PGC-1α is required for AICAR-induced expression of GLUT4 and mitochondrial proteins in mouse skeletal muscle

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Submitted 27 October 2009; accepted in final form 6 July 2010

Leick L, Fentz J, Biensø RS, Knudsen JG, Jeppesen J, Kiens B, Wojtaszewski JF, Pilegaard H. PGC-1α is required for AICAR-induced expression of GLUT4 and mitochondrial proteins in skeletal muscle. Am J Physiol Endocrinol Metab 299: E456–E465, 2010.—We tested the hypothesis that repeated activation of AMP-activated protein kinase (AMPK) induces mitochondrial and glucose membrane transporter mRNA/protein expression via a peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α)-dependent mechanism. Whole body PGC-1α-knockout (KO) and littermate wild-type (WT) mice were given either single or repeated subcutaneous injections of the AMPK activator AICAR or saline. Skeletal muscles were removed either 1 or 4 h after the single AICAR treatment or 24 h after the last injection following repeated AICAR treatment. Repeated AICAR treatment increased GLUT4, cytochrome (cyt) c oxidase I, and (cyt) c protein expression ~10–40% relative to saline in white muscles of WT but not of PGC-1α-KO mice, whereas fatty acid translocase/CD36 (FAT/CD36) protein expression was unaffected by AICAR treatment in both genotypes. GLUT4, cyt c, and FAT/CD36 mRNA content increased 30–60% 4 h after a single AICAR injection relative to saline in WT, and FAT/CD36 mRNA content decreased in PGC-1α-KO mice. One hour after a single AICAR treatment, phosphorylation of AMPK and the downstream target acetyl-coenzyme A carboxylase increased in all muscles investigated independent of genotype, indicating normal AICAR-induced AMPK signaling in the absence of PGC-1α. The hexokinase II (HKII) mRNA and protein response was similar in muscles of WT and PGC-1α-KO mice after single and repeated AICAR treatments, respectively, confirming that HKII is regulated independently of PGC-1α in response to AICAR. In conclusion, here we provide genetic evidence for a role of PGC-1α in AMPK-mediated regulation of mitochondrial and glucose membrane transport protein expression in skeletal muscle.

AMP-activated protein kinase; peroxisome proliferator-activated receptor-γ coactivator-1α; glucose transporter 4; mitochondrial biogenesis

SKELETAL MUSCLE EXHIBITS AN EXCEPTIONAL CAPABILITY to adapt to metabolic perturbations. During metabolic stresses such as hypoxia and muscle contraction, increases in the cellular AMP concentration lead to activation of the cellular energy sensor AMP-activated protein kinase (AMPK) (17). The general function of AMPK is to restore the energy homeostasis of the cell, which is accomplished through signaling to many targets. For instance, AMPK acutely increases glucose uptake and fat oxidation (22) while inhibiting growth-promoting pathways (6, 12). Furthermore, several studies have demonstrated that AMPK regulates expression of genes encoding proteins involved in energy metabolism, including mitochondrial proteins and glucose transporter 4 (GLUT4) (15, 24, 27). In experimental model systems, activation of AMPK can be achieved by 5-aminoimidazole-4-carboxamide-1-β-D-ribonucleoside (AICAR), which is metabolized to the monophosphorylated nucleotide 5-aminoimidazole-4-carboxamide-1-β-D-riboyl monophosphate, an AMP analog. Repeated AICAR-induced activation of AMPK leads to multiple adaptations, including muscular mitochondrial biogenesis and increased expression of GLUT4 (11, 15, 33, 38). These adaptations may at least in part result from cumulative effects of transient increases in gene transcription in response to each AICAR-induced activation of AMPK.

