PGC-1α increases PDH content but does not change acute PDH regulation in mouse skeletal muscle

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The transcriptional coactivator peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)1α regulates the content of pyruvate dehydrogenase (PDH)-E1α and influences PDH activity through regulation of pyruvate dehydrogenase kinase-4 (PDK4) expression and subsequently PDH phosphorylation. PGC-1α whole body knockout (KO), muscle-specific PGC-1α overexpressing mice (MCK PGC-1α), and littermate wild-type (WT) mice underwent two interventions known to affect PDH. Quadriiceps muscles were removed from fed and 24-h fasted mice as well as at 6 h of recovery after 1-h running and from mice that did not run acutely. PDH-E1α protein content and PDH-E1α phosphorylation were lower in PGC-1α KO and higher in MCK PGC-1α mice at rest, but, while MCK PGC-1α had higher PDK4 protein content, KO of PGC-1α had no effect on PDK4 protein content. The differences in phosphorylation partly vanished when expressing phosphorylation relative to the PDH-E1α content with only a maintained elevated phosphorylation in MCK PGC-1α mice. Fasting upregulated PDK4 protein in PGC-1α KO, MCK PGC-1α and WT mice, but this was not consistently associated with increased PDH-E1α phosphorylation. Downregulation of the activity of PDH in the active form (PDHa) at 6 h of recovery from exercise in both the PGC-1α KO and MCK PGC-1α mice and the association between PDH-E1α phosphorylation and PDHa activity in PGC-1α KO mice indicate that PGC-1α is not required for these responses. In conclusion, PGC-1α regulates PDH-E1α protein content in parallel with mitochondrial oxidative proteins, but does not seem to influence PDH regulation in mouse skeletal muscle in response to fasting and in recovery from exercise.

The pyruvate dehydrogenase complex (PDC) is central in regulation of skeletal muscle substrate choice, as it catalyses the irreversible conversion of pyruvate to acetyl-CoA and thereby links glycolysis to carbohydrate oxidation. The activity of pyruvate dehydrogenase (PDH) is regulated by the phosphorylation level of PDH-E1α, which is determined by the upstream PDH kinases (PDK) and PDH phosphatases (PDP) (26). PDP and PDK are regulated by changes in mRNA and protein expression, as shown especially for PDK4 with marked upregulation in skeletal muscle in response to fasting, high-fat diet, and exercise (17, 31, 33, 36, 48). Besides regulation through changes in mRNA and protein expression, the activity of the kinases and phosphatases is also regulated. PDP1 activity is increased by an elevated intracellular Ca2+ concentration (10, 13), while PDK activity is enhanced by increased acetyl-CoA/CoA and NADH/NAD+ ratios via changes in the acetylation and reduction state of the lipooyl-subunit in the E2 subunit of the PDC (2, 40) and downregulated by elevated concentrations of pyruvate (43).

In accordance with the possibility that PGC-1α may affect substrate utilization, cell culture studies have indicated that PGC-1α regulates the expression of PDK4 via coactivation of the orphan nuclear receptor, estrogen-related receptor α (ERRα) (46, 51). Furthermore, elevated PDK4 mRNA and protein content have been reported in mice with chronic (5) and inducible (47) overexpression of PGC-1α in skeletal muscle, suggesting that PGC-1α may regulate substrate utilization in skeletal muscle via changes in PDK4 expression and concomitant regulation of PDH activity. However, the effects of PGC-1α on PDH regulation in skeletal muscle are not known. Therefore, the aim of the present study was to test the hypothesis that PGC-1α affects PDH regulation both by changes in total PDH-E1α content and by changes in PDH-E1α phosphorylation through regulation of PDK4 expression. This was
examined by analyzing PDH regulation in skeletal muscle of PGC-1α KO and MCK PGC-1α mice at rest and in response to fasting and exercise.

**RESEARCH DESIGN AND METHODS**

**Mice**

The generation and phenotype of the whole body PGC-1α KO and the MCK PGC-1α mice used in the present study have been described elsewhere (24, 25). For the PGC-1α KO strain, littermate PGC-1α KO and WT mice were obtained by crossing of heterozygote parents, while littermate MCK PGC-1α and WT mice were obtained by crossing a MCK PGC-1α and a WT parent. The genotypes of the mice were determined by PCR-based genotyping, as previously described (23). Mice were kept on a 11:13-h light-dark cycle and received standard rodent chow (Altromin no. 1324, Chr. Pedersen, Ringsted, Denmark). 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).

