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Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1α in human skeletal muscle

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Gibala MJ, McGee SL, Garnham AP, Howlett KF, Snow RJ, Hargreaves M. Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1α in human skeletal muscle. *J Appl Physiol* 106: 929–934, 2009. First published December 26, 2008; doi:10.1152/japplphysiol.90880.2008.—From a cell signaling perspective, short-duration intense muscular work is typically associated with resistance training and linked to pathways that stimulate growth. However, brief repeated sessions of sprint or high-intensity interval exercise induce rapid phenotypic changes that resemble traditional endurance training. We tested the hypothesis that an acute session of intense intermittent cycle exercise would activate signaling cascades linked to mitochondrial biogenesis in human skeletal muscle. Biopsies (vastus lateralis) were obtained from six young men who performed four 30-s "all out" exercise bouts interspersed with 4 min of rest (<80 kJ total work). Phosphorylation of AMP-activated protein kinase (AMPK; subunits α1 and α2) and the p38 mitogen-activated protein kinase (MAPK) was higher ($P \leq 0.05$) immediately after *bout 4* vs. preexercise. Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) mRNA was increased approximately twofold above rest after 3 h of recovery ($P \leq 0.05$); however, PGC-1α protein content was unchanged. In contrast, phosphorylation of protein kinase B/Akt (Thr^{308} and Ser^{473}) tended to decrease, and downstream targets linked to hypertrophy (p70 ribosomal S6 kinase and 4E binding protein 1) were unchanged after exercise and recovery. We conclude that signaling through AMPK and p38 MAPK to PGC-1α may explain in part the metabolic remodeling induced by low-volume intense interval exercise, including mitochondrial biogenesis and an increased capacity for glucose and fatty acid oxidation.

metabolism; mitochondrial biogenesis; signal transduction; peroxisome proliferator-activated receptor-γ coactivator-1α

FROM A CELL SIGNALING PERSPECTIVE, exercise is often broadly classified as either "strength" or "endurance," with short-duration, intense muscular work usually associated with hypertrophy and prolonged, low- to moderate-intensity work associated with increased mitochondrial mass and oxidative capacity (3, 10). Indeed, the distinct pathways that regulate either cell growth or mitochondrial biogenesis intersect at a number of points in an inhibitory fashion, resulting in a response that is largely exclusive for one type of exercise or the other (3). Studies in animals (1) have shown that electrical stimulation of isolated muscles with either prolonged low-frequency bursts (to mimic endurance training) or short high-frequency bursts (to simulate resistance training) selectively activates signaling cascades associated with mitochondrial biogenesis (e.g., AMP-activated protein kinase, AMPK) or muscle growth (e.g., protein kinase B/Akt; PKB), respectively. In light of these findings, Atherton et al. (1) proposed the "AMPK-PKB switch" hypothesis as a mechanism that partially mediates specific adaptations to endurance and resistance training. However, there is considerable overlap in the signaling response to divergent contractile stimuli in human skeletal muscle (13, 22), and investigators have questioned the veracity of the putative AMPK-PKB switch hypothesis (11).

In contrast to strength or endurance training, little is known about the acute signaling events that mediate skeletal muscle remodeling after sprint or high-intensity interval training (HIT). Like strength training, HIT is characterized by repeated sessions of brief, intermittent exercise performed at a high relative workload. However, HIT is a potent strategy to induce skeletal muscle remodeling that resembles changes usually associated with endurance training (15). As few as six sessions of HIT over 2 wk, totaling <15 min of "all out" cycle exercise (<600 kJ of total work), have been shown to increase the maximal activity of mitochondrial enzymes and improve performance during tasks that rely heavily on aerobic energy provision (8, 14). Other adaptations documented after several weeks of HIT include an increased muscle content of proteins associated with the transport and oxidation of glucose and fatty acids and reduced nonoxidative energy provision during matched-work exercise (15).

Given the oxidative phenotype that is rapidly upregulated by HIT, the present study tested the hypothesis that an acute session of brief, intense interval exercise would activate signaling cascades linked to peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), the transcriptional coactivator that functions as a regulator of mitochondrial biogenesis (2). Needle biopsy samples were obtained before and immediately after exercise and after 3 h of recovery, and protein phosphorylation was determined by immunoblot analyses using phosphospecific antibodies. We assessed the activity of pathways linked to PGC-1α activity and expression including AMPK (α1- and α2-subunits), p38 mitogen-activated protein kinase (p38 MAPK), and Ca^{2+}/calmodulin-dependent protein kinase isoform II (CaMKII). We also measured the mRNA expression and protein content of PGC-1α. Finally, to examine the impact on other signaling pathways linked to skeletal muscle remodeling, we measured phosphorylation of PKB

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(Thr[308] and Ser[473]) and specific downstream targets linked to protein synthesis, including p70 ribosomal S6 kinase (p70S6k) and 4E binding protein 1 (4E-BP1).

