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Ligand-Regulated Heterodimerization of Peroxisome Proliferator-Activated Receptor α with Liver X Receptor α

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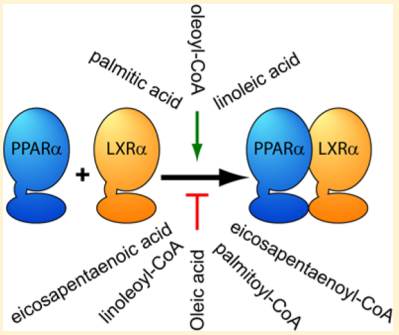
Supporting Information

ABSTRACT: Peroxisome proliferator-activated receptor α (PPARα) and liver X receptor α (LXRα) are members of the nuclear receptor superfamily that function to regulate lipid metabolism. Complex interactions between the LXRα and PPARα pathways exist, including competition for the same heterodimeric partner, retinoid X receptor α (RXRα). Although data have suggested that PPARα and LXRα may interact directly, the role of endogenous ligands in such interactions has not been investigated. Using *in vitro* protein−protein binding assays, circular dichroism, and co-immunoprecipitation of endogenous proteins, we established that full-length human PPARα and LXRα interact with high affinity, resulting in altered protein conformations. We demonstrated for the first time that the affinity of this interaction and the resulting conformational changes could be altered by endogenous PPARα ligands, namely long chain fatty acids (LCFA) or their coenzyme A thioesters. This heterodimer pair was capable of binding to PPARα and LXRα response elements (PPRE and LXRE, respectively), albeit with an affinity lower than that of the respective heterodimers formed with RXRα. LCFA had little effect on binding to the PPRE but suppressed binding to the LXRE. Ectopic expression of PPARα and LXRα in mammalian cells yielded an increased level of PPRE transactivation compared to overexpression of PPARα alone and was largely unaffected by LCFA. Overexpression of both receptors also resulted in transactivation from an LXRE, with decreased levels compared to that of LXRα overexpression alone, and LCFA suppressed transactivation from the LXRE. These data are consistent with the hypothesis that ligand binding regulates heterodimer choice and downstream gene regulation by these nuclear receptors.



Peroxisome proliferator-activated receptor α (PPARα) and liver X receptor α (LXRα) are ligand-activated transcription factors that belong to the steroid hormone receptor superfamily. Both nuclear receptors are known to function as obligate heterodimers with retinoid X receptor α (RXRα) and bind specific DNA sequences [peroxisome proliferator response elements (PPREs) and liver X receptor response elements (LXREs)] in their target genes.[1,2] Moreover, these receptors function as nutrient sensors to affect the regulation of genes involved in metabolism and energy homeostasis.[3,4] High fatty acid levels lead to increased PPARα activity, inducing transcription of genes involved in fatty acid uptake and oxidation.[5] LXRα agonists (including oxysterols and intermediates in the cholesterol biosynthetic pathway) increase the level of transcription of multiple genes in cholesterol elimination, while decreasing that of genes in cholesterol synthesis.[2,6] As important modulators of pathways whose misregulation leads to metabolic disorders, including diabetes, cardiovascular disease, and atherosclerosis, these receptors have been the focus in an attempt to better understand mechanistically how these processes are controlled.

Several studies have shown that cross-talk exists between PPARα and LXRα. Such studies suggest that each receptor can repress genes regulated by the other receptor, presumably through competition for available RXRα.[7,8] This cross-talk may be even more complicated, as PPRE sequences have been found in the LXRα promoter region[9] and PPARα has been identified as an LXRα target gene,[10] suggesting that each receptor may regulate the level of the other. Recent chromatin immunoprecipitation experiments have demonstrated binding of PPARα to LXRα−RXRα response elements, although under the examined conditions only one of these proteins bound to the DNA sequence at a time.[11] Moreover, it has been suggested that PPARα and LXRα themselves may function as heterodimeric partners;[12,13] however, the significance of this finding is unclear, and the effect of endogenous ligands has yet to be elucidated.

As endogenous ligands of PPARα, binding of long chain fatty acids (LCFAs) and their CoA thioesters [long chain fatty acyl-CoA (LCFA-CoA)] induces conformational changes leading to altered cofactor recruitment and increased levels of transactivation of β-oxidation enzymes.[1,14−16] Because ligand-induced conformational changes in protein structure could affect not only cofactor recruitment and binding but also

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interaction with heterodimer partners, binding of LCFA or LCFA-CoA to PPARα could affect PPARα's ability to heterodimerize with RXRα or LXRα. In this case, conformational changes to any of the three proteins could have an effect on PPARα or LXRα activity. Moreover, as LCFA levels are often elevated in metabolic diseases, understanding the role these nutrients play in these regulatory processes is important.

This study focuses on the ability of PPARα and LXRα to heterodimerize in the absence or presence of LCFA or LCFA-CoA. The affinity and overall conformational changes of these receptors were determined *in vitro*, and the resulting effects on DNA binding and transactivation were examined. If PPARα can heterodimerize to either RXRα or LXRα, then ligand binding may provide a mechanism for determining the heterodimer choice.

EXPERIMENTAL PROCEDURES

Chemicals. Coenzyme A, palmitic acid, oleic acid, linoleic acid, eicosapentaenoic acid, palmitoyl-CoA, oleoyl-CoA, linoleoyl-CoA, and clofibrate were from Sigma (St. Louis, MO). Eicosapentaenoyl-CoA was synthesized as previously described[15] and purified by high-performance liquid chromatography (HPLC).[17] All CoA thioesters, whether freshly synthesized or obtained commercially, were >98% undegraded. The human glucocorticoid receptor (hGR) was purchased from Pierce Thermo Scientific (Rockford, IL). Monoclonal antibodies for PPARα and polyclonal antibodies for LXRα (each specific for the α isotype) were purchased from Pierce Thermo Scientific. Polyclonal antibodies for PPARα, RXRα, and GR were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-rabbit IgG secondary antibodies were from Sigma.

Construction of Plasmids for Recombinant Expression of hLXRα and hPPARα. An N-terminal polyhistidine tag (six His residues) was added to the GST open reading frame in the pGEX6P vector (Amersham Biosciences, Piscataway, NJ) by overlap polymerase chain reaction (PCR). The plasmid for expression of recombinant human PPARα (hPPARα) has been described previously.[16] Human LXRα (hLXRα) and human retinoid X receptor α (hRXRα) were amplified from cDNA derived from HepG2 cells using the following primers: 5′-ggatccATGTCCTTGTGGCTGGGGGCCCCTGTG-3′ and 5′-aagcttCTCGAGTCATTCGTGCACATCCCAGATCTC-3′ (hLXRα), 5′-cgaattcATGGACACCAAACATTTCCTGCCG-CT-3′ and 5′-ctcgagCTAAGTCATTGGGTGCGGCGCCT-CC-3′ (hRXRα). In these and subsequent primers, the lowercase letters represent nucleotides outside of the target sequence with restriction sites underlined. Each PCR product was cloned into the pGEM-T easy vector (Promega, Madison, WI) and subsequently transferred into the *Bam*HI−*Hin*dIII or *Eco*RI−*Xho*I sites of the pGEX-6P derivative to produce 6xHis-GST-hLXRα and 6xHis-GST-hRXRα, respectively.

Purification of Recombinant hPPARα and hLXRα Protein. Plasmids for hPPARα, hRXRα, and hLXRα recombinant protein expression were transformed into Rosetta 2 competent cells (Novagen, Gibbstown, NJ) or a DNAK mutant derived from a K12 strain (JW0013, Coli Genetic Stock Center, New Haven, CT). Protein purification was conducted through affinity chromatography with the GST tag and on-column digestion as previously described for hPPARα.[16] Protein concentrations were estimated by the Bradford assay (Bio-Rad, Hercules, CA) and by absorbance spectroscopy using the molar extinction coefficients for each protein. Protein purity was ascertained by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE), followed by Coomassie Blue staining and Western blotting.