The mice were studied at an age of 3.5–4 mo and an equal number of males and females were included in each group. In the PGC-1α KO strain, the WT and PGC-1α KO mice weighed 24.8 ± 1.3 g and 21.3 ± 0.5 g, respectively, and in the MCK PGC-1α strain, WT and MCK-PGC-1α mice weighed 23.6 ± 2.1 g and 24.4 ± 1.3 g, respectively.

**Experimental Protocol**

This study consists of two parts, fasting and recovery from exercise, which were both conducted with the PGC-1α KO strain and the MCK PGC-1α strain.

**Fasting**. Mice were fasting for 24 h with ad libitum access to water (Fast), or having free access to both water and food (Fed). After the 24-h fasting period, the mice were anesthetized with 8% AM, by an intraperitoneal injection of pentobarbital, and quadriiceps muscles were quickly removed and frozen in liquid nitrogen. Samples were stored at −80°C until analyzed.

**Exercise recovery**. Mice were adapted to treadmill running (Exer 4 treadmill; Columbus Instruments, Columbus, OH) by 10 min of running on six consecutive days. Approximately 48 h after the last adaptation session, the mice performed a single 1-h bout at 14 m/min and a 10-degree incline. An air gun was used to encourage the mice to run, if necessary. Mice were killed by cervical dislocation 6 h into the recovery (6 h Post) or without performing the acute running bout (Rest). Quadriceps muscles were quickly removed and frozen in liquid nitrogen. Samples were stored at −80°C until analysis.

**Muscle Glycogen**

Muscle glycogen content was determined as glycosyl units after acid hydrolysis (28) by using an automatic spectrophotometer.

**RNA Isolation and Reverse Transcription**

RNA isolation was performed on ~18 mg muscle tissue with the guanidinium thiocyanate-phenol-chloroform method (7) with modifications (35). Reverse transcription was performed using the Superscript II RNase H− system (Invitrogen) as previously described (35) and was diluted in nuclease-free H2O.

**PCR**

Real-time PCR was performed with an ABI 7900 sequence-detection system (Applied Biosystems, Foster City, CA). Primers and TaqMan probes for amplifying gene-specific mRNA fragments were designed using the mouse-specific database from Ensembl (http://www.ensembl.org/index.html) and Primer Express (Applied Biosystems). All probes were 5′-FAM and 3′-TAMRA labeled, and primers and probes were obtained from TAG Copenhagen (Copenhagen, Denmark) and are shown in table 1. Real-time PCR was performed in triplicates in a total reaction volume of 10 μl using Universal Mastermix (Applied Biosystems). Cycle threshold (Ct) was converted to a relative amount by use of a standard curve constructed from a serial dilution of a pooled RT sample run together with the samples. Target gene mRNA content was for each sample normalized to single-stranded cDNA content determined by OliGreen reagent (Molecular Probes, Leiden, The Netherlands) as previously described (29).

**Antibodies**

The precursor PDH-E1α protein has a 29-long amino acid transit peptide, and when these amino acids are included in the sequence, the previously reported phosphorylation sites (18, 50) are found at Ser232 (site 3), Ser293 (site 1) and Ser300 (site 2). Ser232 (site 3) has been reported to be phosphorylated by PDK1 primarily expressed in heart muscle (4), while the two latter phosphorylation sites are thought to be the dominant in skeletal muscle (17, 19, 32). In addition to these three sites, a new phosphorylation site has been identified at PDH Ser295 (www.phosida.com), but to our knowledge the regulation of this site has never been examined.

Antibodies for phosphorylation of PDH Ser295 and PDK4 were made in sheep. The antibody against phosphorylation on PDH Ser295 was made using a phospho-peptide corresponding to amino acid 289–301 in the COOH-terminal part of the human PDH-E1α subunit (YHGHSMpSDPQVSY). The antibody for PDK4 was made using a peptide corresponding to amino acid 399–412 in the COOH-terminal part of the murine PDK4 (SREPKNLAKEKLAV). The peptides were synthesized with an additional cysteine at the NH2 terminus, coupled to keyhole limpet hemocyanin and used to immunize sheep as previously described (42).

Sera for the phospho-specific antibody were first passed through columns containing immobilized dephospho-peptides, and the antibodies specific for the phosphorylation site were then bound to columns containing immobilized peptides of the same phosphorylation site as the antigen used for immunization and eluted as previously described (42).

