LPS and proinflammatory cytokines decrease lipin-1 in mouse adipose tissue and 3T3-L1 adipocytes


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LPS and proinflammatory cytokines decrease lipin-1 in mouse adipose tissue and 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab 295:E1502–E1509, 2008. First published October 21, 2008; doi:10.1152/ajpendo.90323.2008.—Infection and inflammation affect adipose triglyceride metabolism, resulting in increased plasma free fatty acid (FFA) and VLDL levels during the acute-phase response. Lipin-1, a multifunctional protein, plays a critical role in adipose differentiation, mitochondrial oxidation, and triglyceride synthesis. Here, we examined whether LPS [a Toll-like receptor (TLR)-4 activator], zymosan (a TLR-2 activator), and proinflammatory cytokines regulate lipin-1 in adipose tissue. LPS administration caused a marked decrease in the levels of lipin-1 mRNA and protein in adipose tissue. The decrease in lipin-1 mRNA levels occurred rapidly and lasted for at least 24 h. In contrast, lipin-2 and -3 mRNA levels did not change, suggesting specific repression of lipin-1. Zymosan similarly decreased lipin-1 mRNA without affecting lipin-2 or lipin-3 mRNA levels. To determine the pathways by which LPS repressed lipin-1, we examined the effect of proinflammatory cytokines on cultured adipocytes. In 3T3-L1 adipocytes, TNF-α, IL-1β, and IFN-γ, but not LPS or IL-6, caused a decrease in lipin-1 mRNA levels. Furthermore, TNF-α and IL-1β administration also decreased mRNA levels of lipin-1 in adipose tissue in mice. Importantly, the LPS-induced decrease in lipin-1 mRNA levels was significantly but not totally blunted in TNF-α/IL-1 receptor-null mice compared with controls, suggesting key roles for TNF-α/IL-1β and other cytokines in mediating LPS-induced repression of lipin-1. Together, our results demonstrate that expression of lipin-1, one of the essential triglyceride synthetic enzymes, was suppressed by LPS, zymosan, and proinflammatory cytokines in mouse adipose tissue and in cultured 3T3-L1 adipocytes, which could contribute to a decrease in the utilization of FFA to synthesize triglycerides in adipose tissue, thus promoting the release of FFA into the circulation.

Inflammation; peroxisome proliferator-activated receptor; zymosan; tumor necrosis factor; interleukin-1; fatty acids; lipopolysaccharide

INFLAMMATION INDUCES THE acute-phase response (APR), which results in marked changes in protein synthesis and intermediary metabolism. The APR occurs in response to infectious agents, severe inflammation, or the chronic low-grade inflammation found in atherosclerosis, the metabolic syndrome, and diabetes (13, 15, 18, 22, 39, 40). Many of the changes that occur during the APR are mediated by cytokines, particularly TNF-α and IL-1, which rapidly increase with infections or following lipopolysaccharide (LPS) administration (13, 22). Cytokines induce the APR primarily by increasing or decreasing gene transcription (13, 21, 22). The APR results in dramatic alterations in lipid and lipoprotein metabolism, including enhanced lipolysis, increased circulating free fatty acids (FFAs), hypertriglyceridemia, and decreased HDL cholesterol levels. Enhanced lipolysis increases circulating FFAs that are delivered to the liver and stimulate the synthesis of triglycerides, resulting in increased VLDL secretion and hypertriglyceridemia (18). Therefore, dysregulation of triglyceride metabolism in adipose tissue plays an important role in the dyslipidemia that occurs in association with inflammation.

It has been well documented that LPS and cytokines induce lipolysis mainly by posttranslational phosphorylation of hormone-sensitive lipase (HSL) and its associated protein perilipins (14, 42, 43). Phosphorylation of HSL increases its lipolytic activity (14), whereas phosphorylation of perilipins induces translocation of HSL to the lipid-rich droplet, where lipolysis occurs. Furthermore, a decrease in perilipin protein content may serve as an additional mechanism for TNF-induced lipolysis (36).

