Rapid exercise-induced changes in PGC-1α mRNA and protein in human skeletal muscle

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Mathai AS, Bonen A, Benton CR, Robinson DL, Graham TE. Rapid exercise-induced changes in PGC-1α mRNA and protein in human skeletal muscle. J Appl Physiol 105: 1098–1105, 2008. First published July 24, 2008; doi:10.1152/japplphysiol.00847.2007.—The mRNA of the nuclear coactivator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) increases during prolonged exercise and is influenced by carbohydrate availability. It is unknown if the increases in mRNA reflect the PGC-1α protein or if glycogen stores are an important regulator. Seven male subjects [23 ± 1.3 yr old, maximum oxygen uptake (VO2 max) 48.4 ± 0.8 ml·kg−1·min−1] exercised to exhaustion (∼2 h) at 65% VO2 max followed by ingestion of either a high-carbohydrate (HC) or low-carbohydrate (LC) diet (7 or 2.9 g·kg−1·day−1, respectively) for 52 h of recovery. Glycogen remained depressed in LC (P < 0.05) while returning to resting levels by 24 h in HC. PGC-1α mRNA increased both at exhaustion (3-fold) and 2 h later (6.2-fold) (P < 0.05) but returned to rest levels by 24 h. PGC-1α protein increased (P < 0.05) 23% at exhaustion and remained elevated for at least 24 h (P < 0.05). While there was no direct treatment effect (HC vs. LC) for PGC-1α mRNA or protein, there was a linear relationship between the changes in glycogen and those in PGC-1α protein during exercise and recovery (r = −0.68, P < 0.05). In contrast, PGC-1β did not increase with exercise but rather decreased (P < 0.05) below rest levels at 24 h, and the decrease was greater (P < 0.05) in LC. PGC-1α protein content increased in prolonged exercise and remained upregulated for 24 h, but this could not have been predicted by the changes in mRNA. The β-isofrom declined rather than increasing, and this was greater when glycogen was not resynthesized to rest levels.

carbohydrate; gene expression; glucose; insulin; training signals; metabolism

EXERCISE stimulates transcription of various metabolic genes during exercise and in the subsequent recovery period. However, exercise-induced gene transcription is transient, usually returning to preexercise levels within 24 h following exercise cessation (35, 41). Since peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) was first identified as a regulator of adaptive thermogenesis (43), it has emerged as an important protein in regulating the coordinated response of metabolic gene expression in various tissues, including liver, heart, and skeletal muscle. PGC-1α is implicated in promoting gene expression and muscle morphology characteristic of type I oxidative fibers in skeletal muscle (26). It also plays a role in the regulation of mitochondrial biogenesis (23, 57). Furthermore, PGC-1α mediates upregulation of enzymes involved in lipid utilization and β-oxidation (9, 13, 33), promotes glucose metabolism through upregulating hepatic gluconeogenic genes (15, 44, 59), regulates the PDK-4 gene in rat hepatocytes and cardiac myocytes (28), and increases glucose uptake in L6 muscle cells (32).

Recently, it was shown that PGC-1α mRNA expression was induced by exercise in both rat and human skeletal muscle. Due to the rapid activation of PGC-1α gene transcription and increase in mRNA abundance in skeletal muscle following exercise (36, 42, 48), it has been suggested that PGC-1α may be important in coordinating the transcription of genes following a single exercise bout. In addition, PGC-1α appears to be intimately involved with skeletal muscle adaptations that occur with chronic muscle contraction (17) or exercise training (29, 46). In rat skeletal muscle, PGC-1α mRNA (3) and protein (3, 52) abundance were rapidly increased following two 3-h bouts of swimming exercise. Similarly, a rapid exercise-induced upregulation of PGC-1α mRNA has been demonstrated in human skeletal muscle (12, 36, 41, 47, 48). However, while Russell et al. (46) found that both the mRNA and protein were increased in human muscle after 6 wk of endurance training, two studies that examined human muscle after a single bout of exercise (30, 54) failed to find an increase in PGC-1α protein, although one (54) did observe an increase in PGC-1α mRNA. Thus measures of PGC-1α mRNA do not necessarily reflect PGC-1α protein changes in human muscle.

