Type II nuclear hormone receptors, coactivator, and target gene repression in adipose tissue in the acute-phase response

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Abstract The acute-phase response (APR) leads to alterations in lipid metabolism and type II nuclear hormone receptors, which regulate lipid metabolism, are suppressed, in liver, heart, and kidney. Here, we examine the effect of the APR in adipose tissue. In mice, lipopolysaccharide produces a rapid, marked decrease in mRNA levels of nuclear hormone receptors [peroxisome proliferator-activated receptor γ (PPARγ), liver X receptor α (LXRα) and LXRβ, thyroid receptor α (TRα) and TRβ, and retinoid X receptor α (RXRα) and RXRβ] and receptor coactivators [cAMP response element binding protein, steroid receptor coactivator 1 (SRC1) and SRC2, thyroid hormone receptor-associated protein, and peroxisome proliferator-activated receptor γ co-activator 1α (PGC1α) and PGC1β] along with decreased expression of target genes (adipocyte P2, phosphoenolpyruvate carboxykinase, glycerol-3-phosphate acyltransferase, ABCA1, apolipoprotein E, sterol-regulatory element binding protein-1c, glucose transport protein 4 (GLUT4), malic enzyme, and Spot14) involved in triglyceride (TG) and carbohydrate metabolism. We show that key TG synthetic enzymes, 1-acyl-*sn*-glycerol-3-phosphate acyltransferase-2, monoacylglycerol acyltransferase 1, and diacylglycerol acyltransferase 1, are PPARγ-regulated genes and that they also decrease in the APR. In 3T3-L1 adipocytes, tumor necrosis factor-α (TNF-α) significantly decreases PPARγ, LXRα and LXRβ, RXRα and RXRβ, SRC1 and SRC2, and PGC1α and PGC1β mRNA levels, which are associated with a marked reduction in receptor-regulated genes. Moreover, TNF-α significantly reduces PPAR and LXR response element-driven transcription. Thus, the APR suppresses the expression of many nuclear hormone receptors and their coactivators in adipose tissue, which could be a mechanism to coordinately downregulate TG biosynthesis and thereby redirect lipids to other critical organs during the APR.—Lu, B., A. H. Moser, J. K. Shigenaga, K. R. Feingold, and C. Grunfeld. **Type II nuclear hormone receptors, coactivator, and target gene repression in adipose tissue in the acute-phase response. *J. Lipid Res.* 2006.** 47: **2179–2190.**

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The acute-phase response (APR) refers to an array of biochemical and metabolic changes that are induced by injurious stimuli, including infection and inflammation, trauma, burns, ischemic necrosis, and malignant tumors (1, 2). More recently, it has become widely appreciated that atherosclerosis, obesity, the metabolic syndrome, and diabetes are inflammatory disorders that also induce the APR (3–6). An early and consistent metabolic alteration during the APR is increased plasma FFA and triglyceride (TG) levels, characterized by an increase in VLDL (1, 3). These metabolic changes are thought to be beneficial to the host in the short term in fighting against invading pathogens and in repairing inflamed tissues after injury (1, 7). However, if prolonged, these changes in lipid and lipoprotein metabolism might contribute to dyslipidemia and atherogenesis (3, 8, 9).

In addition to increases in FFA and TG levels, the APR is also accompanied by decreased fatty acid oxidation in tissues and decreased HDL levels (3, 10, 11). Dysregulation of TG lipolysis/synthesis in adipose tissue plays a key role in mediating many of the changes in lipid and lipoprotein metabolism during the APR. For example, an increase in adipose tissue lipolysis leads to increased circulating FFA levels, which provide a source of fatty acids for the increase in hepatic TG synthesis during the APR (3). The increased lipolysis in adipose tissue is thought to be secondary to the

Abbreviations: AGPAT, 1-acyl-*sn*-glycerol-3-phosphate acyltransferase; aP2, adipocyte P2; apoE, apolipoprotein E; APR, acute-phase response; DGAT, diacylglycerol acyltransferase; GLUT4, glucose transport protein 4; GPAT, glycerol-3-phosphate acyltransferase; 11β-HSD, 11β-hydroxysteroid dehydrogenase; IL, interleukin; LPS, lipopolysaccharide; LXR, liver X receptor; LXRE, liver X receptor response element; ME, malic enzyme; MGAT, monoacylglycerol acyltransferase; PEPCK, phosphoenolpyruvate carboxykinase; PGC, peroxisome proliferator-activated receptor γ co-activator 1; PPAR, peroxisome proliferator-activated receptor; PPARE, peroxisome proliferator-activated receptor response element; RXR, retinoid X receptor; SRC, steroid receptor coactivator; SREBP, sterol-regulatory element binding protein; TG, triglyceride; TLR, Toll-like receptor; TNF, tumor necrosis factor; TR, thyroid receptor.

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increased phosphorylation of hormone-sensitive lipase and/or its associated protein, perilipin (12–15). Additionally, high-dose lipopolysaccharide (LPS) treatment decreases adipose tissue lipoprotein lipase activity, which could result in a decrease in the metabolism of TG-rich lipoproteins and the uptake of FFAs, thereby contributing to the increase in plasma TG levels (2). Lastly, many of the enzymes and transporters required for the storage of fatty acids in adipose tissue are reduced during the APR (15, 16). As noted above, adipose tissue lipoprotein lipase is reduced, which decreases the delivery of fatty acids from circulating lipoproteins to adipose tissue. Moreover, during the APR, the expression of fatty acid transport protein and CD36/fatty acid translocase is decreased in adipose tissue, which would reduce the transport of fatty acids into cells (15). Finally, acyl-CoA synthase expression is decreased in adipocytes during the APR (16). Acyl-CoA synthase catalyzes the activation of long-chain fatty acids to acyl-CoA esters, which is an essential step in the metabolism of fatty acids by cells, preventing the efflux of fatty acids from cells, rendering fatty acid transport unidirectional. A decrease in the proteins required for fatty acid uptake in adipose tissue during the APR coupled with an increase in lipolysis would lead to a redistribution of fatty acids away from storage in adipose tissue to increased availability of FFAs for liver to promote TG synthesis and VLDL production.

Although the lipolysis is regulated mainly via a posttranslational mechanism (7, 12, 13), most of the changes in lipid metabolism during the APR are attributed to changes in gene transcription (8, 17). Recent studies by our and other laboratories have shown that many of the changes in lipid metabolism in the liver, heart, and kidney during the APR may be mediated by changes in the levels of type II nuclear hormone receptors that are involved in sensing the lipid milieu of cells and regulating lipid metabolism (liposensors) and the thyroid hormone receptor (18), which regulate fatty acid and cholesterol metabolism (19). Specifically, we have shown in the liver, heart, and kidney that the expression of retinoid X receptor (RXR), liver X receptor (LXR), farnesoid X receptor, thyroid receptor (TR), and peroxisome proliferator-activated receptors (PPARs) is decreased during the APR (20–22). Therefore, we have hypothesized that the expression of these nuclear hormone receptors would also decrease in adipose tissue during the APR. Previous studies have shown that LPS and/or tumor necrosis factor-α (TNF-α) treatment decreases PPARγ expression and function in adipose tissue as well as 3T3-L1 adipocytes in culture (23–25). In this study, we demonstrate that LPS and zymosan, two different inducers of the APR, suppress the expression of several key type II nuclear hormone receptors and their coactivators, which play key roles in regulating lipid metabolism in adipose tissue. Moreover, we demonstrate that changes in the levels of these nuclear hormone receptors are associated with changes in the expression of an array of genes known to be regulated by these transcription factors. Furthermore, we show that the TG-synthesizing enzymes 1-acyl-*sn*-glycerol-3-phosphate acyltransferase 2 (AGPAT2), monoacylglycerol acyltransferase 1 (MGAT1), and diacylglycerol acyltransferase 1 (DGAT1) are regulated by PPARγ in a manner similar to glycerol-3-phosphate acyltransferase (GPAT) and that all are suppressed in the APR.