In addition to lipolysis, LPS and cytokines may also enhance FFA mobilization by decreasing the expression of triglyceride biosynthetic enzymes (4, 27). In adipose tissue, triglyceride synthetic enzymes are highly expressed, and the released FFAs may be quickly reesterified by these abundant enzymes (5, 6). Therefore, repressed triglyceride biosynthesis in addition to activated lipolysis is required for effective fatty acid mobilization during inflammation.

In adipose tissue, triglyceride is synthesized by a stepwise acylation of glycerol phosphate to form phosphatidic acids, essential intermediates for all glycerolipid biosynthesis (3, 5). Phosphatidic acid is then hydrolyzed by Mg2+-dependent phosphatidate phosphatase (PAP1) to form the critical diacylglyceride pool for triglyceride biosynthesis. Because PAP1 is situated at a branch point between monoglycerolipid and polyglycerolipid biosynthetic pathways, regulation of PAP1 is an important control point for glyceride biosynthesis (3). Both genetic and experimental evidence supports this concept. For example, the predominant PAP1 in adipose tissue is lipin-1. Deficiency of lipin-1 leads to lipodystrophy, characterized by a severe deficiency in adipose tissue mass (31, 33). Conversely, lipin-1 overexpression in adipose tissue promotes obesity (32).

In addition to its PAP1 activities, lipin-1 may also serve as a transcriptional coactivator and participate in the hepatic PGC-1α/peroxisome proliferator-activated receptor (PPAR)-α regulatory pathway to upregulate mitochondrial oxidation (12). Recently, the association of lipin-1 gene polymorphisms with energy and glucose metabolism and phenotypes of the metabolic syndrome in humans has been observed (8, 25, 38).

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Furthermore, adipose tissue lipin-1 expression is found to correlate with PPAR-α gene expression and insulin sensitivity in healthy young men (10). Therefore, lipin-1 may have broader effects on energy metabolism and triglyceride biosynthesis (34).

To add another layer of complexity, there are three members in the lipin gene family, namely lipin-1, -2, and -3 (31, 34). These genes share high sequence homology but are encoded by distinct genes. Although all three lipins have been shown to possess PAP1 activities, they exhibit different tissue expression patterns (9, 34). Lipin-1 is preferentially expressed in metabolically active tissues such as adipose tissue and skeletal muscle, accounting for almost all of the PAP1 activity in these tissues (9, 24).

Previously, we have shown that adipose mRNA levels of several triglyceride biosynthetic enzymes, including acyl-CoA synthetase, glycerol-3-phosphate acyltransferase, 1-acylglycerol-3-phosphate acyltransferase, monoacylglyceride acyltransferase, and diacylglyceride acyltransferase, were rapidly decreased by LPS and proinflammatory cytokines in mice (27, 28). Given the critical function of lipin-1 in triglyceride metabolism and energy expenditure, we hypothesized that lipin-1 expression in adipose tissue is also repressed during inflammation. To test this hypothesis, we examined the expression of lipin isoforms in adipose tissue from mice treated with different APR-inducers and in cultured 3T3-L1 adipocytes treated with proinflammatory cytokines.

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 from Sigma (St. Louis, MO). Recombinant mouse TNF-α, IL-β, IL-6, and IFN-γ were from R&D Systems (Minneapolis, MN). Anti-lipin-1 antibody was kindly provided by Dr. John C. Lawrence, Jr. (16).

Animals. Female C57BL/6 mice (8–12 wk of age, ~20 g) were obtained from Charles River Laboratories (Wilmington MA). Tnfrsf1a<sup>−/−</sup> Il1<sup>−/−</sup> mice were from the Jackson Laboratory. 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 either saline, LPS (100 ng/ml), TNF-α (10 ng/ml), IL-1β (10 ng/ml), or IFN-γ (100 ng/ml). The doses of LPS and cytokines used in these experiments are similar to those previously shown to induce metabolic alterations in 3T3-L1 adipocytes (29) and other cells (17, 20).

Isoulation of RNA and cDNA preparation. Total RNA was isolated from tissues or cells by the TRI Reagent method from Sigma. The integrity of RNA was checked on a 1% agarose-formaldehyde gel. First-strand cDNA was reverse transcribed from 1–2 μg of total RNA by using the iScript cDNA synthesis kit (Bio-Rad).

