We determined if fatty acids can regulate the murine Cyp7a1 and human CYP7A1 gene promoters via peroxisome proliferator-activated receptor α (PPARα)/9-cis-retinoic acid receptor α (RXRα). In transfected cells, the murine Cyp7a1 gene promoter displayed markedly lower basal activity, but greater sensitivity to fatty acid or WT 14,645-activated PPARα/RXRα when compared with the human CYP7A1 gene promoter. PPARα/RXRα can bind to a site (Site II) located within the region at nucleotides 412 to 436 of both promoters. Mutagenesis of the human CYP7A1 Site II element abolished the response to activated PPARα/RXRα. The murine Cyp7a1 gene promoter contains an additional PPARα/RXRα-binding site (Site I) located within nucleotides 72 to 57. Replacement of a single residue in human CYP7A1 Site I with that found in the murine Cyp7a1 Site I sequence enabled PPARα/RXRα binding, and this mutation resulted in reduced basal activity, but substantially improved the response to activated PPARα/RXRα in transfected cells. We conclude that fatty acids can regulate the cyp7a gene promoter via PPARα/RXRα. The differential response of the murine Cyp7a1 and human CYP7A1 gene promoters to PPARα activators is attributable to the additional PPARα/RXRα-binding site in the murine Cyp7a1 gene promoter.

Peroxisome proliferator-activated receptors (PPARs) belong to the superfamily of ligand-activated nuclear hormone receptors and regulate a wide spectrum of genes involved in lipid homeostasis (1–3). The regulation of gene transcription by PPARs involves their heterodimerization with 9-cis-retinoic acid receptor α (RXRα) and binding to specific sequences known as peroxisome proliferator response elements (PPREs) in the proximal regions of target genes (2, 4, 5). PPREs consist of a direct repeat of the nuclear receptor hexameric DNA recognition motif (5'-AGGTCA-3') separated by one nucleotide, a configuration termed as DR1. Fatty acids and fibrates, which are known ligands for PPARα (6–11), have been shown to regulate genes involved in cholesterol metabolism such as those encoding apoA-I (12), apoA-II (13), apoC-III (14) and 3-hydroxy-3-methylglutaryl-CoA synthase (15).

The liver plays an important role in maintaining whole body cholesterol homeostasis by regulating the biosynthesis of cholesterol, its uptake from plasma, storage, and catabolism to bile acids (16). The conversion of cholesterol to bile acids in the liver represents an important route by which cholesterol is eliminated from the body. Cholesterol 7α-hydroxylase (cyp7a) is the rate-controlling enzyme of the major pathway involved in this process (17). The gene encoding cyp7a1 has been cloned from the rat (18, 19), mouse (20, 21), human (22, 23), and hamster (24). Several studies have shown that cyp7a gene expression is regulated by a variety of hormonal (25–28) and nutritional (29–36) factors. Although phosphorylation has been suggested to regulate cyp7a3 (30, 37, 38), the abundance of cyp7a mRNA remains the best predictor of cyp7a enzyme activity.

The proximal promoter region of the cyp7a1 gene contains sequences that resemble nuclear hormone response elements. It was shown recently that induction of the rat Cyp7a1 gene in response to oxysterols involves the nuclear receptor LXRα (39), whereas repression of cyp7a gene transcription by bile acids appears to be mediated via the farnesoid X receptor (40). Other nuclear hormone receptors have also been shown to interact with the cyp7a1 gene promoter (41–44). Given the number of nuclear hormone receptor-binding sites in the cyp7a1 gene promoter, it would seem that this class of transcription factors plays a major role in regulating cyp7a gene expression.

We previously demonstrated that dietary fats influence the response of the murine Cyp7a1 gene to dietary cholesterol (35, 36). It is well known that fatty acids regulate gene transcription via PPARα/RXRα (2, 7). Here we evaluated the role of fatty acids in regulating the cyp7a gene expression and whether PPARα/RXRα is involved in the process.