MATERIALS AND METHODS

Materials

LPS (*Escherichia coli* 55:B5) was obtained from Difco Laboratories and diluted in pyrogen-free 0.9% saline. Zymosan A and TRI Reagent were purchased from Sigma (St. Louis, MO). Recombinant mouse TNF-α and interleukin-1β (IL-1β) were purchased form R&D Systems (Minneapolis, MN). [α-^{32}P]dCTP (3,000 Ci/mmol) was purchased from Perkin-Elmer Life Sciences (Boston, MA). The Multiprime DNA labeling system and minispin G-50 columns were obtained from Amersham Pharmacia Biotech. ULTRAhyb Hybridization Buffer was purchased from Ambion (Austin, TX). Fuji medical X-ray film, type Rx, was used for autoradiography.

Animals

Female C57BL/6 mice (8 weeks old) were obtained from Charles River Laboratories (Wilmington, MA). The animals were maintained in a normal-light-cycle room and were fed Purina mouse chow (Ralston Purina, St. Louis, MO) and water ad libitum. Animals were injected with saline, LPS (5 mg/kg body weight, intraperitoneal), or zymosan A (80 mg/kg body weight, intraperitoneal), and food was removed from both control and treated animals after injection. These doses of LPS and zymosan were previously shown to induce the APR in mice (26, 27). At 4 or 16 h after treatment, animals were administered halothane anesthesia and euthanized and fat was snap-frozen in liquid nitrogen, placed in storage tubes in a dry ice bath until the end of experiment, and then stored at −80°C until RNA extraction.

Cell culture and cell differentiation

Murine 3T3-L1 cells (American Type Culture Collection, Manassas, VA) were grown to confluence and differentiated to adipocytes as described (28). Briefly, preadipocytes were cultured in Dulbecco's modified Eagle's medium and 10% fetal bovine serum. When cells became confluent, they were differentiated by treatment with 1.0 μg/ml insulin, 0.5 mM methylisobutylxanthine, and 1 μM dexamethasone in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum for 2 days. Cells were then maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. All experiments were performed after differentiation on day 9 or 10.

Isolation of RNA and Northern blot analysis

Total RNA was isolated from 100–150 mg of snap-frozen white adipose tissue from the periuterine-urinary bladder by the TRI Reagent method from Sigma. Total RNA was then quantified by measuring absorption at 260 nm. Thirty micrograms was denatured and electrophoresed on a 1% agarose/formaldehyde gel. The uniformity of sample loading was checked by visualization of the ethidium bromide staining of the 28S and 18S ribosomal bands before electrotransfer to Nytran membranes. ULTRAhyb Hybridization Buffer was used with [α-^{32}P]dCTP-labeled cDNAs using Amersham's Rediprime II Random Prime Labeling System. mRNA levels were detected by exposure of the membrane to X-ray film or phosphor imaging screens and then quantified using the appropriate machine: densitometer or phos-

phor imager. Dr. Bruce M. Spiegelman kindly provided the adipocyte P2 (aP2) cDNA. Human (h)RXRα cDNA was a gift from Dr. Daniel D. Bikle (University of California, San Francisco). Mouse (m)RXRβ, hLXRα, and hLXRβ cDNAs were kindly provided by Dr. David J. Mangelsdorf (University of Texas Southwestern Medical Center, Dallas, TX). mPPARγ and mPPARδ cDNAs were a gift from Dr. Anthony Bass (University of California, San Francisco). Phosphoenolpyruvate carboxykinase (PEPCK) was provided by Steven Lear (University of California, San Francisco). Apolipoprotein E (apoE) cDNA was kindly provided by Dr. Robert Raffai (University of California, San Francisco). The following mouse probes were generated by RT-PCR, starting from mouse liver [11β-hydroxysteroid dehydrogenase (11β-HSD), ABCA1, malic enzyme (ME), Spot14, and TRβ] and heart (TRα and GLUT4) and using the following primers: 11β-HSD, 5′-CTG AGC CAG GTC CCT GTT TGA T-3′ (upper), 5′-GTG ATG GAC ACG TTG ACC TTG G-3′ (lower); ABCA1, 5′-GCT GCT GTG GAA GAA CCT CAC-3′ (upper), 5′-GAA CAC AGC CAG TTC CTG GAA-3′ (lower); ME, 5′-CCA CCA GCG CGG CTA CCT GCT GAC GCG GGA-3′ (upper), 5′-CCT CTG ACT CGC CGG TGC CGC AGC CCG ATG-3′ (lower); Spot14, 5′-ATG CAA GTG CTA ACG AAA CGC-3′ (upper), 5′-AGA AGT GCA GGT GGA ACT GGG C-3′ (lower); TRβ, 5′-GCC TGG GAC AAG CAG AAG CCC CGT-3′ (upper), 5′-AGC GAC ATT CCT GGC ACT GGT TGC G-3′ (lower); TRα, 5′-ATG GAA CAG AAG CCA AGC AAG GTG GAG-3′ (upper), 5′-CTG CAG CAG AGC CAC TTC CGT GTC A-3′ (lower); GLUT4, 5′-CAA CGT GGC TGG GTA GGC A-3′ (upper), 5′-ACA CAT CAG CCC AGC CGG T-3′ (lower).

PCR primers and quantitative real-time PCR and semiquantitative PCR conditions

One microgram of total RNA was reverse-transcribed with 20 ng of random hexamer (RT-for-PCR kit; Clontech, San Diego, CA) at 42°C for 1 h. All real-time primers were designed using the online source Primer3 (http://frodo.wi.mit.edu/): GPAT, 5′-CAA CAC CAT CCC CGA CAT C-3′ (upper), 5′-GTG ACC TTC GAT TAT GCG ATC A-3′ (lower); AGPAT2, 5′-AGC GGA CAG AAG AAA CTG GA-3′ (upper), 5′-TGA AGT AGA CAC CCC CAA GG-3′ (lower); MGAT1, 5′-CTG GTT CTG TTT CCC GTT GT-3′ (upper), 5′-TGG GTC AAG GCC ATC TTA AC-3′ (lower); DGAT1, 5′-GGC CCA AGG TAG AAG AGG AC-3′ (upper), 5′-GAT CAG CAT CAC CAC ACA CC-3′ (lower); DGAT-2, 5′-AGT GGC AAT GCT ATC ATC ACG T-3′ (upper), 5′-AAG GAA TAA GTG GGA ACC AGA TCA-3′ (lower); cAMP response element binding protein, 5′-TGG AGT GAA CCC CCA GTT AG-3′ (upper), 5′-TTG CTT GCT CTC GTC TCT GA-3′ (lower); SRC1, 5′-TGG GTA CCA GTC ACC AGA CA-3′ (upper), 5′-GAA TGT TTG CGT TTC CAC CT-3′ (lower); SRC2, 5′-ACA GAA CCA GCC AAA CCA AC-3′ (upper), 5′-TGG TTG AGG ATT TCC CTC TG-3′ (lower); thyroid hormone receptor-associated protein, 5′-CCT TCT TTC TCC GCA GTC AC-3′ (upper), 5′-GGA AGA GCA GCG TAA AAT CG-3′ (lower); PGC1α, 5′-TAG GCC CAG GTA CGA CAG C-3′ (upper), 5′-GCT CTT TGC GGT ATT CAT CC-3′ (lower); PGC1β, 5′-CAA GCT CTG ACG CTC TGA AGG-3′ (upper), 5′-TTG GGG AGC AGG CTT TCA C-3′ (lower); internal control 36B4, 5′-GCG ACC TGG AAG TCC AAC TAC-3′ (upper), 5′-ATC TGC TGC ATC TGC TTG G-3′ (lower).