Primer designs and real-time quantitative PCR. The cDNA of each murine lipin was amplified with a pair of specific primers. Primers were designed using online primer3 (http://frodo.wi.mit.edu/). All primers were synthesized by Invitrogen. Sequences of primer set mLipinE6-8 are from Peterfy et al. (30). GenBank accession numbers for the amplified sequences and the primer sequences used for amplifications are summarized in Table 1.

The real-time quantitative PCR reaction contained 20 ng of reverse-transcribed total RNA, 300 nM forward and reverse primers, and 10 μl of 2× iTaq SYBR Green PCR SuperMix (Bio-Rad). PCR was carried out in 96-well plates with the MX3000P real-time PCR (Stratagene). Products were electrophoresed in 1.5% agarose gel to confirm specificity of reactions. Quantification was performed by the comparative cycle of threshold method, with invariant 36B4 used for normalization.

RNA degradation. 3T3-L1 adipocytes were cultured and induced to differentiate as described above. After 10 days, the differentiated adipocytes were washed twice and treated with 10 μg/ml actinomycin D in the presence or absence of TNF-α (10 ng/ml) and IL-1β (10 ng/ml) in 0.2% BSA-supplemented medium for 0, 3, or 6 h. Total RNA was then isolated and lipin-1 mRNA levels were determined by real-time quantitative PCR analysis and normalized with 36B4. Data are expressed as a percentage of saline controls at time 0.

Extract preparation and Western blot analysis. Snap-frozen adipose tissues from control and LPS-treated mice were ground into powder with a porcelain mortar and pestle chilled in liquid nitrogen, and the powdered tissue (0.7 ml of buffer per 0.2 ml of tissue sample) was homogenized in a glass tissue grinder with a Teflon pestle driven by a Homogenizer unit (Bellco Biotechnology, Vineland, NJ). Homogenization buffer was composed of 50 mM NaF, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 10 mM phosphate, 50 mM

Table 1. Primer sequences used for real-time quantitative PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession Number</th>
<th>Forward Primers</th>
<th>Reverse Primers</th>
</tr>
</thead>
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<tr>
<td>mLipin1</td>
<td>AF412811</td>
<td>CCGCAAGAATACCTGGAA</td>
<td>TGAAGACTCCTGTTGATAGG</td>
</tr>
<tr>
<td>mLipin2</td>
<td>NM_022882</td>
<td>CGCTCTACTCTGGCCATCCT</td>
<td>GCCCATCTCTCTCTCTTTAT</td>
</tr>
<tr>
<td>mLipin3</td>
<td>NM_022883</td>
<td>CCGCTCGTTTTCAACGACGCTT</td>
<td>CGACCTCTTGAGAACCTC</td>
</tr>
<tr>
<td>mLipinE6-8*</td>
<td>AF412811</td>
<td>CGCTCGTATTTCACGACGCCTT</td>
<td>CGACCTCTTGGAGAACCTC</td>
</tr>
<tr>
<td>mMCAD</td>
<td>NM_007382</td>
<td>ATGCCCTCGAGAAAGAGAG</td>
<td>CATAGGCTGCCGAAAAATCTC</td>
</tr>
<tr>
<td>mPPARα</td>
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</tr>
<tr>
<td>mCPT1β</td>
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<td>CGTACAGAGAAGACCATC</td>
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<tr>
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<td>NM_013729</td>
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<td>GAACTTGGGAGATGCCCTT</td>
</tr>
<tr>
<td>m36B4</td>
<td>M17885</td>
<td>CGCGAGTCTGAGGCTCAACACTG</td>
<td>ATOGTCCTGCAATGCTT</td>
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</tbody>
</table>

*Primer sequences are obtained from Ref. 30.
β-glycerophosphate, pH 7.4 with 1 unit of proteinase inhibitors (proteinase inhibitor cocktail; Sigma). Subsequently, homogenates were centrifuged at 10,000 g for 10 min at 4°C, and the resulting supernatants were retained for protein and Western blot analysis.

