Sepsis and glucocorticoids downregulate the expression of the nuclear cofactor PGC-1β in skeletal muscle

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Menconi MJ, Arany ZP, Alamdari N, Aversa Z, Gonnella P, O’Neal P, Smith IJ, Tizio S, Hasselgren P. Sepsis and glucocorticoids downregulate the expression of the nuclear cofactor PGC-1β in skeletal muscle. Am J Physiol Endocrinol Metab 299:E533–E543, 2010. First published July 20, 2010; doi:10.1152/ajpendo.00596.2009.—Muscle wasting during sepsis is at least in part regulated by glucocorticoids and is associated with increased transcription of genes encoding the ubiquitin ligases atrogin-1 and muscle-specific RING-finger protein-1 (MuRF1). Recent studies suggest that muscle atrophy caused by denervation is associated with reduced expression of the nuclear cofactor peroxisome proliferator-activated receptor-γ coactivator (PGC)-1β and that PGC-1β may be a repressor of the atrogin-1 and MuRF1 genes. The influence of other muscle-wasting conditions on the expression of PGC-1β is not known. We tested the influence of sepsis and glucocorticoids on PGC-1β and examined the potential link between downregulated PGC-1β expression and upregulated atrogin-1 and MuRF1 expression in skeletal muscle. Sepsis in rats and mice and treatment with dexamethasone resulted in decreased expression of PGC-1β and increased expression of atrogin-1 and MuRF1 in the fast-twitch extensor digitorum longus muscle, with pronounced changes in the slow-twitch soleus muscle. In additional experiments, adenoviral gene transfer of PGC-1β into cultured C2C12 myotubes resulted in a dose-dependent increase in atrogin-1 and MuRF1 mRNA levels. Treatment of cultured C2C12 myotubes with dexamethasone or PGC-1β small interfering RNA resulted in reduced mRNA levels. Taken together, our results suggest that sepsis and glucocorticoids downregulate muscle wasting, possibly by reducing expression of PGC-1β and that PGC-1β is a repressor of atrogin-1 and MuRF1 gene transcription. Additional experiments provided evidence that PGC-1α represses atrogin-1 gene transcription secondary to reduced capacity of FOXO3α to activate the atrogin-1 promoter.

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Sepsis-induced muscle wasting is associated with increased transcription of genes in the ubiquitin-proteasome proteolytic pathway, in particular the genes encoding the ubiquitin ligases atrogin-1 and muscle-specific RING-finger protein-1 (MuRF1) (7, 10, 44). Because stimulated gene transcription is involved in muscle wasting, it is not surprising that increased expression and activity of certain transcription factors, including NF-κB (4, 28, 29, 40), CCAAT-enhancer-binding protein (CEBP)-β and C/EBPα (27, 46), and FOXO1 and FOXO3α (32, 35, 49), play a role in muscle wasting.

In addition to being regulated by transcription factors, gene transcription is also influenced by nuclear cofactors acting as activators or repressors (14). The potential role of nuclear cofactors in muscle wasting was illustrated in recent studies in our laboratory, in which we found evidence that glucocorticoid-induced muscle atrophy may, at least in part, be regulated by the nuclear cofactor p300 (47, 48).

An important group of nuclear cofactors that may also participate in the regulation of muscle mass are members of the peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1 (PGC-1) family (1, 20, 21, 31). Three members of this family have been described: PGC-1α, PGC-1β, and PGC-1-related coactivator (reviewed in Ref. 20). All three family members share a similar basic structure, with an NH2-terminal domain containing nuclear hormone receptor motifs, a COOH-terminal region containing DNA-binding motifs, and serine-arginine-rich domains. In a recent study, muscle atrophy induced by diabetes, uremia, experimental cancer, or denervation was associated with a substantial downregulation of PGC-1α in skeletal muscle (31). In the same study, overexpressing PGC-1α in skeletal muscle blunted the increase in atrogin-1 and MuRF1 caused by denervation or fasting, suggesting that PGC-1α is a repressor of atrogin-1 and MuRF1 gene transcription.

