Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 267–279
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Tumor necrosis factor and interleukin 1 decrease RXRα, PPARα, PPARγ, LXRα, and the coactivators SRC-1, PGC-1α, and PGC-1β in liver cells

Min Sun Kim[1], Trevor R. Sweeney[1], Judy K. Shigenaga, Lisa G. Chui, Arthur Moser, Carl Grunfeld, Kenneth R. Feingold*

Department of Medicine, University of California San Francisco, San Francisco, CA 94143, USA
Metabolism Section, Department of Veterans Affairs Medical Center, San Francisco, CA 94121, USA

Received 20 March 2006; accepted 18 October 2006

Abstract

During the acute phase response, cytokines induce marked alterations in lipid metabolism including an increase in serum triglyceride levels and a decrease in hepatic fatty acid oxidation, in bile acid synthesis, and in high-density lipoprotein levels. Here we demonstrate that tumor necrosis factor (TNF) and interleukin 1 (IL-1), but not IL-6, decrease the expression of retinoid X receptor α (RXRα), peroxisome proliferator–activated receptor α (PPARα), PPARγ, liver X receptor α (LXRα), and coactivators PPARγ coactivator 1α (PGC-1α), PGC-1β, and steroid receptor coactivator 1 (SRC-1) in Hep3B human hepatoma cells. In addition, treatment of mice with TNF and IL-1 also decreased RXRα, PPARα, PPARγ, LXRα, and PGC-1α messenger RNA (mRNA) levels in the liver. These decreases were accompanied by reduced binding of nuclear extracts to RXR, PPAR, and LXR response elements and decreased luciferase activity driven by PPAR and LXR response elements. In addition, the mRNA levels of proteins regulated by PPARα (carnitine palmitoyltransferase 1α) and LXR (sterol regulatory element binding protein) were decreased in Hep3B cells treated with TNF or IL-1. Finally, using constructs of the LXRα promoter or the PGC-1α promoter linked to luciferase, we were able to demonstrate that a decrease in transcription contributes to the reduction in mRNA levels of nuclear hormone receptors and coactivators. Thus, our results suggest that decreased expression of nuclear hormone receptors RXRα, PPARα, PPARγ, and LXRα, as well as coactivators PGC-1α, PGC-1β, and SRC-1 may contribute to the cytokine-induced alterations in hepatic lipid metabolism during the acute phase response.

1. Introduction

The acute phase response (APR), which is induced by infection, inflammation, injury, and stress, results in marked alterations in the synthesis of proteins in the liver [1]. The synthesis of certain proteins, for example, C-reactive protein and serum amyloid A, is increased (positive APR proteins), whereas the synthesis of other proteins, for example, albumin, is decreased (negative acute phase proteins) [2,3]. In addition to changes in hepatic protein synthesis, the APR also induces alterations in hepatic lipid and lipoprotein metabolism including hypertriglyceridemia [4], decreases in serum high-density lipoprotein cholesterol levels [5,6], inhibition of bile acid synthesis [7], and decreases in hepatic fatty acid oxidation and ketogenesis [6,8]. Most of the changes that occur during the APR result from the increased production of cytokines, particularly tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), and IL-6, which mediate these alterations by regulating gene transcription. Although the mechanisms by which cytokines increase the expression of positive acute phase proteins have been extensively studied [9,10], the regulation of the negative acute phase proteins is less well understood.

Nuclear hormone receptors form a large family of transcription factors that regulate many aspects of intermediary metabolism [11,12]. Class II nuclear hormone receptors heterodimerize with retinoid X receptor (RXR) and include the retinoic acid receptors, thyroid hormone receptors, vitamin D receptor, farnesoid X receptors, liver X receptors (LXRs), and peroxisome proliferator–activated receptors

* Corresponding author. Metabolism Section (111F), Department of Veterans Affairs Medical Center, San Francisco, CA 94121, USA. Tel.: +1 415 750 2005; fax: +1 415 750 6927.
E-mail address: kfngld@itsa.ucsf.edu (K.R. Feingold).
[1] These two authors contributed equally to these studies.

0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2006.10.007

(PPARs) [11,13,14]. PPARs, farnesoid X receptor, and LXRα have been shown to play major roles in the regulation of the expression of proteins involved in various aspects of lipid and lipoprotein metabolism. Activation of PPARα stimulates hepatic fatty acid oxidation by increasing the expression of a number of genes important in fatty acid metabolism including acyl-CoA synthetase [15], carnitine palmitoyltransferase (CPT) I [16-18], acyl-CoA oxidase, fatty acid transport protein [15], and fatty acid–binding protein [19]. Fatty acids and products of the cholesterol biosynthetic pathway activate PPARs [20,21]. Activation of LXR stimulates the expression of key enzymes required for bile acid synthesis, the transporters that secrete cholesterol into bile, and the transcription factor SREBP-1c that regulates many of the enzymes that synthesize fatty acids [12,22]. Oxidized cholesterol derivatives activate LXR [22]. Recently, the concept that these nuclear hormone receptors are liposensors monitoring the intracellular levels of various lipids and then modulating the expression of key proteins involved in lipid metabolism to maintain homeostasis has been proposed [12].

Hepatic expression of many of the target genes for PPARs and LXR decrease during the APR [23-28]. We therefore postulated that decreases in RXRα, the obligate heterodimer partner, and/or PPARs and LXRs occur during the APR. We have shown that lipopolysaccharide (LPS) administration to Syrian hamsters results in a decrease in hepatic messenger RNA (mRNA) and/or protein levels of RXRα, -β, and -γ, PPARα and -γ, and LXRα [25]. In addition, hepatic nuclear extracts obtained from animals treated with LPS exhibited reduced binding activity to RXR-RXR, RXR-PPAR, and RXR-LXR response elements (REs) [25]. Moreover, previous studies by other investigators have shown that the administration of LPS to mice or the treatment of HepG2 cells with IL-1β results in the increased transport of RXRα from the nucleus to the cytoplasm where it would not be capable of forming dimers with PPARα and LXR and thereby stimulating transcription [29,30]. A reduction in hepatic nuclear RXR levels alone or in combination with decreases in PPARs or LXRs could contribute to the decrease in the expression of multiple genes involved in lipid metabolism during the APR.

In addition to regulation at the level of nuclear hormone receptors, other factors such as coactivators and corepressors are involved in the regulation of the transcriptional activity of nuclear hormone receptors [31,32]. Coactivators are a class of transcriptional regulators that do not bind DNA directly, but enhance transcription, whereas corepressors inhibit transcription. The binding of ligand to nuclear hormone receptors increases the affinity of the DNA-bound nuclear receptors for coactivators and decreases affinity for corepressors, thereby enhancing gene transcription by modifying the chromatin structure of the target gene or by associating with the RNA polymerase machinery [33,34]. It has been shown that cellular levels of coregulators are crucial for nuclear receptor–mediated transcription [35-37], and many coregulators have been demonstrated to be targets for diverse intracellular signaling pathways and posttranslational modifications [33,34,38]. Given the array of metabolic changes that occur during infection and inflammation, it is also possible that the expression of coactivators and/or corepressors is regulated during the APR.

In the present study, we hypothesized that inflammatory cytokines, TNF, IL-1, or IL-6, exert their effects directly on the hepatocytes and suppress the transcriptional activity of PPAR and LXR by decreasing the expression of RXR, PPARα, and LXRα and coregulators contributing to the alterations in lipid metabolism that occur during the APR.

2. Materials and methods

2.1. Materials

LPS (*Escherichia coli* 55:B5) was obtained from Difco Laboratories (Detroit, MI). Cytokines (hTNFα, hIL-1β, hIL-6, mTNFα, and mIL-1β) were from R&D Systems (Minneapolis, MN). Mimimum essential medium (MEM) was purchased from Fisher Scientific (Houston, TX). For in vivo studies, LPS or cytokines were freshly diluted to desired concentrations in pyrogen-free 0.9% saline. For in vitro studies, LPS or cytokines were freshly diluted to the desired concentration in serum-free MEM containing 0.1% bovine serum albumin (fatty acid free). Pyrrolidinecarbodithioc acid (PDTC) was obtained from Calbiochem (San Diego, CA). Tri-Reagent, WY-14, 643, and fatty acid–free bovine serum albumin were from Sigma (St Louis, MO). [α-^{32}P] deoxycytidine triphosphate (3000 Ci/mmol) and [γ-^{32}P] deoxyadenocine triphosphate (3000 Ci/mmol) were purchased from NEN Life Science Products (Boston, MA).

2.2. Cell cultures

Hep3B cells were maintained in MEM supplemented with 10% fetal bovine serum in 75-cm^2 flasks. Cells were washed twice with PBS (Ca^{2+} and Mg^{2+} free), and trypsinized before seeding. For typical experiments, cells were seeded in 100-mm dishes at a concentration of 2×10^6 cells per dish. After an overnight incubation, cells were washed twice with PBS and the medium was replaced with fresh serum-free MEM plus 0.1% bovine serum albumin and the appropriate cytokine or LPS concentration. For some experiments, PDTC was diluted to 10 μmol/L in fresh serum-free MEM plus 0.1% bovine serum albumin. Cell morphology and viability were not affected by cytokine treatment as assessed by trypan blue staining. For transfection assays, 1.5×10^5 cells were used per well in 6-well plates.