Denatured tissue extracts (~70 µg protein) were loaded onto 10% polyacrylamide precast gels (Bio-Rad) and subjected to electrophoresis. After electrophoresis onto polyvinylidene difluoride membrane (Amersham Pharmacia Biotech), blots were blocked with phosphate-buffered saline containing 0.10% Tween and 5% dry milk for 1 h at room temperature and incubated for 1 h at room temperature with the anti-mouse lipin 1 antibodies at a concentration of 2 µg/ml. Immune complexes were detected by using horseradish peroxidase-linked anti-rabbit IgG (dilution 1:20,000) with a SuperSignal extended duration substrate kit (Pierce, Rockford, IL). Immunoreactive bands were detected by use of Bio-Rad Multi-Imaging System. The blot was then stripped by using Restore Western blot stripping buffer (Pierce) and probed with a monoclonal antibody to GAPDH for normalization of sample loading.

Statistical analysis. The results are presented as means ± SE. Statistical significance between two groups was determined by using the Student’s t-test. When multiple samples were compared, one-way (one independent variable) and two-way (two independent variables) ANOVA were used to determine statistical significance via GraphPad Prism Software version 4. For one-way ANOVA, Tukey-Kramer was used as the post hoc test, and for two-way ANOVA, Bonferroni was used as the post hoc test. A P value < 0.05 was considered significant.

RESULTS

LPS decreases lipin-1 mRNA levels in mouse adipose tissue but not skeletal muscle. We first determined the effect of the administration of LPS, a Toll-like receptor (TLR)-4 agonist, on lipin-1 expression in two lipin-1 dominantly expressed tissues: adipose tissue and gastrocnemius muscle. As expected, treatment with LPS increased serum FFA levels at 16 h and the expression of TNF-α and IL-1β at 4 h (Supplemental Fig. S1; the online version of this article contains supplemental data). As shown in Fig. 1A, 16 h after LPS treatment there was a marked decrease in lipin-1 mRNA levels (>90% decrease) in murine adipose tissue, whereas there was a trend toward an increase in lipin-1 mRNA in skeletal muscle. Due to alternative splicing, lipin-1 has been shown to exist in two isoforms, lipin-1α and -1β. Previous studies suggested that both isoforms have similar PAP1 enzyme activities but demonstrate unique expression patterns in tissues. To determine which isoforms are expressed and contribute to the decrease in lipin-1 mRNA in adipose tissue, a published primer set (30) within two exons (exon 6 and exon 8) was used to simultaneously amplify lipin-1α and -1β (Fig. 1B). As shown in Fig. 1C, lipin-1β was the predominant isoform detected in adipose tissue, whereas lipin-1α was barely detected under the same experimental conditions. Furthermore, a dramatic decrease in the level of lipin-1β mRNA was observed in LPS-treated animals. Therefore, we concluded that the decrease in the levels of lipin-1β, but not lipin-1α, accounted for the observed decrease in adipose tissue during inflammation. Again, lipin-1β was the predominant isoform expressed in skeletal muscle and the levels of lipin-1β in skeletal muscle were not significantly altered (Fig. 1C, bottom), suggesting a tissue-specific repression of lipin-1 by LPS.

LPS decreases lipin-1 expression in a time- and dose-dependent manner in adipose tissue. To further characterize the repression of lipin-1 expression in adipose tissue during the APR, we conducted time and dose experiments. As shown in Fig. 1D, LPS treatment resulted in a ~70% decrease as early as 4 h and reached a maximal decrease (>90% inhibition) at 16 h, which was sustained for at least 24 h. On the basis of our time-course study, an early time point at 4 h was chosen to perform a dose-response experiment. As shown in Fig. 1E, at a dose of 1.0 µg/mouse, LPS resulted in a significant decrease in lipin-1 mRNA levels. We conclude that the repression of
lipin-1 was rapid and the response was very sensitive to LPS treatment.