Circular Dichroism. Circular dichroism was used to examine changes in conformation upon heterodimerization of hPPARα and hLXRα or interaction with ligands, and hGR was used as a negative control. A J-815 spectropolarimeter (JASCO Inc., Easton, MD) was used to record the circular dichroic spectra of individual proteins in the absence of ligand, individual proteins in the presence of ligands, and protein/protein mixtures in the presence and absence of ligands as previously described.[14,15,18] To examine possible protein−protein interactions, spectra of hPPARα (0.42 μM), hGR (0.40 μM), hLXRα (0.44 μM), 0.21 μM hPPARα with 0.22 μM hLXRα, and 0.21 μM hPPARα with 0.20 μM hGR (the final amino acid molarity in each sample was equal to 0.0002 M) were recorded in 0.5 mM HEPES (pH 8.0), 5 μM EDTA, 5 mM KCl, and 0.04% glycerol at 23 °C in a 1 mm cuvette. To examine ligand effects, spectra were recorded in the presence of each ligand at a concentration of 0.5 μM, and the final ethanol concentration in each reaction was <0.05%. Replicate spectra were recorded five times over the far-UV region from 185 to 260 nm at a scan rate of 50 nm/min with a 2 nm bandwidth and a 1 s DIT. Spectra were corrected for buffer and solvent effects, and the spectral result was used to determine the percent composition of α-helices, β-strands, turns, and unordered structures with CONTIN of the CDPro software package (http://lamar.colostate.edu/~sreeram/CDPro).[19] The CD spectrum of the mixed proteins was compared to a theoretical spectrum of combined but noninteracting proteins. This spectrum was calculated by averaging the spectra of each protein analyzed separately at a concentration equal to that in the mixture.[18]

Binding Assays with hPPARα and hLXRα Proteins in the Presence and Absence of PPARα Ligands. Recombinant hPPARα, hLXRα, and hGR proteins were fluorescently labeled with Cy3 or Cy5 dyes using Fluorolink-antibody Cy3 and Cy5 labeling kits (Amersham Biosciences, Pittsburgh, PA). Absorbance measurements were used to determine protein concentrations and dye:protein ratios. Emission spectra (560−700 nm) of 25 nM Cy3-labeled hLXRα were recorded in PBS upon excitation at 550 nm with increasing concentrations of unlabeled hPPARα or hGR protein in a PC1 photon counting spectrofluorometer (ISS Inc., Champaign, IL) at 24 °C. The spectra were corrected for background (buffer, solvent, and each protein individually), and the maximal intensities were measured using Vinci version 1.5 (ISS Inc.). To determine dye effects, emission spectra (660−700 nm) of 25 nM Cy5-labeled hPPARα titrated with increasing concentrations of unlabeled hLXRα were recorded upon excitation at 650 nm. To determine the effect of PPARα ligands on this interaction, these experiments were repeated in the presence of each ligand at a concentration of 25 nM. The dissociation constant (K_d) and the number of binding sites (n) were obtained from a double-reciprocal plot of $1/(1 - F/F_{max})$ and $C_L/F/F_{max}$, where F represents the fluorescence intensity at a given concentration of ligand, F_{max} is the maximal fluorescence obtained, and C_L is the ligand concentration (the slope of the linear line is equal to $1/K_d$, and the number of linear lines is equal to n), as previously described.[14] Binding curves were generated by nonlinear regression analysis using the ligand binding function in Sigma Plot (SPSS Inc., Chicago, IL).

Co-Immunoprecipitation. The co-immunoprecipitation of native proteins from human cells was performed as previously

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described for Co-IP from liver homogenate.[14] HepG2 cells were grown to 95% confluency in DMEM supplemented with 10% FBS at 37 °C and 5% CO_2. Cells were lysed and proteins immunoprecipitated with the ProFound mammalian co-immunoprecipitation kit (Pierce Biotechnology, Rockford, IL) and antibodies to PPARα, LXRα, and GR (negative control). Antibodies specific to the α isotypes of the PPAR and LXR were utilized to ensure only the α forms were precipitated. A no-antibody resin was also used as a negative control. This kit was chosen because antibodies are cross-linked to resin and are not eluted in the samples. Eluted proteins were visualized by Western blotting for PPARα, LXRα, and GR with polyclonal antibodies.

Electrophoretic Mobility Shift Assays. The PPRE sequence from the rat acyl-CoA oxidase (ACOX) promoter[20] was used to identify a similar sequence from the human ACOX promoter, resulting in 5′-GAACTAGAAGGTCAGCTGTCAAGCAGCCA-3′. The LXRE sequence from mouse sterol regulatory element binding protein 1c (SREBP-1c)[21] was used to identify the human sequence,[22] composed of 5′-GCCAGTGACCGCCAGTAACCCCGGAGAC-3′, where underlined sequences represent response element half-sites. Purified recombinant proteins (0.2 μg) were incubated with 40 ng of double-stranded PPRE or LXRE oligonucleotides in binding buffer [2 mM Tris (pH 8.0), 10 mM KCl, 0.5 mM $MgCl_2$, 2.5% glycerol, 0.2 mM DTT, and 0.05% NP-40][21] at room temperature for 20 min in the presence or absence of LCFA. The resulting mixture was cross-linked at 120 mJ/cm^2 in a DNA cross-linker (Stratagene, La Jolla, CA), mixed with gel loading buffer, and electrophoresed in 7% native PAGE gels at 100 V for 50 min. For supershift assays, antibodies to either PPARα or LXRα were added to the mixture prior to cross-linking, and the mixture was electrophoresed in 4% native PAGE gels. Gels were stained for both DNA and protein with a commercially available kit (Invitrogen, Carlsbad, CA) per the manufacturer's instructions and imaged on a cooled charge-coupled device camera (Fujifilm Medical Systems, Stamford, CT). The band intensity was measured as mean 8-bit gray scale density with ImageJ (NIH, available by anonymous FTP).

Construction of Plasmids for Mammalian Expression of hLXRα, hPPARα, and Luciferase Reporters for Cell Assays. hLXRα was amplified from 6xHis-GST-hLXRα using the following primers: 5′-catcggatccaccATGTCCTTGTGGCTGGGGGCCCCTGTG-3′ and 5′-CCGGGAGCTGCATGTGTCAGAGG-3′. The PCR product was cloned into the pGEM-T easy vector (Promega), and a *Bam*HI−end-filled *Xho*I fragment was subsequently transferred into the multiple cloning site of pSG5 (Stratagene; *Bam*HI−end-filled *Bgl*II) to produce pSG5-hLXRα. hPPARα was amplified from 6xHis-GST-hPPARα using the following primers: 5′-catcggatccaccATGGTGGACACGGAAAGCCCA-3′ and 5′-CCGGGAGCTGCATGTGTCAGAGG-3′. The PCR product was cloned into the pGEM-T easy vector, and a *Bam*HI−end-filled *Sal*I fragment was subsequently transferred into the multiple cloning site of pSG5 (*Bam*HI−end-filled *Bgl*II) to produce pSG5-hPPARα.

The human acyl-CoA oxidase (ACOX) promoter (2.3 kb) was amplified from genomic DNA derived from HepG2 cells with the following primers: 5′-cggtaccATGACTCTGTTTTCTATGACCT-3′ and 5′-cgagctcGCTCCGAAGGTCAAGAAACT-3′. The PCR product was cloned into the pGEM-T easy vector and subsequently transferred into the *Kpn*I−*Sac*I sites of pGL4.17 (Promega) to produce ACOX2.3-pGL4.17. The mouse sterol regulatory element binding protein 1c (SREBP-1c) promoter (1.5 kb) was amplified from mouse genomic DNA with the following primers: 5′-cggtaccTCGAGCACTTGCAGGCTGGA and cgagctcGCCCCTAGGGCGTGCAGACG-3′. The PCR product was cloned into the pGEM-T easy vector and subsequently transferred into the *Kpn*I−*Sac*I sites of pGL4.17 (Promega) to produce SREBP1c1.5-pGL4.17. All plasmid constructs were confirmed by DNA sequencing in the Center for Genomics Research at Wright State University.

Transactivation Assays. HepG2 cells (ATCC, Manassas, VA) grown in 24-well culture plates were transfected with 0.4 μg of each full-length mammalian expression vector (pSG5-hPPARα, pSG5-hLXRα, or empty vector, pSG5), 0.4 μg of luciferase reporter construct (ACOX2.3-pGL4.17, SREBP1c1.5-pGL4.17, or empty reporter, pGL4.13), and 0.04 μg of the internal transfection control plasmid pRL-CMV (Promega) with Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. Following transfection incubation, the medium was replaced with serum-free EMEM (Invitrogen) for 2 h, and then ligands were added and cells grown for an additional 20 h. Fatty acids were added to cells as a complex with BSA as previously described,[23,24] and clofibrate was used as a positive control. Each ligand was examined at four concentrations (1, 5, 10, and 20 μM) for each of the reporter constructs. Firefly luciferase activity, normalized to Renilla luciferase (for transfection efficiency), was determined with the dual-luciferase reporter assay system (Promega), according to the manufacturer's instructions. Luminescence was measured with a SAFIRE^2 TECAN 96-well plate reader (Tecan Systems, Inc., San Jose, CA). The sample with 1 μM clofibrate for the ACOX assays was arbitrarily set to 100%.