2.3. Animals

Eight-week-old female C57BL/6 mice were purchased from Jackson Laboratory (Bar Harbor, ME). The animals were maintained in a room with a normal light cycle and were provided with rodent chow and water ad libitum. Anesthesia was induced with halothane. To determine the effect of APR on PPARγ coactivator 1α (PGC-1α),

Table 1

Gene	Accession No.	Forward primers (5′-3′)	Reverse primers (5′-3′)
A. Primer sequences used for generation of cDNA probes for Northern blotting			
CPT1α	NM_001876	TCGGTACTCTCTGAAGATGGC	GAGCAGAGTGGAATCGGGA
SMRT	AF113001	ACTGGCACCAAGAAACACGAC	GCTGCGAGGTGATGTAGTCATT
NCoR	U35312	CCGTAAGTGGCTATGCTCTTTAC	CCCTCTAAAGGTGCTGACACAG
Trap220	AF283812	GCAAGGTGTCTCAGAACCC	CCAGCAGCATCTGCAATAAG
SRC-1	U40396	CCTCAGATGCAGCAGAATGTC	GTGGTTATTCAGTCAGTAGCTG
SRC-2	XM_011633	GCAACCACTCCTCAGGGTAG	CTGCTGGACTCCTGGCTCAG
SRC-3	NM_008679	GCGCCAGAGATATGAAACAATGC	GGCTGCCCATCATCTGTACATTC
CBP	NM_004380	CTGCCTCCCAAGCACTGAATC	CAAACAGGACAGTCATGTCGTG
PGC-1α	AF049330	GACCACAAACGATGACCCTCC	GCCTCCAAAGTCTCTCTCAGG
B. Primer sequences used for real-time QPCR			
mSREBP-1a	NM_011480	GGCCGAGATGTGCGAAC	GTTGATGAGCTGGAGCATGT
mSREBP-1c	NM_011480	AGCTGTCGGGGTAGCGTCTG	GAGAGTTGGCACCTGGGCTG
mSREBP-2	AF374267	GCGTTCTGGAGACCATGGA	ACAAAGTTGCTCTGAAAACAAATCA
hSREBP-1c	BC063281	CGGAGCCATGGATTGCAC	CTTCAAGAGAGGAGCTCAATGTGG
hPGC-1β	NM_133263	CCAAGACCAGCAGCTCCTA	CCACTGTCAAGGTCTGCTCA
mPGC-1β	NM_133249	CAAGCTCTGACGCTCTGAAGG	TTGGGGAGCAGGCTTTCAC
hRXRα	NM_002957	GAGACCTACGTGGAGGCAAA	GATGGAGCGGTGGGAGA
hLXRα	NM_005693	CGCACTACATCTGCCACAGT	TCAGGCGGATCTGTTCTTCT
hPPARα	NM_005037	GGTGGACACGGAAAGCCCAC	GGACCACAGGATAAGTCACC
hPPARγ	L40904	CTCATATCCGAGGGCCAA	TGCCAAGTCGCTGTCATC
h/m36B4	M17885	GCGACCTGGAAGTCCAACTAC	ATCTGCTGCATCTGCTTGG

PGC-1β, and steroid receptor coactivator 1 (SRC-1) mRNA levels, mice were injected intraperitoneally with 100 μg of LPS, 100 ng of TNF, or 80 ng of IL-1 in saline or with saline alone. Food was withdrawn at the time of injection because LPS and cytokines induce anorexia in rodents [7]. Livers were removed after treatment at the time indicated in the text. The doses of LPS or cytokines used in this study have significant effects on triglyceride and cholesterol metabolism [7], but are not lethal, as the LD_{50} for LPS in rodents is approximately 5 mg/100 g body weight.

2.4. Preparation of cell extracts

Nuclear extracts were prepared according to Neish et al [39]. Briefly, cells were disrupted in a sucrose-HEPES buffer containing 0.5% Nonidet P40 as a detergent, protease inhibitors, and dithiothreitol. After disruption by 5-minute incubation on ice and centrifugation, nuclear proteins were separated in a sodium chloride-HEPES buffer and resuspended in a glycerol-containing buffer. Total cell lysates were prepared according to Santa Cruz Biotechnology (Santa Cruz, CA) Western Blotting Protocol. Briefly, cells were washed once with PBS, scraped into 0.6 mL Radio-ImmunoPrecipitation Assay (RIPA) buffer, then incubated for 1 hour with rocking. Lysates were then centrifuged at 10,000*g* for 15 minutes and the supernatant was retained as total cell lysate. All the procedures were carried out on ice. Protein quantification was determined by the Bradford assay (Bio-Rad, Hercules, CA), and yields were similar in control and cytokine-treated groups.

2.5. Western blot analysis

Denatured nuclear protein (10 μg) or denatured total cell lysate (40 μg) was loaded onto 10% polyacrylamide precast gels (Bio-Rad) and subjected to electrophoresis. After electrotransfer onto polyvinylidene difluoride membrane (Amersham Pharmacia Biotech, Piscataway, NJ), blots were blocked with phosphate-buffered saline containing 0.1% Tween and 5% dry milk for 1 hour at room temperature and incubated for 1 hour at room temperature with polyclonal anti-RXRα (Santa Cruz Biotechnology) at a dilution of 1:5000, or polyclonal anti-PGC-1α (Chemicon International, Temecula, CA) at a dilution of 1:1000. Immune complexes were detected by using the appropriate second antibody linked to horseradish peroxidase according to the ECL Plus Western blotting kit (Amersham Pharmacia Biotech). Immunoreactive bands obtained by autoradiography were quantified by densitometry. Other antibodies tried were polyclonal anti-PPARα (Affinity Bioreagents, Golden, CO), polyclonal anti-PPARα and polyclonal anti–SRC-1 (Santa Cruz Biotechnology), and monoclonal anti-LXRα (R&D Systems).

2.6. RNA isolation and Northern blot analysis

Total RNA was isolated from a 100-mm dish by the Tri-Reagent method and resuspended in diethylpyrocarbonate-water. RNA was quantified by measuring the absorption at 260 nm. Twenty micrograms of total RNA was denatured and electrophoresed on a 1% agarose/formaldehyde gel. The uniformity of sample loading was checked by UV visualization of the ethidium bromide–stained 18S and 28S bands before electrotransfer to Nytran membrane (Schleicher & Schuell, Keene, NH). Prehybridization, hybridization, and washing procedures were performed as described previously [24]. Membranes were probed with [α-^{32}P]dCTP–labeled complementary DNAs (cDNAs) by using the random priming technique. mRNA levels were detected by exposure of the membrane to x-ray film and quantification by densitometry. Because traditional housekeeping genes

(actin, GAPDH, and cyclophillin) change during the APR, we did not normalize these data. The responses are specific, as we and others have shown that many genes increase, whereas others decrease. hRXRα cDNA was a gift from Dr Daniel D. Bikle (University of California, San Francisco [UCSF]). rPPAR (α, β, and γ) cDNAs were a gift from Dr Anthony Bass (UCSF). Other mouse and human cDNAs were prepared by polymerase chain reaction (PCR) using the primers shown in Table 1A.

2.7. Quantitative real-time PCR

First-strand cDNA was synthesized from 1 μg of total RNA with random hexamer primers using the Advantage RT-for-PCR kit (BD Biosciences Clontech, Mountain View, CA). The real-time PCR contained, in a final volume of 20 μL, 20 ng of reverse-transcribed total RNA, 300 nmol/L forward and reverse primers, and 10 μL of 2 × SYBR Green PCR Master Mix (Stratagene, La Jolla, CA). PCR

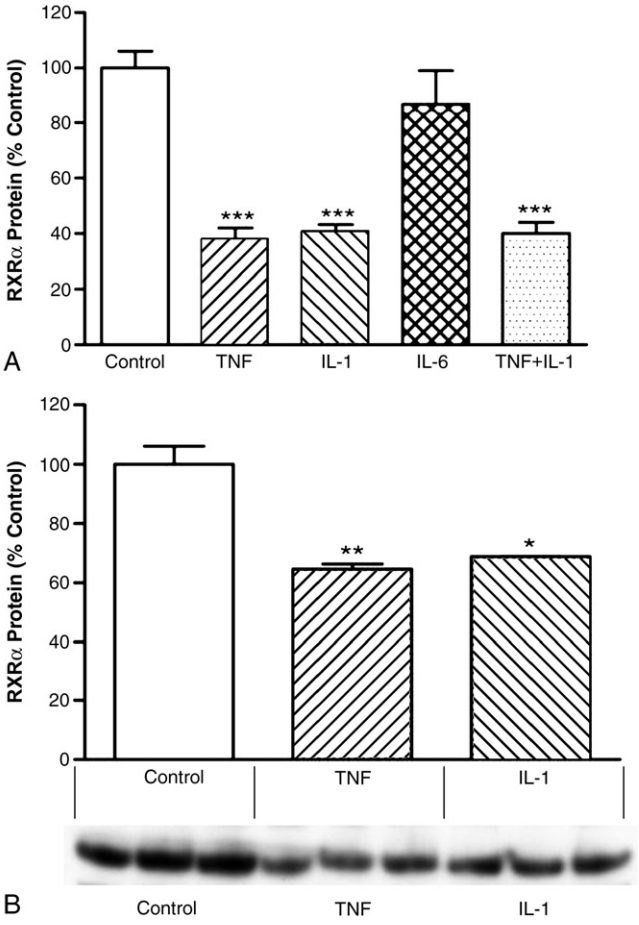

Fig. 1. Effect of cytokines on RXRα protein levels in Hep3B cells. Hep3B cells were plated in 100-mm Petri dishes in culture medium containing 10% serum. After an overnight incubation, cells were treated with the appropriate cytokine at 10 ng/mL. A, After 24 hours' incubation, the nuclei were isolated and extracted, Western blot analysis was performed as described under "Materials and methods." B, After 24 hours' incubation, total cell lysate was extracted and Western blot analysis was performed. Data (mean ± SE, n = 3) are expressed as a percentage of controls. *$P < .5$, **$P < .01$, ***$P < .001$ vs control.

was carried out in 96-well plates using Mx3000P Real-time PCR System (Stratagene). The relative amount of all mRNAs was calculated using the comparative C_T method. 36B4 mRNA was used as the invariant control for all experiments. The primers used are shown in Table 1B.