In addition to exercise, intracellular signaling pathways that mediate metabolic gene upregulation appear to be sensitive to substrate availability. Several studies have demonstrated that specific metabolic genes are upregulated to a greater extent during or following exercise when the initial muscle glycogen content is low (11, 19, 39), suggesting that glycogen acts as a signal to enhance or attenuate gene expression. In addition, recent studies have also shown that substrate availability and glycogen repletion during the recovery period influence exercise-induced gene transcription (2, 41). The increase in PGC-1α mRNA abundance does not appear to be associated with altered free fatty acid (FFA) and/or glucose concentrations, either during exercise or in the first 3–4 h after exercise (12, 47). However, Pilegaard et al. (41) observed that maintaining PGC-1α mRNA upregulation was dependent on limiting carbohydrate (CHO) ingestion during the postexercise recovery phase over a 5-day training period. This suggested that substrate availability, such as skeletal muscle glycogen, might influence PGC-1α mRNA abundance.

Yet, whether intramuscular PGC-1α protein content is altered in response to muscle glycogen remains to be shown. In addition, the relationship between PGC-1α protein and muscle glycogen may be complex. There are large interindividual differences in both muscle glycogen (1) and in skeletal muscle PGC-1α protein (38; Graham and Bonen, unpublished data). Hence, it is possible

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that changes in PGC-1α are most closely related to the interindividual changes in muscle glycogen, such are observed during and after exercise.

The PGC-1β isoform has been less studied, but it too appears to be implicated in the regulation of the transcription of genes of oxidative metabolism. However, there are a number of examples where the response of their mRNAs or that of the proteins themselves do not correspond temporally, qualitatively, or quantitatively (16, 20, 31, 34).

In the present study we examined 1) the effects of exhaustive exercise (~2 h) and 2) the effects of altered changes in muscle glycogen repletion (0–52 h) after exhaustive exercise on PGC-1α mRNA as well as PGC-1α protein in skeletal muscle of healthy men. We also compared the responses of the α- and β-isoforms. The following hypotheses were examined: 1) that exhaustive exercise would induce the upregulation of PGC-1α mRNA and protein abundances, 2) that these changes would be related to the depletion of muscle glycogen, 3) that the restoration of glycogen during the postexercise recovery period would attenuate the upregulation of PGC-1α mRNA and protein, and 4) that the two isoforms would not respond in the same manner. To alter muscle glycogen after exercise, subjects were provided either with a low- or high-CHO diet. This enabled us to examine whether changes in PGC-1α were related to the absolute glycogen concentration or whether the changes in PGC-1α were more closely associated with changes in muscle glycogen.

MATERIALS AND METHODS

Subjects

Seven recreationally active men [age 23 ± 1.3 yr old, height 1.80 ± 0.02 m, mass 78.6 ± 2.6 kg, body mass index 24.3 ± 0.1 kg/m², maximum oxygen uptake (VO_2max) 48.4 ± 0.8 mL·kg⁻¹·min⁻¹] participated in the study after giving their informed written consent. The study conformed to the standards set by the Declaration of Helsinki. The study was approved by the University of Guelph Research Ethics Board.

Preliminary Testing

Prior to the experimental trials, each subject underwent VO_2max tests on a stationary, electronically braked cycle ergometer (Lode Instrument, Groningen, The Netherlands). The fractions and volume of O₂ and CO₂ in the expired air were measured using metabolic measurements systems (Vmax Series 29 and 229, Sensormedics, Yorba Linda, CA); from the data obtained, VO_2max was calculated. To control the muscle glycogen stores, 2 days before each of the two trials, subjects performed 1 h of cycling exercise at 65% VO_2max. Subsequently, the subjects were provided with prepackaged meals that were standardized to supply 2,600 kcal (57% CHO, 18% protein, 19% fat) on the day before the trial. They refrained from consuming alcoholic and caffeinated products and refrained from strenuous exercise until the end of the trials.