The phospho-specific PDH Ser295 antibody was tested for phospho specificity, and the antibody showed full phospho specificity (Fig. 1A).

<table>
<thead>
<tr>
<th>Gene</th>
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<th>Reverse Primer</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDK1</td>
<td>ATCCCCCGATTGAGTGTTCA</td>
<td>GTCGCCGGTTGACTCATCTTT</td>
<td>TCAGATCGTCTGCGCACTGGTG</td>
</tr>
<tr>
<td>PDK2</td>
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<td>CGGATCCAGCAATGCTCCGGA</td>
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<tr>
<td>PDK3</td>
<td>CGCTGCGCAGCTGTCTTCAAA</td>
<td>TGGAGAGAACATAGGAAAGT</td>
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<tr>
<td>PDK4</td>
<td>GAGAATATCGACACCAAATCTGGA</td>
<td>GTGTCGAGAAGATCTTTCG</td>
<td>CATCAGAAAGCACTTGGAGACTCTCAC</td>
</tr>
<tr>
<td>PDP1</td>
<td>GGGGCGACTGACACTCATCTGTT</td>
<td>ACATTGTGAGGCGCTCTTACT</td>
<td>AGTGCTCAAGAAGCACCCATGTGTTCTC</td>
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<td>GCCTGAGATTCTGAAGAGATTT</td>
<td>TGTACGCTCTTCGAGAAGTCTT</td>
</tr>
</tbody>
</table>

PDK, pyruvate dehydrogenase kinase; PDP, PDH phosphatase.

Table 1. Primer and TaqMan probe sequences (5′/3′) used for real-time PCR

References cited in this article include (4, 17, 19, 23, 24, 25, 27, 28, 32, 35, 37, 39, 40, 42).
PDH Ser\textsuperscript{295}

PDK4

Fig. 1. A: validation of pyruvate dehydrogenase (PDH)-E1α Ser\textsuperscript{295} antibody. Human muscle lysate was prepared in the presence or absence of phosphatase inhibitor (PI) and/or λ-phosphatase (λP) and was separated by SDS-PAGE. Dephosphorylated and phosphorylated PDH-E1α was detected by Western blot analysis using the described new antibody PDH-E1α Ser\textsuperscript{295}. MW mkt, molecular weight markers. B: validation of PDH kinase (PDK)4 antibodies. A His-tagged recombinant PDK4 protein expressed in Escherichia coli was used as positive control and loaded with a mouse muscle sample.

The antibody against PDK4 was tested using His-tagged recombinant PDK4 protein expressed in Escherichia coli as positive control (Fig. 1B).

Muscle Lysate

Crushed quadriceps muscles were homogenized in an ice-cold buffer (10% glycerol, 20 mM Na-pyrophosphate, 150 mM NaCl, 50 mM HEPES, 1% NP-40, 20 mM β-glycerophosphate, 10 mM NaF, 1 mM EDTA, 1 mM EGTA, 2 mM PMSF, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 2 mM Na\textsubscript{3}VO\textsubscript{4}, 3 mM benzamidine, pH 7.5) for 2 min using a TissueLyser (TissueLyser II; QIAGEN, Germany) with 30 oscillations/s. Homogenates were rotated end over end for 1 h at 4°C. Lysates were generated by centrifugation (16,000 g) for 20 min at 4°C. Protein content in lysates was measured by the bicinchoninic acid method (Pierce Chemical).

SDS-PAGE and Western Blot Analysis

PDH-E1α, PDK4, and cytochrome c protein expression as well as phosphorylation of PDH-E1α Ser\textsuperscript{293}, Ser\textsuperscript{295}, and Ser\textsuperscript{300} were measured in muscle samples by SDS-PAGE (10% or 15% Tris-HCl gel, BioRad, Hercules, CA) and Western blot analysis by using PVDF membrane and semidy transfer. After the transfer, the PVDF membrane was blocked 2 h at room temperature (TBST+2% skim milk) and then incubated with primary antibody (in TBST+2% skim milk) overnight. The following day, the membrane was incubated with horseradish peroxidase-conjugated secondary antibody (DAKO) for 1 h at room temperature (TBST+2% skim milk). Immobilon Western (Millipore, MA) was used as a detection system. Bands were visualized using an Eastman Kodak Image Station 2000MM. Bands were quantified using Kodak Molecular Imaging Software version 4.0.3, and protein content was expressed as arbitrary units relative to control samples loaded on each gel.