2.8. Electrophoretic mobility shift assay

Ten micrograms of crude nuclear extract were incubated on ice for 60 minutes with 6×10^4 cpm of ^{32}P-labeled oligonucleotides in 15 μL of binding buffer (20% glycerol, 25 mmol/L Tris-HCl [pH 7.5], 40 mmol/L KCl, 0.5 mmol/L $MgCl_2$, 0.1 mmol/L EDTA, 1 mmol/L dithiothreitol, 2 μg of poly deoxyinosine deoxycytidine, and 1 μg of salmon sperm DNA). Double-stranded oligonucleotide probes were end-labeled with T4-polynucleotide kinase (Amersham Pharmacia Biotech) in the presence of 50 μCi of [γ-^{32}P]dATP and purified on a Sephadex G-25 column (Amersham Pharmacia Biotech). Labeled oligonucleotides were added to the nuclear extracts and incubated for 30 minutes on ice before electrophoresis. DNA-protein complexes were separated by electrophoresis (constant voltage of 200 V) on a 5% nondenaturing polyacrylamide gel in 0.5 × Tris-Borate-EDTA at 4°C. The gel was dried, exposed to x-ray film, and quantified by densitometry. In the competition assay, a 100-fold molar excess of the specific or mutated unlabeled oligonucleotide was preincubated on ice for 1 hour with 10 μg of nuclear extract from control cells. The following oligonucleotides were used: peroxisome proliferator response element (PPRE), 5′-GAT CCT CCC GAA CGT GAC CTT TGT CCT GGT CCA-3′ [25]; mut-PPRE, 5′-GAT CCT CCC GAA CGC AGC TGT CAG CTG GGT CCA-3′; RXR response element RXRE, 5′-GAT ACT GCT GTC ACA GGT CAC AGG TCA CAG TTC AA-3′ [25]; mut-RXRE, 5′-GAT ACT GCT GTC ACA GCA CAC AGC ACA CAG TTC AA-3′; LXR response element (LXRE), 5′-GAT CCC TTT GGT CAC TCA AGT TCA AGT GGA TC-3′ [25]; mut-LXRE, 5′-GAT CCC TTT GGT CAC TCA AGA ACA AGT GGA TC-3′.

2.9. Transfection studies

Hep3B cells were grown overnight in 6-well plates (1.5×10^5 per well) and washed twice with serum-free medium. Lipofectin (5 μg/mL) (Invitrogen Life Technologies, Carlsbad, CA) was preincubated in the serum-free medium for 45 minutes. For promoter-luciferase construct, 1.0 μg/mL of PPRE-luciferase (kindly provided by Dr N. Bass, UCSF), nuclear factor κB (NFκB)-luciferase (kindly provided by Dr Arthur Weiss, UCSF), LXRE-luciferase (kindly provided by Dr David Mangelsdorf, University of Texas-Southwestern), LXR promoter-luciferase (kindly provided by Dr David Mangelsdorf), and PGC-1α promoter-luciferase (kindly provided by Dr Bruce M. Spiegelman, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA) were used. Control vectors for the luciferase constructs were not regulated by TNF-α or IL-1β. To form DNA-lipofectin complex, DNA-containing medium was

combined with preincubated lipofectin medium and incubated at room temperature for 15 minutes. At the end of the incubation, the cells were overlaid with the DNA-lipofectin complex and incubated at 37°C for 4 to 6 hours. After cells were washed with serum-free medium, fresh growth medium containing 10% fetal bovine serum was added. After 24 hours of incubation, the cells were washed with serum-free MEM and the experimental medium with or without TNF or IL-1 was added. After 24 hours, the cells were harvested, washed twice with PBS, and treated with passive lysis buffer (Promega, Madison, WI), and aliquots of the lysates were assayed for luciferase activity by using Wallac VICTOR^2 1420 Multilabel Counter (Perkin Elmer Life Sciences, Wellesley, MA). For normalization of transfection efficiency, we tested several vectors including cytomegalovirus β-gal, simian virus 40 β-gal, Rous sarcoma virus β-gal, and thymidine kinase–*Renilla* luciferase (RL). We found that expression of each of these promoters is significantly affected by cytokine treatment in Hep3B cells; some increase and some decrease. Therefore, we used raw data from the luciferase assay, and the results shown herein are representative of similar experiments repeated 3 to 5 times. Our findings therefore represent the effect on the promoters studied.

2.10. Statistical analysis

Data are expressed as mean ± SE of experiments from 3 to 5 dishes or animals per group for each time or concentration point. For transfection experiments, we show the mean of 3 separate experiments that contained 3 dishes per group. The difference between 2 experimental groups was analyzed using the unpaired *t* test. Differences among multiple groups were analyzed using 1-way analysis of variance with the Dunnett posttest correction. A *P* value less than .05 was considered significant.

3. Results

3.1. Cytokines decrease RXR protein and mRNA levels

Our initial experiments examined the effect of TNF, IL-1, and IL-6 alone on RXRα protein levels in the nuclei of Hep3B cells. As shown in Fig. 1A, both TNF and IL-1 decreased RXRα nuclear protein levels, whereas IL-6 had no effect. The combination of TNF and IL-1 did not result in any further reduction in RXRα protein levels compared with TNF or IL-1 alone. Moreover, as shown in Fig. 1B, TNF and IL-1 also decreased the level of RXRα in total cell lysates.

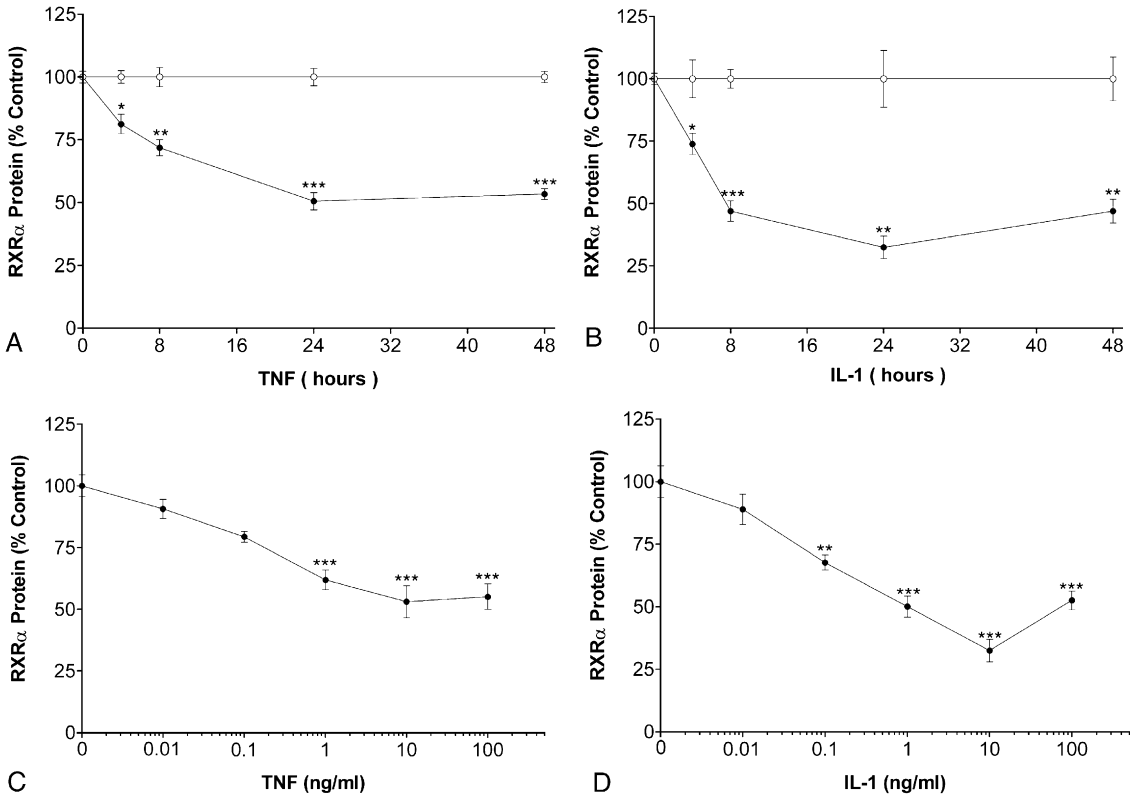

Fig. 2. Time- and dose-dependent changes in RXRα protein levels. Hep3B cells were plated in 100-mm Petri dishes in culture medium containing 10% serum. After an overnight incubation, cells were treated with TNF (10 ng/mL) (A) or IL-1 (10 ng/mL) (B) and nuclear extracts were isolated at the time indicated. In addition, Hep3B cells were treated at various concentrations of TNF (C) or IL-1 (D) for 24 hours and nuclear extracts were isolated. Western blot analysis was performed as described under "Materials and methods." Data (mean ± SE, n = 3) are expressed as a percentage of controls. *$P < .05$, **$P < .01$, ***$P < .001$ vs control.

