Role of PGC-1α in exercise and fasting-induced adaptations in mouse liver

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Haase TN, Ringholm S, Leick L, Bienso RS, Küllrich K, Johansen S, Nielsen MM, Wojtaszewski JF, Hidalgo J, Pedersen PA, Pilegaard H. Role of PGC-1α in exercise and fasting-induced adaptations in mouse liver. Am J Physiol Regul Integr Comp Physiol 301: R1501–R1509, 2011. First published August 10, 2011; doi:10.1152/ajpregu.00775.2010.—The transcriptional coactivator peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α plays a role in regulation of several metabolic pathways. By use of whole body PGC-1α knockout (KO) mice, we investigated the role of PGC-1α in fasting, acute exercise and exercise training-induced regulation of key proteins in gluconeogenesis and metabolism in the liver. In both wild-type (WT) and PGC-1α KO mice, the mRNA content of the gluconeogenic proteins glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) was upregulated during fasting. Pyruvate carboxylase (PC) remained unchanged after fasting in WT mice, but it was upregulated in PGC-1α KO mice. In response to a single exercise bout, G6Pase mRNA was upregulated in both genotypes, whereas no significant changes were detected in PEPCK or PC mRNA. While G6Pase and PC protein remained unchanged, liver PEPCK protein content was higher in trained than untrained mice of both genotypes. The mRNA content of the mitochondrial proteins cytochrome c (Cyt c) and cytochrome oxidase (COX) subunit I was unchanged in response to fasting. The mRNA and protein content of Cyt c and COXI increased in the liver in response to a single exercise bout and prolonged exercise training, respectively, in WT mice, but not in PGC-1α KO mice. Neither fasting nor exercise affected the mRNA expression of antioxidant enzymes in the liver, and knockout of PGC-1α had no effect. In conclusion, these results suggest that PGC-1α plays a pivotal role in regulation of Cyt c and COXI expression in the liver in response to a single exercise bout and prolonged exercise training, which implies that exercise training-induced improvements in oxidative capacity of the liver is regulated by PGC-1α.

Glucose; oxidative proteins; antioxidant enzymes

The Liver is the Major Organ responsible for maintaining plasma glucose levels during prolonged exercise and fasting, which is accomplished by increased hepatic glucose production through elevated hepatic glycolysis and gluconeogenesis. The key gluconeogenic proteins phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate carboxylase (PC), as well as glucose-6-phosphatase (G6Pase), which plays an equally important role in gluconeogenesis and glycogenolysis are thought to be controlled at the transcriptional level. In accordance, the activity of PEPCK, PC, and G6Pase increases during exercise (11, 12) and fasting (23, and it has been demonstrated that the mRNA expression of PEPCK, PC, and G6Pase is upregulated in rodent liver during fasting and/or after a single bout of exercise (3, 9, 18, 20, 29). Several studies have investigated the effects of exercise training on hepatic mRNA and protein expression in rodents (1, 5, 6, 10, 24, 39). For example, endurance exercise training has been shown to increase hepatic glucose release from rat liver perfused in situ with glucagon (13), and this was suggested to be caused by an improved sensitivity to glucagon due to a higher hepatic glucagon receptor density after training (24). However, an increased capability for glucose production with exercise training could also originate from an exercise training-induced increase in gluconeogenic enzymes such as PEPCK, PC, and G6Pase, but this remains to be validated.

The transcriptional coactivator peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α potentially coordinates the expression of proteins that control diverse metabolic pathways in response to metabolic challenges (2, 28, 34). PGC-1α has been suggested to play a pivotal role in the control of gluconeogenesis in the liver (18, 35, 40), coactivating several transcription factors, including hepatocyte nuclear factor (HNF)-4α, forkhead box O1A, and the glucocorticoid receptor, to increase the expression of PEPCK and G6Pase (17, 35, 36). The mRNA expression of PGC-1α in mouse liver has also been demonstrated to be upregulated after a single bout of exercise (20) and with fasting (17, 18, 40), suggesting that PGC-1α mediates the previously reported exercise and/or fasting-induced changes in PEPCK, PC, and G6Pase expression (3, 9, 18, 20, 29). But whether PGC-1α is mandatory in exercise-induced gene responses of gluconeogenic proteins in the liver is not known.