Statistical Analysis. All results are expressed as means ± the standard error. Statistical significance between samples in the presence or absence of ligands was determined by using the Student's t test or analysis of variance with $p < 0.05$.

RESULTS

Protein Expression and Purification. Purified full-length recombinant hPPARα and hLXRα proteins were electrophoresed via 12% SDS−PAGE (Figure 1). Each gel showed a band of approximately 50 kDa, corresponding to the expected size of hPPARα and hLXRα (estimated molecular masses of 52636 and 51768 Da, respectively). Densitometry of these samples indicated >85% purity. Western blot analyses using antibodies for PPARα and LXRα confirmed the identity of

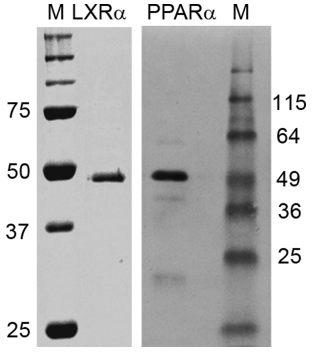

Figure 1. SDS−PAGE and Coomassie blue staining of 4 μg of purified, full-length hLXRα (left) and hPPARα (right) proteins showing the relative purity. The prominent bands at ~52 kDa represent untagged, full-length hLXRα and hPPARα proteins.

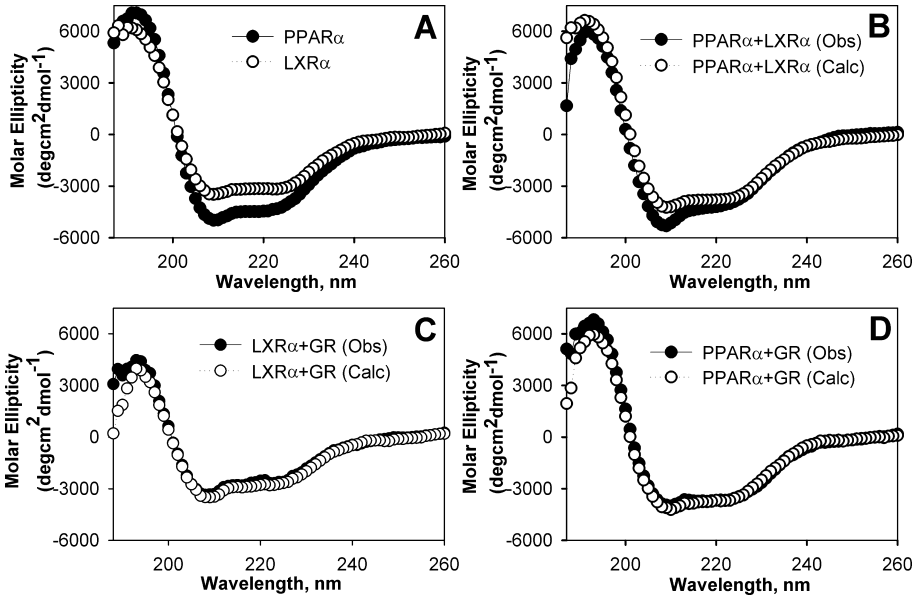

Figure 2. Circular dichroic spectra of hPPARα and hLXRα proteins. (A) Circular dichroic spectra of 0.42 μM hPPARα (●) and 0.44 μM hLXRα (○). (B) Experimentally observed circular dichroic spectrum of a mixture of 0.21 μM hPPARα and 0.22 μM hLXRα (Obs, ●) compared to the calculated average of the individually obtained hPPARα and hLXRα spectra (Calc, ○) representing no interaction between the two proteins. (C) Experimentally observed circular dichroic spectrum of a mixture of 0.22 μM hLXRα and 0.20 μM hGR (Obs, ●) compared to the calculated average of the individually obtained hLXRα and hGR spectra (Calc, ○) representing no interaction between the two proteins. (D) Experimentally observed circular dichroic spectrum of a mixture of 0.21 μM hPPARα and 0.20 μM hGR (Obs, ●) compared to the calculated average of the individually obtained hPPARα and hGR spectra (Calc, ○) representing no interaction between the two proteins. The amino acid molarity for each spectrum was 0.0002 M, and each spectrum represents the average of at least three replicates, scanned 10 times per replicate.

Table 1. Secondary Structure of hPPARα and hLXRα in the Absence of Ligands[a]

protein	α-helix regular H(r) (%)	α-helix distorted H(d) (%)	β-sheet regular S(r) (%)	β-sheet distorted S(d) (%)	turns T (%)	unordered U (%)
PPARα	8.3 ± 0.1	8.5 ± 0.3	18.7 ± 0.4	10.3 ± 0.3	19.4 ± 0.6	34 ± 1
LXRα	6.8 ± 0.4	7.2 ± 0.5	20.1 ± 0.6	11.1 ± 0.3	20.6 ± 0.4	34 ± 1
GR	1.2 ± 0.2	4.6 ± 0.2	24.6 ± 0.4	12.4 ± 0.3	21.6 ± 0.6	36 ± 1
PPARα/LXRα (obs)	6.4 ± 0.5[c]	7.8 ± 0.6	21.6 ± 0.7[b]	11.0 ± 0.3	20.5 ± 0.5	32 ± 1
PPARα/LXRα (calcd)	7.8 ± 0.2	7.9 ± 0.5	18.9 ± 0.5	10.8 ± 0.3	20.4 ± 0.6	34 ± 1
LXRα/GR (obs)	4.0 ± 0.9	6.7 ± 0.6	23 ± 1	11.6 ± 0.4	20.5 ± 0.5	34 ± 1
LXRα/GR (calcd)	3.2 ± 0.6	5.9 ± 0.6	22.8 ± 0.6	11.9 ± 0.3	21.4 ± 0.5	34 ± 1
PPARα/GR (obs)	6.2 ± 0.7	7.5 ± 0.4	21.6 ± 0.7	10.7 ± 0.5	19.5 ± 0.9	34 ± 2
PPARα/GR (calcd)	4.6 ± 0.7	6.8 ± 0.4	22.1 ± 0.5	11.4 ± 0.3	20.7 ± 0.5	34 ± 1

[a]Definitions: obs, obtained experimentally; calcd, calculated average. Significant differences were determined between observed and calcd for each protein mixture (n = 4–6). [b]$p < 0.05$. [c]$p = 0.06$.

these bands (data not shown), further indicating the purification of the full-length, untagged protein.

Circular Dichroism: Effect of the PPARα–LXRα Interaction on Protein Conformation. Although the circular dichroism (CD) spectra of hPPARα and hLXRα were qualitatively similar (Figure 2A), suggesting a similar overall secondary structure, the negative ellipticities at 210 and 222 nm were stronger for hPPARα than for hLXRα. Quantitative analyses of these samples showed that hPPARα had an α-helical content slightly higher than that of hLXRα (Table 1). To examine the possibility that PPARα and LXRα interacted, proteins were mixed in a 1:1 amino acid molar ratio and the experimental, or observed, CD spectrum of this mixture was compared to the calculated average of the two proteins (expected outcome if no conformational change occurred). Minor but significant changes in the spectra (Figure 2B) and the percent composition (Table 1) were noted for the mixture, suggesting that hPPARα and hLXRα interact and undergo a change in conformation upon interaction. In contrast, the experimentally observed spectra of hLXRα with hGR (Figure 2C) and hPPARα with hGR (Figure 2D) both overlaid the calculated average for the two proteins, suggesting no conformational change and no interaction between these proteins. Quantitative analyses confirmed these data, with no significant changes for either protein with hGR (Table 1).