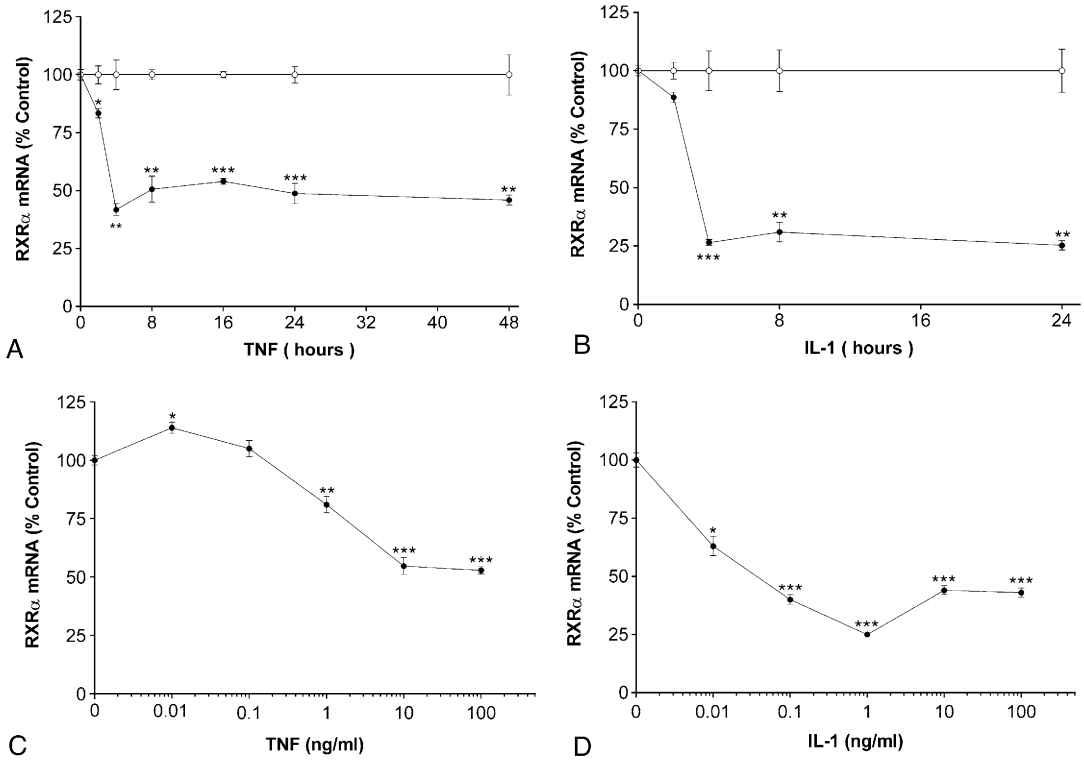

Fig. 3. Time- and dose-dependent changes in RXRα mRNA levels. Hep3B cells were plated in 100-mm Petri dishes in culture medium containing 10% serum. After an overnight incubation, cells were treated with TNF (10 ng/mL) (A) or IL-1 (10 ng/mL) (B) and total RNA was isolated at the time indicated. In addition, Hep3B cells were treated at various concentrations of TNF (C) or IL-1 (D) for 24 hours and total RNA was isolated. Northern blot analysis was performed as described under "Materials and methods." Data (mean ± SE, n = 3) are expressed as a percentage of controls. $*P < .05$, $**P < .01$, $***P < .001$ vs control.

To gain further insight into the effect of cytokines on the expression of RXRα, we next examined the time course of the effect of TNF and IL-1 on RXRα protein levels. As shown in Fig. 2, both TNF (A) and IL-1 (B) produced a decrease in RXRα protein levels as early as 4 hours, which reached a maximal effect at 24 hours and was sustained for 48 hours. We treated Hep3B cells with various doses of cytokines ranging from 0.01 to 100 ng/mL and found that both TNF (Fig. 2C) and IL-1 (Fig. 2D) cause a maximal decrease in RXRα protein levels at a dose of 10 ng/mL with a half-maximal response at approximately 0.2 ng/mL. Together, these results demonstrate that in Hep3B hepatocytes, TNF and IL-1 rapidly decrease the protein levels of RXRα, the major isoform of RXR in the liver, and that this decrease is a very sensitive cytokine response.

We next determined whether the decrease in RXRα protein levels is preceded by a decrease in RXRα mRNA levels. As shown in Fig. 3, both TNF (A) and IL-1 (B) produce a rapid and sustained decrease in RXRα mRNA levels. By 4 hours, a maximal decrease in RXRα mRNA is seen, and this decrease is sustained for 48 hours. It should be noted that the decrease in RXRα mRNA occurred more rapidly than the changes in RXRα protein levels. PDTC, a chemical inhibitor of NFκB, did not block the TNF-induced decrease in RXRα, indicating that increases in NFκB activity are not required for this suppression (data not shown).

In addition to RXRα, there are 2 other RXR isoforms. In our Hep3B cells, we were unable to detect either RXRβ or RXRγ by Western or Northern blotting (data not shown), indicating that the major RXR isoform in Hep3B cells, as in liver, is RXRα.

3.2. TNF and IL-1 decrease PPARα and PPARγ, but not PPARδ, mRNA

We next examined the effect of TNF, IL-1, and IL-6 on LXRs and PPARs. Both TNF and IL-1 decreased LXRα mRNA levels (Fig. 4A) without altering LXRβ mRNA levels (data not shown) The effects of cytokines on PPARs were isoform specific. TNF and, to a lesser extent, IL-1 decreased PPARα (Fig. 4B) and PPARγ (Fig. 4C) mRNA levels. However, IL-1 and, to a lesser extent, TNF increased PPARδ mRNA levels (Fig. 4D). As seen previously for RXRα, IL-6 had no effect on the mRNA levels of LXRs or PPARs. To ensure that the results seen with Northern blotting were correct, we also measured the levels of RXRα, LXRα, PPARα, and PPARγ by using real-time quantitative PCR (QPCR). As shown in Table 2, TNF and IL-1 decreased the mRNA levels of RXRα, LXRα, PPARα, and PPARγ to a similar degree as seen with Northern blotting. Finally, treatment of Hep3B cells with 100 ng/mL

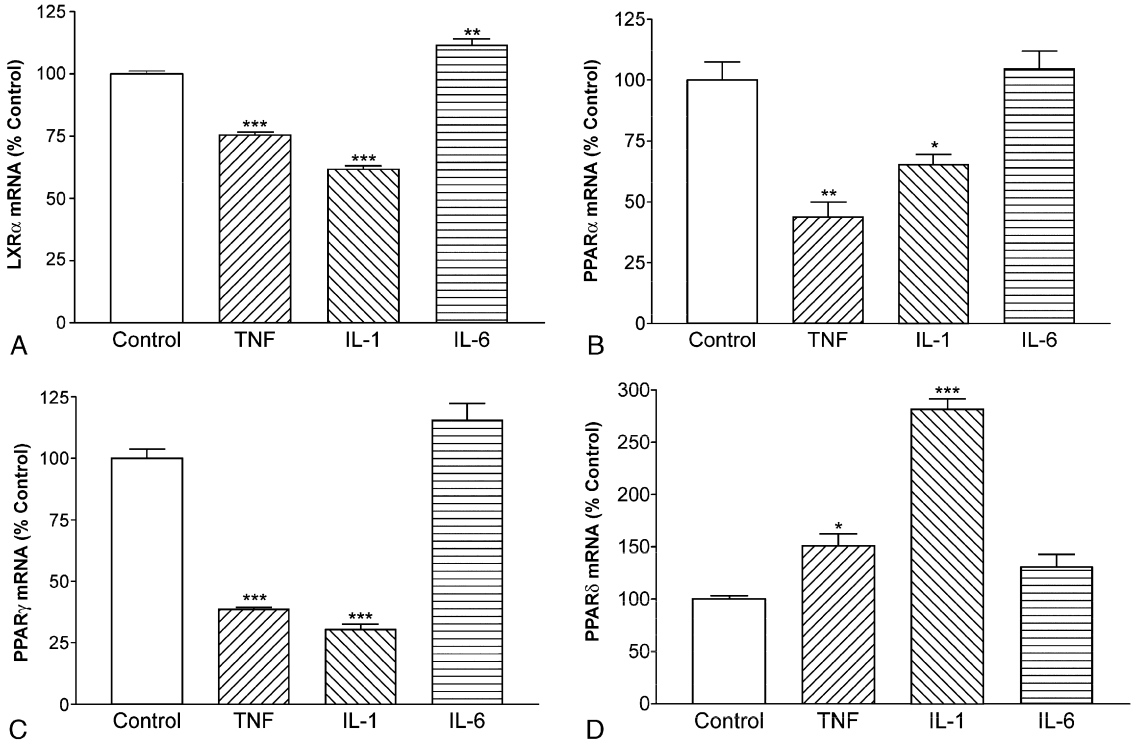

Fig. 4. Cytokines regulate mRNA levels of LXRα and PPARs. Hep3B cells were plated in 100-mm Petri dishes in culture medium containing 10% serum. After an overnight incubation, cells were treated with cytokines (10 ng/mL) as indicated. After 24 hours, total RNA was isolated and Northern blot analysis was performed using cDNAs for LXRα (A), PPARγ (C), and PPARδ (D) as described under "Materials and methods." One microgram of total RNA was also reverse transcribed to cDNA. PPARα mRNA expression (B) was measured by real-time QPCR as described under "Materials and methods." QPCR data were normalized using 36B4 mRNA as the invariant control for all experiments. Data (mean ± SE, n = 3) are expressed as a percentage of controls. *P < .05, **P < .01, ***P < .001 vs control.

LPS did not result in a decrease in the mRNA levels of either RXRα or PPARα (data not shown). These results indicate that in addition to decreasing RXRα, TNF and IL-1 also decreased LXRα, PPARα, and PPARγ mRNA levels. Unfortunately, despite using several different antibodies, we were unsuccessful in measuring LXRα, PPARα, and PPARγ protein levels by Western blotting in Hep3B cells because of a large number of nonspecific bands.

As reported previously in hamsters [25], LPS-treated mice also displayed a decrease in RXRα, PPARα, PPARγ, and LXRα mRNA levels (data not shown), indicating that the APR in vivo decreases their nuclear hormone receptors. Moreover, in TNF- or IL-1–treated mice, RXRα, PPARα, and LXRα mRNA levels decreased in the liver (Fig. 5).