In addition, exercise training has been demonstrated to increase the expression of rodent liver mitochondrial proteins (5) and antioxidant defense-related proteins, such as SOD 2 (6, 10), suggesting that the liver exhibits similar adaptations to regular physical activity as skeletal muscle (4, 15). However, the molecular mechanisms controlling the transcriptional responses of mitochondrial and antioxidant defense proteins in the liver in response to acute interventions, such as exercise and fasting, are still not fully understood. In skeletal muscle, PGC-1α seems to play a dispensable role in the regulation of...
mitochondrial proteins, such as cytochrome c (Cyt c) and cytochrome oxidase (COX) subunit I, in response to exercise (26), because exercise raised the mRNA and protein levels of these proteins in both wild-type (WT) and PGC-1α knockout (KO) mice, although at a lower level in PGC-1α KO mice. Whether this is the case in the liver is not known.

The aim of the present study was to test the hypothesis that 1) fasting and a single bout of exercise induce the mRNA expression of gluconeogenesis-related proteins, as well as mitochondrial proteins in the liver through a PGC-1α-dependent mechanism and 2) PGC-1α is required for exercise training-induced regulation of gluconeogenic and mitochondrial proteins in mouse liver.

MATERIALS AND METHODS

Mice

Experiments for the present study were approved by the Danish Animal Experimental Inspectorate. The study used PGC-1α whole body KO mice (Mus musculus Linnaeus, 1758) and littermate WT mice produced by intercross breeding of heterozygous parents. This mouse strain has previously been described (28). During the experimental period, the mice were housed individually in cages with a 11:13-h light-dark cycle and with free access to standard chow (Altromin nr. 1324, Chr. Pedersen, Ringsted, Denmark) and water.

Experimental Protocols

The present study investigates the role of PGC-1α in both acute and long-term adaptations. It consists of three experimental protocols: two acute (fasting and a single exercise bout) and one prolonged intervention (exercise training). Results from acute exercise- and exercise training-induced regulation in skeletal muscle have previously been published from these experiments (26).

Fasting

Food was removed from PGC-1α KO and littermate WT mice (n = 8 in each group), and the animals were anesthetized after 24 h fasting by an intraperitoneal injection of pentobarbital sodium (6 mg of pentobarbital/100 g body wt). The liver was removed and quickly frozen in liquid nitrogen, and later used for mRNA determinations, which is especially relevant for evaluating effects of such an acute intervention.

Adaptation to Exercise

Prior to both the single treadmill running bout and the treadmill exercise training period, all mice were acclimatized to treadmill exercise 10 min per day (Exer 4 treadmill; Columbus Instruments, Columbus, OH) on three consecutive days. Each 10-min exercise period consisted of 5 min at 8 m/min and 5 min at 14 m/min, with a constant slope of 10%. Running on the treadmill was encouraged by an air pistol when needed.

Single Exercise Bout

Forty-eight hours after the end of adaptation to exercise, PGC-1α KO and littermate WT mice performed a single treadmill running bout at 14 m/min at 10% slope for 1 h. The mice were killed by cervical dislocation immediately (0 h), 2 h after running, while mice not run acutely served as controls (n = 8 in each group). Livers were quickly removed, frozen in liquid nitrogen, and later used for mRNA determinations, which is especially relevant for evaluating effects of acute interventions. The mice were killed between 4 and 6 PM, with food removed 2 h before sacrificing, except for the 2-h group, which because of the running procedure experienced 3 h without food before being killed. The exercise training protocol has previously been described (26).

Exercise Training

Another group of PGC-1α KO and littermate WT mice (n = 16 in each group) performed 5 wk of exercise training (treadmill + wheel running). Treadmill running was performed at 14 m/min and 10% slope for 1 h per day for 5 days each week, and the running distance on the running wheels was controlled [measured by a computer (Sigma Sport, Neustadt, Germany)], so that WT and PGC-1α KO mice completed a similar distance. The total wheel running duration per day was 106 ± 10 min for male WT, 98 ± 13 min for male KO, 92 ± 8 min for female WT, and 88 ± 13 min for female KO. The mice were killed by cervical dislocation 36 h after the last training bout to avoid acute effects of the last exercise bout, and with untrained mice serving as controls. The liver was quickly dissected out and frozen in liquid nitrogen for later determination of protein levels, which is especially relevant for evaluating effects of such a prolonged intervention. The mice were killed between 8 and 10 AM, and food was removed 2 h before. The exercise training protocol has previously been described (26).