Real-time quantitative PCR was performed with the MX300P™ real-time PCR kit (Stratagene, La Jolla, CA) using 2× SYBR Green Master Mix (Stratagene) and 4% of the starting 1 μg of RNA per 20 μl reaction. Forty cycles were conducted, preceded by 10 min at 95°C. Products were electrophoresed on 1.5% agarose gels to confirm the specificity of reactions. Quantification was performed by the comparative threshold cycle method, with invariable 36B4 used for normalization.

Semiquantitative PCR was used for AGPAT1, -3, -4, and -5 amplifications. The validation of the quantitative measurement for each mAGPAT mRNA by RT-PCR was established previously (29). Briefly, under the RT-PCR conditions used, the levels of the PCR products were dependent on the amount of template used in the reaction. At least an 8- to 10-fold difference of cDNA concentration for each mAGPAT was detected.

Western blot analysis

Samples containing 80–100 μg of whole cell extracts from adipose tissues were subjected to SDS-PAGE (10% gel) with prestained protein markers as references as described previously (29). Briefly, protein fractions on the gel were transferred onto polyvinylidene difluoride membranes. The membranes were then incubated with primary antibody for PPARγ (1:500 dilution; Cayman Chemical) and RXRα (1:1,000; Santa Cruz Biotechnology) and then with peroxidase-labeled secondary anti-rabbit IgG antibody (1:5,000; Invitrogen). The protein bands were detected using the SuperSignal West Dura Extended Duration Substrate Kit (Pierce, Rockford, IL) and the Bio-Rad Multi-Imaging System. After nuclear hormone receptor detection, the membranes were stripped and reprobed with monoclonal antibody to GAPDH (Ambion) as an invariable internal control.

Transfection studies and luciferase assay

3T3-L1 cells were plated on six-well plates and differentiated to adipocytes as described above. Before transfection experiments, cells were preincubated in the basal medium without serum and antibiotics for 1 h and were then transfected with DNA-Effectene complex (Qiagen, Inc., Valencia, CA). The DNA-Effectene complex contained 2 μg/ml peroxisome proliferator-activated receptor response element TK-$(PPARE)_3$-luciferase vector (Panomics, Fremont, CA) and liver X receptor response element TK-$(LXRE)_3$-luciferase vector (22) (kindly provided by Dr. David J. Mangelsdorf). Cells were incubated with DNA-Effectene complex in the basal medium at 37°C for 6 h. After the DNA-Effectene complex was removed, the cells were washed once with basal medium and then incubated in the basal medium containing 0.2% BSA in the presence or absence of 10 ng/ml TNF-α. After 24 h of incubation, transfected cells were harvested with lysis buffer (Promega, Madison, WI), and aliquots of the lysates were assayed for luciferase using a Wallac VCTOR^{2}™ 1420 Multilabel Counter (Perkin-Elmer Life Sciences).

Statistical analysis

The results are presented as means ± SEM. Statistical significance between two groups was determined using Student's *t*-test. $P < 0.05$ was considered significant.

RESULTS

LPS and zymosan decrease type II nuclear hormone receptor mRNA levels in mouse adipose tissue

We first determined the effect of the administration of LPS, a Toll-like receptor-4 (TLR-4) agonist, on nuclear hormone receptor expression in adipose tissue. As shown in **Fig. 1A, B**, 16 h after LPS treatment there was a marked decrease in PPARγ mRNA levels (90% decrease) with no change in PPARβ/δ mRNA levels. We did not detect PPARα mRNA in adipose tissue. In addition to the marked reduction in PPARγ mRNA levels, both RXRα and RXRβ, obligate heterodimeric partners of PPARγ, were also greatly reduced (Fig. 1A, B). RXRγ mRNA levels were not detected