Recent in vitro studies have shown that one downstream target of AMPK is peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) (14). PGC-1α is a transcriptional coactivator that has been shown to activate a broad range of transcription factors and to regulate genes encoding mitochondrial proteins and GLUT4 as well as potentially the fatty acid translocase/CD36 (FAT/CD36) (4, 19). Furthermore, acute activation of AMPK by AICAR increases PGC-1α mRNA expression in mouse skeletal muscle (13, 15), and it was demonstrated recently that acute activation of AMPK by AICAR increases cytochrome c (cyt c) and GLUT4 mRNA expression in mouse skeletal muscle via a PGC-1α-dependent mechanism (14). In line with this, AICAR increases the protein content of CD36 in incubated cardiac myocytes and perfused rat hearts (5). However, it is not known whether this mechanism also exists in skeletal muscle or whether PGC-1α may be a signaling intermediate.

Taken together, these observations suggest that AMPK activation leads to increased PGC-1α expression and/or expression and thereby a concomitant induction of muscle phenotypic adaptations. Therefore, the aim of the present study was to test the hypothesis that AICAR-PCG-1α-mediated signaling regulates the expression of GLUT4, FAT/CD36, and mitochondrial proteins in skeletal muscle. This was addressed by performing a repeated AICAR treatment experiment in wild-type (WT) and PGC-1α-knockout (KO) mice.

METHODS

Mice. PGC-1α whole body KO mice on a C57BL/6 background were used in this study. Littermate WT and PGC-1α mice were produced for the experiment by heterozygote crossing. The production of these mice and their phenotype have been described elsewhere (18, 20). All mice were housed individually and received standard rodent chow (Altromin no. 1324; Chr. Pedersen, Ringsted, Denmark), and...
the mice were studied at 2.5–4 mo of age. Experiments were approved by the Danish Animal Experimental Inspectorate and complied with the European Convention for the Protection of Vertebrate Animals used for Experiments and Other Scientific Purposes (Council of Europe, no. 123, Strasbourg, France, 1985).

Single AICAR treatment.

Male and female PGC-1α/H9251-KO and littermate WT mice were given a single subcutaneous injection of saline (0.9% NaCl) or AICAR (500 mg/kg body wt; Toronto Research Chemicals, Toronto, ON, Canada) dissolved in saline, as described previously (15). Female and male mice were divided equally between experimental groups. Mice were euthanized by cervical dislocation at 1 or 4 h after the injection, trunk blood was collected in EDTA-containing tubes, and the oxidative soleus, glycolytic white gastrocnemious (WG), and metabolically mixed quadriceps muscles were removed and quickly frozen in liquid nitrogen.

Table 1. Primer and TaqMan probe sequences used for real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>TaqMan Probe</th>
</tr>
</thead>
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<tr>
<td>PGC-1α</td>
<td>5’AGCCCAAACCAACAACTTTATCTCTTGCC3’</td>
<td>5’TGATCCTGGTCTATGAGCCACACAGCGGCC3’</td>
<td>5’AGATCTACCKAAAATGAAAAAGAGGTC3’</td>
</tr>
<tr>
<td>HKII</td>
<td>5’CTGCTTACCACAGATCCATGACGCTGAC3’</td>
<td>5’CATGACCCGTCATCCATGGC3’</td>
<td>5’CAGTCGGATTCAACGAGACACCC3’</td>
</tr>
<tr>
<td>GLUT4</td>
<td>5’CCGGCCGCTCCCTAATGAGATGTT3’</td>
<td>5’AGGCCACCCGAAAGAAGGTT3’</td>
<td>5’TGGCGCTGTCATAGGCTCAATGAC3’</td>
</tr>
<tr>
<td>COX-I</td>
<td>5’TGCAGCTGTTACACCTGT3’</td>
<td>5’TCTATTTACGCTGAATGACACA3’</td>
<td>5’TCTGAAACCTGGAAACTGAGACCC3’</td>
</tr>
<tr>
<td>Cytc</td>
<td>5’TGCAGCTGCTGCTACCTGT3’</td>
<td>5’CTGCTTCCGCCCAGGACA3’</td>
<td>5’AGGCAAGCTATGAGCTGAGGCA3’</td>
</tr>
<tr>
<td>FAT/CD36</td>
<td>5’CCGATGAAATGACCAGATGCACT3’</td>
<td>5’GTGTCGATGAGCTCTCAT3’</td>
<td>5’TGATAAGCTGCCTCTCTCCGATCG3’</td>
</tr>
<tr>
<td>GS</td>
<td>5’TTCTACCAACAACCTGAGAATTCCA3’</td>
<td>5’GTAGCCTGCAATTTGATGAT3’</td>
<td>5’CCCTGCTGCGCAGAACTACTA3’</td>
</tr>
<tr>
<td>GPhos</td>
<td>5’CCGGAACACACGGCGC3’</td>
<td>5’ATGGAACCTTATACTTGTTG3’</td>
<td>5’AGGCAAGCTATGAGCTGAGGCA3’</td>
</tr>
</tbody>
</table>

PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; HKII, hexokinase II; GLUT4, glucose transporter 4; COX-I, cytochrome c (cyt c) oxidase I; FAT/CD36, fatty acid translocase/CD36; GS, glycogen synthase; GPhos, glycogen phosphorylase.
Repeated AICAR treatment protocol. Female and male PGC-1α-KO and WT littermate mice were given subcutaneous injections of AICAR or saline as described above for 28 days. AICAR was injected every 2nd day during the first 6 days and every day during the rest of the period. Mice were anesthetized 24 h after the last AICAR injection by an intraperitoneal injection of pentobarbital sodium (6 mg pentobarbital sodium/100 g body wt). Trunk blood was collected in EDTA-containing eppendorf tubes, and the soleus, WG, and quadriceps muscles were removed and quickly frozen in liquid nitrogen. All samples were kept at −80°C until further analysis.

Plasma glucose and muscle glycogen. Whole blood was centrifuged for 15 min at 2,600 g to obtain plasma, and plasma glucose concentration was determined by a fluorometric method (28). Glycogen content in quadriceps muscle was determined fluorometrically as glycosyl units after acid hydrolysis (28).

RNA isolation and reverse transcription. Quadriceps muscles obtained 4 h after a single saline or AICAR injection were crushed in liquid nitrogen to ensure homogeneity, and 15–20 mg was weighed out. RNA isolation was performed using a guanidinium thiocyanate-phenol-chloroform method, as described previously (7, 29).

Reverse transcription was performed using the Superscript II RNase H system (Invitrogen, Carlsbad, CA), as described previously (29).

PCR. Real-time PCR was performed using ABI 7900 sequence detection system (Applied Biosystems, Foster City, CA). Primers and TaqMan probes for amplifying gene-specific fragments were designed using mouse-specific database (Ensemble) and Primer Express (Applied Biosystems) and are listed in Table 1. The sequences have in part been published previously (18). Primers and TaqMan probes were obtained from TAG Copenhagen (Copenhagen, Denmark). Real-time PCR was performed as described previously (21).

Muscle lysate preparation. Soleus, WG, and quadriceps muscle lysates were prepared by homogenization (buffer: 10% glycerol, 1% NP-40, 20 mM Na-pyrophosphate, 150 mM NaCl, 50 mM HEPES, 20 mM β-glycerol phosphate, 10 mM NaF, 2 mM PMSF, 1 mM EDTA, 1 mM EGTA, 2 mM sodium orthovanadate, 3 mM benzanidine, 10 μg/ml aprotinin, and 10 μg/ml leupeptin, pH 7.5) using a TissueLyser II (Qiagen) for 2 × 1 min at 30 oscillations/s. The homogenates rotated end over end at 4°C for 1 h. Lysates were prepared from the homogenates by centrifugation for 20 min at 16,000 g and 4°C. To ensure that the high-speed spin did not pellet a fraction of the proteins to be investigated, a Western blot was performed on both the crude homogenate and the corresponding lysate preparations. Identical results were obtained from the two preparations, indicating that none of the proteins of interest were lost in the pellet upon centrifugation and thus confirming the validity of our protocol.
Total protein content was determined as triplicates using the bicinchoninic acid method with BSA as standards (Pierce, Rockford, IL). A maximum coefficient of variance of 5% was accepted between replicates. Unless stated specifically, all chemicals were of analytic grade from Sigma-Aldrich.