Less is known about the influence of catabolic conditions on the expression of PGC-1β in skeletal muscle. This is important, because although PGC-1α and -1β are close homologs and share extensive sequence identity, PGC-1α and -1β can influence the transcription of different genes and regulate individual metabolic pathways differentially in various tissues (20, 25, 36). Importantly, the influence of sepsis on the expression of PGC-1β in skeletal muscle is not known. We tested the hypothesis that sepsis downregulates the expression of PGC-1β in skeletal muscle. Because the catabolic effects of sepsis are, at least in part, mediated by glucocorticoids, we also examined the potential role of glucocorticoids in the regulation of PGC-1β expression in skeletal muscle.

MATERIALS AND METHODS

Animal experiments. Sepsis was induced in male Sprague-Dawley rats (50–70 g) or C57BL/6 mice (18–24 g) by cecal ligation and puncture (CLP), as described previously (7, 27, 28, 44). Other animals underwent sham operation (laparotomy and manipulation, but no ligation or puncture of the cecum). Saline (10 ml/kg body wt sc) was injected on the back at the time of surgery to prevent hypovolemia and septic shock. Animals had free access to water, but food was withheld after the surgical procedures to avoid the influence of differences in food intake on metabolic changes in sham-operated and septic animals. Extensor digitorum longus (EDL) and soleus muscles were harvested 8 and 16 h after sham operation or CLP, immediately frozen in liquid nitrogen, and stored at −80°C. The septic model used here resulted in a reproducible increase in muscle protein breakdown and...
Table 1. Primers used for TaqMan real-time PCR analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence, 5′–3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGC-1α</td>
<td></td>
</tr>
<tr>
<td>Forward primer</td>
<td>GGCDCGTACAGTGAAGTGT TT</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>ATTGGCTCCGGCCCTTCTT</td>
</tr>
<tr>
<td>TaqMan probe</td>
<td>TGGTACGCAAGCAGCCTAC</td>
</tr>
<tr>
<td>PGC-1β</td>
<td></td>
</tr>
<tr>
<td>Forward primer</td>
<td>TACATCCTGGATTCGAGAG</td>
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<tr>
<td>Reverse primer</td>
<td>TTTTCTAAGGCTGCGCCT</td>
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<tr>
<td>TaqMan probe</td>
<td>ATGCCGGCCTGTTCGAGG</td>
</tr>
<tr>
<td>Atrogin-1</td>
<td></td>
</tr>
<tr>
<td>Forward primer</td>
<td>CTTGCCACAGACTGAGGTCTCCGA</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>CAAGCTCCAGACGGTTACTGCT</td>
</tr>
<tr>
<td>TaqMan probe</td>
<td>TGCCATCTCTGAGATACG ACC</td>
</tr>
<tr>
<td>MuRF1</td>
<td></td>
</tr>
<tr>
<td>Forward primer</td>
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</tr>
<tr>
<td>Reverse primer</td>
<td>CAGCTCAAACAGCCTGGCATG</td>
</tr>
<tr>
<td>TaqMan probe</td>
<td>AGGGAAAGCCAGCAGTGAGG</td>
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Gene, peroxisome proliferator-activated receptor-γ coactivator; MuRF1, muscle-specific RING-finger protein-1.

Table 2. Primers used for SYBR Green real-time PCR analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence, 5′–3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat cox5b</td>
<td>GGAGATCATGATAGCGCACAG</td>
</tr>
<tr>
<td>Forward primer</td>
<td>GGTTCGTACAGAATCAGC</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>ATGCCAGCGCAGCC</td>
</tr>
<tr>
<td>Rat cycs</td>
<td></td>
</tr>
<tr>
<td>Forward primer</td>
<td>CTTGCCATAGTTGGATGAGT</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>CACATAGGTTGGAGGCGAC</td>
</tr>
<tr>
<td>Rat atp5f</td>
<td></td>
</tr>
<tr>
<td>Forward primer</td>
<td>GGGAGAAGCCGGGCAACAGC</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>CATACGTCTGAGATACGC</td>
</tr>
<tr>
<td>Rat atp5o</td>
<td></td>
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<tr>
<td>Forward primer</td>
<td>GGGAGAAGCCGGGCAACAGC</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>CATACGTCTGAGATACGC</td>
</tr>
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cox5b and cox8h, members of the cytochrome oxidase complex; cycs, somatic cytochrome c; nduf5, member of the NADH dehydrogenase complex; atp5o, member of the ATPase complex.
was then used for quantitative PCR, using the indicated oligonucleotide pairs, in a real-time PCR system (model 7300, Applied Biosystems). Each sample was run in duplicate, and all runs included a negative control. Amplification of hypoxanthine-guanine phosphoribosyl transferase RNA was used as endogenous control to normalize the mRNA concentrations. Analysis was performed using the ΔΔC_{T} method.