To determine whether LPS regulated the levels of other lipin isoforms, we also measured lipin-2 and lipin-3 mRNA levels. In skeletal muscle and adipose tissues, lipin-2 and lipin-3 were expressed at much lower levels, ~30- and ~250-fold less than lipin-1 RNA in skeletal muscle and ~10 and ~80-fold less than lipin-1 mRNA in adipose tissues, respectively (data not shown). After LPS administration, there was no significant alteration in the levels of lipin-2 and -3 mRNA in adipose tissue at the 4-h time point (Fig. 2A). In contrast, a small but significant ~30% increase in lipin-1 mRNA levels in skeletal muscle at 4 h was observed (data not shown) with no significant change in either lipin-2 or -3 mRNA levels in the same tissue (data not shown). These results suggest that the LPS decrease in lipin-1 mRNA levels occurs in a tissue- and isoform-specific manner.

We next assessed whether LPS treatment would also result in a parallel decrease in lipin-1 protein levels in adipose tissue. As shown in Fig. 2B, lipin-1 protein levels decreased by ~70%, 16 h following LPS treatment.

**LPS decreases PPAR-α and its target gene mRNA levels in murine adipose tissues.** Lipin-1 has been shown to be an inducible amplifier to increase PPAR-α levels as well as stimulate PGC-1α/PPAR-α regulatory pathways in mouse liver (12). Recently, lipin-1 mRNA levels were found to correlate with PPAR-α and median chain acyl-CoA dehydrogenase (MCAD) expression levels in adipose tissue of healthy young men (10). Because LPS decreased lipin-1 expression in adipose tissue, we next examined whether the expression of PPAR-α and several of its target genes were also decreased in adipose tissue. As shown in Fig. 3, LPS treatment resulted in a significant decrease in the levels of PPAR-α and several of its target genes, including carnitine palmitoyltransferase-1β (CPT1β), acyl-CoA oxidase (ACO), and MCAD, which are involved in fatty acid utilization and oxidation. Thus a decrease of lipin-1 expression is associated with a decrease in PPAR-α and its target gene mRNA levels in adipose tissue during the APR.

**Zymosan and proinflammatory cytokines decrease lipin-1 mRNA in murine adipose tissues.** We next determined whether other inducers of inflammation also affect the expression of lipin-1 in adipose tissue. As shown in Fig. 4, zymosan treatment (80 mg/kg body wt ip), which activates TLR-2, resulted in a decrease in lipin-1 levels but not lipin-2 and -3 mRNA (data not shown), similar to what was observed following LPS administration. Thus induction of the APR, regardless of the mechanism, resulted in decreased expression of lipin-1 in adipose tissue.

Many of the metabolic changes induced in adipose tissues during inflammation are mediated by proinflammatory cytokines, such as TNF-α and IL-1β, which are rapidly induced by LPS and zymosan treatment. As shown in Fig. 4, administration of either TNF-α (1 μg/mouse ip) or IL-1β (80 ng/mouse ip) decreased lipin-1 mRNA levels by ~70 and ~50%, respectively, demonstrating that treatment of mice with proinflammatory cytokines decreases lipin-1 expression in adipose tissue in vivo.

**Effects of LPS and inflammatory cytokines on the expression of lipin-1α and -1β splice variants in 3T3-L1 adipocytes.** To determine whether the effects of LPS and cytokines on lipin 1 expression in adipocytes is a direct effect or is mediated by the secretion of other bioactive agents either systemically or in a paracrine fashion, we next determined the effect of LPS, TNF-α, IL-1, and other cytokines on lipin 1 mRNA levels in 3T3-L1 adipocytes. Alternatively spliced lipin isoforms (lipin-1α and -1β) exhibited a distinct expression pattern during the differentiation from preadipocytes to mature adipocytes (30). In our culture condition, 3T3-L1 preadipocytes had similar expression levels of lipin-1α and β (Fig. 5A). During differentiation, lipin-1β levels increased whereas lipin-1α transiently increased at day 1 and then gradually decreased below undifferentiated levels, which is similar to the in vivo situation in which lipin-1β is the predominant isoform in adipocytes. Thus fully differentiated adipocytes, which predominantly express lipin-1β, were used in our subsequent experiments.