MATERIALS AND METHODS

Cloning and Construction of Recombinant Plasmids—Genomic clones containing the human CYP7A1 gene were isolated from a human chromosome 8 library (American Type Culture Collection, Manassas, VA). A genomic clone containing the entire murine Cyp7a1 gene was described previously (21). Gene chimeras containing the proximal promoter region of the human CYP7A1 gene (nt −372 to +61) or the murine Cyp7a1 gene (nt −412 to +77) were generated from the cloned cyp7a gene fragments and linked to the chloramphenicol acetyltransferase (CAT) structural gene sequence in pCAT-Basic (Promega Corp., Madison, WI). The primary structures of the in vitro amplified human CYP7A1 and murine Cyp7a1 gene fragments were confirmed by sequencing. The rabbit antisem to PPARα and plasmids encoding murine PPARα and RXRα were described previously (45).

Mutant derivatives of the human CYP7A1 gene promoter were con-
structured using mutagenic primers by in vitro DNA amplification and the plasmid containing the wild-type gene chimera as template. The 3'-half-site of the human CYP7A1 Site II regulatory element was converted into an XhoI site, and a point mutation was introduced into the 3'-half-site of the human CYP7A1 Site I element. The primary structure of the putative transactivated promoter was verified by sequence analysis.

Cell Culture and Transfections—Mc Ardle RH-7777 rat hepatoma cells (46) were maintained in Dulbecco's modified Eagle's medium supplemented with 20% fetal calf serum at 37 °C in a humidified atmosphere of 5% CO₂ and 95% air. Cells were plated (60-mm culture dishes) at equal densities 16–18 h prior to transfection and grown in medium containing 20% fetal calf serum. Cells were transfected when cultures were at 50–60% confluency using the calcium phosphate coprecipitation procedure (47) with a mixture of plasmids that contained 2.5 μg of plasmid pCMV-β-gal (encoding β-galactosidase) as a control for transfection efficiency, cyp7a-CAT gene chimeras, and expression vectors encoding PPARα and RXRα. All samples were complemented with sonicated salmon sperm DNA to equalize the mass of total DNA (20 μg) added to each dish. One hour after the introduction of DNA, the transfected cells were treated with either fatty acids (stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2); Sigma-Aldrich, Oakville, Ontario, Canada) complexed to lipid-free bovine serum albumin (BSA) or WY 14,643 (BIOMOL Research Labs Inc., Plymouth Meeting, PA) dissolved in dimethyl sulfoxide at a final concentration of 100 μM. Control cells received either lipid-free BSA or Me₂SO alone. In initial experiments, we found that fatty acids dissolved in ethanol were toxic when added to cultures of human transfected Mc Ardle RH-7777 cells at concentrations ≥100 μM. However, the equivalent concentrations of fatty acids were well tolerated when added to cell culture medium as a complex with BSA and were therefore used in all subsequent experiments. Extracts of transfected cells were prepared 36–48 h after the introduction of DNA and assayed for both CAT and β-galactosidase activities by standard methods. The CAT reaction products were separated by TLC, and radioactivity of acetylated [14C]chloramphenicol was quantitated using a Fuji BAS1000 phosphomager. The activity values were normalized to the β-galactosidase activity, which was not affected by the treatments.