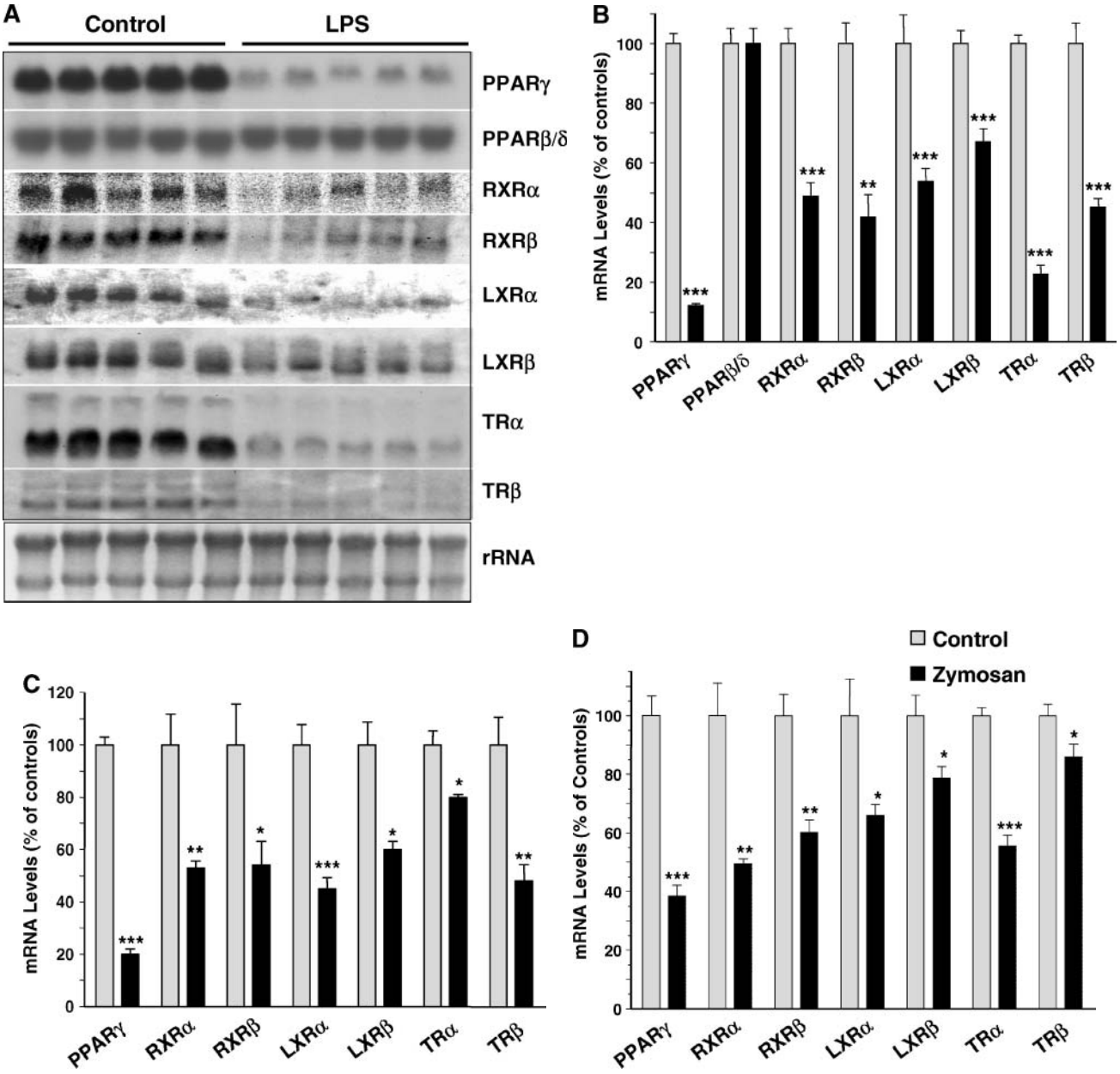

Fig. 1. Effect of lipopolysaccharide (LPS) on type II nuclear hormone receptors in mouse adipose. Mice were injected intraperitoneally with either saline or LPS (5 mg/kg body weight) or zymosan (80 mg/kg body weight), and the animals were euthanized at 16 h after LPS administration. Total RNA was isolated from white adipose tissue from the urinary bladder-uterine area, and Northern blot analysis was performed at the 16 h time point (A, B) or the 4 h time point (C) as described in Materials and Methods. Each gel was stained by ethidium bromide to verify equal loading of total RNA (18S and 18S rRNA in A). D shows nuclear hormone receptor expression in mouse adipose tissue at 16 h after zymosan treatment. LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; TR, thyroid receptor. Data are expressed as a percentage of controls (means ± SEM; n = 5). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus controls.

in adipose tissue. Finally, LXRα and LXRβ and TRα and TRβ were decreased at 16 h after LPS treatment. Farnesoid X receptor mRNA levels were not detected in adipose tissue. Thus, the APR reduces the expression of a subset of type II nuclear hormone receptors that play important roles in lipid metabolism in adipose tissue.

To determine whether this decrease is a rapid early effect, we next examined the levels of these nuclear hormone receptors at 4 h after LPS treatment. As shown in Fig. 1C, LPS treatment resulted in a rapid decrease in the mRNA levels of PPARγ, RXRα and RXRβ, LXRα and LXRβ, TRβ, and to a lesser extent TRα. PPARβ/δ mRNA levels showed no change at the 4 h time point.

To determine whether other inducers of the APR also affect the expression of nuclear hormone receptors in adipose tissue, we next measured mRNA levels of nuclear hormone receptors 16 h after treatment with zymosan A, a fungal wall product that interacts with TLR-2, thereby inducing cytokine production and the APR. As shown in Fig. 1D, zymosan treatment resulted in a decrease in

PPARγ, RXRα and RXRβ, LXRα and LXRβ, and TRα and TRβ levels, similar to what was observed with LPS administration. Thus, induction of the APR, regardless of the mechanism, resulted in decreased expression of a subset of type II nuclear hormone receptors.

We next determined whether the repression of receptor RNA also causes a decrease of receptor protein levels. As shown in **Fig. 2**, LPS treatment resulted in a significant decrease of PPARγ and its obligatory partner RXRα protein, suggesting that the transcriptional repression has pathophysiological significance.

LPS decreases a number of nuclear hormone receptor coactivators

We next examined the effect of LPS administration on the mRNA levels of several coactivators that interact with type II nuclear hormone receptors and are required for active gene transcription. As shown in **Fig. 3**, the mRNA levels of a number of coactivators were significantly decreased by 40–90% at 16 h after LPS administration. Thus, a decrease in coactivators, coupled with the decrease in type II nuclear hormone receptors, could result in a greater repression of receptor-mediated gene transcription.

LPS decreases the expression of type II nuclear hormone receptor target genes

Previous studies have shown that several proteins that are regulated by PPARγ are decreased in adipose tissue during the APR (lipoprotein lipase, fatty acid transport protein 1, CD36, acyl-CoA synthase) (15, 16). Therefore, we next determined whether the mRNA levels of other genes known to be regulated by PPARγ are suppressed during the APR. As shown in **Fig. 4A**, both aP2 and PEPCK were decreased at 16 h after LPS administration. Moreover, the expression of a gene known to be downregulated by PPARγ activation, 11β-HSD, was increased after LPS treatment. Glycerol kinase has been shown to be a PPARγ target gene, but we did not detect its mRNA in adipose tissue, consistent with its very low activity in adipose tissue (30).

We next determined whether the expression of genes known to be regulated by LXR/RXR heterodimers was also altered during the APR. As shown Fig. 4B, there were decreases in the mRNA levels of ABCA1, apoE, GLUT4, and sterol-regulatory element binding protein-1c (SREBP-1c) (SREBP-1a and SREBP-2 were unchanged) during the APR. Finally, we determined whether the expression of genes regulated by TR was also regulated during the APR. As shown in Fig. 4C, both ME and Spot14 decreased during the APR. Thus, the expression of a large number of genes that are regulated by PPARs, LXR, and TR was altered during the APR in adipose tissue.

LPS decreases the expression of TG synthetic/resynthetic enzymes in adipose tissue

We next examined the effect of LPS treatment on the mRNA levels of several enzymes that catalyze TG synthesis in adipose tissue. As shown in **Fig. 5A**, 16 h after LPS treatment there was a marked decrease in the mRNA levels of GPAT, AGPAT2, MGAT1, and DGAT1 (GPAT decreased by 68%, AGPAT2 by 81%, MGAT1 by 56%, and DGAT1 by

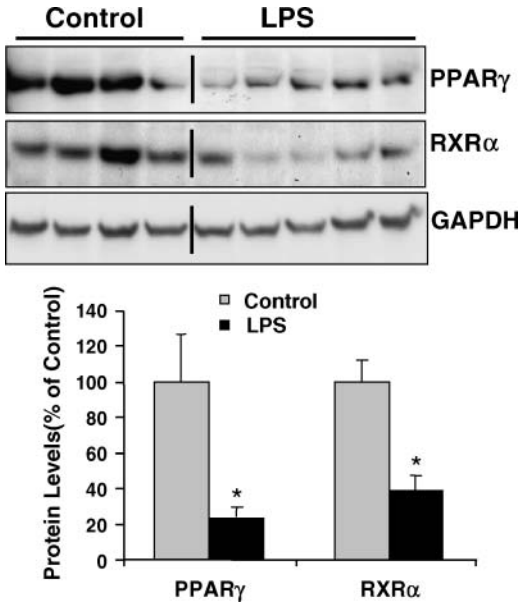

Fig. 2. Effect of LPS on PPARγ and RXRα proteins in mouse adipose tissue. Mice were injected intraperitoneally with either saline or LPS (5 mg/kg body weight), and the animals were euthanized at 16 h after LPS administration. PPARγ and RXRα protein levels were determined by Western blot analysis of white adipose tissue from urinary bladder-uterine area as described in Materials and Methods (top two gels). GAPDH levels were probed with a monoclonal antibody to GAPDH to show equal loading of proteins (bottom gel). Data in the bottom panel are expressed as a percentage of controls (means ± SEM; n = 4–5). * $P < 0.05$ versus controls.

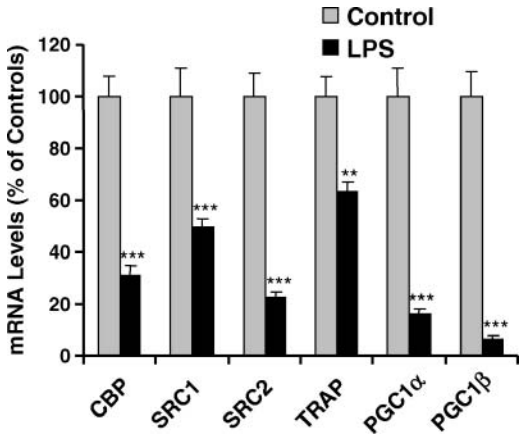

Fig. 3. Effect of LPS on nuclear hormone receptor coactivators in mouse adipose. Mice were injected intraperitoneally with either saline or LPS (5 mg/kg body weight), and the animals were euthanized at 16 h after LPS administration. Total RNA was isolated from white adipose tissue from the urinary bladder- uterine area, and quantitative real-time PCR analysis was performed as described in Materials and Methods. CBP, cAMP response element binding protein; PGC, peroxisome proliferator-activated receptor γ activator; SRC, steroid receptor coactivator; TRAP, thyroid hormone receptor-associated protein. Data are expressed as a percentage of controls (means ± SEM; n = 5). ** $P < 0.01$, *** $P < 0.001$ versus controls.