As shown in Fig. 5B, the proinflammatory cytokines TNF-α, IL-1β, or IFN-γ decreased lipin-1 mRNA by ~90, ~70, or ~75%, respectively. In contrast, LPS and IL-6 treatment did
not significantly reduce lipin-1 mRNA. It has been shown that 3T3-L1 adipocytes have fewer TLR-4 compared with other tissues and respond relatively poorly to LPS treatment (23). Although even higher doses of LPS might have been able to induce a more robust decrease in lipin-1 mRNA, the dose of 100 μg/ml LPS that we used was a high dose. Together, these results suggest that the repression of lipin-1 by LPS was due to the secretion of proinflammatory cytokines such as TNF-α, IL-1β, and/or IFN-γ, which in turn affect adipocytes.

To determine the mechanism that accounts for the TNF- and IL-1-induced decrease in lipin 1 mRNA, we next carried out mRNA degradation studies. As shown in Fig. 6 the half-life of lipin 1 mRNA was ~4 h in 3T3-L1 cells. Moreover, neither TNF nor IL-1 treatment significantly altered the half-life, suggesting that the decrease in lipin 1 mRNA levels induced by TNF or IL-1 treatment was due to repression of gene transcription.

TNF and IL-1 signaling is involved in repressing lipin-1 expression in adipose tissue. To further explore the possibility that the LPS-induced repression of lipin-1 expression is mediated through TNF-α and/or IL-1β signaling in vivo, LPS repression of adipose lipin-1 expression was examined in mice deficient in both TNF-α p55 and IL-1β receptor type 1. As shown in Fig. 7, LPS administration caused a marked ~90% reduction in lipin-1 mRNA levels in control wild-type mice compared with saline-injected controls. In contrast, LPS administration resulted in a moderate ~50% reduction in lipin-1 mRNA levels in TNF-α and IL-1β receptor double knockout mice, a significant attenuation of the LPS-induced repression of lipin-1 gene. These results suggest that the repression of adipose lipin-1 following LPS administration is at least partially mediated through TNF-α and IL-1β receptors.

Elimination of TNF-α and IL-1β signaling did not fully inhibit the LPS repression of adipose lipin-1, and there was a significant decrease in lipin-1 mRNA levels (~75%) following IFN-γ treatment. These results suggest that other cytokines, in addition to TNF-α and IL-1β, are also involved in the repression of lipin-1 in adipocytes following LPS treatment.

**Fig. 3.** Effect of LPS on peroxisome proliferator-activated receptor (PPAR-α) and its target genes. Mice were injected intraperitoneally with either saline or LPS (100 μg/mouse) and the animals were euthanized at 16 h after LPS administration. Total RNA was isolated from adipose tissue; subsequently RT and PCR were performed as described in MATERIALS AND METHODS. The mRNA levels of PPAR-α, carnitine palmitoyltransferase-1 (CPT1), acyl-CoA oxidase (ACO), and median chain acyl-CoA dehydrogenase (MCAD) were analyzed. QPCR data were normalized to 36B4 mRNA as an invariant control. Data (means ± SE, n = 5–10) are expressed as a percentage of controls. *P < 0.05, **P < 0.01 vs. controls.

**Fig. 4.** Effect of zymosan and proinflammatory cytokines on lipin-1 levels in adipose tissue. Mice were injected intraperitoneally with zymosan (80 mg/kg body wt), TNF-α (1 μg/mouse), or IL-1β (80 ng/mouse). The animals were euthanized 16 h after administration. Total RNA was isolated from adipose tissue; subsequently RT and PCR were performed as described in MATERIALS AND METHODS. QPCR data were normalized to 36B4 mRNA as an invariant control. Values are means ± SE, n = 5. Data are expressed as a percentage of controls. One-way ANOVA analysis was done to compare all treatments to controls using Tukey-Kramer as the post hoc test. **P < 0.01, ***P < 0.001 vs. control.