Electrophoretic Mobility Shift Assays—The murine PPARα and RXRα proteins were synthesized in vitro using a coupled transcription/translation system (Promega Corp.) with a standard scale of synthesis consisting of 25 μl of reaction mixture and 0.5 μg of DNA template. Fragments produced by restriction enzyme digestion of the human CYP7A1 gene promoter were first tested for binding to PPARα/RXRα by electrophoretic mobility shift assays. The sequences of the promoter fragments shown to bind to PPARα/RXRα were used to design probes that were used to screen genomic regions that bore similarity to the consensus PPRE sequence as described in Methods. The following synthetic double-stranded oligonucleotides were synthesized and shown to bind to PPARα and RXRα via electrophoretic mobility shift assays: 5'-CTCTGGACATTGTCAAAGGTGAG-3' (human Site I, nt 158–200); 5'-CTCTGGACATTGTCAAAGGTG-3' (murine Site I, nt 192–240); 5'-GTTAGAAGAAGCTCTCTGAAAGTCAG-3' (human Site II, nt 54–96); and 5'-GTTAGAAGAAGCTCTCTGAAAGTC-3' (murine Site II, nt 76–118). The altered residues in the mutated derivatives of these sequences are described in the figure legend. The oligonucleotides were synthesized with a 4-nt 5'-extension (5'-AATT-3') to allow for radioolabeling of annealed oligonucleotides by fill-in reaction catalyzed by the Klenow fragment of Escherichia coli DNA polymerase I. Radioabeled double-stranded oligonucleotides (0.1 pmol, ±15,000 cpm) were incubated in a total volume of 40 μl for 15 min at 25 °C with 4 μg of poly(dI-dC), 4 μg of BSA, and 1 μl of recombinant PPARα and RXRα in binding buffer (10 mM HEPES, pH 7.9, 150 mM NaCl, 1 mM EDTA, 7% glycerol, 150 μM phenylmethylsulfonyl fluoride, and 1 mM dithiothreitol). Double-stranded oligonucleotides containing the rat acyl-CoA oxidase promoter (45) and the rat tyrosine aminotransferase glucocorticoid response element (48) were used as competitors for PPARα/RXRα binding. In competition experiments, increasing amounts (5–50-fold molar excess) of unlabeled acyl-CoA oxidase PPRE, murine Cyp7a1 Site I, or human CYP7A1 Site I were added to reaction mixtures and incubated at room temperature for 10 min prior to the addition of radioabeled double-stranded oligonucleotides. After an additional 15 min of incubation at room temperature, DNA-protein complexes were separated by electrophoresis on a 4% polyacrylamide gel in 0.5X Tris borate electrophoresis buffer at 4 °C and visualized by autoradiography or phosphoimaging. In some experiments, 1–2 μl of rabbit anti-PPARα antisem (45) was added after incubation of the radiolabeled probes with the reticulocyte lysates. The reaction was left on ice for an additional 30–60 min prior to electrophoresis.

RESULTS

Fatty Acids Stimulate the Murine Cyp7a1 and Human CYP7A1 Gene Promoters via PPARα/RXRα—We observed that mice consuming a semipurified high fat (20%) diet in the absence of exogenous bile acids had a significantly higher (2–3-fold, p < 0.01) abundance of Cyp7a1 mRNA compared with mice consuming a chow diet (5% fat content). To investigate whether fatty acids directly regulate cyp7a gene transcription through PPARα/RXRα, gene chimeras containing the murine Cyp7a1 or human CYP7A1 proximal promoter regions linked to the CAT structural gene were transfected into rat hepatoma cells in the absence or presence of plasmids encoding PPARα and RXRα. In the presence of PPARα/RXRα, the basal activity of the murine Cyp7a1 gene promoter was significantly lower (p < 0.001) than that of the human CYP7A1 gene promoter (Fig. 1A). Addition of stearic acid (18:0), oleic acid (18:1), or linoleic acid (18:2) complexed to lipid-free BSA to the culture medium at a concentration of 100 μM increased the activities of both the murine Cyp7a1 and human CYP7A1 gene promoters as reflected by the rise in CAT activity in lysates of treated cells (Fig. 1B). These results indicate that fatty acids stimulate the cyp7a gene promoter of both species.

Interestingly, the response of the murine Cyp7a1 gene promoter to fatty acids was consistently greater than that of the human CYP7A1 gene promoter. To verify the observed difference in the response of the murine Cyp7a1 and human CYP7A1 gene promoters to fatty acids, cells transfected with the chimeric plasmids were treated with WY 14,643, a potent and well-characterized synthetic activator of PPARα (6, 8, 10). As shown in Fig. 1C, the response of the murine Cyp7a1 gene promoter to WY 14,643 was significantly greater (11-fold activation) compared with the human CYP7A1 gene promoter (1.5-fold activation). These results show that although the human CYP7A1 gene promoter has a higher basal activity compared with the murine Cyp7a1 gene promoter, the human CYP7A1 gene is less responsive to regulation by activated PPARα/RXRα.