SDS-PAGE and Western blotting. Muscle lysate proteins were separated using Tris-HCl gels (Bio-Rad) and transferred (semidyry) to PVDF membranes (Immobilon Transfer Membrane; Millipore). An equal amount of total protein was loaded in each well, and standard Western blotting procedures were used for detection of specific proteins, as described previously (2). Following detection and quantification using a charge-coupled device image sensor and 1D software (Kodak Image Station, 2000 MM; Kodak), the protein content was expressed in arbitrary units relative to control samples loaded in duplicates on each separate gel.

Primary antibodies used for Western blotting were anti-phospho (p)-AMPK Thr172 (no. 2535; Cell Signaling Technology, Boston, MA), anti-p-acetyl-coenzyme A carboxylase (ACC; no. 07-303; Millipore, Billerica, MA), which was raised against a peptide corresponding to the sequence in rat ACC1 containing the Ser79 phosphorylation site, but the antibody also recognized the mouse ACC2 when phosphorylated most likely at the corresponding Ser227, anti-hexokinase II (HKII; no. 2867; Cell Signaling Technology), anti-GLUT4 (no. PA1–1065; Affinity Bioreagents, Rockford, IL), anti-cyt c oxidase I (COX-I; no. 459600; Invitrogen, Carlsbad, CA), anti-cyt c (no. 556433; BD Pharmigen, Franklin Lakes, NJ), anti-CD36 (no. AF2519; R & D Systems, Minneapolis, MN), anti-glycogen synthase (gift from Professor Oluf Pedersen), and anti-glycogen phosphorylase (no. 7370-9808; AbD Serotec, Oxford, UK). Nonvisible contaminants of fat tissue in the muscle samples may contribute a significant amount of FAT/CD36 protein. To evaluate the extent of this potential problem, Western blot analyses were performed with muscle lysate using the fat-specific marker perilipin (anti-perilipin, anti-goat, no. GP33; Progen Biotechnik, Heidelberg, Germany). However, perilipin was present in very low and similar amounts in muscle samples from both genotypes, and because both FAT/CD36 and perilipin were also expressed similarly in subcutaneous adipose tissue in the two genotypes (data not shown), we conclude that fat contamination of the muscle preparations has not been a contributing factor in the FAT/CD36 determinations in muscle. Secondary antibodies used were all species-specific horseradish peroxidase-conjugated immunoglobulins (DakoCytomation, Glostrup, Denmark, and Invitrogen).

Statistics. All data are presented as means ± SE. Two-way analysis of variance was applied to evaluate the effect of genotype and AICAR treatment on plasma glucose, muscle glycogen, mRNA, and protein content. A Student-Newman-Keuls post hoc test was used to locate differences. If homogeneity of variances was not obtained, the data were transformed. However, in two instances [1) soleus p-AMPK Thr172 1 h after a saline or AICAR injection and 2) cyt c mRNA expression after chronic AICAR or saline treatment] homogeneity of variances was not reached after log transformation, and a nonparametric test (Dunn’s) was used. Differences are considered significant at $P < 0.05$, and a tendency is reported for $0.05 \leq P < 0.1$. Statistical
RESULTS

Single AICAR treatment. To examine the effects of a single AMPK activation when PGC-1α is lacking, a single AICAR injection was given to WT and PGC-1α-KO mice with determinations of plasma glucose concentration as well as muscle glycogen concentration, AMPK signaling, and mRNA responses.

Plasma glucose and quadriceps muscle glycogen content. The plasma glucose concentration was in both genotypes 60% lower (P < 0.05) 1 h after a single AICAR injection than 1 h after a single saline injection. No differences were apparent in plasma glucose concentration 4 h after injection of saline or AICAR (Fig. 1A).