**Fig. 5.** Effect of LPS and proinflammatory cytokines on lipin-1 levels in 3T3-L1 adipocytes. Murine 3T3-L1 cells were cultured and induced to differentiate into adipocytes as described in MATERIALS AND METHODS. A: expression of lipin-1 isoforms during 3T3-L1 differentiation. mLpin-1E6-8 primer set was used to amplify both lipin-1α and -1β, and the PCR products (37 cycles) were analyzed by a 1% agarose gel electrophoreses. Lipin-1β levels increased with differentiation whereas lipin-1α levels exhibit a transient increase at day 1, then decreased afterward. B: effect of LPS, TNF-α, IL-1β, IL-6, or IFN-γ on the expression of total lipin-1 mRNA in 3T3-L1 adipocytes. After 10 days of differentiation, the differentiated adipocytes were washed once and treated with either LPS or the indicated cytokines in 0.2% BSA supplemented medium for 24 h with LPS (100 ng/ml), TNF-α (10 ng/ml), IL-1β (10 ng/ml), IL-6 (10 ng/ml), and IFN-γ (100 ng/ml). Total RNA was isolated. Levels of lipin-1 mRNAs were determined by real-time quantitative PCR analysis. Data are expressed as a % of saline controls (set as 100%). QPCR data were normalized to 36B4 mRNA as an invariant control. One-way ANOVA analysis was done to compare all treatments to controls using Tukey-Kramer as the post hoc test. *P < 0.05, **P < 0.01 vs. controls.
Infection and inflammation induce the APR, which leads to dramatic changes in lipid metabolism (18). Increased triglyceride lipolysis and subsequent release of FFAs from adipose tissues are characteristic changes that occur during the APR (18). These changes contribute to the increased circulating FFA levels, which provide a rich source of FFAs for the increase in hepatic triglyceride synthesis and secretion of VLDL. These metabolic changes increase the levels of circulating VLDL, which provide much needed energy to the host when glucose is limited and thus benefit the host in fighting against invading pathogens and in repairing inflamed and injured tissues (18). However, if prolonged, these changes in lipid and lipoprotein metabolism might also contribute to dyslipidemia and insulin resistance (35, 40).

In mammals, adipose lipolysis and FFA release depend on the precisely regulated balance of triglyceride lipolysis and biosynthesis within adipocytes (41, 44). It is well documented that stimulation of lipolysis in adipose tissue during inflammation is mainly regulated via posttranslational mechanisms. Stress hormone- and cytokine-mediated phosphorylation of hormone sensitive lipase and perilipin has been shown to play a key role in activating lipolysis (7, 11, 42). Equally important but much less understood is the decrease in triglyceride biosynthesis during the APR. Without simultaneous repression of triglyceride biosynthesis, FFAs will undergo a futile recycling within adipocytes (5, 37). In the present study, we provide evidence for repression of a key triglyceride synthetic enzyme, lipin-1, in adipose tissue during the APR. Both lipin-1 mRNA and protein were decreased and the decrease in mRNA was not due to an increase in mRNA degradation, suggesting that repression is transcriptionally regulated. We found that repression of lipin-1 was induced by different inflammatory inducers, including LPS (a model of bacterial infections) and zymosan (a model of fungal infections). Furthermore, we demonstrated that proinflammatory cytokines TNF-α, IL-1β, and IFN-γ are important inflammatory mediators in suppressing lipin-1 in adipose tissue during the APR.

Lipin-1 was the first member identified in a family of three genes: lipin-1, -2, and -3. These three genes have distinct tissue expression profiles, but all encode proteins that have PAP-1 activities (9, 34). In the mouse, lipin-1 is expressed at high levels and constitutes the major PAP1 activity in adipose tissue (9). Indeed, adipocytes in lipin-1-deficient mice fail to accumulate triglyceride, whereas adipocytes in lipin-1 transgenic mice accumulate more triglyceride per cell (31, 32). Thus modulation of lipin-1 expression is an important regulator of triglyceride biosynthesis in adipose tissue. However, it should be recognized that, in addition to decreases in the expression of lipin-1, we have previously shown that LPS and proinflammatory cytokines decrease multiple enzymes in adipose tissue important in the synthesis of triglycerides, including acyl-CoA synthetase, glycerol-3-phosphate acyltransferase, 1-acyl-glycerol-3-phosphate acyltransferase, monoaacylglyceride acyltransferase, and diacylglyceride acyltransferase (27, 28). Therefore the decrease in lipin-1 can be considered as part of a multifaceted, coordinated response that results in a decrease in triglyceride synthesis in adipose tissue. Coupled with the increase in lipolysis induced by LPS and cytokines, which also is due to multiple enzymatic changes, this decrease in triglyceride biosynthesis would result in an increase in FFA release from adipocytes (11, 18, 29).