Protein–Protein Binding. As the CD spectrum shows only a change in conformation, protein–protein binding experiments were conducted to determine the affinity of hPPARα for hLXRα. Each protein was fluorescently labeled with either Cy3 or Cy5 dye at essentially one dye per protein molecule. Upon titration of Cy5-labeled hPPARα protein with nonfluorescent hLXRα, the fluorescence intensity decreased, suggesting either a conformational change in Cy5-hPPARα or quenching of the Cy5 fluorophore upon hLXRα binding. This change in fluorescence intensity plotted as a function of hLXRα concentration resulted in a strongly saturable binding curve [K_d

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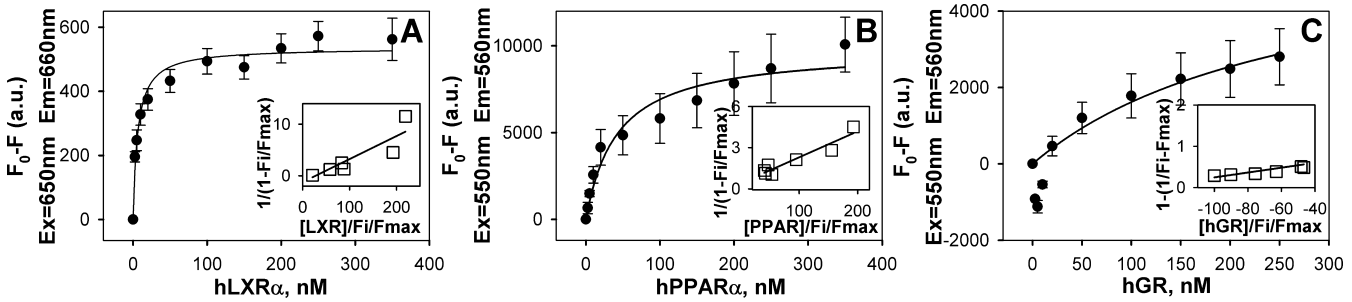

Figure 3. Fluorescent protein−protein binding assays with labeled protein titrated against increasing concentrations of unlabeled protein. (A) Change in the fluorescence intensity of 25 nM Cy5-labeled hPPARα titrated with increasing hLXRα concentrations of 0−250 nM. (B) Change in the fluorescence intensity of 25 nM Cy3-labeled hLXRα titrated with increasing concentrations of hPPARα. (C) Change in the fluorescence intensity of 25 nM Cy5-labeled hPPARα titrated with increasing hGR concentrations of 0−250 nM as a control. Insets represent double-reciprocal linear plots of each binding curve. Values represent means ± the standard error (n = 3−6).

= 6 ± 2 nM (Figure 3A)]. Transformation of these values into a double-reciprocal plot resulted in a single straight line, suggesting a single binding site (Figure 3A, inset). To ensure that the fluorophore did not alter protein−protein binding, the reverse experiment was conducted. Upon titration of Cy3-labeled hLXRα with nonfluorescent hPPARα, the fluorescence intensity increased. This change in fluorescence intensity plotted as a function of hPPARα concentration also resulted in a saturable binding curve (Figure 3B) at a single binding site (inset), but with slightly weaker affinity (K_d = 42 ± 16 nM). To determine whether this binding was specific for LXRα and PPARα, Cy5-labeled hLXRα was titrated with nonfluorescent hGR (Figure 3C); however, the shape of the curve was nonsaturable and almost linear, suggesting only weak or nonspecific binding.

With regard to the small differences noted between the binding affinities calculated from the changes in the fluorescence intensity of Cy5-labeled hPPARα versus Cy3-labeled LXRα, differences in labeling efficiency or location could contribute to some of the observed differences. Even though dye labeling of each protein occurred in an essentially 1:1 ratio, slight differences in labeling efficiency were noted, with the labeling of hPPARα (1.0:1) being slightly more efficient than the labeling of hLXRα (0.93:1). This slightly lower labeling efficiency for Cy3-hLXRα could yield an underestimation of the binding affinity and explain in part some of the differences noted between the two assays. It is also possible that the addition of the fluorophore on the hLXRα protein was more inhibitory than the addition of the fluorophore on the hPPARα protein. Because of this possibility, we chose to use the Cy5-PPARα for subsequent assays to examine ligand effects. Regardless of any inhibitory effects of the fluorophore, both assays resulted in strong, saturable binding, demonstrating a direct, high-affinity interaction between the two receptors, which was not observed with hGR.

Co-Immunoprecipitation. To demonstrate that these proteins could interact *in vivo*, co-immunoprecipitation of native proteins from HepG2 cells was performed. Samples that co-immunoprecipitated with the PPARα antibody showed a prominent band for both PPARα and LXRα, but no band was seen for GR (Figure 4, column 1). While the resultant PPARα band was present as a doublet, this was expected, as both the monoclonal PPARα antibody used for the co-immunoprecipitation (specific for the α isotype[25]) and the polyclonal PPARα antibody used for the Western blot have both been shown to produce a doublet from liver samples, presumably because of

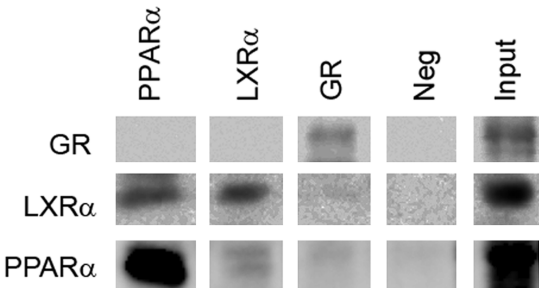

Figure 4. Co-immunoprecipitation of endogenous hPPARα, hLXRα, and hGR proteins from HepG2 cells. The cell lysate from HepG2 cells was immunoprecipitated with antibodies to PPARα, LXRα, or GR or no antibody as a negative control (Neg). The total protein attached to each antibody was separated via 12% SDS−PAGE and then subjected to Western blot analysis for the presence of GR, LXRα, and PPARα (indicated at the left). An input sample (equivalent to 10% of that used for the co-immunoprecipitation) was used as a positive control.

the phosphorylation of PPARα.[26,27] Similar results were obtained for the LXRα antibody (Figure 4, column 2), suggesting that native PPARα and LXRα can interact in cells but that GR does not. To further confirm these results, a GR specific antibody was used, and this resulted in only precipitation of GR (Figure 4, column 3), further confirming a specific interaction of hPPARα with hLXRα.

Effect of PPARα Ligands on PPARα−LXRα Secondary Structure. Because ligand-activated nuclear receptors undergo conformational changes upon ligand binding, and because significant differences were noted for the interaction of hPPARα with hLXRα, CD was used to determine whether ligand binding affected the overall conformation of the hPPARα−hLXRα heterodimer. For this study, eight known endogenous hPPARα ligands were utilized. These ligands were chosen on the basis of similar binding affinities but variations in chemical structure, including a saturated LCFA (C16:0), monounsaturated LCFA (C18:1), two polyunsaturated LCFAs (C18:2 and C20:5), and their CoA thioesters. While each of the examined ligands has been shown to bind hPPARα with similar affinity (K_d values of 12−34 nM for LCFA and 11−16 nM for LCFA-CoA), binding of each ligand results in slightly different hPPARα conformational changes.[16]

To distinguish effects on the heterodimer pair from effects on only hPPARα, or even hLXRα, spectra of hPPARα with hLXRα in the presence of solvent or ligand were compared to the calculated average of each individual protein in the presence of ligand (Figure 5). The spectrum of hPPARα with hLXRα in the