Experimental Protocol

Each subject completed two trials, separated by 2 wk, in which the CHO consumption during the recovery period was assigned by randomized crossover design. On experimental days, subjects ate a standardized breakfast (440 kcal: CHO 65%, protein 16%, fat 19%) 2 h before exercise commencement. Following breakfast, a venous catheter was placed percutaneously into a medial antecubital vein and was kept patent by infusion of saline. Subjects cycled on a stationary cycle ergometer at 65% VO_2max until voluntary exhaustion. This exercise intervention was repeated on the second test day, and subjects were instructed to cycle for the same length of time on both days. Respiratory variables were taken at 15-min intervals during exercise to ensure that subjects were cycling at appropriate intensities. Each subject cycled on the same cycle ergometer (Lode Excalibur, Groningen, The Netherlands; or Monark 894 E, Vansbro, Sweden) for both trials. Antecubital venous blood was drawn from a catheter at rest, at 30-min intervals during exercise, during the first 2 h of recovery, and by venipuncture at 24 h and 52 h of recovery.

Using a percutaneous needle muscle biopsy technique (7) with suction, muscle biopsies were taken from the vastus lateralis under local anesthetic at rest, exhaustion (Exh), and at 2, 24, and 52 h after exercise. Immediately following the Exh biopsy and again 1 h later, subjects consumed either 1 g CHO/kg body wt [high-CHO trial (HC)], provided in the form of a maltodextrin-dextrose solution (Gatorlode, Gatorade, Chicago IL), or an equivalent volume of water [low-CHO trial (LC)]. Following the 2-h time point, subjects were provided with meals for the remaining 50-h recovery period. Subjects consumed either a HC (7 g CHO/kg body wt, 1 g protein/kg body wt, 0.8 g fat/kg body wt per day) or LC diet (2.9 g CHO/kg body wt, 1.4 g protein/kg body wt, 2.4 g fat/kg body wt per day) during the recovery period. HC provided a daily macronutrient composition of 72% CHO, 10% protein, and 18% fat while LC averaged a daily macronutrient composition of 30% CHO, 14% protein, and 56% fat.

Analysis

Blood samples. Whole blood lactate and glucose concentrations were determined by autoanalyzer (YSI 2300 STAT Plus, Yellow Springs, OH). In addition, nonheparinized blood samples were allowed to clot at room temperature and subsequently were centrifuged at 1,340 g for 10 min. Serum was stored at −20°C until analyzed for concentrations of fatty acids (NEFA C kit, Wako Chemicals, Richmond VA) and insulin (Coat-a-count, Diagnostics Products, Los Angeles CA).

Muscle samples. The muscle samples (n = 5 per subject in each of 2 experimental trials) were dissected free of connective tissue and fat, weighed, frozen in liquid N₂, and stored at −80°C until subsequent isolation of glycogen (<1.5–3 mg dry wt), protein (<40–60 mg wet wt), and total RNA (<25–50 mg wet wt). RNA measurements were only performed on the 10 biopsies from each of four subjects, due to limitations in tissue availability in some of the muscle samples from the other three subjects.

Muscle glycogen. Muscle glycogen was analyzed as previously described (1). Briefly, 1.5–3 mg freeze-dried tissue was powdered and separated into acid-insoluble (proglycogen) and acid-soluble (macroglycogen) fractions by addition of 1.5 M perchloric acid. Following the hydrolysis of glycogen with HCl in each of these two fractions, the enzymatic method of Bergmeyer et al. (6) was used to measure glucosyl units. Total glycogen was calculated as the sum of pro- and macroglycogen.