The cyp7a Gene Promoter Contains a PPARα/RXRα-binding Site—Analysis of the human CYP7A1 and murine Cyp7a1 gene promoter fragments produced by restriction enzyme digestion revealed that PPARα/RXRα can bind to the cyp7a promoter somewhere in the region between nt –255 and –47 (data not shown). Inspection of the sequence revealed that this region of the cyp7a gene promoter contains a DR1 motif that resembles a PPARα/RXRα-binding site. This DR1 motif spans nt –144 to –132 and nt –158 to –146 of the human CYP7A1 and murine Cyp7a1 gene promoters, respectively. The sequence of this site, which we have designated as Site II, is invariant among the promoter regions of the human, murine, rat, and hamster cyp7a genes (Fig. 2A). To determine if Site II could function as a PPRE, we tested its ability to interact with PPARα/RXRα by electrophoretic mobility shift assay. Double-stranded oligonucleotides containing the Site II sequence displayed strong binding to PPARα/RXRα heterodimers (Fig. 2B, left arrow). Interaction of the probe with the recombinant transcription factors was not observed when either PPARα or RXRα was omitted from the binding reactions (data not shown). Addition of anti-PPARα antibodies to the binding reaction containing PPARα/RXRα resulted in the formation of a complex with greatly reduced mobility (Fig. 2B, right arrow), demonstrating that the complex contains PPARα. The binding of Site II to PPARα/RXRα was competed efficiently by the well-characterized acyl-CoA oxidase PPRE (45), but not by a glucocorticoid response element (48) (Fig. 2C, left panel). Mutagenesis of the 3'-half-

V. Drover and L. B. Agellon, unpublished results.
The CAT activity was normalized to cotransfected plasmids encoding PPARα, RXRα, and β-galactosidase (internal reference standard) were cotransfected into hepatoma cells. Fatty acids (stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2)) complexed with greatly reduced mobility (right arrow). C, the specificity of the binding was tested by adding a 5- or 50-fold molar excess of competitor (glucocorticoid response element (GRE) or acyl-CoA oxidase PPRE) relative to the radiolabeled probe in the binding reactions. The binding of PPARα/RXRα heterodimers to wild-type human CYP7A1 Site II (arrows) relative to mutant Site II is shown in the right panel. D, mutagenesis of the 3′-half-site of the human CYP7A1 Site II element abolished responsiveness to WY 14,643 (black bars). The wild-type and mutant cyp7a-CAT gene chimeras were introduced into hepatoma cells along with plasmids encoding PPARα, RXRα, and β-galactosidase. The normalized CAT activity values in the absence of PPARα/RXRα ligands (white bars) were assigned a value of 1. Results shown represent the means ± S.E. of three independent experiments performed in triplicate.

Fig. 2. The cyp7a gene promoter contains a conserved PPARα/RXRα-binding site. A, shown is a schematic (top) illustrating the approximate location of the Site II element of the cyp7a gene promoter. The sequences of the Site II elements of the human (GenBankTM/EBI Data Bank accession number L20569), murine (this work and L20569), rat (J02929), and hamster (L04690) cyp7a gene promoters and of the mutant human CYP7A1 Site II element (mut Site II) are shown below. The dot represent identical sites, and the lowercase letters represent residues in the DR1 spacer region. bp, base pairs. B, PPARα/RXRα binding to the cyp7a Site II element (left arrow) was demonstrated by electrophoretic mobility shift assay. Addition of anti-PPARα antisera (last lane) resulted in the formation of a complex with greatly reduced mobility (right arrow). C, the specificity of the binding was tested by adding a 5- or 50-fold molar excess of competitor (glucocorticoid response element (GRE) or acyl-CoA oxidase PPRE) relative to the radiolabeled probe in the binding reactions. The binding of PPARα/RXRα heterodimers to wild-type human CYP7A1 Site II (arrows) relative to mutant Site II is shown in the right panel. D, mutagenesis of the 3′-half-site of the human CYP7A1 Site II element abolished responsiveness to WY 14,643 (black bars). The wild-type and mutant cyp7a-CAT gene chimeras were introduced into hepatoma cells along with plasmids encoding PPARα, RXRα, and β-galactosidase. The normalized CAT activity values in the absence of PPARα/RXRα ligands (white bars) were assigned a value of 1. Results shown represent the means ± S.E. of two independent experiments performed in triplicate.