In addition to its direct role in triglyceride biosynthesis, lipin-1 has been shown to function as a coactivator interacting with PPAR-α and PGC-1-α in the liver (12). Increasing the expression of lipin-1 in hepatocytes enhanced the expression of PPAR-α/PGC-1-α target genes such as acyl CoA oxidase and CPT1, leading to an increase in fatty acid oxidation (12). Studies have further shown that lipin-1 also can interact with other transcription factors including HNF4-α, PPAR-δ, and PPAR-γ (12). Thus the decrease in lipin-1 expression in adipose tissue by inflammatory stimuli could affect the expression of PPAR-α target genes and in the present study we...
demonstrate that CPT1β, ACO, and MCAD are decreased. Because CPT1β, ACO, and MCAD are critical enzymes involved in fatty acid oxidation in mitochondria, the decrease in lipin-1 together with previously shown decreases in PPAR-α and PG C1-α may significantly decrease fatty acid oxidation via these effector enzymes. Similarly, the decrease in lipin-1 could contribute to the decrease in PPAR-γ target genes such as GPAT, AGPAT2, and MGAT (27), which we have previously shown to be repressed by LPS and other inflammatory stimuli. However, we have previously shown in both adipose tissue and liver that there are decreases in multiple nuclear hormone receptors, including PPAR-α and -γ, retinoid X receptor, farnesoid X receptor, and thyroid receptor, as well as coactivators, including PGC1-α and -β and SRC 1 and 2, in response to inflammatory stimuli (1, 2, 19, 27), all of which could regulate the expression of the genes of interest here. Other members of what we now know are large transcription complexes (>30 proteins) are also likely decreased. Therefore the decrease in lipin-1 expression, rather than being a primary driving force, is more likely to be part of a coordinated change in multiple transcription factors and coactivators that together induce the alterations in gene expression that characterize the APR.

LPS-induced alterations in lipid metabolism in adipose tissue are mainly mediated by proinflammatory cytokines such as TNF-α, IL-1β, or IL-6 (18). In the present study, we provide evidence that proinflammatory cytokines, TNF-α and IL-1β, are involved in repressing lipin-1 expression in adipose tissue. First, two different APR-inducers (LPS and zymosan), which induce a rapid increase in serum TNF-α and IL-1β levels, repressed lipin-1 expression in adipose tissue. Second, TNF-α or IL-1β repressed lipin-1 expression in adipose tissue in vivo and in cultured 3T3-L1 adipocytes in vitro, suggesting a direct effect of these cytokines. Most importantly, we demonstrated the lack of both TNF-α and IL-1β receptors greatly attenuated the LPS-repression of lipin-1. These results strongly suggest that TNF-α and IL-1β receptor-mediated signaling is important in repressing lipin-1 during the APR. However, it should be recognized that LPS still decreased lipin-1 mRNA levels in adipose tissue of receptor-null mice, suggesting that there are other mediators in addition to TNF-α and IL-1β that contribute to the decrease in lipin-1 expression during the APR. In fact, IFN-γ can also cause a decrease in the levels of lipin-1, suggesting a role for other cytokines or signaling molecules in addition to TNF-α and IL-1β.

In summary, our study showed that expression of lipin-1, one of the key triglyceride synthetic enzymes, was suppressed by LPS, zymosan, and proinflammatory cytokines in mouse adipose tissue and in cultured 3T3-L1 adipocytes. Furthermore, our findings reveal that proinflammatory cytokines such as TNF-α and IL-1β play important roles in suppressing lipin-1. Fat mobilization is one of the predominant metabolic alterations during the APR, and a decrease in lipin-1 is part of the coordinated metabolic response that leads to the increase in serum FFA that characterizes the APR.

GRANTS

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