transcription factors. Finally,
plasma triglyceride levels in mice treated with a synthetic LXR
agonist correlated with increased FAS expression more closely
than with SREBP-1c expression. Taken together our results
strongly suggest that direct actions of LXR on the FAS pro-
moter contribute to regulation of lipogenesis by LXR ligands
in vivo.

The identification of the FAS promoter as a direct target for
LXR/RXR heterodimers fits well with the previously hypothe-
sized role for LXR as a cholesterol sensor (19). The LXR path-
way provides a mechanism whereby cholesterol and fatty acid
metabolism can be coupled. Under conditions where cellular
cholesterol levels are high, it is appropriate that the substrate
for cholesterol esterification, fatty acids, be readily available.
Our results indicate that LXRs have the ability to control FAS
expression in peripheral cells such as macrophages as well as
hepatocytes. The ability to up-regulate FAS expression in re-

The LXR and SREBP binding sites independently confer LXR-
extension of human lipid metabolism.

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