PGC-1β gene silencing with siRNA and PGC-1β overexpression by adenoviral-mediated gene transfer. The role of PGC-1β in the regulation of protein degradation and expression of atrogin-1 and MuRF1 was tested by knocking down the expression of PGC-1β using siRNA technology or by overexpressing PGC-1β using adenoviral-mediated gene transfer. Mouse PGC-1β siRNA (PPARGC1B, ON-TARGET plus SMART pool, Dharmacon, Lafayette, CO) and control siRNA (NON-TARGET plus SMART pool; Dharmacon) were used for gene silencing experiments. Myotubes grown in six-well culture plates were transfected with 200 nM PGC-1β siRNA or control siRNA using DharmaFECT 3 reagent (4%) in DMEM without FBS or antibiotics for 24 h. The effectiveness of the siRNA treatment was assessed by measuring PGC-1β mRNA levels by real-time PCR.

In other experiments, adenoviral-mediated gene transfer was used to overexpress PGC-1β. Recombinant adenovirus particles containing PGC-1β were generated by cloning the gene into Lentilox vector pLL3.7 followed by transfection into 293FT cells, resulting in the production of intact adenoviral particles. Recombinant adenovirus particles containing green fluorescent protein were also generated for use as control adenovirus particles. Lysates of the 293FT cells containing the adenoviral particles were then reinfected into larger numbers of 293FT cells, resulting in amplification of the adenovirus. For infection, differentiated myotubes were incubated with adenovirus at 10–50 multiplicity of infection in differentiation medium.

Protein degradation in cultured myotubes. The role of PGC-1β in the regulation of protein degradation was examined by measuring protein degradation rates in myotubes transfected with PGC-1β siRNA and in myotubes in which PGC-1β expression was increased by adenoviral gene transfer, as described above. After transfection with PGC-1β siRNA or adenoviral gene transfer, myotube proteins were labeled with 1-[3,5-3H]tyrosine (1.0 μCi/ml) for 48 h. After the [3H]tyrosine-containing medium was removed, the myotubes were rinsed, and fresh differentiation medium containing a high concentration (2 mM) of unlabeled tyrosine (to limit reincorporation of [3H]tyrosine into proteins) was added. Protein degradation rates were determined by measuring the release of [3H]tyrosine into the medium and were calculated as described in detail previously (23, 42).

Statistical analysis. Student’s t-test was used for comparisons between two experimental groups. When comparisons were made between more than two groups, ANOVA followed by Tukey’s or Student-Newman-Keuls test was used as appropriate. P < 0.05 was considered statistically significant. Most experiments were repeated at least three times to provide evidence of reproducibility. Values are means ± SE.

RESULTS

In initial experiments, PGC-1β mRNA levels were reduced by ~70% and 80% in EDL muscles 8 and 16 h after induction of sepsis, respectively (Fig. 1A). These time points were based on previous experiments in which protein breakdown rates and the expression and activity of the ubiquitin-proteasome pathway were increased in EDL muscles 8 and 16 h after CLP in rats (7, 38, 44).

To test whether sepsis downregulates the expression of PGC-1α, similar to PGC-1β, we next determined PGC-1α mRNA levels in EDL muscles 8 and 16 h after induction of sepsis. PGC-1α mRNA levels were reduced in EDL muscles 16 h, but not 8 h, after CLP (Fig. 1B), suggesting that PGC-1α expression may be less sensitive to the effects of sepsis than PGC-1β expression (cf. Fig. 1A).

Results in previous studies suggest that the catabolic effects of sepsis are more pronounced in white, fast-twitch skeletal (e.g., EDL) muscle than in red, slow-twitch (e.g., soleus) muscle.
To test whether the response of PGC-1α and -1β to sepsis is different in slow- and fast-twitch muscle, we next measured PGC-1α and -1β mRNA levels in soleus muscles of sham-operated and septic rats (Fig. 1, C and D). Interestingly, PGC-1α mRNA levels were not affected by sepsis in soleus muscles at any of the time points studied, and PGC-1β mRNA was reduced only at 16 h after CLP in soleus muscles. Taken together, the results shown in Fig. 1 suggest that PGC-1β is more sensitive to the effects of sepsis than PGC-1α and that sepsis-induced changes in PGC-1β expression occur earlier in fast- than in slow-twitch muscle.