Fig. 1. Human CYP7A1 and murine Cyp7a1 gene promoters are stimulated by PPARα/RXRα activators. A, CAT activity in hepatoma cells transfected with equal molar amounts of the human or murine cyp7a-CAT gene chimera (white and black bars, respectively). The CAT activity was normalized to β-galactosidase activity encoded by a cotransfected β-galactosidase expression vector. The results shown represent the means ± S.E. of three independent experiments performed in triplicate. B, response of the human CYP7A1 and murine Cyp7a1 gene promoters (white and black bars, respectively) to fatty acids in the presence of PPARα/RXRα. The cyp7a-CAT gene chimeras and plasmids encoding PPARα, RXRα, and β-galactosidase (internal reference standard) were cotransfected into hepatoma cells. Fatty acids (stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2)) complexed to lipid-free BSA were added to the cell culture medium to a final concentration of 100 μM. Control cells received lipid-free BSA (no FA). C, response of the human CYP7A1 and murine Cyp7a1 gene promoters to WY 14,643 in the presence of PPARα/RXRα. WY 14,643 was added to culture medium to a final concentration of 50 μM (black bars). Control cells received MeSO (carrier) alone (white bars). For both B and C, the average normalized CAT activity value in the absence of ligands was assigned a value of 1. The results are the means ± S.E. of two independent experiments performed in triplicate.
The murine Cyp7a1 gene promoter contains an additional PPARα/RXRα-binding Site—The LXRα/RXRα heterodimers bind to a tandem repeat of a nuclear hormone-binding sequence motif separated by four unconserved residues (i.e., a DR4 element termed the LXR response element) (49). Region −72 to −57 of the murine Cyp7a1 gene promoter contains a DR4 element that is similar to the LXR response element found in the rat Cyp7a1 gene promoter (38). Alignment of murine and rat DR4 elements shows a single nucleotide difference in the spacer region, but the 5′- and 3′-half-sites are identical (Fig. 3A). Inspection of the murine DR4 sequence (designated as Site I) suggests the existence of a possible DR1 element embedded in the DR4. The DR1 element is configured in such a way that it shares a common 3′-half-site with the DR4.

Comparison of the murine, human, rat, and hamster cyp7a gene promoters revealed that the sequence of Site I is not conserved (Fig. 3A). Electrophoretic mobility shift assays using oligonucleotides corresponding to the murine Site I element demonstrated a strong interaction with PPARα/RXRα, whereas the corresponding Site I element of the human CYP7A1 gene promoter was unable to bind PPARα/RXRα (Fig. 3B, left arrow). Binding of PPARα was confirmed by the formation of a complex with greatly reduced mobility in the presence of anti-PPARα antibodies (Fig. 3B, right arrow). Consistent with these results, the binding of PPARα/RXRα to murine Cyp7a1 Site I could be competed with the acyl-CoA oxidase PPRE, but not with the human CYP7A1 Site I element (Fig. 3C). These results indicate that the Site I element of the murine Cyp7a1 gene promoter is configured to enable binding of PPARα/RXRα.

Alteration of a Single Nucleotide in the Human CYP7A1 Site I Regulatory Element Allows PPARα/RXRα Binding—The human CYP7A1 Site I differs from the murine Cyp7a1 Site I by three nucleotide residues (Fig. 4A). We designed three different double-stranded oligonucleotides containing the human CYP7A1 Site I sequence in which each of the variant residues was replaced with the corresponding residue in the murine Cyp7a1 Site I sequence. Changes from T-to-G and from A-to-C in the 5′-half-site of the DR4 element and in the spacer region, respectively, did not promote binding of human CYP7A1 Site I to PPARα/RXRα (Fig. 4B, left panel). In contrast, the C-to-T substitution in the 3′-half-site of human CYP7A1 Site I enabled binding to PPARα/RXRα (Fig. 4B, left panel), and the binding was competed by both the acyl-CoA oxidase PPRE and murine Cyp7a1 Site I element (Fig. 4B, right panel).