Muscle homogenization and immunoblotting. Proteins were isolated from the muscles, separated using SDS/PAGE, and the presence of PGC-1α and -β were detected (band sizes of approximately 98 and 113 kDa, respectively) using Western blotting procedures as previously described (5, 16). Equal quantities of protein were loaded into each lane for each muscle, and a common standard was included in all blots. In addition, variations in protein loading were normalized based on Ponceau-S stains. Commercially available antibodies for the PGC-1 isoforms (Calbiochem, La Jolla, CA, and Abnova, Hornby, Ontario, Canada, respectively) were employed and were visualized on film (Kodak, Fisher Scientific, Ontario, Canada) using chemiluminescent detection reagents (Amersham Biosciences, Oakville, Canada), and band densities were determined using the ChemiGenius2 Bioimaging system (SynGene, Perkin-Elmer, Woodbridge, Ontario, Canada) as per manufacturer’s directions. These routine procedures have been described by us previously (5, 8, 10, 27).
Real-time RT-PCR. RNA was isolated from muscle using Trizol reagent (Invitrogen, Burlington, Ontario, Canada). Approximately 25–50 mg of frozen muscle was added to 1 ml of ice-cold Trizol and homogenized for two 10-s bursts at 15,000 rpm using a Polytron 3100 homogenizer (Kinematica, Littau, Switzerland). Homogenates were centrifuged at 12,000 g for 10 min at 4°C to pellet cellular debris. Chloroform (200 µl) was added to the supernatant fraction and shaken vigorously for 15 s. The aqueous and organic phases were separated by centrifugation at 12,000 g for 15 min. The aqueous phase was removed, and 600 µl of isopropanol was added. After mixing thoroughly, the solution was added to an RNAeasy Mini spin column (Qiagen, Mississauga, Ontario, Canada), and RNA was isolated as per manufacturer’s instructions. DNase (Qiagen) treatment was performed according to the manufacturer’s instructions. Invitrogen, Burlington, Ontario, Canada. Approximately 1100 µg of RNA in a total reaction step of 10 min at 4°C was added to the supernatant fraction and shaken for 10 min at 4°C to pellet cellular debris. Glucose, Fatty Acids, and Insulin

Blood glucose, serum free fatty acids (FFA), and plasma insulin concentrations were summarized in Table 1. Exercise resulted in similar blood glucose and insulin concentrations during both trials. Ingestion of 1 g CHO/kg body wt at the start of recovery and again 1 h later resulted in significant increases in blood glucose and insulin concentrations (P < 0.05) within 30 min and throughout the first 2 h of recovery relative to Exh. Serum FFA increased to similar concentrations during the two exercise sessions. Serum FFA, relative to concentrations observed at Exh, were depressed from 1 h to 52 h recovery in HC. In LC, FFA concentrations remained high during the first 2 h of recovery and again 1 h later resulted in significant increases in blood glucose and insulin concentrations (P < 0.05) within 30 min and throughout the first 2 h of recovery relative to Exh. With the exception of 24 h postexercise, FFA concentrations in LC were higher (P < 0.05) at 1 h and at all subsequent times during recovery relative to HC. There was no effect of the postexercise experimental treatments on blood lactate concentrations. In both trials, lactate concentrations increased significantly (P < 0.05) throughout exercise to 3.31 ± 0.30 mM relative to rest and thereafter were not different from the concentrations at rest at any time during recovery (data not shown).

Table 1. Blood glucose, serum insulin, and serum FFA concentrations

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<th>Exercise</th>
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<td></td>
<td>Rest</td>
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<td>Glucose, mM</td>
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<td>HC trial</td>
<td>4.41±0.55</td>
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| LC trial   | 3.82±0.18| 3.70±0.19| 3.50±0.12| 3.25±0.20| 3.24±0.15*| 3.25±0.08†| 3.41±0.07†| 3.30±0.10†| 4.71±0.37†| 4.38±0.09
| Insulin, pM|          |          |          |           |         |          |          |          |         |         |
| HC trial   | 110±47   | 32±8     | 15±4     | 14±5     | 132±17*  | 142±25*  | 224±47*  | 201±41*  | 110±45*  | 99±23   |
| LC trial   | 99±17    | 17±4     | 18±4     | 22±12    | 21±5†    | 23±9†    | 14±2†    | 19±7†    | 48±16†   | 49±16   |
| FFA, µM    |          |          |          |           |         |          |          |          |         |         |
| HC trial   | 328±108  | 545±221  | 700±134  | 1,283±199| 1,409±156| 639±63*  | 293±34*  | 169±47*  | 220±85*  | 88±15*  |
| LC trial   | 225±61   | 320±47   | 625±103  | 1,211±183| 1,549±173| 1,646±198†| 1,715±244†| 1,523±193†| 480±65*  | 635±12†|

Values are means ± SE. HC, high carbohydrate; LC, low carbohydrate; FFA, free fatty acids. *Significantly different from Exh (P < 0.05). †Significantly different from HC at the corresponding time point (P < 0.05).