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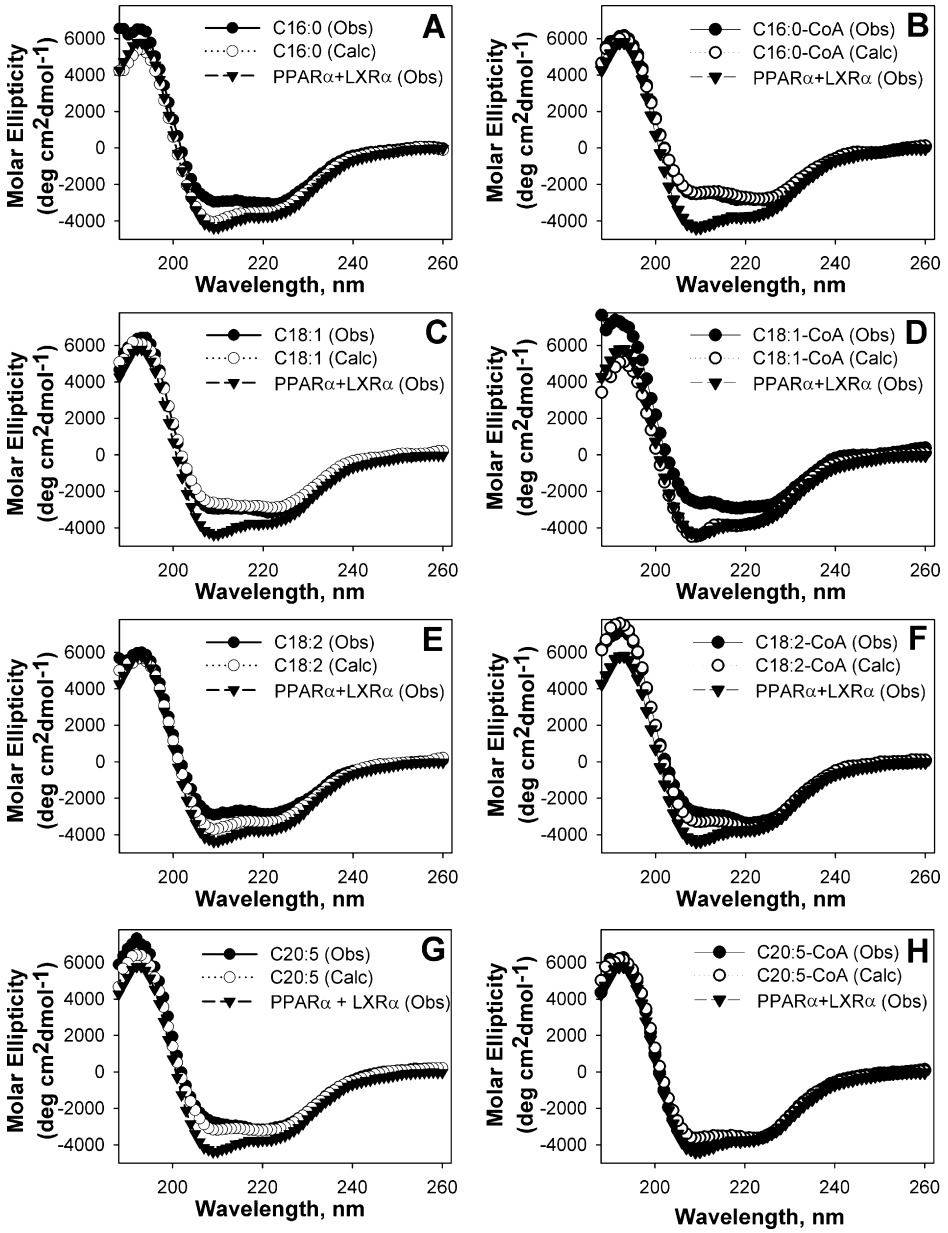

Figure 5. Circular dichroic spectra of a mixture of hPPARα and hLXRα in the absence and presence of LCFA and LCFA-CoA. Far-UV spectra obtained experimentally of a mixture of equal amino acid molarities of hPPARα and hLXRα in the absence of ligands (▼), experimentally observed spectra of a mixture of equal amino acid molarities of hPPARα and hLXRα in the presence of ligands (Obs, ●), and the calculated average of the two proteins individually examined in the presence of ligand (Calc, ○). Ligands include (A) palmitic acid (C16:0), (B) palmitoyl-CoA (C16:0-CoA), (C) oleic acid (C18:1), (D) oleoyl-CoA (C18:1-CoA), (E) linoleic acid (C18:2), (F) linoleoyl-CoA (C18:2-CoA), (G) eicosapentaenoic acid (C20:5), and (H) eicosapentaenoyl-CoA (C20:5-CoA). Each spectrum is representative of an average of 10 scans taken from at least three replicates.

presence of palmitic acid [Figure 5A (●)] showed strong changes compared to the spectrum of hPPARα with hLXRα and solvent [Figure 5A (▼)], suggesting an effect of palmitic acid. Furthermore, the spectrum of hPPARα with hLXRα in the presence of palmitic acid was also different from the spectrum of the calculated average of each protein in the presence of palmitic acid [Figure 5A (○)], suggesting that the two proteins are still interacting and the new spectrum is a result of palmitic acid altering the heterodimer secondary structure. Quantitative analyses of these samples showed a lower estimated percentage of α-helices and slightly higher β-sheet contents for hPPARα with hLXRα and palmitic acid than for either hPPARα with hLXRα and solvent or the calculated average of hPPARα with hLXRα and palmitic acid (Table 2). On the contrary, the spectrum of hPPARα with hLXRα and palmitoyl-CoA completely overlaid the spectrum of the calculated average for these proteins (Figure 5B), suggesting that the addition of palmitoyl-CoA might weaken the interaction of hPPARα with hLXRα. The estimated percent composition further supported this, with the hPPARα–hLXRα–palmitoyl-CoA structure having significant changes in α-helical and β-sheet content compared to those of the hPPARα–hLXRα structure in the presence of solvent, but no changes compared to the calculated average (Table 2). For all of the examined ligands, each ligand seemed to have some effect on structure, with eicosapentaenoyl-CoA resulting in the smallest spectral changes (Figure 5H). Addition of oleoyl-CoA (Figure 5D) and linoleic acid (Figure 5E) resulted in spectral changes similar to those of

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Table 2. Secondary Structures of hPPARα and hLXRα, Individually and as a Mixture (corrected for the solvent effect) in the Presence and Absence of Fatty Acids[a]

protein	α-helix regular H(r) (%)	α-helix distorted H(d) (%)	β-sheet regular S(r) (%)	β-sheet distorted S(d) (%)	turns T (%)	unordered U (%)
PPARα/LXRα/solvent	5.6 ± 0.6	7.2 ± 0.5	21.2 ± 0.9	11.1 ± 0.3	20.4 ± 0.6	35 ± 1
PPARα/LXRα/16:0 (obs)	3.1 ± 0.9[b]	6.0 ± 0.9	23 ± 2	11.8 ± 0.6	21.4 ± 0.7	35 ± 1
PPARα/16:0 and LXRα/16:0 (calcd)	4 ± 1	6.7 ± 0.7	22 ± 2	11.5 ± 0.5	21.5 ± 0.7	34 ± 1
PPARα/LXRα/16:0-CoA (obs)	2.3 ± 0.7[c]	5.6 ± 0.6[d]	24.7 ± 0.7[b]	12.0 ± 0.2[b]	21.3 ± 0.4	34 ± 1
PPARα/16:0-CoA and LXRα/16:0-CoA (calcd)	3 ± 1	5.8 ± 0.7	24.3 ± 0.7	12.1 ± 0.3	21.3 ± 0.4	34 ± 1
PPARα/LXRα/18:1 (obs)	4 ± 1	6.2 ± 0.7	23 ± 1	11.7 ± 0.4	21.1 ± 0.7	34 ± 1
PPARα/18:1 and LXRα/18:1 (calcd)	1.7 ± 0.4	5.1 ± 0.2	25.4 ± 0.5[d]	12.2 ± 0.1	21.7 ± 0.4	34.1 ± 0.7
PPARα/LXRα/18:1-CoA (obs)	4 ± 1	6.2 ± 0.8	23 ± 1	11.3 ± 0.4	21.5 ± 0.5	34 ± 1
PPARα/18:1-CoA and LXRα/18:1-CoA (calcd)	6 ± 1	7.7 ± 0.7	21 ± 1	11.0 ± 0.4	20.9 ± 0.7	34 ± 2
PPARα/LXRα/18:2 (obs)	3.4 ± 0.7[d]	5.9 ± 0.7	24 ± 1	11.7 ± 0.4	21.1 ± 0.6	34 ± 1
PPARα/18:2 and LXRα/18:2 (calcd)	4 ± 1	6.2 ± 0.6	23 ± 1	11.4 ± 0.2	20.4 ± 0.5	35 ± 1
PPARα/LXRα/18:2-CoA (obs)	3 ± 1	6.0 ± 0.9	19 ± 3	11.7 ± 0.5	21.5 ± 0.7	35 ± 1
PPARα/18:2-CoA and LXRα/18:2-CoA (calcd)	5 ± 1	6.8 ± 0.5	22.4 ± 0.7	11.4 ± 0.2	21.0 ± 0.6	35 ± 1
PPARα/LXRα/20:5 (obs)	4.1 ± 0.9	6.3 ± 0.7	22 ± 1	11.4 ± 0.5	21.2 ± 0.8	34 ± 1
PPARα/20:5 and LXRα/20:5 (calcd)	4 ± 1	6.4 ± 0.7	23 ± 1	11.4 ± 0.4	21.2 ± 0.8	34 ± 1
PPARα/LXRα/20:5-CoA (obs)	5 ± 1	6.9 ± 0.7	22 ± 1	11.1 ± 0.3	20.4 ± 0.7	34 ± 2
PPARα/20:5-CoA and LXRα/20:5-CoA (calcd)	5 ± 1	6.9 ± 0.8	22 ± 1	11.2 ± 0.3	20.8 ± 0.7	35 ± 2

[a]Definitions: obs, observed; calcd, calculated average. Significant differences were determined between the obs value and the PPARα/LXRα/solvent value for each protein mixture (n = 3–6). [b]$p < 0.05$. [c]$p < 0.01$. [d]$p = 0.08$.

palmitic acid, suggesting that these ligands affect the heterodimer. Oleic acid (Figure 5C), linoleoyl-CoA (Figure 5F), eicosapentaenoic acid (Figure 5G), and eicosapentaenoyl-CoA (Figure 5H) resulted in spectra that were similar to the calculated average of the individual proteins, similar to that of palmitoyl-CoA, suggesting that these ligands may be affecting the individual proteins (rather than the heterodimer) and possibly inhibiting heterodimer formation.