Commercially available antibodies were used to detect cytochrome c (cat. no. 556433; BD Biosciences). Protein levels of the PDH-E1α subunit and PDH-E1α phosphorylation at Ser\textsuperscript{293} and Ser\textsuperscript{300} were determined using in-house made antibodies as previously described (32), and PDK4 protein as well as phosphorylation of PDH-E1α Ser\textsuperscript{295} were determined using new in-house made antibodies as described above.

PDH Activity in the Active Form

The activity of PDH in the active form (PDHa) was determined as previously described (6, 8, 39) with modifications (32). Total creatine was determined fluorometrically (3), and the PDHa activity was adjusted to total creatine in each muscle sample as previously described (41).

Calculations and Statistics

As PDH may be regulated both by changes in the content of PDH-E1α and by changes in the phosphorylation state of PDH-E1α, data for the PDHa activity and phosphorylation on the three PDH-E1α sites are presented as both absolute values and relative to the total amount of PDH-E1α protein. To study the regulation of PDK4 expression relative to the PDH-E1α content, PDK4 protein is also presented as both absolute and relative to PDH-E1α. The absolute data may be considered the physiologically relevant measure, while the relative data reflect the regulation of the single PDH-E1α molecule in terms of PDH-E1α phosphorylation, PDK4 and PDHa activity.

Values presented are means ± SE. Two-way ANOVA was applied to evaluate the effect of genotype and either fasting or exercise within each mouse strain. The Student-Newman-Keuls’s post hoc test was used to locate differences. Differences were considered significant at P < 0.05, and a tendency is reported when 0.05 < P ≤ 0.1. Statistical calculations were performed using SigmaStat version 3.11.

RESULTS

Fasting

As metabolism shifts toward higher fat oxidation during fasting, the effect of 24-h fasting on muscle glycogen and PDH regulation was examined in MCK-PGC-1α and PGC-1α KO mice.

Muscle glycogen. The muscle glycogen concentration decreased (P ≤ 0.05) in quadriceps muscle in response to fasting in PGC-1α KO, MCK-PGC-1α, and WT littermate mice. The PGC-1α KO mice had lower (P ≤ 0.05) muscle glycogen level after fasting than WT, and MCK PGC-1α mice had higher (P ≤ 0.05) muscle glycogen content than WT, both in the fed and the fasted condition (Tables 2 and 3).

PDK and PDP mRNA expression. As expected, a pronounced upregulation (P ≤ 0.05) of PDK4 mRNA content was observed in both genotypes and their WT littermates in response to 24 h of fasting, whereas the pattern for the mRNA content...
content of PDK1–3 was less clear. Surprisingly, an upregulation (P ≤ 0.05) of the PDP2 mRNA was evident in response to fasting (Tables 4 and 5).

**PDH-E1α content.** There was no effect of fasting on the PDH-E1α protein content in any of the examined mice. But PGC-1α KO mice had a lower (P ≤ 0.05) level of PDH-E1α protein in quadriceps than WT, and accordingly, MCK PGC-1α mice had a higher (P ≤ 0.05) level than WT littermates (Figs. 2 and 6A).

**PD4 protein.** PD4 protein was increased (P ≤ 0.05) in PGC-1α KO and WT mice in response to fasting. The PD4 protein to PDH-E1α protein ratio was higher (P ≤ 0.05) in PGC-1α KO than WT mice in the fasted state (Figs. 3A and 6A). In the MCK PGC-1α strain, PD4 protein was upregulated (P ≤ 0.05) in both WT and MCK PGC-1α mice in response to fasting. When normalizing the PD4 protein to PDH-E1α protein, the PD4 protein content was lower (P ≤ 0.05) in MCK PGC-1α mice than in WT in the fasted state (Figs. 3B and 6A).

**PDH-E1α phosphorylation.** Fasting only affected PDH-Ser293 in the PGC-1α KO mice, where phosphorylation was higher (P ≤ 0.05) after fasting, but when normalizing phosphorylation to PDH-E1α protein, both PDH-Ser293 (0.05 < P ≤ 0.1) and PDH-Ser293 (P ≤ 0.05) were higher in fasted PGC-1α KO mice than fed PGC-1α KO mice. In the WT, only PDH-Ser293 was more (P ≤ 0.05) phosphorylated after fasting than in the fed state. Absolute phosphorylation of these three phosphorylation sites was lower (P ≤ 0.05) in PGC-1α KO than in WT in both the fed and fasted states, but when expressed relative to the amount of PDH-E1α protein, these differences largely disappeared (Figs. 3A and 6A).