Statistical Analyses

Two-way ANOVAs for repeated measures were used to determine the effect of time and treatment (HC or LC) on blood parameters, mRNA and protein content, and glycogen content. A Student-Newman–Keuls post hoc comparison was used when appropriate. Statistical analyses were performed using SigmaStat 3.1 (Richmond, CA). PGC-1α mRNA was normalized to 18S rRNA, and both PGC-1α mRNA and protein content were expressed relative to the corresponding trial control sample (rest), which was set to 1. Linear relationships between glycogen content and PGC-1α mRNA and protein expression were examined using Pearson product-moment correlation analyses. Statistical significance was accepted at P < 0.05, and all values are presented as means ± SE.

RESULTS

Performance

The duration of exhaustive cycling exercise on days 1 and 2 was not significantly different (day 1: 113.3 ± 4.8 min; day 2: 115.7 ± 5.5 min). Similarly, the time to exhaustion for HC and LC trials was not significantly different from each other (HC: 115.1 ± 5.5 min; LC: 113.9 ± 4.8 min).
**Muscle Glycogen**

Muscle glycogen concentrations are presented in Fig. 1. At rest the glycogen concentrations were similar (HC 449 ± 13 mmol glucosyl units/kg dry wt; LC 451 ± 24 mmol glucosyl units/kg dry wt), and they were decreased significantly (P < 0.05) by exhaustive exercise in both trials (HC 82 ± 25 mmol glucosyl units/kg dry wt; LC 146 ± 37 mmol glucosyl units/kg dry wt). Despite careful replication of the exercise intensity and cycling times to exhaustion, the mean glycogen concentration at exhaustion was significantly (P < 0.05) lower in HC relative to LC (decreases of 82 and 68%, respectively). Glycogen concentration had returned to resting levels 24 h after exercise in HC. In contrast, in LC, glycogen concentrations remained depressed throughout the recovery period relative to the concentrations observed before exercise (P < 0.05) and relative to HC at the corresponding time points (P < 0.05). Specifically, muscle glycogen content was 32%, 41%, and 35% lower in LC than in HC at 2, 24, and 52 h of recovery, respectively.

**PGC-1α Gene and Protein Expression**

PGC-1α mRNA and protein data are depicted in Figs. 2 and 3, respectively. Exhaustive exercise induced a two- to threefold increase in PGC-1α mRNA relative to rest (P ≤ 0.05). PGC-1α mRNA abundance continued to increase to ~6.2-fold by 24 h of recovery (P < 0.001 vs. all other time points). By 24 h of recovery, PGC-1α mRNA abundance was repressed and corresponded to levels observed at rest. In contrast, PGC-1α protein concentration was increased significantly (23%, P < 0.05) at the end of exhaustive exercise, and it remained elevated (23 ± 8%, P < 0.05) at 2 h of recovery, despite the large increase in mRNA at this time. At 24 h after exercise, the PGC-1α protein was still 16 ± 9% greater (P = 0.05) than at rest, while the mRNA had returned to resting levels.

**Relationship Between Muscle Glycogen and PGC-1α**

There were no differences in PGC-1α mRNA or protein between the treatments either at the end of exercise, or during the 52-h recovery period when glycogen depots were markedly altered in HC and LC. However, when the changes (increase) in PGC-1α protein from rest to exhaustion as well as from exhaustion to 52 h of recovery (decrease) were analyzed vs. the respective decrease and subsequent increase in muscle glycogen, a strong relationship (r = 0.68; P < 0.001) was observed (Fig. 4). Similar analysis with the mRNA and glycogen did not result in a significant relationship.
This is the first study to examine both PGC-1α gene transcription in various tissues. As far as we can ascertain, muscle (3, 48), is involved in the coordination of metabolic well as for a prolonged period (52 h) during the recovery period in relation to altered muscle glycogen content. The major novel findings of the present study were 1) that PGC-1α protein expression in human skeletal muscle increased significantly (+23%) during prolonged exhaustive exercise; 2) that PGC-1α protein expression remained upregulated for at least 24 h; 3) that the quantitative and temporal patterns of change in PGC-1α mRNA did not reflect the changes in PGC-1α protein; 4) that across the entire exercise and recovery period, there was a strong association between the changes in the PGC-1α protein and muscle glycogen; and 5) that the response of PGC-1β bore no relationship to that of PGC-1α and was affected by the low-carbohydrate diet.