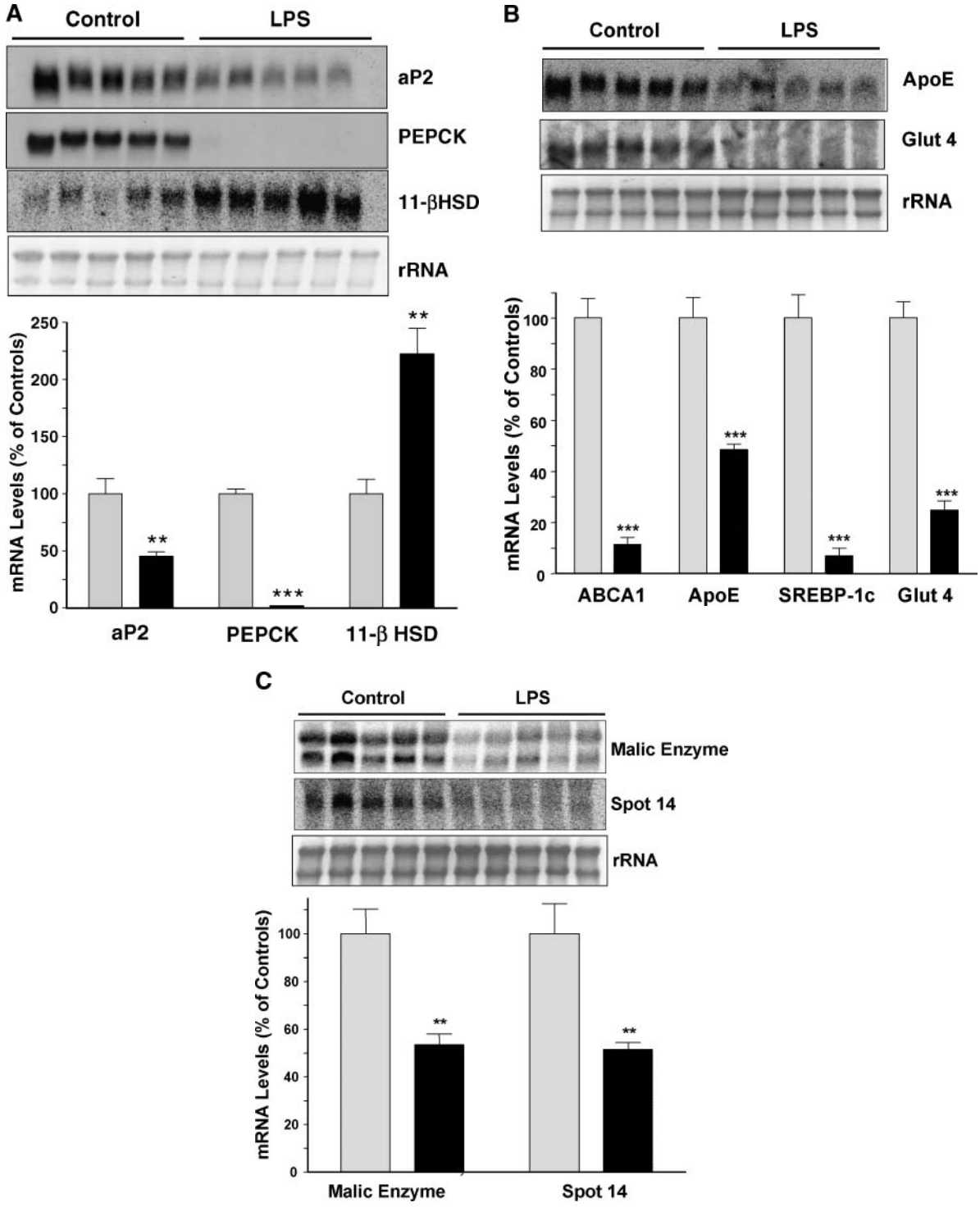

Fig. 4. Effect of LPS on nuclear hormone receptor target genes in mouse adipose tissue. Mice were injected intraperitoneally with either saline or LPS (5 mg/kg body weight), and the animals were euthanized at 16 h after LPS administration. Total RNA was isolated from white adipose tissue from the urinary bladder-uterine area, and Northern blot analysis [for adipocyte P2 (aP2), phosphoenolpyruvate carboxykinase (PEPCK), 11β-hydroxysteroid dehydrogenase (11β-HSD), apolipoprotein E (apoE), glucose transport protein 4 (GLUT4), malic enzyme, and Spot14] and quantitative real-time PCR [for ABCA1 and sterol-regulatory element binding protein (SREBP-1c)] were performed as described in Materials and Methods. Regulation of nuclear hormone receptor target genes is shown in A for PPARγ target genes, in B for liver X receptor (LXR) target genes, and in C for TR target genes. Data are expressed as a percentage of controls (means ± SEM; n = 5). ** $P < 0.01$, *** $P < 0.001$ versus controls.

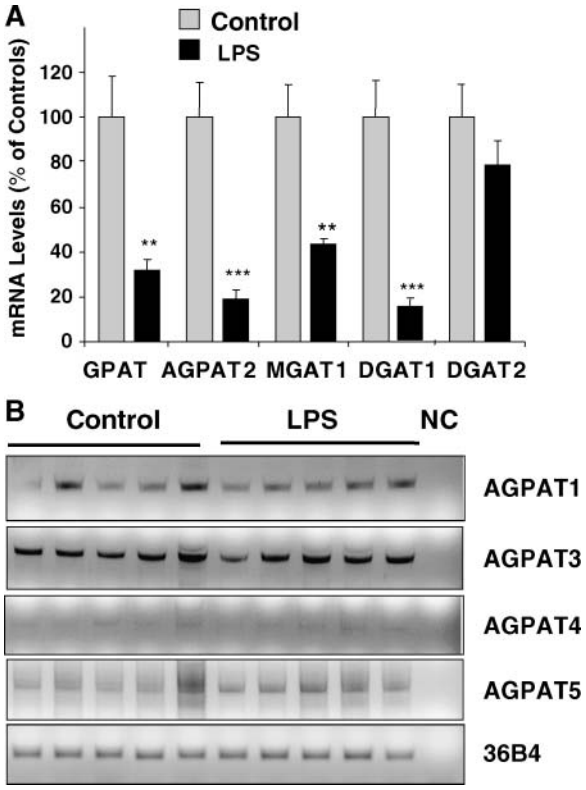

Fig. 5. Effect of LPS on triglyceride (TG) synthetic/resynthetic enzymes in mouse adipose tissue. Mice were injected intraperitoneally with either saline or LPS (5 mg/kg body weight), and the animals were euthanized at 16 h after LPS administration. Total RNA was isolated from white adipose tissue from the urinary bladder-uterine area. Either quantitative real-time PCR [for glycerol-3-phosphate acyltransferase (GPAT), 1-acyl-*sn*-glycerol-3-phosphate acyltransferase 2 (AGPAT2), monoacylglycerol acyltransferase 1 (MGAT1), diacylglycerol acyltransferase 1 (DGAT1), and DGAT2, in A] or semi-quantitative RT-PCR (for AGPAT1, AGPAT3, AGPAT4, AGPAT5, and internal control 36B4, in B) was performed as described in Materials and Methods. Data are expressed as a percentage of controls (means ± SEM; n = 4–5). ** $P < 0.01$, *** $P < 0.001$ versus controls.