PPARα Ligands Affect hPPARα's Affinity for hLXRα. To determine whether the structural changes noted by CD affected the affinity of PPARα for LXRα, the protein–protein binding experiments were repeated in the presence of LCFA and LCFA-CoA. Because previous experiments have shown that hPPARα binds LCFA and LCFA-CoA with high affinity and at a single binding site,[16] equal molarities of hPPARα and ligand were mixed and allowed to bind prior to elucidation of hPPARα's affinity for hLXRα. Titration of Cy5-hPPARα with hLXRα in the presence of palmitic acid resulted in a sharply saturable change in the fluorescence intensity (Figure 6A), suggesting high-affinity binding similar to that seen in the absence of palmitic acid (Table 3). Transformation of these data into a double-reciprocal plot resulted in a single straight line, indicating a single binding site (Figure 6A, inset). Although the change in fluorescence intensity was not as sharp in the presence of palmitoyl-CoA (Figure 6B), oleic acid (Figure 6C), oleoyl-CoA (Figure 6D), or linoleoyl-CoA (Figure 6F), binding was saturable with binding affinities between 27 and 53 nM (Table 3). However, the changes in fluorescence intensity in the presence of linoleic acid (Figure 6E), eicosapentaenoic acid (Figure 6G), and eicosapentaenoyl-CoA (Figure 6H) were not saturable at 300 nM hLXRα, suggesting very weak or nonspecific binding. These data further confirmed the structural changes seen by CD and indicated that some LCFA decrease the affinity of hPPARα for hLXRα (Table 3).

Effect of PPARα Ligands on the Ability of the PPARα–LXRα Structure To Bind Response Elements. Electrophoretic mobility shift assays were used to determine whether the hPPARα–hLXRα heterodimer could bind to either PPRE or LXRE sequences. As the RXRα homodimer binds to both response elements,[20,21] hRXRα binding to each response element was used as a positive control. This binding resulted in the strongest band observed for either response element (Figure 7A). PPARα (in the absence of RXRα or LXRα) showed no binding to either response element. However, a very weak band was noted for LXRα (in the absence of RXRα or PPARα) binding to the PPRE, and a stronger band was noted for LXRE binding, suggesting that LXRα homodimers may be able to bind to the LXRE. Although only weak binding by the PPARα–LXRα heterodimer was noted for PPRE binding, LXRE binding was stronger (Figure 7A). Supershift assays were conducted to ensure that this observed binding was due to the PPARα–LXRα heterodimer (and not just LXRα binding). The addition of either a PPARα or LXRα antibody resulted in supershifted bands (Figure 7A). While the LXRα antibody resulted in a single supershifted band, two bands were noted with the addition of the PPARα antibody: one shifted band and one supershifted band (Figure 7A). It is possible that the two bands represent DNA bound by the PPARα–LXRα heterodimer (top band) and DNA bound by LXRα homodimers (lower band). As PPARα is unable to bind either response element alone, these data further indicate DNA binding by the PPARα–LXRα heterodimer.

Because many PPARα ligands altered the PPARα–LXRα conformation and several decreased the protein–protein binding affinity, whether these changes also altered DNA binding was examined. Neither LCFA nor clofibrate, a known PPARα agonist, had any effect on the binding of the PPARα–LXRα heterodimer to the PPRE from the ACOX promoter (Figure 7B,C). As the level of binding of the PPARα–LXRα

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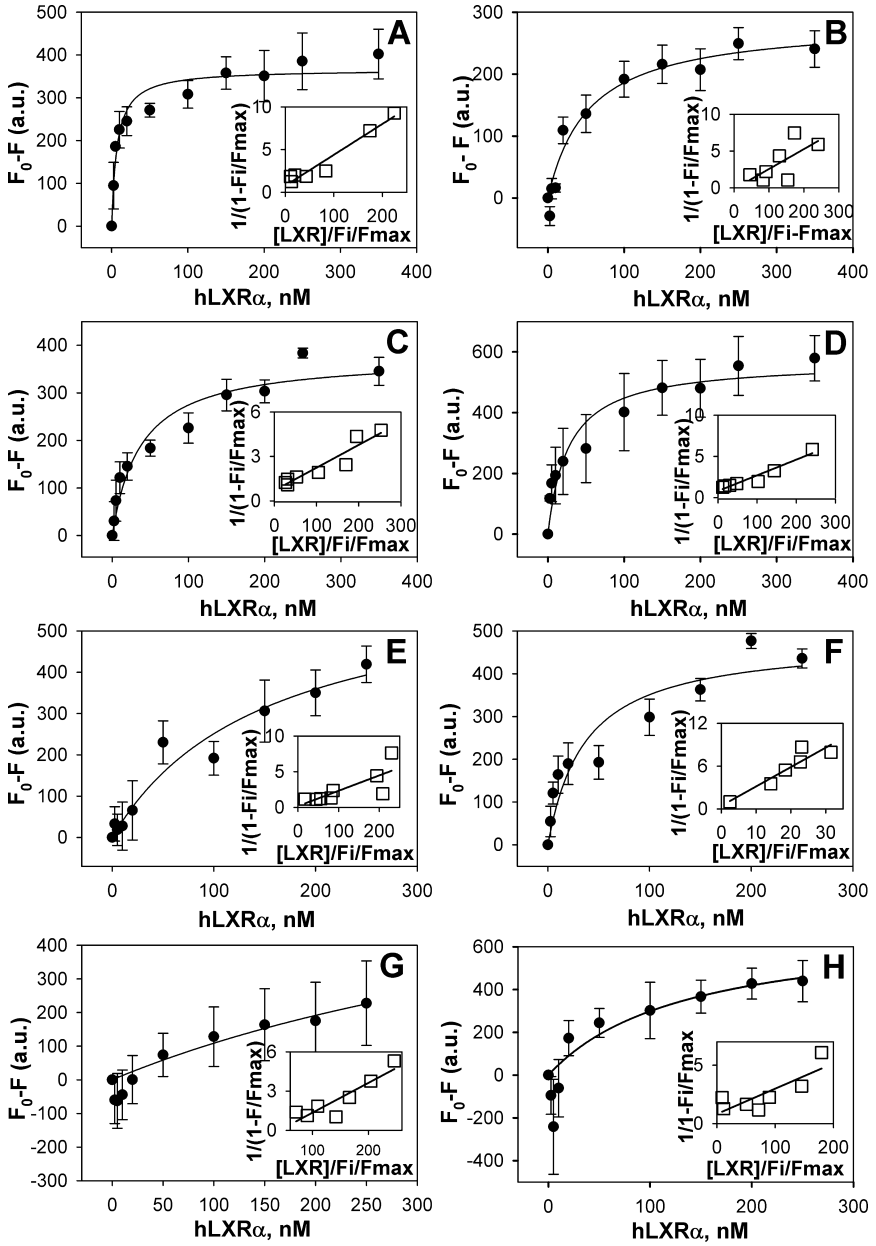

Figure 6. Fluorescent protein−protein binding assays of Cy5-labeled hPPAR*α* titrated against increasing concentrations of unlabeled hLXR*α* in the presence of LCFA and LCFA-CoA. The change in fluorescence intensity of 25 nM Cy5-labeled hPPAR*α* titrated with increasing concentrations (0−250 nM) of hLXR*α* in the presence of 25 nM (A) palmitic acid, (B) palmitoyl-CoA, (C) oleic acid, (D) oleoyl-CoA, (E) linoleic acid, (F) linoleoyl-CoA, (G) eicosapentaenoic acid, and (H) eicosapentaenoyl-CoA. Insets represent double-reciprocal linear plots of each binding curve. Values represent means ± the standard error (n = 3−5).