Whereas quadriceps muscle glycogen increased ~40% in WT mice 1 and 4 h after a single AICAR injection, no significant AICAR-induced changes were detected in muscle glycogen in mice lacking PGC-1α. Furthermore, muscle glycogen was ~30–120% higher (0.05 ≤ P < 0.1) in WT mice than in PGC-1α-KO mice after a single injection (Fig. 1B).

These data suggest that, whereas lack of PGC-1α does not influence AICAR-induced changes in plasma glucose, lack of PGC-1α attenuates AICAR-induced glycogen resynthesis in skeletal muscle.

AMPK-related signaling. One hour after a single AICAR injection, phosphorylation of AMPK Thr172 and ACC Ser227 was induced similarly in quadriceps and WG (40–400%) of the two genotypes (Fig. 2, B, C, E, and F). AMPK and ACC phosphorylation had returned to baseline 4 h after a single AICAR injection (Fig. 2, B, C, E, and F).

Four hours after a single injection, soleus and quadriceps p-AMPK Thr172 was ~30–40% higher (P < 0.05), and quadriceps p-ACC Ser227 was 120% higher (P < 0.05) in PGC-1α-KO than in WT mice (Fig. 2, A, C, and F). These findings may indicate that at least some muscles of the PGC-1α-KO mouse were more metabolically stressed than muscles of WT mice 4 h after the injection.

Metabolic mRNA expression. To verify that AICAR treatment elicited a transient mRNA response, metabolic mRNA expression was determined in quadriceps muscle 4 h after a single AICAR injection. The PGC-1α mRNA content increased (P < 0.05) 200% in quadriceps muscle of WT mice 4 h after a single AICAR treatment (Fig. 3A).

Whereas HKII mRNA expression increased similarly in WT (150%, P < 0.05) and PGC-1α-KO mice (100%, P = 0.08), and COX-I, glycogen synthase (GS), and glycogen phosphorylase

Fig. 4. Glycogen synthase (GS; A) and glycogen phosphorylase (GPhos; B) mRNA content in quadriceps muscle from WT and PGC-1α-KO mice 4 h after a single SA or AICAR injection as well as GS (C) and GPhos protein content (D) in quadriceps muscle from WT and PGC-1α-KO mice 1 and 4 h after injections. Target mRNA content is normalized to the GAPDH mRNA content, and protein content is expressed as AU. Results are given relative to WT saline (1h for protein). Values are means ± SE; n = 5–6. (#)Tendency for significant difference between genotypes, 0.05 ≤ P < 0.1. Horizontal line indicates a main effect.
(GPhos) mRNA did not change, both GLUT4 and cyt c mRNA content tended to increase (0.05 < P < 0.1) 40–50% after a single AICAR treatment only in WT mice (Figs. 3, B–E, and 4, A and B). In addition, the FAT/CD36 mRNA content tended to increase (0.05 < P < 0.1) 70% in WT and decline (0.05 < P < 0.1) 40% in PGC-1α-KO mice 4 h after a single AICAR injection (Fig. 3F).

Whereas the HKII and COX-I mRNA content was similar in the two genotypes after a single saline injection, the mRNA levels of GLUT4 (P = 0.06) and cyt c (P < 0.05) were after AICAR injections lower, and FAT/CD36 mRNA content was after saline higher (P < 0.05) in muscles of PGC-1α-KO mice than of WT mice (Fig. 3, B–F). In addition, whereas the GS mRNA content was similar, the GPhos mRNA content tended to be higher (0.05 < P < 0.1) in PGC-1α-KO mice than in WT (Fig. 4, A and B).