Table 2
Gene expression changes in Hep3B cells by real-time QPCR

	Control	TNF	IL-1
RXRα	100 ± 14.8	52.6 ± 3.4**	77.1 ± 3.7*
LXRα	100 ± 11.7	46.4 ± 2.1**	63.9 ± 4.7*
PPARα	100 ± 2.5	45.7 ± 9.6**	72.4 ± 7.5*
PPARγ	100 ± 5.5	53.4 ± 2.1**	63.2 ± 5.5*
PGC-1α	100 ± 20.8	47.1 ± 6.2*	87.5 ± 14.3

Hep3B cells were plated in 100-mm Petri dishes in culture medium containing 10% serum. After an overnight incubation, cells were treated with cytokines (10 ng/mL) for 24 hours. Total RNA was isolated and real-time QPCR was performed as described under "Materials and methods." QPCR data were normalized using 36B4 mRNA as the invariant control for all experiments. Data are mean ± SEM; n = 3.

* P < .05.

** P < .01.

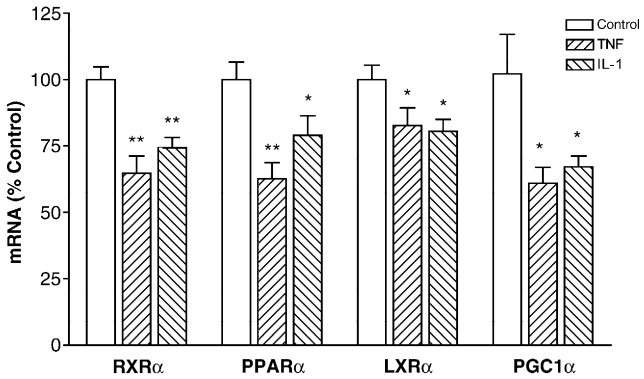

Fig. 5. Cytokines decrease the expression of RXRα, PPARα, LXRα, and PGC-1α in vivo. C57BL/6 mice were injected intraperitoneally with either saline (Control), TNF (100 ng per mouse), or IL-1 (80 ng per mouse), and the animals were killed after 16 hours. Total RNA was prepared from liver, and expression levels for RXRα, PPARα, LXRα, and PGC-1α were measured by real-time QPCR as described under "Materials and methods." QPCR data were normalized using 36B4 mRNA as the invariant control for all experiments. Data (mean ± SE, n = 4) are expressed as a percentage of controls. *P < .05, **P < .01, ***P < .001 vs control.

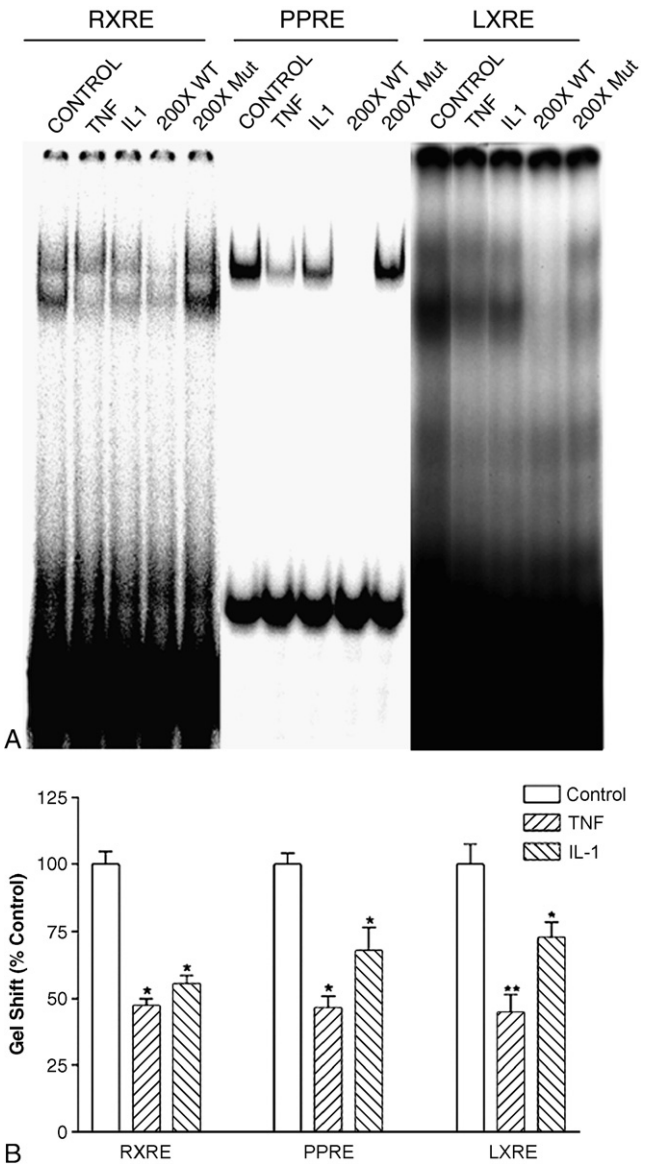

Fig. 6. Cytokines decrease the binding of nuclear extracts to RXRα, PPARα, and LXRα. Hep3B cells were plated in 100-mm Petri dishes in culture medium containing 10% serum. After an overnight incubation, cells were treated with cytokines at 10 ng/mL. After 24 hours, nuclear extracts were isolated and 10 μg of the nuclear proteins was used for electrophoretic mobility shift assay with oligonucleotides for RXRα-, PPARα-, and LXRα-specific REs as described under "Materials and methods." A, Representative electrophoretic mobility shift assays for nuclear receptors studied. B, Quantification of electrophoretic mobility shift assays from individual experiments. Data (mean ± SE, n = 5) are expressed as a percentage of controls. $*P < .05$, $**P < .01$ vs control.

3.3. Cytokines decrease the binding of nuclear extracts to RXRα, PPARα, and LXR REs

To determine if the decrease in RXRα, LXRα, and PPARα and -γ induced by TNF and IL-1 treatment results in decreased binding of nuclear extracts to the respective REs, we next carried out electrophoretic mobility shift assays. The REs used in these studies were a direct repeat spaced by one oligonucleotide (direct repeat [DR] 1) from the retinol-binding protein type II for the RXR:RXR homodimers, a DR-1 PPRE from the acyl-CoA oxidase promoter for the PPAR:RXR heterodimers, and a DR-4 from the cholesterol 7α-hydroxylase promoter for the LXR:RXR heterodimer. As shown in Fig. 6, treatment of Hep3B cells with either TNF or IL-1 significantly reduced the binding of nuclear extracts to the RXRE, PPRE, and LXRE. These data demonstrate that decreases in the levels of RXRα, LXRα, PPARα, and PPARγ resulted in reduced binding activity to known cognate REs for their target genes.

3.4. Cytokines decrease the expression of PPARα- and LXRα-regulated transcripts

To determine the effect of cytokine treatment on the expression of genes regulated by PPARα, the mRNA level of CPT1α was assayed in Hep3B cells after cytokine treatment. TNF and IL-1 significantly decreased mRNA levels of CPT1α (Fig. 7A), a well-known PPARα target gene, whereas 18S mRNA levels are not affected by cytokine treatment (data not shown). In addition, TNF and

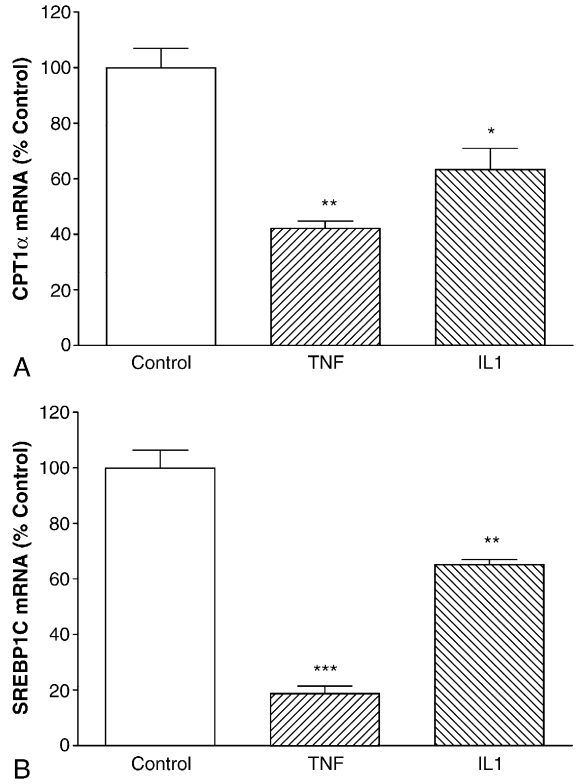

Fig. 7. Cytokines decrease the mRNA level of CPT1α and SREBP-1c. Hep3B cells were plated in 100-mm Petri dishes in culture medium containing 10% serum. After an overnight incubation, cells were treated with cytokines (10 ng/mL) as indicated. After 24 hours, total RNA was isolated and Northern blot analysis was performed using cDNA for CPT1α (A) as described under "Materials and methods." One microgram of total RNA was also reverse transcribed to cDNA. SREBP-1c mRNA expression (B) was measured by real-time QPCR as described under "Materials and methods." QPCR data were normalized using 36B4 mRNA as the invariant control for all experiments. Data (mean ± SE, n = 3) are expressed as a percentage of controls. $*P < .05$, $**P < .01$, $***P < .001$ vs control.