Table 3. Binding Affinities of hPPAR*α* for hLXR*α*, in the Absence and Presence of LCFA or LCFA-CoA

	K_d (nM)	
ligand	LCFA	LCFA-CoA
none	6 ± 2	
C16:0	7 ± 2	53 ± 17
C18:1	37 ± 10	27 ± 12
C18:2	209 ± 89	36 ± 11
C20:5	>600	135 ± 85

heterodimer to the PPRE was already very low, any changes may be below the limit of detection. In contrast, the presence of LCFA or T0901317, a known LXR*α* agonist, decreased the level of binding of the PPAR*α*−LXR*α* heterodimer to the LXRE from the SREBP-1c promoter (Figure 7C), possibly because of weakened heterodimer interactions. This binding of the PPAR*α*−LXR*α* heterodimer was compared to the binding of the respective RXR*α* heterodimer to the same sequences in the presence of LCFA (Figure S1 of the Supporting Information). Although DNA binding was weaker for the PPAR*α*−LXR*α* heterodimer than for either the PPAR*α*−RXR*α* or LXR*α*−RXR*α* heterodimer, LCFA effects were similar for each response element (Figure S1A,C of the Supporting Information). LCFA addition had no effect on PPRE binding by either heterodimer pair (Figure S1B,D of the Supporting Information), suggesting that these ligand-induced conformational changes do not affect PPRE binding. However, LXRE

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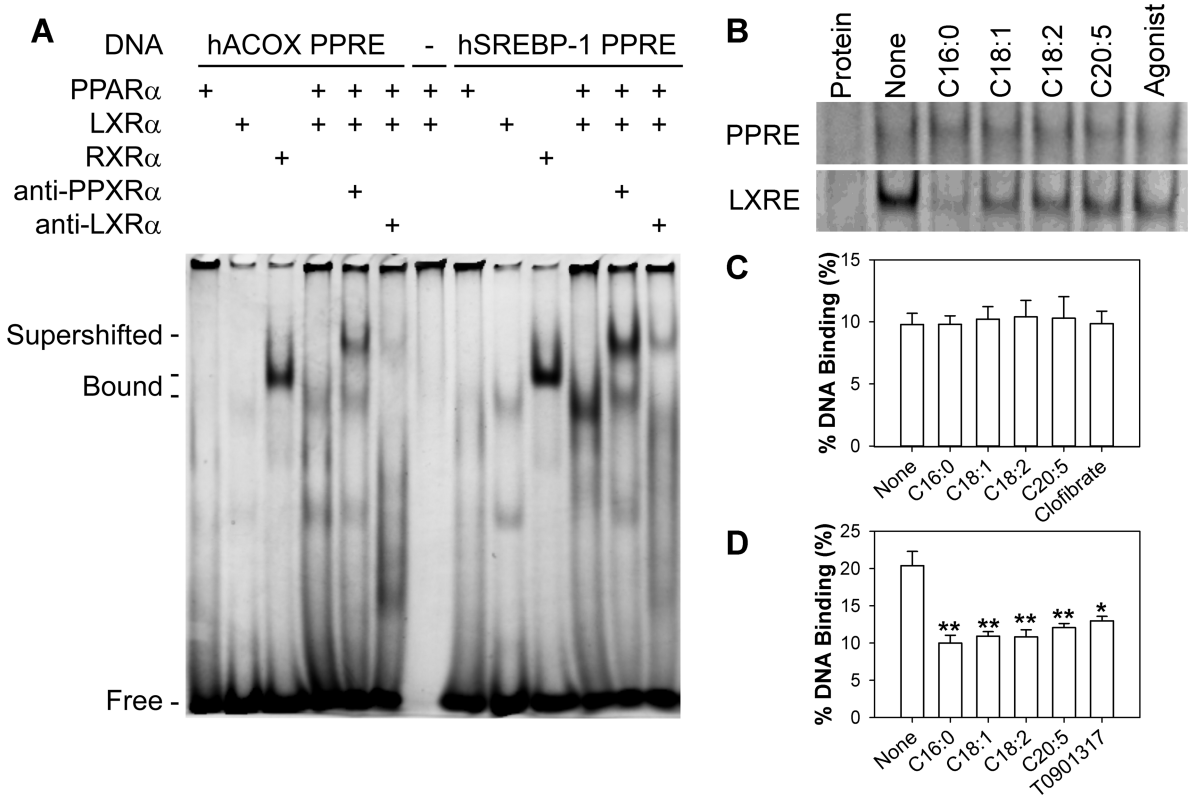

Figure 7. (A) Electrophoretic mobility shift assays of DNA binding by each individual protein (hPPARα, hLXRα, and hRXRα), a mixture of hPPARα and hLXRα proteins, and a mixture of hPPARα and hLXRα proteins in the presence of anti-PPARα or anti-LXRα. The left side of the gel shows binding to the hACOX PPRE; the middle lane is a no DNA control, and the right side of the gel shows binding to the hSREBP-1c LXRE. (B) Representative electrophoretic mobility shift assays showing DNA binding. The top gel shows PPRE binding for the hPPARα–hLXRα heterodimer in the presence of LCFA or clofibrate (agonist). The bottom gel shows LXRE binding for the hPPARα–hLXRα heterodimer in the presence of LCFA or T0901317 (agonist). (C) Relative DNA binding to a PPRE sequence by hPPARα–hLXRα heterodimers in the absence (none) or presence of LCFA. Values are presented relative to binding to the same response element by hPPARα–hRXRα heterodimers in the presence of clofibrate, a known PPARα agonist. (D) Relative DNA binding to an LXRE sequence by hPPARα–hLXRα heterodimers in the absence (none) or presence of LCFA. Values are presented relative to binding to the same response element by hLXRα–hRXRα heterodimers in the presence of T0901317, a known LXRα agonist. DNA binding was determined by electrophoretic mobility shift assays, and resulting bands were quantified by densitometry. Values represent means ± the standard error (n = 4 or 5). Asterisks denote significant differences due to the addition of ligand: *p < 0.05, and **p < 0.01.

binding by both LXRα–PPARα and LXRα–RXRα heterodimers was weakened by the addition of LCFA, suggesting either a direct consequence of the altered PPARα affinity for LXRα or an altered affinity for DNA in the presence of LCFA.

Effect of PPARα Ligands on PPARα–LXRα Transactivation of the PPRE or LXRE. Because the addition of ligand had only minor effects on DNA binding, but altered protein conformation, the ability of PPARα ligands to affect transactivation was examined. For PPARα activity, the ACOX promoter was cloned into a promoter-less luciferase reporter, while the SREBP-1c promoter was used as an LXRα target. HepG2 cells were transfected to overexpress hPPARα, hLXRα, hPPARα, and hLXRα or an empty vector (pGS5), and the effects of LCFA were examined. Overexpression of hPPARα or hLXRα alone had no effect on ACOX-luciferase activity compared to that with the empty vector, while overexpression of both slightly increased hPPARα activity (Figure 8A,B). Although LCFA have been shown to strongly transactivate ACOX-luciferase reporters in cells overexpressing both PPARα and RXRα,[24,28] no significant changes were noted for the addition of LCFA or clofibrate to cells overexpressing hPPARα and hLXRα (Figure 8A,B). Because binding of the hPPARα–hLXRα heterodimer to this PPRE sequence was weak, the effect of the hPPARα–hLXRα heterodimer on PPARα transactivation may not be as significant as the effect of the hPPARα–hRXRα heterodimer. However, HepG2 cells endogenously express each of these proteins, as well as hRXRα, so if LXRα and PPARα were only competing for available RXRα, it would be expected that repression would be observed in cells ectopically expressing LXRα. These data suggest that the increased activity seen in cells overexpressing both receptors may be due to hPPARα–hLXRα interactions.