Table 2. Effect of repeated AICAR treatment on mRNA content

<table>
<thead>
<tr>
<th>Gene</th>
<th>Saline</th>
<th>AICAR</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>WT</td>
<td>KO</td>
</tr>
<tr>
<td>HKII</td>
<td>1.00 ± 0.09</td>
<td>0.73 ± 0.12 (#)</td>
</tr>
<tr>
<td>GLUT4</td>
<td>1.00 ± 0.12</td>
<td>0.81 ± 0.09</td>
</tr>
<tr>
<td>COX-I</td>
<td>1.00 ± 0.06</td>
<td>0.34 ± 0.05#</td>
</tr>
<tr>
<td>Cyt c</td>
<td>1.00 ± 0.05</td>
<td>0.41 ± 0.05#</td>
</tr>
<tr>
<td>FAT/CD36</td>
<td>1.00 ± 0.16</td>
<td>0.40 ± 0.05#</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9–10. AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribonucleoside; WT, wild type; KO, knockout. HKII, GLUT4, COX-I, cyt c, and FAT/CD36 mRNA content in quadriceps muscle from WT and PGC-1α-KO mice after a 28-day program of saline or AICAR injections. Target mRNA content is normalized to the GAPDH mRNA content and given relative to WT saline. *Significantly different from saline within given genotype, P < 0.05; #significantly different from WT mice within given group, P < 0.05; (#)tendency for being significantly different from WT mice within given group, 0.05 ≤ P < 0.1.

**Fig. 5.** HKII (A–C) and GLUT4 protein content (D–F) in soleus, WG, and quadriceps of WT and PGC-1α-KO mice after a 28-day program of SA or AI injections. Protein content is expressed as AU and given relative to WT saline. Values are means ± SE; n = 9–10. *Significantly different from saline within that genotype, P < 0.05; #significantly different from WT mice within given group, P < 0.05. Horizontal line indicates main effect. Representative blots are shown in A–F, top.
Repeated AICAR treatment. To investigate whether repeated AMPK activation induces mRNA/protein expression through a PGC-1α-dependent mechanism, a repeated AICAR treatment experiment was performed.

Metabolic mRNA expression. Repeated AICAR treatment did not induce a significant change in the HKII, GLUT4, or cyt c mRNA content in quadriceps of either genotype but reduced (P < 0.05) the COX-I (30%) and FAT/CD36 (40%) mRNA content in quadriceps of WT mice (Table 2), showing that repeated AICAR treatment did not elicit a detectable increase in the mRNA content of these metabolic proteins 24 h after the last injection.

The COX-I and cyt c mRNA content was 50–60% lower (P < 0.05) and the HKII content ~30% lower (0.05 ≤ P < 0.1) in PGC-1α-KO mice than in WT mice after both repeated saline and AICAR treatments, whereas the FAT/CD36 mRNA content was 60% lower (P < 0.05) in PGC-1α-KO mice than in WT mice after repeated saline injections, and GLUT4 mRNA tended to be ~20% lower (0.05 ≤ P < 0.1) in PGC-1α-KO mice than in WT mice after AICAR treatment (Table 2).

Metabolic protein expression. Repeated AICAR treatment induced a similar 50–150% increase (P < 0.05) in HKII protein content in WG and quadriceps in the two genotypes compared with repeated saline treatment (Fig. 5, B and C). No changes were observed in HKII protein content in soleus with AICAR treatment in either genotype (Fig. 5A). GLUT4, COX-I, and cyt c protein expression increased (P < 0.05) 20–50% after repeated AICAR treatment in WG and quadriceps muscles of WT mice but not of the PGC-1α-KO mice (Figs. 5, E and F, and 6, B, C, E, and F). However, no changes were detected in cyt c, GLUT4 or COX-I protein expression in soleus after AICAR treatment (Figs. 5D and 6, A and D). The protein content of FAT/CD36 in quadriceps and WG muscles (not measured in soleus) did not change in response to repeated AICAR treatment (Fig. 7).

HKII and FAT/CD36 protein expression was independent of genotype, but COX-I and cyt c protein content was in all three muscles 20–100% lower (P < 0.05) in PGC-1α-KO mice than in WT. In soleus, GLUT4 protein expression was 10–20% lower (P < 0.05) in PGC-1α mice than in WT mice (Fig. 5, 6, and 7).