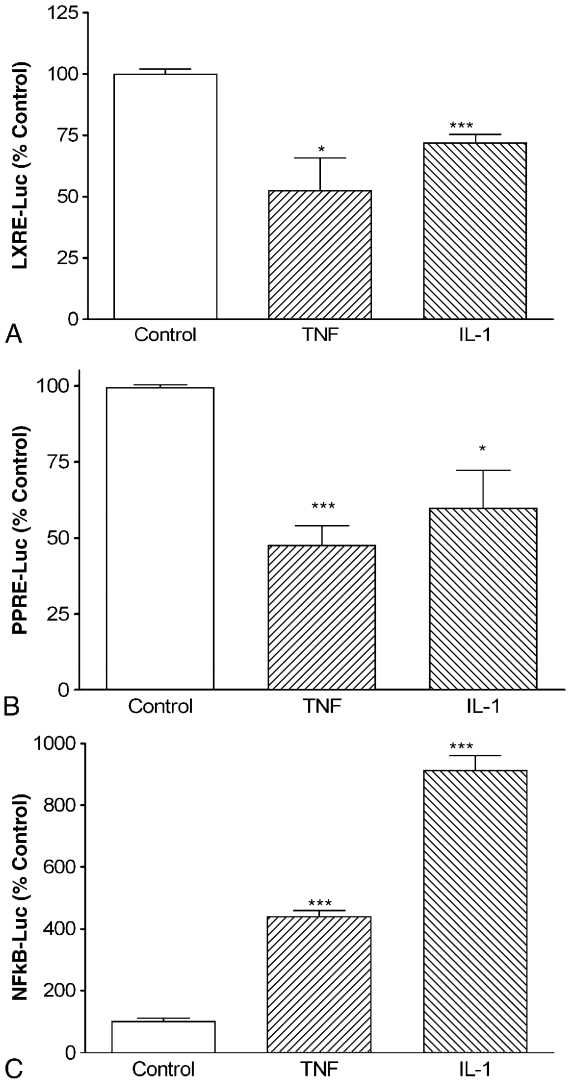

Fig. 8. Cytokines regulate LXRα-, PPARα-, and NFκB-regulated transcription. Hep3B cells were plated in 6-well plates. Transfections were performed as described under "Materials and methods." The next day, cells were treated with cytokines (10 ng/mL) as indicated. At the end of the incubation, cells were harvested in 0.5 mL passive lysis buffer and luciferase activity was determined (Promega). Data (mean ± SE, n = 3) are expressed as a percentage of controls. $*P < .05$, $***P < .001$ vs control.

IL-1 both decrease the mRNA levels of SREBP-1c, an LXR-regulated gene (Fig. 7B). Similarly, LPS administration to intact mice also decreased CPT1α and SREBP-1c mRNA levels (data not shown). In contrast, mRNA levels of SREBP-1a and SREBP-2 were not altered by cytokine or LPS treatment (data not shown).

We next carried out transient transfection studies by using an LXRE or PPRE construct linked to luciferase. As shown in Fig. 8A and B, both IL-1 and TNF significantly decrease the expression of luciferase driven by an LXRE or PPRE promoter construct transfected into Hep3B cells. We also determined the effect of cytokines on the ability of a PPRE promoter construct to induce expression in cells treated with an exogenous activator of PPARα (WY14,643). Surprisingly, WY14,643 had only modest effects on luciferase activity driven by a PPRE (mean increase in luciferase activity in 5 separate experiments was 25%). These results suggest that Hep3B cells in our culture system have significant levels of endogenous ligands that activate the expression of PPRE and, therefore, the addition of exogenous ligands has only minimal effects. Nevertheless, TNF and IL-1 inhibited the expression of luciferase driven by the PPRE-containing promoters even in the presence of the exogenous ligand, WY14,643 (data not shown). Similarly, we determined the ability of cytokines to inhibit the expression of the LXRE in cells treated with the LXR activator, 22R-OH cholesterol. The effect of 22R-OH cholesterol on the expression of the LXRE varied; in some experiments, luciferase activity was doubled, whereas in other experiments there were only minimal changes. Regardless of the level of expression, both TNF and IL-1 decreased the expression of luciferase driven by an LXRE (data not shown).

TNF and IL-1 are key regulators of inflammatory responses and stimulate the expression of genes that contain NFκB or activator protein 1 binding sites in their promoters. As shown in Fig. 8C, TNF and IL-1 increase the transcriptional activity mediated by NFκB by about 5- and 10-fold, respectively. These results indicate that the inhibition by TNF and IL-1 of the expression of LXRE- and PPRE-regulated genes in Hep3B cells is specific and not due to a generalized repression of transcription.

3.5. LPS and cytokines decrease levels of coactivators PGC-1α, PGC-1β, and SRC-1

Because we found that protein and mRNA levels of nuclear hormone receptors are significantly decreased during the APR, we next determined whether LPS administered to intact animals or cytokine treatment of Hep3B cells also has an effect on the expression of coactivators and corepressors. The expression of PGC-1α was markedly decreased by LPS and by TNF and IL-1 during the APR in mouse liver and Hep3B cells (Figs. 5 and 9A, D; Table 2). Similarly, PGC-1β mRNA levels were also markedly reduced in liver by LPS treatment (Fig. 9B). However, in Hep3B cells, TNF greatly decreased PGC-1β mRNA levels, whereas IL-1 had no effect (Fig. 9E). Finally, mRNA levels of SRC-1, one of the p160 family coactivators, were decreased during the APR (Fig. 9C and F). In contrast we found that LPS, TNF, or IL-1 do not affect mRNA levels of corepressors silencing mediator of retinoid and thyroid hormone receptor (SMRT) or nuclear hormone corepressor (NCoR) or coactivators Trap220, SRC-2, and SRC-3, and cAMP response element–binding protein (CBP) in either the liver with LPS treatment or Hep3B cells with TNF or IL-1 treatment (data not shown). These results indicate that the mRNA levels of the coactivators PGC-1α, PGC-1β, and SRC-1 are specifically reduced during the APR, which could contribute to the alteration in gene expression and lipid metabolism during the APR.

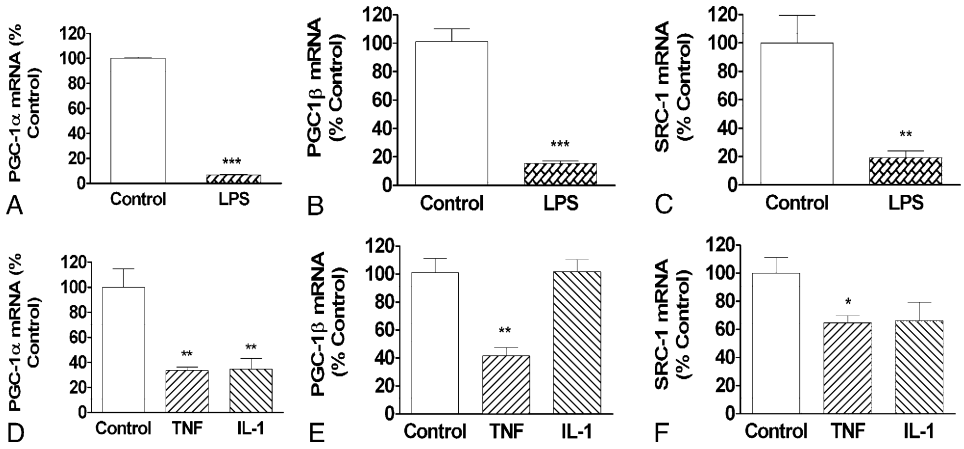

Fig. 9. Cytokines decrease the expression of PGC-1α, PGC-1β, and SRC-1 in vivo and in vitro. C57BL/6 mice were injected intraperitoneally with either saline (Control) or LPS (100 μg of LPS per mouse) and the animals were killed at 16 hours after LPS administration. Total RNA was prepared from liver, and expression levels for PGC-1α (A), PGC-1β (B), and SRC-1 (C) were determined. In addition, Hep3B cells were treated with cytokines (10 ng/mL). After 24 hours, total RNA was isolated and expression levels for PGC-1α (D), PGC-1β (E), and SRC-1 (F) were determined. Expression levels of PGC-1α and SRC-1 were measured by Northern blot, whereas expression levels of PGC-1β were measured by real-time QPCR as described in "Materials and methods." QPCR data were normalized using 36B4 mRNA as the invariant control for all experiments. Data (mean ± SE; n = 4 to 5 [in vivo]; n=3 [in vitro]) are expressed as a percentage of controls. *P < .05, **P < .01, ***P < .001 vs control.

We next determined the effect of cytokines on PGC-1α protein levels. As shown in Fig. 10, both TNF and IL-1 decrease the protein levels of PGC-1α. Unfortunately, no antibodies against PGC-1β are commercially available, so we were unable to determine if the protein levels of this coactivator also decrease. With regard to SRC-1, the presence of nonspecific bands made analysis problematic.

3.6. Cytokines decrease the expression of LXRα and PGC-1α

We next determined if the reduction in mRNA levels of LXRα and PGC-1α that is induced by cytokines occurs via changes in transcription of these genes. As shown in Fig. 11A, transcription of an LXRα promoter-luciferase construct is decreased by both TNF and IL-1 treatment. Similarly, the transcription of a PGC-1α promoter-luciferase

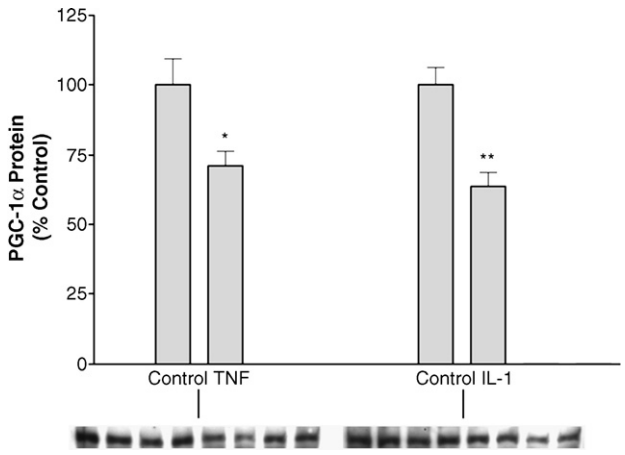

Fig. 10. TNF and IL-1 decrease PGC-1α protein levels. Hep3B cells were plated in 100-mm Petri dishes in culture medium containing 10% serum. After an overnight incubation, cells were treated with TNF (10 ng/mL) or IL-1 (10 ng/mL) and nuclear extracts were isolated. Western blot analysis was performed as described under "Materials and methods." Data (mean ± SE, n = 5) are expressed as a percentage of controls. *P < .05, **P < .01 vs control.