Although we reported previously that the gene expression of atrogin-1 and MuRF1 was increased in skeletal muscle after CLP in rats (7, 44), we wanted to confirm the same response in the present experiments. mRNA levels for atrogin-1 and MuRF1 were increased 8 and 16 h after CLP in the same EDL muscles that were analyzed in Fig. 1, A and B, suggesting a strong correlation between reduced PGC-1β expression and increased expression of atrogin-1 and MuRF1 (Fig. 2, A and B). As expected, the sepsis-induced changes in atrogin-1 and MuRF1 expression were less pronounced in soleus than EDL muscles (Fig. 2, C and D). Atrogin-1 and MuRF1 mRNA levels were increased 8 h after induction of sepsis, a time point at which PGC-1α mRNA was not altered in EDL or soleus muscles, and PGC-1β mRNA was not altered in soleus muscle (Fig. 1). Taken together, the results shown in Figs. 1 and 2 suggest (but do not prove) that downregulation of PGC-1β may be involved in the upregulation of atrogin-1 and MuRF1 in sepsis-induced wasting of white, fast-twitch skeletal muscle.

Whereas the atrogin-1 and MuRF1 genes may be negatively regulated by PGC-1β, there is evidence that PGC-1β (as well as PGC-1α) can increase the expression of other genes, in particular genes involved in energy metabolism and mitochondrial biogenesis and function (1, 20, 31). To examine whether sepsis-induced downregulation of PGC-1β is associated with reduced expression of genes regulating energy metabolism and mitochondrial abundance, mRNA levels for several of these genes were measured in EDL muscles from sham-operated and septic rats. The expression of cox5b, cycs, nduf5, atp5o, and cox8h was reduced by 20–50% in EDL muscles 16 h after CLP (Fig. 3A), suggesting that inhibited expression of PGC-1β (and perhaps PGC-1α as well) may result in reduced levels of gene products involved in mitochondrial biogenesis and oxidative metabolism in fast-twitch muscle. Similar, but less pronounced, changes in the expression of cox5b, cycs, atp5o, and cox8h were seen in soleus muscles from septic rats (Fig. 3B). The expression of nduf5 was not altered in septic soleus muscles. Thus, reduced expression of PGC-1β may be accompanied by multiple changes in gene expression in different types of skeletal muscle, in addition to increased expression of atrogin-1 and MuRF1, many of which may participate in the development of muscle wasting. Because PGC-1α mRNA levels were not altered in soleus muscles during sepsis (Fig. 1D), it is possible that reduced expression of PGC-1β is more important for the downregulation of genes involved in mitochondrial biogenesis and oxidative metabolism, at least in red, slow-twitch muscle.

Because several sepsis-induced catabolic responses are regulated by glucocorticoids (13, 33), we next wanted to determine whether glucocorticoids can regulate the expression of PGC-1β in skeletal muscle. First, we examined the effects of glucocorticoids on muscle PGC-1β expression by treating rats with dexamethasone (10 mg/kg). We found in previous reports that this treatment resulted in increased expression of the ubiquitin-proteasome pathway and stimulated muscle proteolysis (37). Here we found that treatment of rats with dexametha-

**Fig. 2.** Sepsis in rats results in upregulated expression of the ubiquitin ligases atrogin-1 and muscle-specific RING-finger protein-1 (MuRF1). A and B: atrogin-1 and MuRF1 mRNA levels in EDL muscles 8 and 16 h after sham operation or CLP in rats. C and D: atrogin-1 and MuRF1 mRNA levels in soleus muscles 8 and 16 h after sham operation or CLP in rats. Values are means ± SE; n = 8 in each group. *P < 0.05 vs. sham.
Dexamethasone reduced the expression of PGC-1α in EDL muscles by ~70% and upregulated atrogin-1 and MuRF1 mRNA levels approximately fivefold (Fig. 4A). Interestingly, the expression of PGC-1α in EDL muscles was not influenced by dexamethasone treatment (Fig. 4A). In addition, treatment of rats with dexamethasone increased the expression of atrogin-1 and MuRF1 five- to sixfold in soleus muscles but did not result in changes in PGC-1α or -1β mRNA levels in the same muscles (Fig. 4B).