85%). In contrast, we did not detect changes in the mRNA levels of DGAT2 (Fig. 5A) and several other AGPAT isoforms, including AGPAT1, -3, and -5, whereas AGPAT4 was barely detected in adipose tissue (Fig. 5B). Thus, several key genes that are responsible for TG synthesis/resynthesis were decreased by LPS treatment in the adipose tissue, and the repression appeared to be specific for certain enzyme isoforms, such as AGPAT2, MGAT1, and DGAT1.

GPAT, AGPAT2, MGAT1, and aP2 are adipose abundant transcripts and are similarly regulated by PPARγ agonist

During adipocyte differentiation of 3T3-L1 cells, there is a coordinated increase in the activity of several key enzymes situated in TG biosynthetic pathways, including GPAT, AGPAT, and DGAT (31–34). In parallel with the increase in enzyme activities, the mRNA levels of GPAT and DGAT1 have been shown to increase markedly in 3T3-L1 cells during their differentiation into adipocytes (32, 33). To determine whether an increase in MGAT1 and AGPAT2 mRNA levels also occurs during the course of 3T3-L1 adipocyte differentiation, quantitative real-time PCR was conducted to monitor the mRNA levels of MGAT1 and AGPAT2. As shown in **Fig. 6A**, AGPAT2 and MGAT1 mRNA levels were increased by 15- to 35-fold during differentiation, an increase in magnitude similar to that of the well-known adipose differentiation marker aP2. These data demonstrate that several key TG synthetic/resynthetic enzymes are coordinately upregulated during the differentiation of 3T3-L1 cells into adipocytes, supporting their critical role in TG biosynthesis.

PPARγ has been shown to directly regulate genes that promote the storage of fat in adipose tissue, including aP2, lipoprotein lipase, fatty acid binding protein, and fatty acid synthase (35). In a previous study using comprehensive mRNA profiling, Way et al. (36) identified the committed TG synthetic enzyme GPAT as a PPARγ-regulated gene in adipose tissue. To explore the possibility that the other TG synthetic/resynthetic enzymes are also potentially PPARγ-regulated, we differentiated 3T3-L1 cells into adipocytes and then treated the adipocytes with the PPARγ

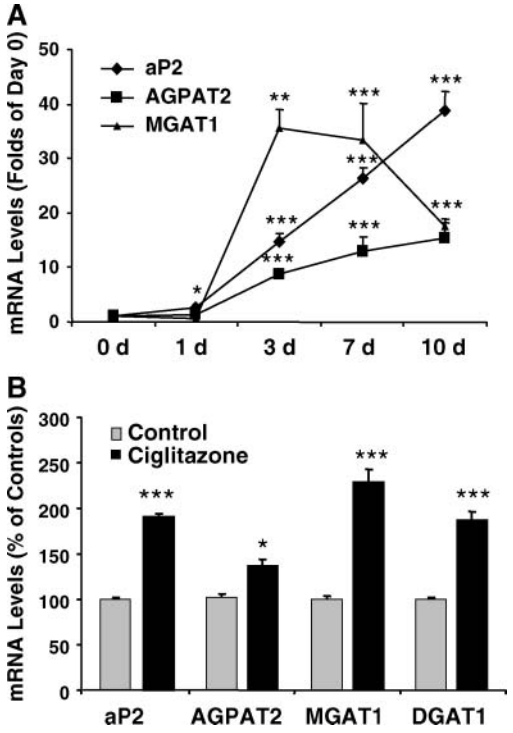

Fig. 6. aP2, AGPAT2, and MGAT1 are adipose abundant proteins and are similarly upregulated by the PPARγ ligand ciglitazone. A: 3T3-L1 cells were induced to differentiate into adipocytes as described in Materials and Methods. Cells at different stages of differentiation were collected, and total RNAs were prepared. Levels of aP2, AGPAT2, and MGAT1 mRNAs were determined by real-time quantitative PCR analysis. B: In another set of experiments, differentiated adipocytes were washed once and treated with 15 nM ciglitazone in 0.2% BSA-supplemented medium for 24 h. Total RNA was isolated, and levels of aP2, AGPAT2, MGAT, and DGAT mRNAs were determined by real-time quantitative PCR analysis. Data are expressed as fold increase of undifferentiated controls (day 0) in A (means ± SEM; n = 4) or as a percentage of controls in B (means ± SEM; n = 8). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

agonist ciglitazone. As shown in Fig. 6B, after 24 h of treatment with 15 nM ciglitazone, there was a marked increase in the mRNA levels of AGPAT2, MGAT1, and DGAT1. The levels of upregulation were comparable to the increase of a well-known PPARγ target gene, aP2. Thus, our data have identified these key TG synthetic/resynthetic enzymes as PPARγ-regulated genes in adipocytes.

TNF-α decreases the mRNA levels of a subset of type II nuclear hormone receptors, coactivators, and their target genes in 3T3-L1 adipocytes

LPS may exert direct effects on adipocytes via TLR-4 (37) or indirectly through various inflammatory cytokines, including TNF-α and IL-1β, which are secreted by various immune cells, including macrophages. Because PPARγ is highly expressed and plays a key role in lipid/carbohydrate metabolism in adipocytes, we first determined whether LPS, TNF-α, or IL-1β has a suppressive effect on PPARγ expression. As shown in **Fig. 7A**, TNF-α treatment (10 ng/ml) resulted in a 50% reduction in the levels of PPARγ mRNA. In addition to a decrease in PPARγ mRNA levels, the mRNA levels of the receptor's obligatory partner RXR were also decreased (RXRα decreased by 50% and RXRβ by 25%). In contrast, no change of PPARγ expression in 3T3-L1 adipocytes was detected even in the presence of a very high concentration of LPS (100 ng/ml LPS), suggesting no direct effect of LPS treatment (data not shown). Similarly, no change in PPARγ expression was observed when 3T3-L1 adipocytes were treated with the proinflammatory cytokine IL-1β (10 ng/ml; data not shown). Thus, TNF-α but not LPS or IL-1β suppressed the expression of PPARγ and its obligatory partner RXR in adipocytes.

We next examined whether the expression of coactivators is also repressed by TNF-α treatment. As shown in Fig. 7B, TNF-α treatment for 24 h significantly decreased steroid receptor coactivator 1 (SRC1; by ~35%), SRC2 (by ~50%), peroxisome proliferator-activated receptor γ activator 1 (PGC1α; by ~65%) and PGC1β (by ~70%), whereas cAMP response element binding protein and thyroid hormone receptor-associated protein mRNA levels remained unchanged.