Liver Glycogen and Plasma Glucose

Liver glycogen and plasma glucose in the exercise protocol were determined as previously described (31) by using a Fluoroscan (Thermo Scientific, Finland) with liver glycogen content determined as glycosyl units after acid hydrolysis. Plasma glucose in the fasting experiment was determined using Contour glucose strips (Bayer Diabetes Care, Stockholm, Sweden).

RNA Isolation and Reverse Transcription

RNA isolation was performed on 25–30 mg liver tissue by the phenol-chloroform extraction method (8) with modifications (32). The final pellet was resuspended in 0.1 mM EDTA in DEPC water (5 μl/mg liver tissue). A total of 3 μg RNA was reverse transcribed using the SuperScript II (Invitrogen, Carlsbad, CA) and oligo dT, as previously described (32).

Real-Time PCR

The amount of specific mRNAs was quantified by fluorescence-based real-time PCR (ABI7900; Applied Biosystems, Foster City, CA) using Taqman probes. Primers and Taqman probes to amplify a specific fragment of the mRNAs were designed using Primer Express (Applied Biosystems). Primer and Taqman probe sequences are given in Table 1. The primers and probes were optimized as previously described (33). The PCR was performed in triplicates, as previously described (30). The amount of specific mRNA was normalized to total cDNA content determined by Oligreen reagent (Molecular Probes, Leiden, The Netherlands), as previously described (30).

Western Blot Analysis

Extraction of protein from liver tissue was performed in cold lysis buffer [10% glycerol, 20 mM Na-pyrophosphate, 150 mM NaCl, 50 mM HEPES, 1% NP-40, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM PMSF, 1 mM EDTA, 1 mM EGTA 10 μg/ml aprotinin, 10 μg/ml leupeptin, 2 mM NaN3, 3 mM benzamidin (pH 7.5)]. The protein content of the lysate samples was determined by the bicinchoninic acid method (Pierce, Rockford, IL).

Liver lysate proteins were separated using Tris-HCl gels (Bio-Rad, Stockholm, Sweden), and transferred (semi-dry) to PVDF membranes (Immobilon Transfer Membrane, Millipore A/S, Copenhagen, Denmark) and standard Western blotting procedures were used for detection of specific proteins. Following detection and quantification using a charge-coupled device-image sensor and 1D software (Kodak Image Station, 2000MM; Kodak, Brondby, Denmark), the protein content...
Table 1. Primer and probe sequences used in real time PCR