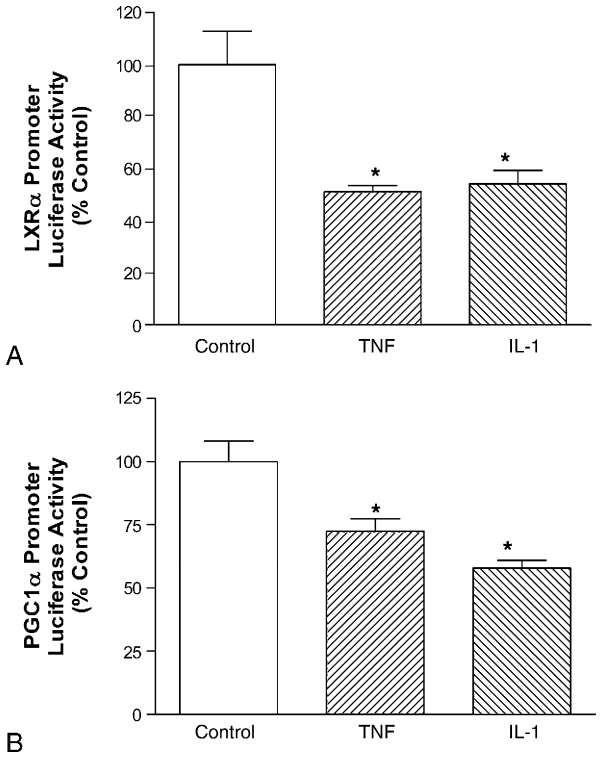

Fig. 11. Cytokines decrease LXRα and PGC-1α promoter activity. Hep3B cells were plated in 6-well plates. Transfections were performed as described under "Materials and methods." The next day, cells were treated with cytokines (10 ng/mL) as indicated. At the end of the incubation, cells were harvested in 0.5 mL passive lysis buffer and luciferase activity was determined (Promega). Data (means ± SE, n = 3) are expressed as a percentage of controls. *P < .05 vs control.

construct is also decreased by TNF and IL-1 (Fig. 11B). These data indicate that decreases in transcription contribute to the reduction in mRNA levels observed with cytokine treatment.

4. Discussion

Infection, inflammation, and injury induce the APR, which results in a wide range of metabolic changes that are mediated by altered gene expression, caused primarily by the production of inflammatory cytokines TNFα, IL-1β, and IL-6 [4,7]. The mechanism of induction of the positive acute phase proteins has been studied in detail [9,10,40]. Activation of the TNF/IL-1 receptors leads to activation of transcription factors such as NFκB and activator protein 1 inducing class I positive acute phase proteins, whereas activation of the IL-6 receptor activates Janus kinase tyrosine kinase, which subsequently phosphorylates tyrosine residues of signal transducer and activator of transcription proteins inducing the transcription of class II positive acute phase proteins. However, the underlying mechanisms of the negative acute phase proteins are less well understood. Data indicate that decreases in expression of genes involved in the metabolism of fatty acids, cholesterol, and bile acids are responsible for many of the changes in lipid metabolism that occur during the APR [4].

The characteristic changes in lipid metabolism that occur during the APR include hypertriglyceridemia, decreased hepatic fatty acid oxidation and ketogenesis, inhibition of bile acid synthesis, and decreases in serum high-density lipoprotein levels. We previously showed that genes such as fatty acid transport protein, CD36/FAT, liver fatty acid binding protein, acyl CoA oxidase, and CPT1α that are involved in triglyceride and fatty acid metabolism are down-regulated in the liver during the APR [4]. Because these genes have been identified as target genes of the nuclear hormone receptor PPARα, it is reasonable to speculate the involvement of PPARα/RXR in the altered lipid metabolism during the APR. Similarly, several of the genes involved in reverse cholesterol transport, bile acid synthesis, and hepatic cholesterol secretion, including CETP, cholesterol 7α hydroxylase, ABCG5, and ABCG8, also decrease during the APR [4]. These genes are well-recognized target genes of LXR and, therefore, it is reasonable to speculate the involvement of LXR/RXR in the alterations of lipid metabolism that occur during the APR.

In support of this hypothesis, previous studies have shown that the levels and DNA-binding activity of PPARα, LXRα, and their obligate heterodimer partners, RXRα, -β, and -γ, are significantly decreased during the LPS-induced APR in the livers of Syrian hamsters [25]. This decrease was accompanied by the reduced expression of their target genes such as acyl Co-A synthase in the case of PPARα and cholesterol 7α hydroxylase for LXR. In the present manuscript, we extend these findings and demonstrate that TNF and IL-1, but not IL-6, decrease the levels of RXRα, PPARα, PPARγ, and LXRα in Hep3B cells. The decrease in RXRα was a very sensitive response with a half-maximal effect seen at approximately 0.2 ng/mL for both TNF and IL-1. Moreover, the response occurred very rapidly, as decreases in RXRα mRNA levels were seen as early as 2 hours after cytokine treatment. These results indicate that proinflammatory cytokines can directly induce changes in the levels of RXRα, PPARα, PPARγ, and LXRα in hepatic tissue. In the case of LXRα, we were able to further demonstrate that decreases in transcription at least partially account for the decrease in mRNA levels. Whether RXRα, PPARα, and PPARγ are also regulated at the level of gene transcription remains to be determined. Of note is that IL-6 is the proinflammatory cytokine primarily responsible for stimulating increases in many of the positive acute phase proteins [9]. The results of the present study suggest that TNF and IL-1, rather than IL-6, are the key cytokines for regulating the expression of many of the negative acute phase proteins involved in lipid metabolism. Of course, although the present study demonstrates the ability of TNF and IL-1 to directly decrease the levels of RXRα, PPARα, PPARγ, and LXRα in Hep3B cells, these results do not rule out the possibility that other cytokines or signaling molecules contribute to the decreases observed in vivo after the induction of the APR.

LPS in vivo and TNF and IL-1 in cultured hepatocytes differentially regulated PPAR isoforms. As noted above, PPARα and PPARγ levels decreased, whereas PPARβ/δ levels were unchanged or even increased. The molecular basis for this difference is not understood, but it could have physiologic ramifications. Activation of PPARα is well recognized to stimulate fatty acid oxidation in liver and other tissues [41]. Very recent studies have unexpectedly shown that in the liver, PPARβ/δ stimulates many of the key genes involved in fatty acid synthesis, including acetyl-CoA carboxylase β, fatty acid synthase, acyl-CoA thioesterase 1, and ATP citrate lyase [42]. These alterations in expression of PPAR isoforms are compatible with the changes in lipid metabolism observed during the APR, that is, a decrease in fatty acid oxidation and an increase in fatty acid synthesis [4].

In addition to demonstrating a decrease in RXRα, PPARα, PPARγ, and LXRα in Hep3B cells after TNF and IL-1 treatment, the present study also demonstrates that cytokines decreased the binding activity of Hep3B cell nuclear proteins to RXR, PPAR, and LXR REs. Moreover, the mRNA levels of a PPARα target gene, CPT1α, and an LXRα target gene, SREBP-1c, were reduced by TNF and IL-1. Finally, the expression of both a PPRE and an LXRE was decreased in cytokine-treated cells. Together, these results indicate that the decreases in RXR, PPAR, and LXR that occur during the APR are associated with changes in gene expression.

However, it is very likely that factors in addition to these nuclear hormone receptor transcription factors also play a

role in regulating gene expression during the APR. Transcriptional activity of nuclear hormone receptors involves many cofactors that interact with the transcriptional activation function domains. These cofactors may suppress or activate transcriptional activity [31,32]. In the present study, we demonstrate that several cofactors that activate transcription are decreased both in intact liver during the LPS-induced APR and in Hep3B cells treated with TNF or IL-1. Specifically, PGC-1α, PGC-1β, and SRC-1 were decreased. In the case of PGC-1α, we were able to demonstrate that the decrease was at least partially accounted for by a decrease in gene transcription. Whether PGC-1β and SRC-1 are also regulated at the level of transcription remains to be determined. In contrast, the corepressors NCoR and SMRT and the coactivators SRC-2, SRC-3, CBP, and thyroid receptor–associated protein (TRAP) 220 were not altered by cytokine treatment. Although we studied several cofactors, it should be recognized that the number of proteins that interact with nuclear hormone receptors can be quite large and the interactions are poorly understood. For example, Surapureddi et al [43] identified a complex of cofactors for PPARα named the PPARα-interacting cofactor (PRIC). PRIC was composed of 25 polypeptides that interact with PPARα, and 18 of these proteins contain one or more LXLL motifs. Those with the LXLL motifs include CBP, SRC-1, PPAR-binding protein, PPAR-interacting protein (PRIP), PRIP-interacting protein with methyltransferase domain, TRAP 100, suppressor of Ras-2 (SUR-2), PCG-1, and several previously unknown cofactors. The molecular mechanisms by which these cofactors interact to regulate transcription activity of nuclear hormone receptors are not understood. It has been proposed that CBP/E1A-associated factor (P300) anchors a large multiprotein complex that performs the acetylation-methylation function to enhance transactivation by nuclear receptors, whereas SRC-1 and -3 possess intrinsic acetyltransferase activity. Other cofactors may perform their function by contacting the basal transcriptional machinery. Regardless of the precise mechanisms involved, our results indicate that during the APR several of these proteins are down-regulated. Thus, it is likely that the decreased expression of PPAR and LXR target genes is not only due to decreases in PPAR, LXR, and RXR, but may also be due to changes in cofactors that also regulate transcription.