In the MCK PGC-1α strain, PDH-Ser293 was in WT mice more (P ≤ 0.05) phosphorylated after fasting than in the fed state, while the MCK PGC-1α mice showed no response to fasting. Phosphorylation was higher (P ≤ 0.05) in MCK PGC-1α than WT on all three sites at both time points, and in the fed state, phosphorylation remained higher (P ≤ 0.05) after normalizing to PDH-E1α protein (Figs. 3B and 6A).

**Pyruvate dehydrogenase activity.** Fasting reduced (P ≤ 0.05) the PDHa activity in WT mice of the PGC-1α KO strain. The PGC-1α KO mice had a lower (P ≤ 0.05) PDHa activity than WT, both in the fed and fasted state. When the PDHa activity was related to PDH-E1α protein content, there was no genotype differences (Fig. 3A).

A tendency for an overall decrease (0.05 < P ≤ 0.1) in PDHa activity was observed in the MCK-PCG-1α strain in response to fasting. The same overall pattern was also evident when normalizing the PDHa activity to the PDH-E1α content. PDHa activity tended to be higher (0.05 < P ≤ 0.1) in MCK PGC-1α mice than in WT, but when the PDHa activity was related to PDH-E1α protein content, there was no genotype difference (Fig. 3B).

**Exercise Recovery**

Because recovery from endurance exercise is characterized by increased fat oxidation, ensuring glycogen restoration, muscle glycogen and PDH regulation were examined in skeletal muscle of PGC-1α KO and MCK PGC-1α mice at 6 h of recovery from exercise.

**Muscle glycogen.** Quadriceps muscle glycogen content was lower (P ≤ 0.05) at 6 h Post than at Rest in both strains and independent of genotype. MCK PGC-1α mice had higher (P ≤ 0.05) muscle glycogen content than WT at both time points (Table 2).

**PDK and PDP mRNA expression.** The PD4 mRNA content increased (P ≤ 0.05) 6 h Post relative to Rest in both the PGC-1α KO and MCK PGC-1α mice. Changes in the mRNA content of the other PDKs and PDPs were less pronounced or absent (Table 3).

**PDH-E1α content.** PGC-1α KO mice had a lower (P ≤ 0.05) PDH-E1α protein content and PDH-E1α phosphorylation than WT, and MCK PGC-1α mice had a higher (P ≤ 0.05) PDH-E1α protein content and PDH-E1α phosphorylation than WT KO mice. In the WT, only PDH-E1α phosphorylation was higher (P ≤ 0.05) after fasting, but when normalizing the PDHa activity to the PDH-E1α content, PDHa activity tended to be higher (0.05 < P ≤ 0.1) in MCK PGC-1α mice than in WT, but when the PDHa activity was related to PDH-E1α protein content, there was no genotype difference (Fig. 3B).

**Table 4.** Muscle glyogen during exercise recovery

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>KO</th>
<th>WT</th>
<th>KO</th>
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<tr>
<td></td>
<td>REST</td>
<td>6 h POST</td>
<td>REST</td>
<td>6 h POST</td>
</tr>
<tr>
<td>Glycogen</td>
<td>20.4 ± 1.0</td>
<td>14.8 ± 0.8*</td>
<td>19.1 ± 1.8</td>
<td>13.4 ± 1.0*</td>
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Values are means ± SE (mmol/kg wet wt). The muscle glycogen content in quadriceps muscles from PGC-1α KO and WT littermates as well as from MCK PGC-1α mice and WT littermates in the fed state and after 24 h of fasting. The mRNA content is normalized to the content of single-stranded DNA. *Significantly different from FED, P ≤ 0.05; †significantly different from WT littermates at given time point, P ≤ 0.05.
WT. No effect of exercise was evident on PDH-E1α protein content (Figs. 4 and 6B).

**PDK4 protein.** Exercise did not affect PDK4 protein content significantly at 6 h Post in any of the groups, but PDK4 protein normalized to PDH-E1α protein was higher (P ≤ 0.05) at 6 h Post than at Rest in the PGC-1α KO mice (Figs. 5A and 6B).