The increase in PGC-1α was of similar magnitude to that observed previously in training studies with rodents and humans, suggesting that it was physiologically meaningful. Consistent with previous studies of exercising humans, we observed that PGC-1α mRNA content increased during and immediately after exercise (12, 36, 41, 42, 48, 54) and returned to resting levels by 24 h after exercise. The present data clearly demonstrate that changes in mRNA do not necessarily reflect similar changes (temporally or quantitatively) in the protein. Watt et al. (54) reported a 12-fold increase in PGC-1α mRNA abundance, but no increase in PGC-1α protein content after prolonged (3 h) exercise and 3 h of recovery. We found that by the end of the exercise there was a two- to threefold increase in mRNA as well as a significant, 23% increase in PGC-1α protein. The latter remained at this level at 2 h of recovery even though the abundance of mRNA had doubled compared with the end of exercise. Furthermore, while the PGC-1α mRNA declined to rest levels within 24 h, the protein content was still 16% greater than rest. While the PGC-1α mRNA concentration after 52 h of recovery approximated that of rest, it is uncertain at which point between 24 and 52 h PGC-1α protein had returned to preexercise levels. The PGC-1α protein results in the present study are comparable to those reported by Wright et al. (55) for rodent muscle following 6 h of swimming. While they did not find an increase at exhaustion, the protein was elevated ~40% at both 3 and 12 h of recovery. They also observed that compared with rest, the PGC-1α protein was increased by 80% at 18 h of recovery. Due to ethical limitations, the number of biopsies permitted was restricted in the present experiments. In the future it would be interesting to investigate the interval between 2 and 24 h in more detail. These previous studies (54, 55) failed to observe an increase in

PGC-1β Protein Expression

The β isoform had both a time and a treatment effect (P ≤ 0.05) (Fig. 5). In contrast to PGC-1α, there was no increase in PGC-1β with exercise, and in fact it decreased (P ≤ 0.05) over time in both treatments. In the HC diet trial, the level at 24 h was less (P ≤ 0.05) than rest, exhaustion, and 2 h of recovery. The 2-h and 24-h concentrations were less (P ≤ 0.05) than exhaustion. Similarly, with the LC treatment at both 24 and 52 h, the data were lower (P ≤ 0.05) than rest, exhaustion, and also less than 2 h of recovery. In addition, at both 24 and 52 h, the data were lower (P ≤ 0.05) in the LC diet than in the HC treatment, and this corresponded to when the between-trials differences in muscle glycogen were greatest.

DISCUSSION

PGC-1α, an exercise-induced gene in rat and human skeletal muscle (3, 48), is involved in the coordination of metabolic gene transcription in various tissues. As far as we can ascertain, this is the first study to examine both PGC-1α mRNA and protein abundance in human skeletal muscle during exercise as well as for a prolonged period (52 h) during the recovery period in relation to altered muscle glycogen content. The major novel findings of the present study were 1) that PGC-1α protein expression in human skeletal muscle increased significantly (+23%) during prolonged exhaustive exercise; 2) that PGC-1α protein expression remained upregulated for at least 24 h; 3) that the quantitative and temporal patterns of change in PGC-1α mRNA did not reflect the changes in PGC-1α protein; 4) that across the entire exercise and recovery period, there was a strong association between the changes in the PGC-1α protein and muscle glycogen; and 5) that the response of PGC-1β bore no relationship to that of PGC-1α and was affected by the low-carbohydrate diet.