<table>
<thead>
<tr>
<th>Primer/Probe</th>
<th>Gene Sequences</th>
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<tbody>
<tr>
<td>PGC-1α Forward primer</td>
<td>5’AGCGAACAAGAAGAGGTTGTTCG 3’</td>
</tr>
<tr>
<td>PGC-1α Reverse primer</td>
<td>5’TCAAGATCGTGGATCAAGATTTCTTCG 3’</td>
</tr>
<tr>
<td>PGC-1α Probe</td>
<td>5’AGAGTACCAAAGTGGAGGTTGGTCG 3’</td>
</tr>
<tr>
<td>G6Pase Forward primer</td>
<td>5’TGGCCATGTCGACGTTTCTA 3’</td>
</tr>
<tr>
<td>G6Pase Reverse primer</td>
<td>5’TGGCCATGTCGACGTTTCTA 3’</td>
</tr>
<tr>
<td>G6Pase Probe</td>
<td>5’AGGCGAACAAGAAGAGGTTGTTCG 3’</td>
</tr>
<tr>
<td>SOD1 Forward primer</td>
<td>5’GGCCGATGGAAAGGAAGCGA 3’</td>
</tr>
<tr>
<td>SOD1 Reverse primer</td>
<td>5’TCCACAGATCTCCATAGCCACA 3’</td>
</tr>
<tr>
<td>SOD1 Probe</td>
<td>5’TGGCGGAAGGAGGGCTGCC 3’</td>
</tr>
<tr>
<td>Cyt c Forward primer</td>
<td>5’TGCCACTAGTCGACGTTTCTA 3’</td>
</tr>
<tr>
<td>Cyt c Reverse primer</td>
<td>5’TGCCACTAGTCGACGTTTCTA 3’</td>
</tr>
<tr>
<td>Cyt c Probe</td>
<td>5’ACCTGATGCTGTTTGGACAATGG 3’</td>
</tr>
<tr>
<td>Coxi Forward primer</td>
<td>5’TGGAAATGCAAGATCCGATGTCAGTCG 3’</td>
</tr>
<tr>
<td>Coxi Reverse primer</td>
<td>5’TGGAAATGCAAGATCCGATGTCAGTCG 3’</td>
</tr>
<tr>
<td>Coxi Probe</td>
<td>5’ACCTGATGCTGTTTGGACAATGG 3’</td>
</tr>
<tr>
<td>Catalase Forward primer</td>
<td>5’TGGAAATGCAAGATCCGATGTCAGTCG 3’</td>
</tr>
<tr>
<td>Catalase Reverse primer</td>
<td>5’TGGAAATGCAAGATCCGATGTCAGTCG 3’</td>
</tr>
<tr>
<td>Catalase Probe</td>
<td>5’ACCTGATGCTGTTTGGACAATGG 3’</td>
</tr>
<tr>
<td>Gpx1 Forward primer</td>
<td>5’GACGGTGTTGCTGCTGTTGCTG 3’</td>
</tr>
<tr>
<td>Gpx1 Reverse primer</td>
<td>5’GACGGTGTTGCTGCTGTTGCTG 3’</td>
</tr>
<tr>
<td>Gpx1 Probe</td>
<td>5’AATCGAGTTGCAAGACGACAAGAGACG 3’</td>
</tr>
<tr>
<td>Gpx4 Forward primer</td>
<td>5’GTGGGAGAAGACCGTTTCTA 3’</td>
</tr>
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<td>Gpx4 Reverse primer</td>
<td>5’GTGGGAGAAGACCGTTTCTA 3’</td>
</tr>
<tr>
<td>Gpx4 Probe</td>
<td>5’GTGGGAGAAGACCGTTTCTA 3’</td>
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was expressed in arbitrary units relative to control samples loaded in duplicates on each separate gel. Primary antibodies used for Western blot analysis were Cyt c (no. 556433; BD Biosciences, San Jose, CA), COXI (no. 459600; Invitrogen), SOD2 (no. 06–984, Upstate, Lake Placid, NY), PEPCK (no. 10004943; Cayman Chemical, Ann Arbor, MI), G6Pase (no. sc-27198 (c14), Santa Cruz Biotechnology, Santa Cruz, CA) PC (no. 2500845; Sigma–Aldrich, St. Louis, MO), AMPK phosphorylation Thr172 (no. 2535S; Cell Signaling Technology, Beverly, MA), and AMPK α2 (kindly donated by D. G. Hardie, University of Dundee, Dundee, UK).

Production of Recombinant Mouse G6Pase in E. coli

A full-length G6Pase cDNA clone (# 432711) for mammalian expression was purchased from Invitrogen. Using Gateway technology, we initially transferred the cDNA to pDONOR221 (Invitrogen) and subsequently to the E. coli expression vector pET-DEST42 (Invitrogen). Recombinant G6Pase was produced in BL21 (DE3) (Invitrogen) grown in LB medium at 30°C after addition of 0.5 mM IPTG and 3% ethanol at OD450 = 0.5.

Statistics

The data are presented as means ± SE. The effect of fasting and genotype, acute exercise and genotype, as well as exercise training and genotype on mRNA and protein content, and of fasting and acute exercise on plasma glucose and liver glycogen was tested with the use of a two-way ANOVA test. The effect of acute exercise was also tested separately for each genotype by a one-way ANOVA test. When a main effect was detected, a Student Newman-Keuls post hoc test was performed to localize significant differences. P < 0.05 is used as a significance level, and a tendency is reported when 0.05 ≤ P ≤ 0.1 (actual P values are shown on graphs).