To further examine the role of PGC-1β in the regulation of muscle atrogin-1 and MuRF1 expression, we next overexpressed PGC-1β in cultured C2C12 myotubes by adenoviral transfer of PGC-1β cDNA. The adenoviral gene transfer resulted in a dose-dependent increase in PGC-1β mRNA levels (Fig. 7A) and an opposite, dose-dependent decrease in atrogin-1 and MuRF1 expression, respectively (Fig. 5A). The response to sepsis was similar in soleus muscles with regard to PGC-1α and -1β expression, whereas the increase in atrogin-1 mRNA levels was less pronounced and MuRF1 expression did not change in soleus muscles during sepsis (Fig. 5B).

Also, in EDL muscles, the response of PGC-1β, atrogin-1, and MuRF1 mRNA expression to dexamethasone (10 mg/kg) was similar in mice and rats (cf. Fig. 6A with Fig. 4A). Similar to the observation in dexamethasone-treated rats, PGC-1α and -1β expression was not influenced by dexamethasone in mouse soleus muscles (cf. Fig. 6B with Fig. 4B). Atrogin-1 expression was not altered in soleus muscles of dexamethasone-treated mice, whereas MuRF1 mRNA levels were increased ~2.2-fold (Fig. 6B).

The experiments described above were performed in rats. To test whether sepsis-induced downregulation of PGC-1α and -1β expression in skeletal muscle is species-specific, we next determined the influence of sepsis on muscle PGC-1α and -1β mRNA levels in mice. Similar to our observation in septic rats, sepsis induced by CLP in mice resulted in a ~60% reduction of PGC-1α and a ~80% reduction of PGC-1β mRNA in EDL muscles, and these effects on PGC-1α and -1β were accompanied by a 2.5- and 5-fold increase in atrogin-1 and MuRF1 expression, respectively (Fig. 5A). The response to sepsis was similar in soleus muscles with regard to PGC-1α and -1β expression, whereas the increase in atrogin-1 mRNA levels was less pronounced and MuRF1 expression did not change in soleus muscles during sepsis (Fig. 5B).

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gin-1 and MuRF1 mRNA levels (Fig. 7, B and C). These results strongly suggest that PGC-1β is a repressor of atrogin-1 and MuRF1 gene transcription in skeletal muscle. Interestingly, the reduction of atrogin-1 expression (~30%) was less pronounced than the reduction of MuRF1 expression (~70%) and required a higher level of PGC-1β expression, suggesting that atrogin-1 gene transcription is less sensitive to PGC-1β-mediated repression than MuRF1 gene transcription. In an additional experiment, we measured protein degradation in myotubes transfected with PGC-1β, hypothesizing that overexpression of PGC-1β would reduce protein degradation (perhaps secondary to reduced expression of atrogin-1 and MuRF1). Surprisingly, protein degradation was not influenced by PGC-1β overexpression but was 23 ± 1.3%/24 h in PGC-1β-transfected myotubes (50 multiplicity of infection) and 20 ± 0.96%/24 h in myotubes transfected with control vector (not significant). This result suggests that increased expression of PGC-1β (and reduced atrogin-1 and MuRF1 expression) is not sufficient to inhibit basal protein degradation in cultured muscle cells.

Although increased PGC-1β expression (resulting in reduced atrogin-1 and MuRF1 expression) was not sufficient to reduce protein breakdown rates in the present experiments, the results, of course, do not rule out that PGC-1β may regulate muscle protein breakdown. To further test that notion, we next tested whether downregulation of PGC-1β expression may increase muscle protein degradation. We first examined whether there is a correlation between PGC-1β expression and protein degradation in dexamethasone-treated cultured C2C12 myotubes. We and others reported previously that dexamethasone-treated cultured myotubes can be used as an in vitro model of muscle wasting characterized by increased protein degradation and upregulated expression and activity of atrogin-1 and MuRF1 (2, 12, 23, 42). Here, we found that treatment of C2C12 myotubes with 1 μM dexamethasone for 24 h resulted in a ~25% reduction of PGC-1β mRNA and a ~25% increase in protein degradation (Fig. 8, A and B). To more definitively test the link between reduced PGC-1β expression and protein degradation, we next measured protein degradation in myotubes in which the PGC-1β expression was downregulated by PGC-1β siRNA. This treatment resulted in a 50% reduction of PGC-1β mRNA and stimulated protein degradation (Fig. 8, C and D). The increase in protein degradation was less pronounced in myotubes treated with PGC-1β siRNA than...
dexamethasone-treated myotubes may be that mechanisms cultured C2C12 myotubes by adenoviral transfer of PGC-1α. PGC-1α downregulation of PGC-1α mRNA levels (Fig. 8, A and B). These observations suggest that FOXO3α expression is, at least in part, regulated by PGC-1α in skeletal muscle and that some of the PGC-1α-mediated changes in atrogin-1 and MuRF1 mRNA levels may be secondary to changes in FOXO3α expression.