The PDK4 protein/PDH-E1α level was higher in PGC-1α KO mice than in WT at Rest (0.05 < P ≤ 0.1) and at 6 h Post (P ≤ 0.05). In the MCK PGC-1α strain, absolute PDK4 protein content was higher (P ≤ 0.05) in MCK PGC-1α than WT mice, but PDK4 protein normalized to PDH-E1α protein was lower (P ≤ 0.05) in MCK PGC-1α than WT (Figs. 5B and 6B).

**PDH-E1α phosphorylation.** In the PGC-1α KO strain, exercise increased (P ≤ 0.05) PDH-Ser293 and PDH-P295 phosphorylation in WT mice and PDH-Ser293 and PDH-Ser300 phosphorylation in PGC-1α KO mice at 6 h Post. PDH-Ser300 and PDH-P295 phosphorylation was lower (P ≤ 0.05) in PGC-1α KO than WT, but this difference partly disappeared when expressing PDH-E1α phosphorylation relative to the amount of PDH-E1α protein (Figs. 5A and 6B).

No effect of exercise on PDH-E1α phosphorylation was evident in the MCK-PGC-1α strain, while MCK PGC-1α mice had a higher (P ≤ 0.05) phosphorylation than WT mice (Figs. 5B and 6B).

**PDHa activity.** In the PGC-1α KO strain, quadriceps PDHa activity tended to increase (0.05 < P ≤ 0.1) in WT mice, but was reduced (P ≤ 0.05) in PGC-1α KO mice at 6 h Post relative to Rest. When normalizing PDHa activity to PDH-E1α protein, this downregulation (P ≤ 0.05) in response to exercise was still evident in the PGC-1α KO mice. PDHa activity was lower (P ≤ 0.05) in PGC-1α KO mice than in WT at 6 h Post, but when normalizing PDHa activity to PDH-E1α protein, PGC-1α KO mice had a higher (P ≤ 0.05) PDHa activity than WT at Rest (Fig. 5A).

In the MCK PGC-1α mice, the PDHa activity was reduced (P ≤ 0.05) at 6 h Post relative to Rest. In addition, MCK PGC-1α mice had higher (P ≤ 0.05) PDHa activity than WT at Rest, and PDHa activity normalized to total PDH-E1α was lower (P ≤ 0.05) in MCK PGC-1α mice than WT both at Rest and 6 h Post (Fig. 5B).

**Basal oxidative protein content.** To compare the PDH-E1α protein content with the content of mitochondrial oxidative proteins, cytochrome c protein content was measured in quadriceps of both mouse strains. At basal state cytochrome c protein content was ~50% lower (P ≤ 0.05) in the PGC-1α KO mice than in WT, while MCK-PGC-1α mice had a three-fold higher (P ≤ 0.05) cytochrome c protein level than the WT littermates.

**DISCUSSION**

The main findings of the present study are that PGC-1α expression regulates both the total amount of PDH-E1α as well as the phosphorylation state and activity of PDH in skeletal muscle at rest. However, fasting- and exercise-induced PDH regulation in skeletal muscle does not require PGC-1α.

The present findings that the PDH-E1α protein content in skeletal muscle was elevated in MCK PGC-1α mice and reduced in PGC-1α KO mice provide evidence that PGC-1α regulates the expression of PDH-E1α in skeletal muscle. Moreover, the similar changes in PDH-E1α protein and cytochrome c protein content in each of the strains suggest a PGC-1α-mediated coordinated regulation of these mitochondrial proteins. These observations indicate that when the PGC-1α content is chronically modified in skeletal muscle, the capacity for carbohydrate oxidation follows the general changes in mitochondrial oxidative enzymes (5, 11, 23–25, 47). This is in accordance with the previous finding that the respiratory exchange ratio value during maximal performance is > 0.9 in MCK PGC-1α mice, reflecting predominant carbohydrate oxidation (5).

The observed higher phosphorylation level of PDH-E1α in MCK PGC-1α mice and lower phosphorylation in PGC-1α KO mice in the resting state is overall in accordance with the higher and lower content of PDH-E1α, respectively, in these mice.
However, the observed elevated PDHa activity in the MCK PGC-1α mice relative to WT indicates that despite the markedly higher PDH-E1α phosphorylation in skeletal muscle of MCK PGC-1α mice, more dephosphorylated PDH-E1α molecules were still present in the MCK PGC-1α muscles than in WT. Likewise, the similar PDHa activity in PGC-1α KO and WT mice in the exercise recovery experiment, although the phosphorylation level was markedly reduced in PGC-1α KO, suggests that an equal number of PDH-E1α molecules were in the dephosphorylated state, corresponding to a larger proportion of the total PDH-E1α molecules in PGC-1α KO than in WT mice. Although a previous study has indicated that increased PDHa activity is not necessarily associated with increased flux through the PDC during exercise (9), the present observation is expected to reflect that at rest PGC-1α KO mice maintain the level of carbohydrate flux into the mitochondria.