RESULTS

Fasting

Liver glycogen and plasma glucose. To confirm that animals were metabolically challenged, liver glycogen content and plasma glucose levels were measured. The liver glycogen content was reduced (P < 0.05) ~60% in WT mice and ~70% in PGC-1α KO mice in response to 24-h fasting relative to fed animals (Table 2). No genotype difference was evident.

Similarly plasma glucose was reduced (P < 0.05) ~45% in WT mice and ~33% in PGC-1α KO mice in response to 24-h fasting relative to fed animals (Table 2). In addition, the fasting plasma glucose concentration was 30% higher (P < 0.05) in PGC-1α KO mice than in WT.

PGC-1α. Fasting increased the liver PGC-1α mRNA content 2.5-fold (P < 0.05) in WT mice (Fig. 1A).

G6Pase, PEPCK, and PC. Fasting induced a ~2-fold increase in liver G6Pase mRNA content in WT mice (P < 0.05) and tended to in PGC-1α KO mice (P = 0.095). No significant difference was found in G6Pase mRNA content between genotypes in either state (Fig. 1B).

The liver PEPCK mRNA content increased (P < 0.05) 5- to 6-fold with fasting in both genotypes, with no significant difference between genotypes (Fig. 1C).

Liver PC mRNA content was unchanged in WT mice in response to fasting, whereas PC mRNA content in PGC-1α KO mice increased ~60% (P < 0.05) in response to fasting (Fig. 1D). There was a tendency for a main genotype difference (P = 0.052) in the PC mRNA content.

Cyt c and COXI. Fasting did not affect the liver Cyt c mRNA content in either genotype, and there was no genotype difference in the Cyt c mRNA content (Fig. 1E).

Table 2. Liver glycogen content and plasma glucose levels in fed and 24-h fasted wild-type and PGC-1α knockout mice

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>PGC-1α KO</th>
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<tr>
<td>Liver glycogen, mmol/kg liver</td>
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</tr>
<tr>
<td>Fed</td>
<td>308.0 ± 20.6</td>
<td>343.6 ± 15.8</td>
</tr>
<tr>
<td>Fast</td>
<td>122.4 ± 25.7*</td>
<td>110.8 ± 23.5*</td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>6.333 ± 0.289</td>
<td>6.786 ± 0.350</td>
</tr>
<tr>
<td>Fast</td>
<td>3.422 ± 0.291*</td>
<td>4.514 ± 0.310*#</td>
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Values are expressed as means ± SE; n = 7–8. WT, wild-type; KO, knockout. *Significantly different from Fed within given genotype, P < 0.05. #Significantly different from WT at given time point, P < 0.05.
Similarly, fasting did not affect the liver COXI mRNA content in either genotype, and no genotype difference was observed (Fig. 1F).

Antioxidant enzymes. Fasting did not change the liver mRNA content of uncoupling protein (UCP) 2, SOD1, SOD2, catalase, glutathione peroxidase (Gpx) 1, and Gpx4 in either genotype, and no significant genotype difference was evident in these mRNAs (data not shown).

Single Exercise Bout

Liver glycogen and plasma glucose. To confirm that animals were metabolically challenged by the single exercise bout intervention, liver glycogen content and plasma glucose levels were measured. The liver glycogen content was reduced ($P < 0.05$) $\sim$14% in WT mice and $\sim$40% in PGC-1α KO mice in response to the single exercise bout relative to resting animals (Table 3). The liver glycogen content immediately after acute exercise was $\sim$40% lower ($P < 0.05$) in PGC-1α KO mice than in WT.

The single exercise bout induced no statistically significant difference in plasma glucose immediately after exercise compared with before in either genotype (Table 3). However, the plasma glucose concentration was $\sim$20% lower ($P < 0.05$) in PGC-1α KO mice relative to WT mice immediately after exercise.

AMPK phosphorylation. The level of AMPK phosphorylation (AMPK phosphorylation relative to total AMPKα2) was unchanged immediately after the single exercise bout in both WT mice ($0.85 \pm 0.09$ before and $0.94 \pm 0.06$ after exercise) and PGC-1α KO mice ($0.98 \pm 0.07$ before and $0.85 \pm 0.02$ after exercise). No genotype difference was found.

PGC-1α. A single exercise bout induced no statistically significant change in liver PGC-1α mRNA content (Fig. 2A).