**DISCUSSION**

In the present study, induction of sepsis in rats or mice by CLP or treatment with dexamethasone resulted in reduced expression of the nuclear cofactor PGC-1α in white, fast-twitch skeletal muscle. In the same experiments, expression of the muscle-specific ubiquitin ligases atrogin-1 and MuRF1 was increased, consistent with the concept that PGC-1α is a repressor of atrogin-1 and MuRF1 gene transcription. More direct evidence for a role of PGC-1α in the regulation of the atrogin-1 and MuRF1 genes was found in cultured myotubes in which overexpression of PGC-1α resulted in downregulation of atrogin-1 and MuRF1 mRNA levels, and silencing of the PGC-1α gene resulted in increased protein degradation. The present observations are important, because they provide the first evidence that sepsis results in reduced expression of PGC-1α in skeletal muscle and that downregulated PGC-1α expression may be involved in increased expression of atrogin-1 and MuRF1 in skeletal muscle.

Further evidence for a role of PGC-1α in the regulation of muscle mass was reported in a recent report published after the submission of the current work (3). In that report (3), PGC-1α overexpression reduced FOXO3α-induced increases in atrogin-1 and MuRF1 gene transcription. Interestingly, in the same report (3), overexpression of PGC-1α resulted in a 15–25% reduction of protein degradation, which differs from the observation in the current report of unchanged protein degradation rates in myotubes overexpressing PGC-1α. We do not have an explanation for these apparently contradictory results.

Although not reported previously in sepsis- and glucocorticoid-induced muscle wasting, reduced PGC-1α expression was reported recently in muscle atrophy caused by denervation (30). Thus it is possible that downregulation of PGC-1α...
expression is a generalized response to muscle-wasting conditions, suggesting that reduced PGC-1β may play an important role in the loss of muscle mass during various catabolic conditions.

In addition to determining the influence of sepsis on PGC-1β expression, we also examined PGC-1α mRNA levels in the same experimental model. Reduced muscle PGC-1α expression was reported previously in several conditions characterized by muscle atrophy, including diabetes, uremia, cancer, starvation, and denervation (21, 30, 31), but the influence of sepsis on muscle PGC-1α expression is not well understood. Although sepsis resulted in reduced PGC-1α mRNA levels in EDL muscles, several observations reported here suggest that reduced PGC-1β expression may be more important than reduced PGC-1α expression in sepsis-induced muscle wasting.

For example, the expression of PGC-1β, but not PGC-1α, was reduced in EDL muscles 8 h after induction of sepsis, a time point at which atrogin-1 and MuRF1 mRNA levels were increased six- to eightfold in the present experiments and muscle protein breakdown rates and ubiquitin mRNA levels were increased in previous reports from our laboratory (16, 39). In addition, PGC-1α mRNA levels were not affected in soleus muscles, whereas PGC-1β expression was reduced by 70%. Although the response to various catabolic conditions is typically less pronounced in slow-twitch (e.g., soleus) than in fast-twitch (e.g., EDL) muscle (15, 24, 39), in previous experiments, we found that total protein breakdown rates and ubiquitin mRNA levels were increased in soleus muscle of rats made septic by CLP, although those changes were substantially less pronounced than in EDL muscles (39). Thus the correla-
tion between changes in PGC-1β expression and muscle wasting-related changes seems to be more robust than the corresponding correlation for PGC-1α.