We next examined whether the expression of genes that are known to be regulated by PPARγ is also repressed by TNF-α treatment. As shown in Fig. 7C, TNF-α treatment for 24 h decreased aP2 expression by 50% and PEPCK

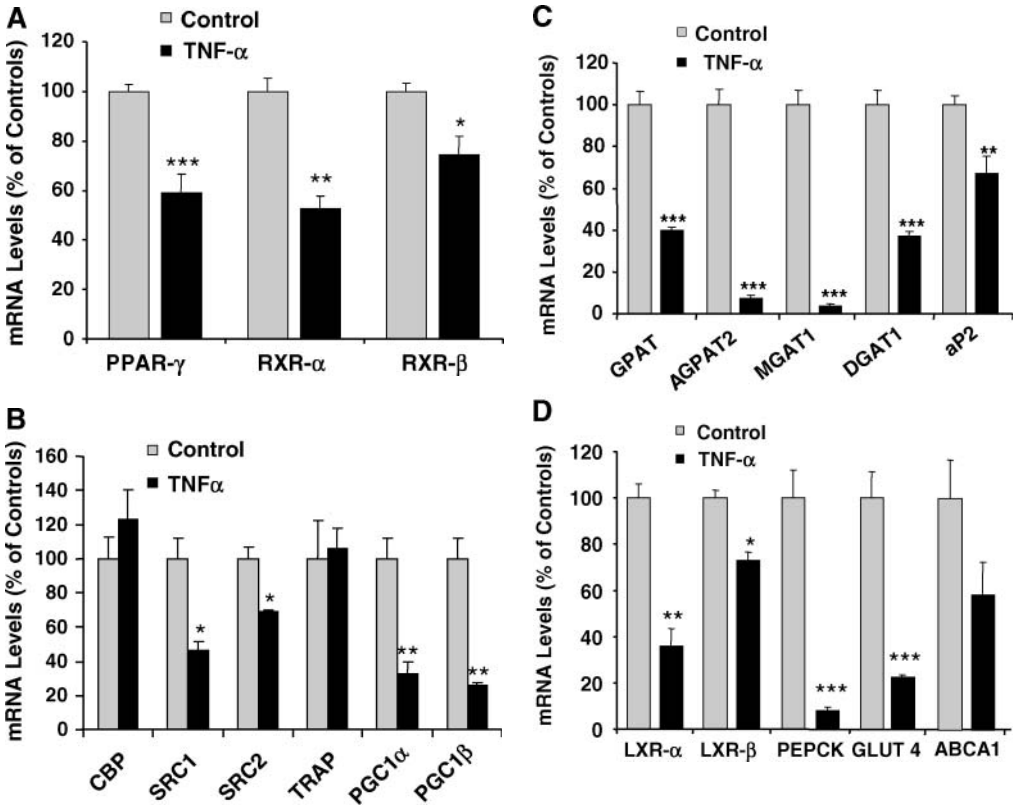

Fig. 7. Effect of tumor necrosis factor-α (TNF-α) on the expression of type II nuclear hormone receptors, coactivators, and their regulated genes in 3T3-L1 adipocytes. 3T3-L1 cells were fully differentiated into adipocytes as described in Materials and Methods. Differentiated adipocytes were washed once and treated with 10 ng/ml TNF-α in 0.2% BSA-supplemented medium for 24 h. Total RNA was isolated. A: mRNA levels of PPARγ, RXRα, and RXRβ were determined by Northern blot analysis. B: mRNA levels of coactivators (CBP, SRC1 and SRC2, TRAP, and PGC1α and PGC1β) were determined by quantitative real-time PCR. C: mRNA levels of PPARγ-regulated genes (GPAT, AGPAT2, MGAT1, DGAT1, and aP2) were determined by real-time quantitative PCR. D: mRNA levels of LXR and their target genes were determined by Northern blot analysis. Data are expressed as a percentage of controls (means ± SEM; n = 4–~8). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus controls.

expression by >90%. Moreover, the mRNA levels of de novo TG/phospholipid synthetic enzymes such as GPAT and AGPAT2 were decreased by 60% and 92% of control levels, respectively. Likewise, the TG resynthetic enzymes MGAT1 and DGAT1 were decreased by 96% and 63%, respectively. These results demonstrate that TNF-α could be one of the mediators in suppressing PPARγ and its obligatory partners RXRα and RXRβ, as well as receptor coactivators. This repression is associated with a marked decrease in the expression of their target genes in adipocytes.

Therefore, we next determined whether the mRNA levels of other type II nuclear hormone receptors are also suppressed in adipocytes after TNF-α treatment. As shown in Fig. 7D, 24 h after TNF-α treatment there was a significant decrease in LXR mRNA expression (LXRα decreased by 64% and LXRβ by 27%), with no change in TRα mRNA levels (data not shown). We did not detect TRβ mRNA in 3T3-L1 adipocytes. The marked reduction in LXR mRNA levels was associated with a reduction in an LXR target gene, GLUT4, that plays an essential role in glucose metabolism in adipocytes (Fig. 7D). These results collectively suggest that TNF-α may be one of the mediators of LPS action in repressing gene expression in adipocytes.

TNF-α decreases PPAR and LXR transcriptional activities

To determine whether TNF-α's effects on PPAR and LXR levels are associated with the decreased transcriptional activity of those nuclear hormone receptors, we next performed transfection studies in 3T3-L1 adipocytes using PPARE- and LXRE-luciferase constructs (pTK-PPARE$_3$-LUC and pTK-LXRE$_3$-LUC). As shown in **Fig. 8A**, treatment of transfected cells with TNF-α caused an ~70% reduction in the activity of luciferase linked to PPARE compared with controls. TNF-α also reduced LXR-driven luciferase activity by ~60% (Fig. 8B). TNF-α treatment had no effect on either pTK-PPARE(−)LUC or pTK-LXRE(−)LUC backbone plasmids. Therefore, TNF-α suppressed the expression of both PPARE- and LXRE-mediated transcription in 3T3-L1 adipocytes.

DISCUSSION

Infection, inflammation, and trauma induce the APR, which leads to dramatic alterations in lipid and lipoprotein metabolism (3), such as increased FFA levels (38, 39), hypertriglyceridemia (11, 40), and decreased HDL (41, 42). Alterations in lipid metabolism in adipose tissue play a key role in mediating many of these changes. During the APR, lipolysis in adipocytes is activated by hormones and cytokines, such as catecholamines, TNF, and interferon (18, 43). Hormone/cytokine binds to cell surface receptors and results in the activation of cAMP-dependent protein kinase (protein kinase A), which in turn phosphorylates two important proteins: hormone-sensitive lipase and its associated protein perilipin (18, 39). This modification induces the migration of lipase from the cytoplasm to the lipid droplet, where efficient hydrolysis occurs. These changes contribute to increased circulating FFA levels, which provide a source of fatty acids for the increase in hepatic TG synthesis and secretion of VLDL (3).

Recently, we and others have shown that a number of changes in lipid metabolism in the liver, heart, and kidney during the APR may be mediated in part by changes in the levels of type II nuclear hormone receptors (3, 20, 22, 44). Because many of the proteins regulated by these nuclear hormone receptors are involved in the metabolism of TG, we determined whether the repression of type II nuclear hormone receptors during the APR was one of the mechanisms that could account for the changes in lipid metabolism in adipose tissue.