G6Pase, PEPCK, and PC. The acute exercise bout increased ($P < 0.05$) liver G6Pase mRNA content 9.5 fold in WT mice and 4.5-fold in PGC-1α KO mice immediately after exercise.

Table 3. Liver glycogen content and plasma glucose levels in WT and PGC-1α KO mice at rest (pre) and immediately after exercise (0 h)

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>PGC-1α KO</th>
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<tr>
<td><strong>Liver glycogen, mmol glycogen/kg liver</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>277.2 ± 29.3</td>
<td>248.0 ± 23.1</td>
</tr>
<tr>
<td>0 h</td>
<td>240.4 ± 18.3*</td>
<td>151.2 ± 28.0#</td>
</tr>
<tr>
<td><strong>Plasma glucose, mmol/l</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>7.64 ± 0.34</td>
<td>6.97 ± 0.4</td>
</tr>
<tr>
<td>0 h</td>
<td>8.67 ± 0.52</td>
<td>6.91 ± 0.7#</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; $n = 7$ or 8. *Significantly different from pre within given genotype, $P < 0.05$. #Significantly different from WT at given time point, $P < 0.05$. 

Fig. 1. The mRNA content of PGC-1α (A), G6Pase (B), PEPCK (C), PC (D), Cyt c (E), and COXI (F) in liver from wild-type (WT) and PGC-1α knockout (KO) in fed (Fed) and 24-h fasted (Fast) mice. Target mRNA content is normalized to total cDNA. Values are expressed as means ± SE; $n = 6–8$. *Significantly different from Fed within given genotype, $P < 0.05$. The $P$ value shown in B indicates the difference from Fed within KO. Horizontal line indicates a tendency for a main genotype effect ($P$ value shown) in D.
However, no significant difference was evident in G6Pase mRNA content between genotypes. The G6Pase mRNA content returned to resting level at 2 h of recovery in both genotypes (Fig. 2B).

Application of a two-way ANOVA did not reveal any effect of acute exercise on the liver PEPCK mRNA content in either genotype. But a Student’s t-test revealed a difference (P < 0.05) in PEPCK mRNA content between resting mice and immediately after exercise in WT mice. In addition, there was a tendency for a main genotype difference (P = 0.089) in the PEPCK mRNA content (Fig. 2C).

PC mRNA content remained unchanged in response to a single exercise bout in both genotypes, and no genotype difference was observed at any time point (Fig. 2D).

Cyt c and COXI. The acute exercise bout induced a 40% increase (P < 0.05) in liver Cyt c mRNA content immediately after exercise in WT mice, but not in PGC-1α KO mice, giving rise to a difference between genotypes (P < 0.05). In both genotypes, no differences were observed in liver Cyt c mRNA content at 2 h and 6 h of recovery relative to resting mice (Fig. 2E).

Liver COXI mRNA content increased (P < 0.05) 45% immediately after exercise in WT mice, but not in PGC-1α KO mice. This gave rise to a tendency for a difference between genotypes (P = 0.073) immediately after exercise. In both genotypes, no differences were observed in liver COXI mRNA content at 2 h and 6 h of recovery relative to resting mice (Fig. 2F).

**Antioxidant enzymes.** The acute exercise bout did not affect the liver UCP2, SOD1, SOD2, catalase, Gpx1, or Gpx4 mRNA content in either genotype, and no significant genotype difference was observed in these mRNAs (data not shown).

**Exercise Training**

**G6Pase, PEPCK, and PC.** G6Pase protein content in the liver remained unchanged with prolonged exercise training in both genotypes, and no genotype difference was evident in G6Pase protein content either in untrained or trained mice (Figs. 3A and 4A). Band specificity of G6Pase was verified by use of a recombinant G6Pase protein as a positive control (Fig. 4B). The two bands detected for G6Pase were analyzed together and used for protein quantification.

In response to exercise training, PEPCK protein content was ~40% higher (P < 0.05) in trained WT mice and tended to be 30% higher (P = 0.1) in PGC-1α KO mice than in the corresponding untrained mice. Furthermore PEPCK protein content was 45% higher (P < 0.05) in untrained PGC-1α KO mice than in untrained WT mice, and tended to be 20% higher (P = 0.058) in trained PGC-1α KO mice than in trained WT mice (Figs. 3B and 4A).
PC protein content in the liver remained unaffected by prolonged exercise training in both genotypes, and no genotype difference was evident in PC protein content either in untrained or trained mice (Figs. 3C and 4A).