The increase in PGC-1α was of similar magnitude to that observed previously in training studies with rodents and humans, suggesting that it was physiologically meaningful. Consistent with previous studies of exercising humans, we observed that PGC-1α mRNA content increased during and immediately after exercise (12, 36, 41, 42, 48, 54) and returned to resting levels by 24 h after exercise. The present data clearly demonstrate that changes in mRNA do not necessarily reflect similar changes (temporally or quantitatively) in the protein. Watt et al. (54) reported a 12-fold increase in PGC-1α mRNA abundance, but no increase in PGC-1α protein content after prolonged (3 h) exercise and 3 h of recovery. We found that by the end of the exercise there was a two- to threefold increase in mRNA as well as a significant, 23% increase in PGC-1α protein. The latter remained at this level at 2 h of recovery even though the abundance of mRNA had doubled compared with the end of exercise. Furthermore, while the PGC-1α mRNA declined to rest levels within 24 h, the protein content was still 16% greater than rest. While the PGC-1α mRNA concentration after 52 h of recovery approximated that of rest, it is uncertain at which point between 24 and 52 h PGC-1α protein had returned to preexercise levels. The PGC-1α protein results in the present study are comparable to those reported by Wright et al. (55) for rodent muscle following 6 h of swimming. While they did not find an increase at exhaustion, the protein was elevated ~40% at both 3 and 12 h of recovery. They also observed that compared with rest, the PGC-1α protein was increased by 80% at 18 h of recovery. Due to ethical limitations, the number of biopsies permitted was restricted in the present experiments. In the future it would be interesting to investigate the interval between 2 and 24 h in more detail. These previous studies (54, 55) failed to observe an increase in
PGC-1α at the end of the exercise, suggesting that that exercise intensity rather than duration could be an important factor.

The PGC-1β isoform has been less studied, but it often appears to act in a complementary fashion to PGC-1α in the regulation of the transcription of genes of oxidative metabolism. However, there are a number of examples where the response of their mRNAs or that of the proteins themselves do not correspond temporally, qualitatively, or quantitatively. Holloway et al. (16) recently reported that there was no correlation between the α- and β-isofoms in muscle from either lean or obese women. There are several reports that the mRNA β-isofom is not responsive to exercise training (20, 31). Mortensen et al. (34) reported that mRNA for PGC-1β did not respond to acute exercise although that for the α-isofom increased. Furthermore in response to training the mRNA of PGC-1α increased but that of PGC-1β decreased in muscle. Thus the present data showing a decrease in PGC-1β protein is consistent with these previous reports. Not only is the direction of change in contrast to that for PGC-1α, but the slower and longer response is also markedly different. Our present data strongly suggest that the postexercise regulation of these isofoms is very different.

The upregulation of PGC-1α may be important in orchestrating gene transcription in the recovery period. While it is impressive that a single bout of exercise resulted in an elevation of PGC-1α protein for at least 24 h, the 23% increase is fairly modest. It could be queried whether this increase is physiologically significant. However, it is of a similar magnitude as that found in a variety of investigations in conditions that enhance muscle oxidative capacity. In a recent study using rodents, a 25–30% increase in PGC-1α protein expression, induced by transfecting this gene into muscle, increased the expression of a wide variety of metabolic proteins, including FAT/CD36, MCT-1, GLUT-4, and COX-4, as well as increasing the oxidation of palmitate by isolated mitochondria (4). The rapid upregulation of PGC-1α protein in the present study is consistent with the concept that this nuclear coactivator may be involved with coordinating the activation and expression of exercise-induced genes during exercise and in the recovery period. It is possible that the increase in PGC-1α associated with one bout of prolonged exercise initiates the induction of “metabolic genes” involved in fatty acid and glucose metabolism that have been shown to increase with a single bout of exercise (21, 22, 29, 40, 42, 58). While it is speculative, it may be that regular exercise/training results in repeated increases in PGC-1α, and this in turn promotes the well-known enhanced oxidative capacity of muscle. Indeed, the PGC-1α that remained upregulated for 24 h after exercise would provide the new, elevated baseline for the next exercise-induced increase in PGC-1α. Training of either obese (49) or high-fat-fed (24) rodents has been shown to increase PGC-1α protein by 150% and 30%, respectively. In addition, Russell et al. (46) reported that PGC-1α was increased in humans who trained for 6 wk in all muscle fiber types. Clearly, repeated bouts of exercise (training) contribute to the accumulation of PGC-1α, a process that commences in rodents (55) and humans (present study), with the first bout of exercise.