Fig. 3. Content of PDK4 protein, phosphorylation level of PDH-E1α Ser293, PDH-E1α Ser295, and PDH-E1α Ser300, as well as the activity of PDH in the active form (PDHa) in quadriceps muscles from PGC-1α KO and WT littermates as well as from MCK PGC-1α mice and WT littermates in the fed state and after 24 h of fasting. The results are both represented as absolute values (A) and relative to the total amount of PDH-E1α (B). Protein content and phosphorylation are presented as arbitrary units. Values are means ± SE. *Significantly different from Fed, \( P \leq 0.05 \); †significantly different from WT littermates at given time point, \( P \leq 0.05 \). Symbols in brackets indicate a tendency for a significant difference, \( 0.05 < P \leq 0.10 \).
as WT mice, despite the lower total PDH capacity. However, the reduced PDHa activity in the PGC-1α KO mice relative to WT in the fasting experiment shows that maintenance of PDHa activity in skeletal muscle, when PGC-1α is lacking, depends on the specific circumstances. It cannot be ruled out that this may be related to the euthanization method used, but could also potentially be the death time of the day, with related effects of circadian rhythm where PGC-1α has been suggested to play a role (27).

Because total phosphorylation and PDHa activity will be determined by a combination of the total number of PDH-E1α molecules and the proportion of these being phosphorylated, a further understanding of the regulation of PDH by PGC-1α may be obtained by expressing PDH-E1α phosphorylation and PDHa activity relative to the PDH-E1α content. The findings that skeletal muscle PDH-E1α phosphorylation relative to PDH-E1α protein content consistently remained higher in MCK PGC-1α than WT mice indicate that PGC-1α overexpression increases the proportion of the available PDH-E1α molecules being phosphorylated. These observations suggest that high PGC-1α levels change the balance between phosphorylation and dephosphorylation of PDH, which might be through PGC-1α-mediated regulation of PDK4 expression as previously reported in C2C12 cell culture studies (46).

However, the present observation that PGC-1α-associated modification of PDK4 protein content only was observed in the MCK PGC-1α mice in the exercise recovery experiment indicates that PGC-1α does not consistently regulate PDK4 protein expression. This is further supported by the findings that PDK4 protein content relative to the content of PDH-E1α was reduced in MCK PGC-1α mice and increased in PGC-1α KO mice. Taken together, PGC-1α-mediated regulation of PDK4 expression cannot be the sole reason for the observed PGC-1α-associated regulation of PDH-E1α phosphorylation. Based on the present mRNA analyses it may be speculated that PGC-1α-mediated regulation of PDK2, known to be relatively high expressed in skeletal muscle (4), could play an additional role, although the genotype associated PDK2 mRNA differences only reached statistical significance for the PGC-1α KO strain in the fasting experiment. Such a possibility for PGC-1α-mediated PDK2 regulation is in line with a previous study showing that PDK2 protein increased in human skeletal muscle with exercise training (21). In addition, the observed PGC-1α related PDH-E1α phosphorylation may also be through effects on PDK activity, as PDK activity is known to be influenced by the reduction and acetylation state of the PDH-E2 subunit as well as by the pyruvate concentration in the cell (12). Hence it cannot be ruled out that PGC-1α-associated modifications of metabolic intermediates can mediate such changes in PDH-E1α phosphorylation and PDHa activity.

The present findings that fasting increased skeletal muscle PDK4 protein content and lowered the PDHa activity in WT mice are in accordance with previous studies showing an upregulation of PDK4 expression and/or downregulation of the PDHa activity in rodent and human skeletal muscle in response to fasting (37, 44, 48, 49). But the fasting-induced upregulation of PDK4 protein in both PGC-1α KO and MCK PGC-1α mice as observed in the present study has not previously been reported and shows that PGC-1α is neither necessary nor does PGC-1α prevent the fasting-induced regulation of PDK4 protein content in skeletal muscle. This is in line with the previous observations that fasting-induced PDK4 upregulation is regulated through PPARα (48, 49) and that PGC-1α-mediated PDK4 regulation is through ERRα and not PPARα (46). However, the finding that fasting reduced the PDHa activity in WT, but not PGC-1α KO mice, suggests that PGC-1α ablation impairs the fasting-induced downregulation of PDHa activity, and thus potentially, the normal switch from carbohydrate to fat metabolism as usually observed. In line with this suggestion is the observed larger muscle glycogen use in the PGC-1α KO mice than in WT, but the lower fasting PDHa activity level in PGC-1α KO than in WT indicates that carbohydrate oxidation was smaller in the PGC-1α KO than WT mice. Together this may reflect that the PGC-1α KO mice were unable to remove all of the produced pyruvate via the TCA cycle, leading to a relatively larger anaerobic glycolysis in PGC-1α KO mice than in WT mice in the fasted state.