Cyt c and COXI. Cyt c protein content increased (P < 0.05) 30% with exercise training in WT, but with no change in PGC-1α KO mice, resulting in 35% lower (P < 0.05) Cyt c protein content in trained PGC-1α KO mice than trained WT mice (Figs. 3D and 4A). COXI protein content increased (P < 0.05) 25% with exercise training in WT, but with no change in PGC-1α KO mice. There was, however, no significant genotype difference in either untrained or trained mice (Figs. 3E and 4A).

Antioxidant enzymes. SOD2 protein content remained unchanged with prolonged exercise training in both genotypes, and no genotype difference was evident in the untrained or in the trained mice (Figs. 3F and 4A).

DISCUSSION

The main findings of the present study are that exercise training increased Cyt c, COXI, and PEPCK protein in mouse liver, and while the PEPCK response was independent of PGC-1α, the changes in Cyt c and COXI required PGC-1α. In accordance, acute exercise increased liver Cyt c and COXI mRNA content only in WT mice, while G6Pase mRNA increased in both genotypes. Together, this indicates that PGC-1α is required for exercise training-induced adaptations of mitochondrial oxidative proteins in mouse liver. In addition, PGC-1α KO mice had lower liver glycogen and plasma glucose content after acute exercise than WT mice, suggesting that the PGC-1α KO mice relied more on carbohydrates than WT mice during exercise.

One aim of the present study was to investigate the role of PGC-1α in fasting and exercise-induced mRNA and protein expression of metabolic proteins in the liver. The present observation that WT mice increased Cyt c and COXI protein content in the liver with exercise training is in accordance with a previous study showing that prolonged exercise training increased liver mitochondrial enzyme activities and proteins (5). But here, we show for the first time that PGC-1α is required for both acute exercise to induce an increase in Cyt c and COXI mRNA and for training to upregulate Cyt c and COXI protein content in the liver. A previous study from our laboratory revealed that exercise training increased Cyt c and COXI protein content in skeletal muscle of young mice at least partly independent of PGC-1α (26). However, the present findings show that PGC-1α is mandatory for the training-induced adaptations in Cyt c and COXI in the liver and hence...
potentially, in general, for oxidative proteins in the liver. These observations support previous studies demonstrating the necessity of PGC-1α for regulation of metabolism and energy homeostasis (14, 27).

Our results also show for the first time that prolonged exercise training increased PEPCK protein content, but not PC and G6Pase protein in the liver, while the finding that G6Pase mRNA increased much more potently than PEPCK in response to a single exercise bout is in accordance with previous studies (3, 9, 18, 20). Both PEPCK and G6Pase are important enzymes in gluconeogenesis, while G6Pase also is a key enzyme in glycogenolysis. These findings may therefore together suggest that acute exercise is associated with more marked induction of enzymes in glycogenolysis than of enzymes in gluconeogenesis, while prolonged exercise training improves gluconeogenic capacity.