Here, we show that induction of the APR by either LPS or zymosan decreases the levels of a number of type II nuclear hormone receptors, including PPARγ, RXRα and

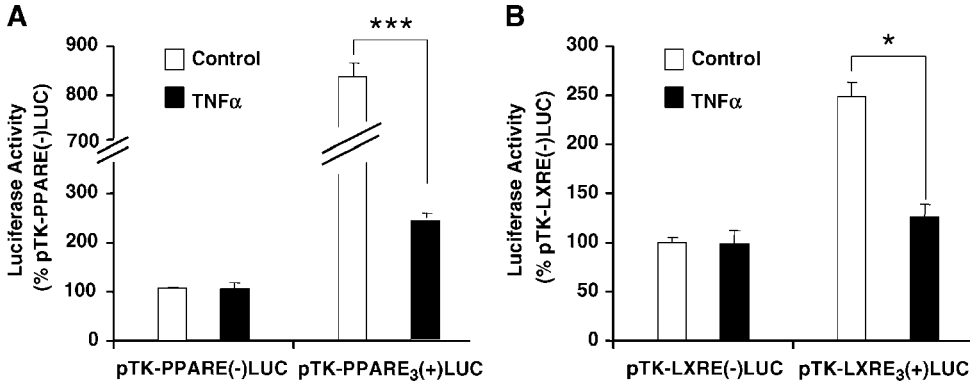

Fig. 8. Effect of TNF-α on peroxisome proliferator-activated receptor response element (PPARE)- and liver X receptor response element (LXRE)-linked luciferase activity in transfected 3T3-L1 adipocytes. 3T3-L1 cells were plated on six-well plates and fully differentiated into adipocytes. The 3T3-L1 adipocytes were then transfected with either PPARE- and LXRE-linked luciferase reporter constructs (LUC) or their respective backbone plasmids as described in Materials and Methods. After 6 h, cells were washed and incubated in basal medium with 0.2% BSA in the presence or absence of 10 ng/ml TNF-α for 24 h. At the end of the incubation, cells were harvested in a lysis buffer and luciferase activity was determined. Data are expressed as a percentage of controls (means ± SEM; n = 3–6). * $P < 0.05$, *** $P < 0.001$ versus controls.

RXRγ, LXRα and LXRβ, and TRα and TRβ. The decrease in receptor mRNA levels was significant as early as ~4 h after LPS administration and was sustained in the late stage of the APR (16 h). Of note, LPS and zymosan decreased PPARγ mRNA levels by ~90% and ~60%, respectively, the largest degree of reduction among all type II nuclear hormone receptors we examined. Notably, the key receptor protein levels were also decreased in adipose tissues, suggesting pathophysiologically significant repression during the APR. In addition, our in vitro experiments with 3T3-L1 adipocytes also showed significant repression of PPARγ and its obligatory partner RXRs by the inflammatory cytokine TNF-α. Thus, our in vivo and in vitro findings demonstrate a downregulation of type II nuclear hormone receptors during inflammation. Nuclear hormone receptor-mediated gene transcription requires the concerted action of transcription factors and coactivator proteins. The simultaneous repression of a number of coactivators represents a multifactorial process (nuclear hormone receptors, their obligatory partner RXRs, and cofactors). We recognize that altered activity of other adipogenic transcription factors may also occur during the APR (45, 46); nevertheless, these studies support the hypothesis that downregulation of type II nuclear hormone receptors and their coactivators may be one of the important mechanisms to coordinately regulate lipid metabolism in adipose tissue during the APR.

The decrease in type II nuclear hormone receptors and their coactivators could affect the transcription of a variety of genes that are involved in lipid metabolism in adipose tissue during the APR (19). In this study, we demonstrate that the mRNA levels of a number of proteins whose expression is regulated by these receptors were decreased by LPS administration. Specifically, we demonstrate that mRNA levels of several known PPARγ target genes, aP2, PEPCK, and GPAT, were decreased by LPS in adipose tissue. We also show for the first time that AGPAT2, MGAT1, and DGAT1 are PPARγ-regulated proteins whose expression in adipose tissue is also repressed by LPS administration. In addition, our in vitro experiments with 3T3-L1 adipocytes demonstrate that TNF treatment caused a similar marked reduction in the expression of PPARγ-regulated genes (aP2, PEPCK, GPAT, AGPAT2, MGAT1, and DGAT1). Furthermore, LXR-regulated proteins (ABCA1, apoE, SREBP-1c, and GLUT4) and TR-regulated proteins (ME and Spot14) were decreased in adipose tissue by LPS administration. In previous studies, we have shown that LPS and/or cytokine treatment decreases the expression of lipoprotein lipase (47), FATP1, CD36 (15), and acyl-CoA synthase (16). Finally, our transfection study showed that TNF-α suppressed the expression of both PPARE- and LXRE-mediated transcription in 3T3-L1 adipocytes. The repression of PPARγ function is probably via a combination of the reduction of PPARγ expression (25) and the blockage of PPARγ binding to its response elements and its coactivator PGC (24). Therefore, downregulation of the transcription factors PPAR and LXR during the APR provides a plausible explanation for the reduction in receptor-targeted gene transcription.

Given the large number of genes that type II nuclear hormone receptors can regulate, a decrease in type II nuclear hormone receptors and receptor coactivators during the APR could provide an underlying mechanism to coordinately decrease the expression of a cohort of proteins required for TG biosynthesis. In fact, many of these proteins are either transporters or enzymes that are involved directly in fatty acid uptake and fatty acid activation as well as TG synthesis and/or resynthesis. For example, during the APR, the expression of fatty acid transport protein and CD36 (fatty acid translocase) is reduced (15), which decreases the transport of fatty acids into adipocytes. In addition, acyl-CoA synthase expression is also reduced (16), which inhibits the activation of long-chain fatty acids to acyl-CoA, a required substrate for TG synthesis. In this study, we further show that the expression of other key TG synthetic/resynthetic enzymes is also decreased. These enzymes are situated at both the de novo glycerol-3-phosphate pathway (GPAT, AGPAT2, and DGAT1) and the monoglycerol pathway (MGAT1 and DGAT1), which catalyze sequential acylations of the glycerol backbone. Indeed, linkage studies in humans have revealed that mutations in hAGPAT2 are one of the causes of congenital generalized lipodystrophy (48, 49), whereas both GPAT-deficient (50) and DGAT1-deficient (51) mice have reduced amounts of white adipose tissue. Thus, the reduction of these key enzymes greatly reduces TG formation in adipose tissue. Coupled with accelerated lipolysis, a simultaneous inhibition of lipogenesis prevents the futile intracellular cycling of fatty acids and thus enhances the mobilization of fatty acid from TG in adipose tissue during the APR. Finally, the decrease in ABCA1 could contribute to the decrease in HDL levels seen in the APR.

In addition to changes in lipid metabolism, glucose metabolism also is altered in adipose tissue during the APR. Because glucose metabolism is tightly linked to the lipogenetic process in adipose tissue, alterations in glucose metabolism may in turn affect TG biosynthesis during the APR. In this study, we show that the insulin-regulated glucose transporter GLUT4 (52), a target gene of LXR, is decreased during the APR. Downregulation of GLUT4 inhibits insulin-mediated glucose uptake, thus contributing to the insulin resistance of adipose tissue during the APR. Once inside adipocytes, glucose is readily converted into glucose-6-phosphate that is used for glycolysis (30). Because adipocytes do not express significant amounts of glycerol kinase (data not shown), glycerol-3-phosphate, which is required for TG formation, is derived primarily from glycolysis within adipocytes. Thus, a reduction in glucose uptake can reduce the supply of glycerol-3-phosphates and TG synthesis is compromised. In addition, PEPCK is also reduced markedly (>90% reduction in its mRNA level both in vivo and in vitro). PEPCK is the rate-limiting enzyme in converting oxaloacetate to phosphoenolpyruvate, which is another important source of glycerol-3-phosphate within adipocytes. Reduced PEPCK would further decrease glycerol-3-phosphate concentration. Thus, the two main pathways that produce glycerol-3-phosphate in adipose tissue are decreased during the APR. This decrease in glycerol-

3-phosphate production would be expected to limit the reesterification of fatty acids and reduce TG synthesis.

In summary, this study demonstrates that the expression of type II nuclear hormone receptors and their coactivators is suppressed in mouse adipose tissue during the APR. This suppression is associated with the reduced expression of many receptor-regulated proteins, including a number of transporters and enzymes, that are involved in lipid and carbohydrate metabolism. This study suggests that downregulation of type II nuclear hormone receptors and their coactivators may represent an underlying mechanism in the resetting of energy homeostasis and fuel metabolism in adipose tissue during the APR.

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