Substrate availability, altered by differences in the diet during recovery from exercise, influences transcription (41) and mRNA content (2, 41) of various metabolic genes in the recovery period. As anticipated in the present study, ingestion of 1 g CHO·kg body wt \(^{-1} \cdot \text{h}^{-1}\) for the first 2 h of recovery led to increased circulating insulin and glucose concentrations and large differences in the rate of glycogen restoration. There were no treatment effects of HC vs. LC on PGC-1α protein during the postexercise recovery period; however, as individual differences were apparent, we used linear regression analysis to test for a possible relationship between the changes in PGC-1α protein and the changes in glycogen across exercise and 52 h of recovery (Fig. 4). The strong \((r = −0.68)\) relationship between the change in PGC-1α protein and the change in glycogen during exercise and recovery suggests that glycogen stores could be a regulatory factor. It is noteworthy that Pilegaard et al. (41) have demonstrated that following 5 days of training, exercise-induced upregulation of PGC-1α mRNA was sustained over a longer time period by the ingestion of a low-CHO diet. Unfortunately, they did not examine the impact of this on PGC-1α protein. The fact that changes in PGC-1α protein content were not evident between the HC and LC treatments but were evident when we examined individual changes may seem discordant. While a mechanistic explanation is presently lacking, we speculate that PGC-1α may be responsive to relative changes in muscle glycogen within an individual rather than to absolute concentrations of muscle glycogen among individuals. Glycogen concentrations among individuals are known to be highly variable (1), and we (Graham and Bonen, unpublished data) and others (38) have also observed that skeletal muscle PGC-1α protein content among individuals is also highly variable; thus on an absolute basis there may be no obvious relationship between these two parameters. We acknowledge that this is speculative and requires further examination.

Although not assessed in this study, factors associated with calcium- and AMPK-signaling pathways present putative mechanisms by which PGC-1α transcription and expression are upregulated during an acute bout of exhaustive exercise (37). Increased intracellular calcium is associated with PGC-1α mRNA and protein upregulation in cell line (17) and animal models (52). Indeed, in C2C12 cells, the calcium/calcmodulin dependent proteins kinase family (especially CaMKII in muscle) emerges as a putative regulator (14, 56). In human skeletal muscle, both knee extension exercise (36) and cycling exercise (47) induced myocyte-enriched calcineurin interacting protein 1 (MCIP-1) mRNA, a marker of activated calcineurin in vivo, concomitant with increases in PGC-1α mRNA in the early recovery period. Collectively, these findings suggest that in exercising human skeletal muscle, the calcium signaling pathway may be involved in PGC-1α gene transcription.

In addition to calcium-signaling pathways, activation of AMPK, an energy-sensing kinase, has been implicated in PGC-1α regulation. Pharmacological activation of AMPK increased PGC-1α protein in rat muscle cells in vitro (17, 50–52). In addition, transgenic mice expressing a dominant negative mutant of AMPK were unable to induce expression of PGC-1α as occurred in their wild-type counterparts (60), further supporting a role for AMPK in PGC-1α upregulation. Conversely, PGC-1α mRNA upregulation was not affected in α1- and α2-AMPK knockout mice following acute exercise, suggesting that AMPK is not required for exercise-induced PGC-1α regulation (18). In addition, Wright et al. (55) demonstrated that 2 h of swimming markedly activated (phosphorylated) p38 MAPK in rodent muscle. This was accompa-
nied with an increased activation of its substrate (ATF-2), and they (55) speculated that these actions resulted in activation of PGC-1α and its translocation to the nucleus. To date, the mechanisms inducing the upregulation of PGC-1α in exercising human muscle have not been determined. However, it is possible that several mechanisms exist that regulate increases in PGC-1α expression during exercise and recovery.

Recently, it was suggested that activation of PGC-1α could mediate the initial phase of the exercise-induced adaptive increase in metabolic gene expression, while the subsequent postexercise increase in PGC-1α protein sustains and enhances the increase in mitochondrial biogenesis (55). Furthermore, it has also been reported that methylation (53), acetylation (45), and phosphorylation (25) can each effect the activation of PGC-1α, but it is not known if these covalent modifications are important in active skeletal muscle.

In conclusion, the present study demonstrated a significant increase in PGC-1α protein upregulation during exercise that persisted for at least 24 h. The fast upregulation of PGC-1α is consistent with its putative role in coordinating metabolic gene expression during exercise and continues in the recovery period. This response is very consistent with its putative role in coordinating metabolic gene expression during exercise and recovery.

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