PGC-1α has been suggested to function as a transcriptional coactivator for the expression of the gluconeogenic enzymes G6Pase and PEPCK (37, 40). However, the present observation that the G6Pase mRNA content in the liver increased after a single exercise bout, as well as after 24-h fasting in both WT and PGC-1α KO mice, shows that PGC-1α is not obligatory for the regulation of G6Pase mRNA in response to fasting and exercise. Similarly, the present finding of a similar exercise training-induced increase in PEPCK protein in WT and PGC-1α KO mice does not support that PGC-1α plays a role in the training adaptations in PEPCK. Likewise, the observed increase in hepatic PEPCK mRNA content in both WT and PGC-1α KO mice after 24 h of fasting demonstrates, that PGC-1α is not required for this response. This finding is somewhat in contrast to previous studies showing that whole body PGC-1α KO mice had elevated PEPCK mRNA in the fed state and no further change upon fasting (17, 28), but the reason for this difference between the previous and the current finding is not clear. Interestingly, the fasting-induced increase in PC mRNA content in PGC-1α KO mice but not in WT mice, as well as the higher PEPCK protein content in PGC-1α KO mice in the exercise training study, may indicate that lack of PGC-1α leads to a compensatory increase in the expression of PC and PEPCK and thus potentially an elevated hepatic glucose production. The observation that the fasting plasma glucose concentration was higher in PGC-1α KO mice than in WT mice is in accordance with this suggestion. A possible reason for an increased need for hepatic glucose output could be the reduced ability of skeletal muscle to oxidize fat. Hence, muscle-specific PGC-1α overexpression has been shown to elevate the mRNA expression of proteins important for fatty acid oxidation and transport in muscle (7, 38), while knockout of PGC-1α has been shown to reduce the mRNA expression of proteins in fat metabolism (16, 25). A reduced ability for fat oxidation in skeletal muscle of PGC-1α KO mice, may, therefore, lead to elevated carbohydrate use and a higher demand of glucose derived from the liver in these mice. In line with this possibility are the present findings that hepatic glycogen content in WT mice was reduced only ~14% in response to a single exercise bout, but ~40% in PGC-1α KO mice, and that plasma glucose was lower in PGC-1α KO mice than WT mice immediately after exercise. An increased demand of hepatic glucose could also explain the compensating increase in the gluconeogenic protein PEPCK in the PGC-1α KO mice observed in the exercise study.

The combined fasting and exercise results in the present study suggest that more than one signaling pathway is implicated in fasting and exercise-induced PEPCK, PC, G6Pase, COXI, and Cyt c regulation in the liver. Hence, the present observations show that signaling pathways leading to regula-
tion of gluconeogenic proteins in the liver do not require PGC-1α, while PGC-1α is obligatory for exercise and exercise training-induced increases in Cyt c and COXI mRNA and protein content, respectively. Because exercise has been shown to increase AMPK phosphorylation in mouse liver (22), and in vitro studies have demonstrated that PGC-1α is a downstream target for AMPK (21), an AMPK-PGC-1α pathway could be a possible player in the observed exercise-induced increases in Cyt c and COXI mRNA in the liver in WT mice. However, the lack of significant changes in the level of AMPK phosphorylation after acute exercise in the present study does not support that AMPK was involved in mediating the observed responses to acute exercise.

No effect of exercise or fasting on antioxidant enzymes was detected in either genotype in this study, suggesting that these interventions do not affect antioxidant enzyme expression in the liver. In addition, the present finding that a lack of PGC-1α does not affect the basal mRNA and protein level of the examined antioxidant enzymes is different from previous findings in skeletal muscle (25), suggesting tissue-specific PGC-1α dependency in regulation of antioxidant enzyme expression.

The present findings also demonstrate that the exercise-induced mRNA responses in the liver are fast and peak immediately after a single exercise bout with resting levels restored already 2 h after the exercise stops. This rapid induction in hepatic mRNA expression was also observed in previous studies (3, 20); however, we add to these previous findings by providing a time course of hepatic mRNA expression after a single exercise bout. Thus, the kinetics of exercise-induced hepatic mRNA responses diverge from exercise-induced mRNA responses of metabolic proteins observed in muscle tissue, which have been shown typically to peak some hours into recovery (19, 26, 32).

In conclusion, the present study shows that PGC-1α is mandatory for acute exercise and exercise training-induced increases in Cyt c and COXI mRNA and protein expression in the liver. However, PGC-1α is not required for fasting, acute exercise, and/or exercise training-induced regulation of the gluconeogenic proteins PEPCK and PC or the gluconeogenic and glycolysis enzyme G6Pase. Furthermore, the divergent responses of the liver in response to exercise and fasting suggest that although similar adaptations are needed, different signaling pathways are involved.

**Perspectives and Significance**

The current finding that an exercise training-induced increase in mitochondrial oxidative protein content in the liver is impaired in PGC-1α KO mice, adds to the evidence that decreased level of this transcriptional coactivator could play a role in the mitochondrial impairment and decreased ability to cope with metabolic stress observed in obesity and type 2 diabetes patients. Understanding the actions and functions of PGC-1α may conceivably contribute to future treatment strategies of metabolic diseases.

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