To demonstrate the functional significance of the human CYP7A1 Site I regulatory element, a cyp7a-CAT gene chimera containing mutagenized human CYP7A1 Site I was created and analyzed for its response to WY 14,643-activated PPARα/RXRα in hepatoma cells. Analysis of CAT activity in extracts of cells transfected with the gene chimera carrying this mutation revealed a marked reduction of the basal promoter activity as compared with cells transfected with the wild-type version of the human CYP7A1 promoter (Fig. 4C, left panel). This result, taken together with the results shown in Fig. 1A, suggests that the low basal activity of the murine Cyp7a1 gene promoter compared with the wild-type human CYP7A1 gene promoter is attributable to the sequence of the Site I element. As observed before, the wild-type human CYP7A1 gene promoter showed a small (1.5-fold) but consistent stimulation in the presence of WY 14,643. In contrast, the human CYP7A1 gene promoter containing the Site I C-to-T mutation responded significantly better (3.5-fold, \( p < 0.01 \)) (Fig. 4C, right panel). These results illustrate that a single nucleotide difference in the 3′-half-site of Site I can alter the basal activity of the cyp7a promoter as

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**Fig. 3.** The murine Cyp7a1 gene promoter contains an additional PPRE. A, shown is a schematic (top) illustrating the approximate location of the Site I element of the cyp7a gene promoter. The sequences of the Site I elements of the cyp7a gene promoters from different species are shown below. The dots represent identical residues, and the lowercase letters represent residues in the spacer regions. B, the PPARα/RXRα heterodimers bound to murine Cyp7a1 Site I (left arrow) were demonstrated by electrophoretic mobility shift assay. Addition of anti-PPARα antiserum (last lane) resulted in the formation of a complex with greatly reduced mobility (right arrow). C, the specificity of PPARα/RXRα binding (left arrow) was tested by adding a 5–50-fold molar excess of the rat acyl-CoA oxidase (AOx) PPRE or the human Site I element relative to the radiolabeled probe in the binding reactions.
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**DISCUSSION**

Both the murine Cyp7a1 and human CYP7A1 gene promoters were induced when the culture medium of cells transfected with gene chimeras containing these promoters was supplemented with fatty acids. The stimulatory effect was observed even with stearic acid, a fatty acid that interacts weakly with PPARs (9, 11). This could be explained by the fact that metabolism of fatty acids in the transfected cells can produce metabolites that are more potent activators of PPARs than the parental fatty acids. Inspection of the proximal region of the cyp7a gene promoters from various mammalian species revealed the existence of an invariant DR1 motif that is a possible binding site for PPARα/RXRα. Electrophoretic mobility shift assays using recombinant PPARα and RXRα revealed that this sequence (termed Site II) was capable of binding PPARα/RXRα in vitro. Since mutagenesis of the human Site II element abolished the stimulatory effect of fatty acids on the human CYP7A1 gene promoter, we surmised that this site is capable of acting as a target for PPARα-mediated signaling.

Although both fatty acids and WY 14,643 reproducibly stimulated the human CYP7A1 gene promoter in our experimental system, the magnitude of the stimulation was modest. Fibrates have generally been shown to increase the concentration of cholesterol in human and rat bile, and this effect appears to be correlated with the reduction of cholesterol 7α-hydroxylation activity in both species (50–53). However, it should be noted that some fibrates have only minimal effects on the lithogenic index of bile or cyp7a activity (50, 53). Significant changes in the activities of other cholesterol-metabolizing enzymes have also been demonstrated (50, 51), and these effects likely also contribute to changes in the lithogenic index of bile.

Recently, it was discovered that human liver contains significantly lower levels of PPARα mRNA compared with murine liver (54). Measurement of PPARα/RXRα DNA-binding activity in liver extracts suggested that human liver contains an order of magnitude less activity compared with murine liver (54). These observations raise the possibility that PPARα may not play a significant role in regulating human CYP7A1 gene expression. The conserved Site II element of the cyp7a gene promoter is also capable of binding other transcription factors. The reduction of cholesterol 7α-hydroxylation activity observed in humans and rats after fibrate therapy may therefore be due to an indirect effect of PPARα-mediated signaling. A transgenic mouse strain bearing the human CYP7A1 gene should aid in clarifying the significance of PPARα function in regulating human CYP7A1 gene expression.