However, striking differences were noted for effects on LXRα activity. In the absence of ligand, cells overexpressing only hLXRα or hLXRa and hPPARa showed increased activity versus cells with the empty vector (Figure 8C,D). Because HepG2 cells endogenously express hPPARα, hLXRα, and hRXRα, the activity seen in cells overexpressing only hLXRα may be due in part to hLXRα–hRXRα interactions. With the addition of 1 μM ligand, activity levels for cells overexpressing hPPARα alone and cells overexpressing both hPPARα and hLXRα significantly decreased and were similar to those for cells with the empty vector (Figure 8C). The addition of 10 μM ligand resulted in decreased activity with each of the examined ligands for all cells, with eicosapentaenoic acid resulting in the largest changes (Figure 8D). These results were consistent with

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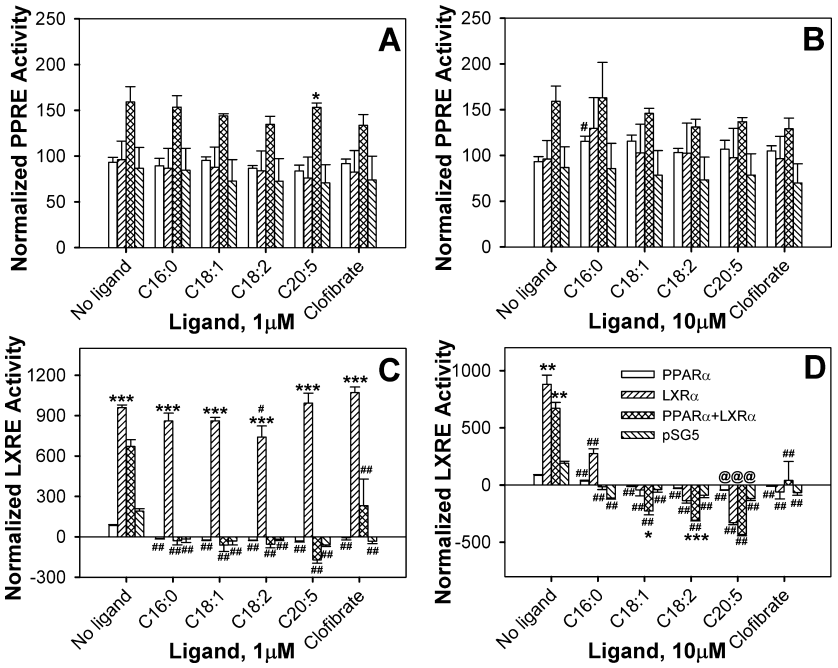

Figure 8. Normalized firefly luciferase levels driven by promoters containing response elements for PPARα or LXRα. Transactivation of the PPARα-regulated gene, ACOX, in the absence and presence of 1 μM (A) and 10 μM LCFA (B). Transactivation of the LXRα-regulated gene, SREBP-1c, in the absence and presence of 1 μM (C) and 10 μM LCFA (D). Number symbols denote significant differences due to ligand as compared to no-ligand controls for all panels: #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ Asterisks denote significant differences between overexpression cell lines for a given ligand treatment: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. @@@ indicates all overexpression cell lines were significantly different from each other at the $p < 0.001$ level.

both the decreased affinity of PPARα for LXRα and the decreased level of LXRE binding seen in the presence of eicosapentaenoic acid. These data suggested that LCFA decrease LXRα activity, in the presence and absence of hPPARα. This further suggests that such repression may be due to more than just competition between PPARα and LXRα for RXRα.

DISCUSSION

Nuclear receptor-mediated metabolic regulation is complex. Both PPARα and LXRα play important roles in such regulation through transcriptional control of genes involved in fatty acid oxidation, cholesterol metabolism, and fatty acid synthesis, yet how these receptors coordinate such regulation is not fully understood. Previous experiments have indicated that cross-talk occurs between PPARα and LXRα, and it has been suggested that these two receptors may even directly interact.[12,29] However, the significance of this finding is unclear, and the effect of endogenous ligands remains to be elucidated. To clarify the role that PPARα ligands play in the PPARα–LXRα interaction, tag-free, full-length human PPARα and LXRα proteins were used for these studies. These studies provide several new insights into PPARα–LXRα cross-talk and the importance of ligand binding on heterodimerization.

These data demonstrate a direct, very high-affinity interaction between hPPARα and hLXRα, with binding affinities in the low nanomolar range. Further, our data show that endogenous, high-affinity PPARα ligands could alter hPPARα–hLXRα binding. While only a subset of known endogenous hPPARα ligands were examined, the structural changes noted by CD suggested that ligand binding either altered the secondary structure of the heterodimer or suppressed heterodimerization. Protein–protein binding experiments confirmed these results, with high-affinity binding of hPPARα to hLXRα in the presence of the shorter chain saturated LCFA, palmitic acid, and decreased hPPARα–hLXRα affinity in the presence of the longer, unsaturated LCFA, eicosapentaenoic acid. As each of the examined ligands has been shown to bind hPPARα with similar affinity (K_d values of 12–34 nM for LCFA and 11–16 nM for LCFA-CoA),[16] altered heterodimer formation may stem from unique ligand-induced conformational changes.

While we clearly observed binding of the hPPARα–hLXRα heterodimer to DNA, previous experiments have suggested that the heterodimer of mouse PPARα (mPPARα) and hLXRα is incapable of binding DNA.[12] Because PPARα is an obligate heterodimer, requiring dimerization to bind the PPRE, it is possible that the hPPARα–hLXRα interaction is stronger than the mPPARα–hLXRα interaction, resulting in more stable DNA binding. It is also possible that mPPARα and hPPARα preferentially bind different degenerate PPRE sequences, as previous experiments have shown species variation in rodent and human PPRE sequence binding.[30] Because previous studies examined the binding of the mPPARα–hLXRα heterodimer to a rat PPRE and this work examined binding of the hPPARα–hLXRα heterodimer to a human PPRE, this may explain some of the observed differences. However, the existence of a specific, high-affinity hPPARα–hLXRα response element, separate from the PPRE or LXRE, remains to be identified.

These data suggest a specific role for a PPARα–LXRα heterodimer rather than just competition between the two proteins for heterodimerization with RXRα. Previous experiments have shown that LXRα can repress an ACOX-luciferase reporter, presumably through competition for RXR.[8] Data presented herein actually show an elevated level of expression from an ACOX-luciferase reporter in cells overexpressing both

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PPARα and LXRα, and no effect on cells overexpressing only LXRα. These activity differences are likely due to differences in the constructs used for the expression assays. The previous experiment used a synthetic hybrid of the TK promoter possessing three copies of the PPRE from rat ACOX. Our studies used a 2.3 kb fragment of human DNA comprising the endogenous PPRE and ACOX promoter. Thus, a human PPRE within the native environment of an ACOX promoter displays regulation by the human PPARα−LXRα heterodimer and strengthens the idea that there is cross-talk between the PPARα- and LXRα-regulated pathways.

Previously published data have suggested that LCFA decrease SREBP-1c levels due to activation of PPARα, leading to an increased level of PPARα−RXRα heterodimer formation and consequently fewer LXRα−RXRα heterodimers.[7] However, data included herein suggest that other mechanisms may be responsible. In addition to the presence of an LXRα−PPARα heterodimer, ligand interactions influence the activities of the nuclear receptors involved. For example, the level of binding of the LXRα−RXRα heterodimer to DNA was reduced in the presence of LCFA, even in the absence of PPARα, suggesting that LCFA may directly affect the LXRα−RXRα heterodimer. This idea is further supported by studies showing that the RXRα−LBD species can bind polyunsaturated LCFA.[31]

In summary, these data show for the first time a direct, high-affinity interaction between full-length human PPARα and human LXRα proteins. Furthermore, this interaction could be altered by the addition of PPARα ligands (LCFA or LCFA-CoA), with polyunsaturated fatty acids abolishing the high-affinity interaction. Although DNA binding was weak compared to that of the RXRα heterodimers, binding did occur, suggesting a specific role for the PPARα−LXRα heterodimer. In addition, cells overexpressing both PPARα and LXRα showed altered transactivation of both a PPARα and LXRα target reporter, with LCFA decreasing the extent of LXRE transactivation. Taken together, these data suggest that ligand binding may determine heterodimer choice and downstream gene regulation of these nuclear receptors.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Electrophoretic mobility shift assays showing DNA binding by the hPPARα−hLXRα heterodimer versus that of the hPPARα−hRXRα or hLXRα−hRXRα heterodimer. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

PPAR, peroxisome proliferator-activated receptor; LXR, liver X receptor; RXR, retinoid X receptor; PPRE, peroxisome proliferator response element; LXRE, liver X receptor response element; PPARα, peroxisome proliferator-activated receptor α; LXRα, liver X receptor α; LCFA, long chain fatty acid; LCFA-CoA, long chain fatty acyl-CoA; hGR, human glucocorticoid receptor; hPPARα, human PPARα; hLXRα, human LXRα; ACOX, acyl-CoA oxidase; SREBP-1c, sterol regulatory element binding protein-1c; CD, circular dichroism; LBD, ligand binding domain; mPPARα, mouse PPARα.

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