The observation that fasting-induced PDK4 mRNA and protein increases and PDP1 mRNA reduction was not associated with increased PDH-E1α phosphorylation was unexpected (12). However, changes in PDK activity may have occurred and in addition the finding that PDP2 mRNA, in contrast to previous findings (14, 20), increased in response to fasting in the present study, may indicate that the increased PDK content was balanced by an increased PDP content and concomitantly leading to unchanged phosphorylation state. Furthermore, the lack of association between fasting-induced changes in PDHa activity and PDH-E1α phosphorylation state also indicates that phosphorylation of these sites are not the only determinant of the PDHa activity at rest as previously stated (15, 36).

Previous studies have shown that exercise induces a marked upregulation of skeletal muscle PDK4 mRNA in recovery from exercise (35, 36) and increased PDK4 protein content specifically at 6 h of recovery in humans (16), and it has been suggested that such regulation plays a role in sparing carbohydrate for glycogen rebuilding in the recovery phase (34). As PGC-1α mRNA expression and potentially PGC-1α activity are increased in the early part of recovery (30) and in vitro studies have shown that PGC-1α can regulate the expression of PDK4 through ERRα (46), exercise-induced PGC-1α regulation may play a role in the exercise-induced PDK4 expression. However, the present findings that the similar increase in PDH-E1α phosphorylation in PGC-1α KO and WT mice at 6

![Graph showing content of PDH-E1α protein in quadriceps muscles from PGC-1α KO and WT littermates before (Rest) and 6 h after (6 h Post) 1 h of treadmill running. Values are means ± SE. *Significantly different from WT littermates at given time point, p ≤ 0.05.](http://ajpregu.physiology.org/)
h of recovery only was associated with increased PDK4 protein expression in the PGC-1α KO mice do not support that PGC-1α is required for exercise-induced PDK4 protein expression and PDH-E1α phosphorylation. Similarly, the observation that the increased PDH-E1α phosphorylation at 6 h of recovery was associated with reduced PDHa activity only in PGC-1α KO mice further supports that PGC-1α is not needed for exercise-induced regulation of PDHa activity in recovery. These findings may even indicate that PGC-1α interferes with an associated regulation of PDK4 expression, PDH-E1α phosphorylation, and PDHa activity. A discrepancy between exercise-induced PDK4 expression and PDH-E1α phosphorylation may be due to changes in expression of the other PDKs or the PDPs or by changes in PDK activity as observed in response to...
acutely prolonged exercise and exercise training (21, 45). Furthermore, the observed exercise-induced downregulation of PDHa activity in MCK PGC-1α mice, without changes in PDH-E1α phosphorylation indicates that high-PGC-1α expression influences PDHa activity in recovery from exercise by other means than changing the phosphorylation state of PDH-E1α.

**Perspectives and Significance**

PGC-1α increases the expression of PDH-E1α approximately to the same extent as other mitochondrial proteins involved in oxidative metabolism, and PGC-1α has an impact on the basal level of PDH-E1α phosphorylation with an increased level when PGC-1α expression is high and a reduced level when PGC-1α is lacking. Still high-PGC-1α expression is associated with elevated resting PDHa activity reflecting that carbohydrate oxidation is maintained with PGC-1α overexpression. However, PGC-1α does not appear to affect fasting and exercise-induced PDH regulation in mouse skeletal muscle. Thus taken together, the observed PGC-1α-mediated effects on PDH regulation appear mainly to be a consequence of modifications in the amount of PDH-E1α protein.

Because the elevated PDH-E1α phosphorylation in MCK PGC-1α mice could not be explained by increased PDK4 protein, the potential role of changes in PDK activity and/or expression of other PDK’s should be elucidated in future studies.

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