Upregulation of the ubiquitin-proteasome pathway and stimulation of protein degradation in muscle wasting caused by sepsis and several other catabolic conditions are, at least in part, regulated by glucocorticoids (13, 33, 37). Results in the present study suggest that glucocorticoids can regulate muscle PGC-1α and -1β expression as well. Evidence for hormonal control of PGC-1 cofactors has been reported by others (11, 20, 22), but the present study provides the first evidence that glucocorticoids downregulate the expression of PGC-1β in skeletal muscle.

Although our experiments in septic and dexamethasone-treated rats and mice suggest that downregulated PGC-1β expression may be involved in the upregulation of atrogin-1 and MuRF1, this conclusion was based on circumstantial evidence. The observations in cultured myotubes that overexpression of PGC-1β downregulated atrogin-1 and MuRF1 mRNA levels and silencing of PGC-1β resulted in increased protein degradation provided more direct evidence for a role of PGC-1β in the expression of atrogin-1 and MuRF1 and in the regulation of muscle protein degradation.

The less pronounced increase in protein degradation noticed in PGC-1β siRNA- than in dexamethasone-treated myotubes may seem surprising considering the more pronounced reduction in PGC-1β mRNA levels in PGC-1β siRNA-treated myotubes. The observation, however, may have several potential explanations. First, it is likely that dexamethasone-induced protein degradation is mediated by multiple mechanisms in addition to reduced PGC-1β expression, including increased expression of the transcription factors FOXO1 and FOXO3a (32, 35) and C/EBPβ and C/EBPδ (27, 46), as well as increased expression and activity of the nuclear cofactor p300 (47, 48). When these (and other) factors work in concert, the increase in protein degradation may be more pronounced than if only PGC-1β is downregulated. In addition, it is possible that silencing of the PGC-1β gene by PGC-1β siRNA was counteracted by a compensatory increase in the expression of PGC-1α, as suggested by results in PGC-1β-knockout mice (18). The important conclusion from the experiments in PGC-1β siRNA-treated myotubes and in myotubes overexpressing PGC-1β is that downregulation of PGC-1β may result in increased protein degradation, possibly secondary to increased atrogin-1 and MuRF1 expression.

Whereas the present experiments were focused on the potential role of PGC-1β in the regulation of atrogin-1, MuRF1, and protein degradation in skeletal muscle, it should be noted that PGC-1 nuclear cofactors may also regulate other cellular functions of potential significance for muscle wasting. Importantly, reduced PGC-1β (and PGC-1α) levels may result in impaired mitochondrial biogenesis, fusion, and function (19, 20), which may be of significance, in light of recent reports of mitochondrial dysfunction in skeletal muscle during sepsis (8, 9).

An important limitation of the present study is the fact that it is not known if the reduced expression of PGC-1α and -1β noticed in skeletal muscle of septic rodents reflects similar changes in septic patients. Fredriksson et al. (9) reported recently that the expression of PGC-1α and -1β and several mitochondrial genes was not altered in intensive care unit patients with sepsis induced by multiple organ failure. Although that report may suggest that the findings in the present study are not relevant for the situation in patients with sepsis, there may be other explanations for the apparent discrepancies between the present study and the report by Fredriksson et al. First, among the 17 patients included in the study by Fredriksson et al., only 3 patients were listed as being septic (see Table 1 in Ref. 9). Second, only 2 of the 3 patients with sepsis had abdominal sepsis (which corresponds to the CLP model used here). Therefore, at this point, it is not known if downregulated expression of PGC-1α and -1β, as noted in rats and mice with septic peritonitis in the present study, reflects the situation in patients with septic peritonitis. More studies are needed to address this important question.

Regardless of limitations, the present report is important, because it further supports the concept that muscle wasting in various catabolic conditions is regulated at the molecular level by transcription factors and nuclear cofactors (14, 34). Previous studies suggest that PGC-1 cofactors can interact with transcription factors involved in muscle wasting, such as FOXO transcription factors (31), the glucocorticoid receptor (17), and C/EBPβ (43). In addition, there is evidence that PGC-1α may interact with nuclear cofactors regulating the acetylation and deacetylation of nuclear proteins, including p300 (41) and the deacetylating enzymes SIRT1 (26) and histone deacetylase (HDAC5) (6). It will be important in future
experiments to determine whether interactions between PGC-1β and various nuclear regulatory proteins regulate muscle mass.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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