DISCUSSION

The main findings of the present study are that PGC-1α is required for repeated AICAR treatment-induced increases in GLUT4, cyt c, and COX-I protein expression in mouse skeletal muscle, supporting the observation that AMPK-mediated regulation of GLUT4 and mitochondrial proteins occurs through PGC-1α.
PGC-1α has been suggested to be an important factor in regulating exercise training-induced adaptations in mitochondrial proteins (10, 16, 30). Nevertheless, a recent study from our laboratory using whole body PGC-1α-KO mice demonstrated that PGC-1α is redundant in training-induced increases in expression of several mitochondrial proteins in skeletal muscles of young mice (18). But exercise is known to elicit numerous intracellular signaling cascades in skeletal muscle (8, 25, 31, 32, 34, 37), and furthermore, several unknown exercise-induced signaling pathways may exist. These pathways may exert compensatory effects when PGC-1α is not present (18). However, the current results showing that repeated AICAR treatment induced increases in muscle mitochondrial proteins as well as GLUT4 protein in WT mice but not in PGC-1α-KO mice do suggest that PGC-1α is required for AMPK-induced metabolic protein changes. In line with such an AMPK-PGC-1α signaling pathway are the present and the previous findings (14) that PGC-1α is required for a single AICAR treatment-induced increase in GLUT4 mRNA and cyt c mRNA expression in mouse skeletal muscle. AICAR is not a specific AMPK activator, and AICAR stimulation may exert AMPK-independent effects (9). However, the previous finding that repeated AICAR treatment upregulates mitochondrial proteins as well as GLUT4 protein expression in WT mice but not AMPKα2-KO mice (15) provides evidence that the AICAR-mediated effects are via AMPK. In addition, the observation that repeated treatment with β-guanadinopropionic acid (which reduces the intramuscular ATP/AMP ratio) increases mitochondrial content and PGC-1α mRNA expression in WT mice but not in transgenic mice expressing a dominant-negative mutant of AMPK in muscle (39) further supports that AICAR-induced increases in mitochondrial proteins and GLUT4 are through an AMPK-PGC-1α signaling pathway.

Although several studies have indicated that PGC-1α regulates skeletal muscle FAT/CD36 mRNA content (4) and that AICAR has been shown to induce FAT/CD36 protein in rat heart muscle (5), the present findings do not support the observation that PGC-1α is required for FAT/CD36 protein expression and that repeated AICAR treatment upregulates FAT/CD36 protein content in mouse skeletal muscle. However, the lower skeletal muscle FAT/CD36 mRNA content observed in PGC-1α-KO mice than in WT mice after 28 days of injections with saline and the increased FAT/CD36 mRNA content in response to a single AICAR injection only in WT mice are in accord with the previously reported higher FAT/CD36 mRNA levels in muscles of PGC-1α overexpression mice (4, 36). The different impact of PGC-1α on FAT/CD36 mRNA and protein expression indicates that additional regulatory mechanisms are involved, but this remains to be elucidated.

Interestingly, opposite of the findings in WG and quadriceps muscles, no changes were detected in metabolic proteins in soleus with repeated AICAR treatment, which suggests that oxidative fibers and glycolytic fibers display a different response to repeated AICAR treatment. However, it should be noted that these findings are in contrast to a previous report in rats (38), but these different observations may be due to species differences in muscle fiber characteristics.

Unlike the findings at the protein level, there was no detectable cumulative effect observed at the mRNA level of HKII, GLUT4, cyt c, or COX-I in either genotype after repeated AICAR treatment. Although this observation is in contrast to a previous study reporting that 6 days of AICAR treatment of mice (250 mg·kg⁻¹-day⁻¹ ip) increased the mRNA expression of 32 genes (determined by microarray analysis) encoding proteins involved in oxidative metabolism (26), it should be noted that muscles in that study were sampled only 4 h after the last AICAR injection, and thus no attempts were made to distinguish between the effect of single and repeated injections of AICAR.