In summary, the present study demonstrates that in liver cells, TNF and IL-1 cause a marked decrease in RXRα, PPARα, PPARγ, LXRα, PGC-1α, PGC-1β, and SRC-1. The cytokine-induced decreases were associated with decreased DNA-binding activity and decreased expression of PPARα- and LXR-regulated genes. This study suggests that the decreased expression of nuclear hormone receptors PPARα, PPARγ, LXRα, and RXRα and their coactivators PGC-1α, PGC-1β, and SRC-1 may be, at least in part, responsible for some of the changes in lipid metabolism that occur during the APR.

Acknowledgment

This work was supported by grants from the Research Service of the Department of Veterans Affairs and by grant R01 HD29706 from the National Institutes of Health.

References

[1] Kushner I. The phenomenon of the acute phase response. Ann N Y Acad Sci 1982;389:39-48.
[2] Baumann H, Gauldie J. The acute phase response. Immunol Today 1994;15:74-80 [see comments].
[3] Moshage H. Cytokines and the hepatic acute phase response. J Pathol 1997;181:257-66.
[4] Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, et al. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. J Lipid Res 2004;45:1169-96.
[5] Sammalkorpi K, Valtonen V, Kerttula Y, Nikkila E, Taskinen MR. Changes in serum lipoprotein pattern induced by acute infections. Metabolism 1988;37:859-65.
[6] Memon RA, Feingold KR, Moser AH, Doerrler W, Adi S, Dinarello CA, et al. Differential effects of interleukin-1 and tumor necrosis factor on ketogenesis. Am J Physiol 1992;263:E301-9.
[7] Hardardottir I, Grunfeld C, Feingold KR. Effects of endotoxin on lipid metabolism. Biochem Soc Trans 1995;23:1013-8.
[8] Memon RA, Feingold KR, Moser AH, Doerrler W, Grunfeld C. In vivo effects of interferon-alpha and interferon-gamma on lipolysis and ketogenesis. Endocrinology 1992;131:1695-702.
[9] Kishimoto T, Taga T, Akira S. Cytokine signal transduction. Cell 1994;76:253-62.
[10] Zhong Z, Wen Z, Darnell Jr JE. Stat3 and Stat4: members of the family of signal transducers and activators of transcription. Proc Natl Acad Sci U S A 1994;91:4806-10.
[11] Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, et al. The nuclear receptor superfamily: the second decade. Cell 1995;83:835-9.
[12] Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. Nuclear receptors and lipid physiology: opening the X-files. Science 2001;294:1866-70.
[13] Blumberg B, Evans RM. Orphan nuclear receptors—new ligands and new possibilities. Genes Dev 1998;12:3149-55.
[14] Kliewer SA, Lehmann JM, Willson TM. Orphan nuclear receptors: shifting endocrinology into reverse. Science 1999;284:757-60.
[15] Martin G, Schoonjans K, Lefebvre AM, Staels B, Auwerx J. Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. J Biol Chem 1997;272:28210-7.
[16] Yu GS, Lu YC, Gulick T. Co-regulation of tissue-specific alternative human carnitine palmitoyltransferase Ibeta gene promoters by fatty acid enzyme substrate. J Biol Chem 1998;273:32901-9.
[17] Mascaro C, Acosta E, Ortiz JA, Marrero PF, Hegardt FG, Haro D. Control of human muscle–type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator–activated receptor. J Biol Chem 1998;273:8560-3.
[18] Brandt JM, Djouadi F, Kelly DP. Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator–activated receptor alpha. J Biol Chem 1998;273:23786-92.
[19] Issemann I, Prince R, Tugwood J, Green S. A role for fatty acids and liver fatty acid binding protein in peroxisome proliferation? Biochem Soc Trans 1992;20:824-7.
[20] Hanley K, Komuves LG, Ng DC, Schoonjans K, He SS, Lau P, et al. Farnesol stimulates differentiation in epidermal keratinocytes via PPARalpha. J Biol Chem 2000;275:11484-91.

[21] Gearing KL, Gottlicher M, Widmark E, Banner CD, Tollet P, Stromstedt M, et al. Fatty acid activation of the peroxisome proliferator activated receptor, a member of the nuclear receptor gene superfamily. J Nutr 1994;124:1284S-8S.
[22] Steffensen KR, Gustafsson JA. Putative metabolic effects of the liver X receptor (LXR). Diabetes 2004;53(Suppl 1):S36-S42.
[23] Memon RA, Bass NM, Moser AH, Fuller J, Appel R, Grunfeld C, et al. Down-regulation of liver and heart specific fatty acid binding proteins by endotoxin and cytokines in vivo. Biochim Biophys Acta 1999;1440:118-26.
[24] Memon RA, Fuller J, Moser AH, Smith PJ, Feingold KR, Grunfeld C. In vivo regulation of acyl-CoA synthetase mRNA and activity by endotoxin and cytokines. Am J Physiol 1998;275:E64-E72.
[25] Beigneux AP, Moser AH, Shigenaga JK, Grunfeld C, Feingold KR. The acute phase response is associated with retinoid X receptor repression in rodent liver. J Biol Chem 2000;275:16390-9.
[26] Bagby GJ, Spitzer JA. Lipoprotein lipase activity in rat heart and adipose tissue during endotoxic shock. Am J Physiol 1980; 238:H325-30.
[27] Gouni I, Oka K, Etienne J, Chan L. Endotoxin-induced hypertriglyceridemia is mediated by suppression of lipoprotein lipase at a post-transcriptional level. J Lipid Res 1993;34:139-46.
[28] Memon RA, Feingold KR, Moser AH, Fuller J, Grunfeld C. Regulation of fatty acid transport protein and fatty acid translocase mRNA levels by endotoxin and cytokines. Am J Physiol 1998;274:E210-7.
[29] Ghose R, Zimmerman TL, Thevananther S, Karpen SJ. Endotoxin leads to rapid subcellular re-localization of hepatic RXRalpha: A novel mechanism for reduced hepatic gene expression in inflammation. Nucl Recept 2004;2:4.
[30] Zimmerman TL, Thevananther S, Ghose R, Burns AR, Karpen SJ. Nuclear export of retinoid X receptor alpha in response to interleukin-1beta–mediated cell signaling: roles for JNK and SER260. J Biol Chem 2006;281:15434-40.
[31] McKenna NJ, O'Malley BW. Minireview: nuclear receptor coactivators—an update. Endocrinology 2002;143:2461-5.
[32] Rosenfeld MG, Glass CK. Coregulator codes of transcriptional regulation by nuclear receptors. J Biol Chem 2001;276:36865-8.
[33] Chawla S, Hardingham GE, Quinn DR, Bading H. CBP: a signal-regulated transcriptional coactivator controlled by nuclear calcium and CaM kinase IV. Science 1998;281:1505-9.
[34] Ait-Si-Ali S, Ramirez S, Barre FX, Dkhissi F, Magnaghi-Jaulin L, Girault JA, et al. Histone acetyltransferase activity of CBP is controlled by cycle-dependent kinases and oncoprotein E1A. Nature 1998;396:184-6.
[35] Weiss RE, Xu J, Ning G, Pohlenz J, O'Malley BW, Refetoff S. Mice deficient in the steroid receptor co-activator 1 (SRC-1) are resistant to thyroid hormone. EMBO J 1999;18:1900-4.
[36] Xu J, Qiu Y, DeMayo FJ, Tsai SY, Tsai MJ, O'Malley BW. Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. Science 1998;279:1922-5.
[37] Ito M, Yuan CX, Okano HJ, Darnell RB, Roeder RG. Involvement of the TRAP220 component of the TRAP/SMCC coactivator complex in embryonic development and thyroid hormone action. Mol Cell 2000;5:683-93.
[38] Wang Z, Rose DW, Hermanson O, Liu F, Herman T, Wu W, et al. Regulation of somatic growth by the p160 coactivator p/CIP. Proc Natl Acad Sci U S A 2000;97:13549-54.
[39] Neish AS, Khachigian LM, Park A, Baichwal VR, Collins T. Sp1 is a component of the cytokine-inducible enhancer in the promoter of vascular cell adhesion molecule-1. J Biol Chem 1995;270: 28903-9.
[40] Kolesnick R, Golde DW. The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. Cell 1994;77:325-8.
[41] Lee CH, Olson P, Evans RM. Minireview: lipid metabolism, metabolic diseases, and peroxisome proliferator–activated receptors. Endocrinology 2003;144:2201-7.
[42] Lee CH, Olson P, Hevener A, Mehl I, Chong LW, Olefsky JM, et al. PPAR{delta} regulates glucose metabolism and insulin sensitivity. Proc Natl Acad Sci U S A 2006;3444-9.
[43] Surapureddi S, Yu S, Bu H, Hashimoto T, Yeldandi AV, Kashireddy P, et al. Identification of a transcriptionally active peroxisome proliferator–activated receptor alpha–interacting cofactor complex in rat liver and characterization of PRIC285 as a coactivator. Proc Natl Acad Sci U S A 2002;99:11836-41.