In contrast, the murine Cyp7a1 gene promoter was highly responsive to fatty acids and WY 14,643 in transfected cells. Since the murine Cyp7a1 gene promoter did not contain other sequences that resemble the typical PPARα/RXRα-binding site (a DR1 motif) (2, 5, 45), the basis for the enhanced response of the murine Cyp7a1 gene promoter in transfected cells was not immediately obvious. Mapping of the murine proximal gene promoter using restriction fragments as probes in gel mobility shift assays surprisingly revealed that the fragment containing the LXRα/RXRα-binding site (LXR response element, a DR4 motif) (49) could also bind PPARα/RXRα in vitro. Unlike Site II, the sequence that makes up the murine DR4 element (termed
The expression of the murine Site I variant enables PPARα/RXRα binding. In addition, this substitution substantially improved the response of the human CYP7A1 gene promoter to WY 14,643 in transfected cells. The magnitude of the response of the modified human CYP7A1 gene promoter did not reach that observed for the murine Cyp7a1 gene promoter, but this may be due to other sequence differences between the two promoters. Thus, we attribute the robust response of the murine Cyp7a1 gene promoter to fatty acids and WY 14,643 to the existence of a second PPARα/RXRα-binding site. Studies done on mice that are homozygous for a disrupted Scp2 gene (encodes sterol carrier protein 2 and sterol carrier protein X) (55) provide additional support for the involvement of PPARα in the regulation of murine Cyp7a1 gene expression. The expression of the Cyp7a1 gene in these mice is increased, coincident with the enhanced expression of hepatic genes known to be regulated by PPARα (55). This effect has been ascribed to the accumulation of phytanic acid, a metabolite that can act as a ligand for and activator of PPARα (56).

Previously, we demonstrated that the fat background of the diet had a significant impact on the response of the murine Cyp7a1 gene promoter to dietary cholesterol (35, 36). The stimulatory effect of dietary cholesterol on the murine Cyp7a1 gene is likely mediated via LXRα, a nuclear hormone receptor that is activated by oxysterols (39, 57), since inactivation of the LXRα gene in mice abolishes the response (58). Based on the present study, it appears that PPARα/RXRα and LXRα/RXRα bind to overlapping sequences in the murine Cyp7a1 gene promoter. The exclusive interaction of these transcription factors with the murine Site I element may explain why fats have an apparent influence on the stimulatory effect of dietary cholesterol. The exact requirements dictating the preferential binding of either of these transcription factors to the murine Site I element remain to be elucidated. In any case, the data suggest that fats are important physiological regulators of murine Cyp7a1 gene expression.

The inhibitory effect of bile acids on cyp7a1 gene expression is a well documented phenomenon (17). The discovery that bile acids serve as ligands for the farnesoid X receptor provides an explanation for the repressive effect of bile acids on the transcription of bile acid-responsive genes (40, 59, 60). A recent study demonstrated that ligand-bound farnesoid X receptor is capable of antagonizing transactivation by LXRα, a nuclear hormone receptor that is known to be regulated by PPARα and LXRα (55). This effect has been ascribed to the accumulation of phytanic acid, a metabolite that can act as a ligand for and activator of PPARα (56).

In summary, we demonstrated that the murine Cyp7a1 and human CYP7A1 gene promoters are differentially responsive to regulation by fatty acids via PPARα/RXRα in transfected cells. The basis for the differential sensitivity is apparently due to a variation in a critical residue in the Site I element of the cyp7a1 gene promoter. The murine Site I variant enables PPARα/RXRα binding and allows the murine Cyp7a1 gene promoter to be more responsive to regulation by fats. Mice may have either maintained or acquired more sophisticated control of cyp7a1 gene expression through evolution to allow greater flexibility in regulating bile acid synthesis.
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