The present observation that PGC-1α-KO mice had a higher p-AMPK Thr172 level than WT in both soleus and quadriceps 4 h after a single saline/AICAR treatment may be related to PGC-1α-KO mice exhibiting exaggerated responses to unexpected stimuli (20). Hence, it cannot be ruled out that the injections have agitated the PGC-1α-KO mice more than the WT mice and that the PGC-1α-KO mice were therefore more active in the cage than WT mice 4 h after the saline/AICAR injection. However, this is only speculative.

![Image](http://ajpendo.physiology.org/ by 10.220.33.5 on October 14, 2017)
The result that in vivo AICAR treatment increased the muscle glycogen content in WT mice is in agreement with previous studies in mice (1, 3, 11, 35, 38), and the observation that this increase is abolished when PGC-1α is absent supports previous indications that PGC-1α is implicated in glycogen use and storage (4, 23, 36). Thus, overexpression of PGC-1α leads to increased glycogen content in cell culture (23) and in mouse skeletal muscles (4, 36), and the postexercise replenishment of muscle glycogen is delayed in PGC-1α-KO animals compared with WT animals (18, 36). Several mechanisms may be implicated in the impaired ability to increase muscle glycogen when PGC-1α is lacking. For example, potentially a reduced glucose delivery related to a reduction in circulating plasma glucose levels, but no differences were evident in plasma glucose concentration between WT and PGC-1α-KO mice in the present study. In addition, the findings that GS mRNA and protein were similar in WT and PGC-1α-KO animals and that GPhos mRNA was higher in PGC-1α-KO mice are in accord with previous results in PGC-1α overexpression mice (4, 36). However, the similar GPhos protein content is unexpected based on the previous report of lower GPhos protein level in inducible PGC-1α overexpression mice (36), but this discrepancy may be due to the different mouse models. Thus, the similar protein expression of GLUT4, HKII, GS, and GPhos in WT and PGC-1α KO-animals indicates that the different resynthesis pattern of glycogen in the two genotypes after the acute AICAR treatment is not caused by differences in the content of these key proteins in glucose uptake, glycogen storage, and glycogen use. It may be speculated that acute regulation of GS and GPhos activity could be involved, but this remains to be determined. Alternatively, a reduced ability to spare glucose and consequently an increased glucose oxidation when PGC-1α is lacking may contribute to the observed glycogen level. Reduced expression of pyruvate dehydrogenase kinase-4 (PDK4), which is a negative regulator of glucose oxidation, might be involved in eliciting such change, because PGC-1α has been shown to stimulate the expression of PDK4 (35). However, because a single AICAR treatment has been shown to increase PDK4 mRNA expression in PGC-1α-KO mice, a PDK4-mediated inhibition of carbohydrate oxidation does not appear to be a likely explanation for the present observations either (14). Instead, a reduced ability to fat oxidation in PGC-1α-KO mice may prevent a normal increase in fat oxidation, leading to elevated carbohydrate use (4), and the concomitant lack of muscle glycogen increase after AICAR treatment in PGC-1α-KO mice.

In conclusion, the results show that PGC-1α is required for AICAR-induced expression of mitochondrial proteins and GLUT4 protein, suggesting that PGC-1α mediates AMPK-induced regulation of these proteins in response to metabolic perturbations such as exercise training. In addition, lack of PGC-1α attenuates an AICAR-induced increase in muscle glycogen.

ACKNOWLEDGMENTS

We thank Prof. B. Spiegelmann for providing the PGC-1α-KO mice initially to start breeding.

GRANTS

The study was supported by the Lundbeck Foundation, the Novo Nordisk Foundation, and the Danish Medical Research Council and the EXGENESIS consortium of the European Commission (LSHM-CT-2004-005272). The Centre of Inflammation and Metabolism was supported by Danish National Research Foundation (Grant no. 02-512-555).

DISCLOSURES

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

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