METHODS

Subjects

Six healthy active young men (23 ± 2 yr, 78 ± 3 kg) agreed to take part in the study after being advised of the procedures and potential risks. The project was approved by the Human Research Ethics Committee of the University of Melbourne, and all subjects provided written informed consent. All subjects were engaged in recreational exercise pursuits such as jogging and cycling several times per week but were not specifically training to compete in these activities. Their peak oxygen uptake, assessed using a standardized incremental cycle test to exhaustion, was 3.7 ± 0.3 l/min. Before the experiment, subjects also performed one 30-s all out cycling bout on an air-braked ergometer (Repco, Melbourne, Australia) to become familiar with the high-intensity exercise intervention.

Experimental Protocol

At least 1 wk following the familiarization session, subjects reported to the laboratory in the morning 2–3 h after consuming a light meal of their own choosing. The area over the lateral aspect of both thighs was anesthetized and prepared for the extraction needle muscle biopsy samples from the vastus lateralis muscle of each leg. A total of four muscle biopsies were obtained over the course of the experiment, with each biopsy obtained from a separate incision site (5 mm) made through the skin and underlying fascia. Two biopsies were obtained from each leg, and the two incision sites on each leg were spaced 2–3 cm apart. The relative arrangement of the four biopsy samples was varied between subjects to avoid potential order bias. The first biopsy was obtained at rest, and the corresponding incision was closed with a suture. Two additional incisions were made before exercise and covered with sterile gauze and surgical tape. Subjects then performed four 30-s all out cycling bouts on the air-braked ergometer (Repco) with 4 min of rest between bouts. Immediately following the first and last exercise bout, the gauze was quickly removed and a biopsy sample was obtained through one of the incisions (within 10 s following the completion of the exercise bout). The final incision site was prepared during recovery, and the fourth biopsy sample was obtained 3 h after the final bout of exercise. During the 3-h period between the last exercise bout and the final biopsy, subjects remained in the laboratory and read or worked on a computer while seated. Subjects did not ingest anything except for water during the exercise and recovery period. After each biopsy procedure, the muscle sample was immediately frozen by plunging the needle into liquid nitrogen. All samples were subsequently divided into several pieces while still frozen and stored at −80°C before analyses were performed.

Muscle Analyses

Metabolites. A 25- to 30-mg piece of frozen muscle was freeze-dried, powdered, and dissected free of blood and connective tissue. Aliquots of freeze-dried muscle were extracted and analyzed for ATP, phosphocreatine, creatine, and lactate and glycogen (measured as glucosyl units) using enzymatic assays adapted for fluorometry as previously described (16).

Immunoblotting. A 40- to 50-mg piece of frozen muscle was added to 10 volumes of homogenizing buffer (50 mM Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1% Triton X-100, 50 mM NaF, 5 mM Na-pyrophosphatase, and 1 mM DTT) with 2 μl of protease inhibitor cocktail and homogenized on ice using a Polytron PT 1200E homogenizer. The sample was centrifuged at 13,000 rpm for 5 min, and the supernatant was collected. The protein content of the supernatant was determined using a commercial bicinchoninic acid assay (BCA Protein Assay; Pierce, Rockford, IL), and all samples were subsequently diluted to a standard concentration using homogenizing buffer. Samples were further diluted with 4× Laemmli buffer and heated at 100°C for 5 min. For each blot, a standard and an internal control were loaded along with 40 μl of each sample onto a 5% polyacrylamide stacking gel and separated using a 10% polyacrylamide separating gel of 1.5-mm thickness at 180 V with a running time of 45 min in Tris-glycine electrophoresis buffer. The gels were electroblotted onto nitrocellulose membranes in transfer buffer (37 mM Tris base, 140 mM glycine, and 20% methanol) for 90 min at 90 V at 4°C. Membranes were incubated in Tris-buffered saline-Tween (TBST; 10 mM Tris base, 150 mM NaCl, and 0.05% Tween 20) with 5% skim milk for 1 h and washed with TBST. Membranes were incubated overnight at 4°C with primary antibodies against the following: pT172 AMPK, pS79 acetyl-CoA carboxylase (ACC), pT286 CaMKII, pT180/Y182 p38 MAPK, pT308 Akt, pS473 Akt, pT389 S6K, and pT37/46 4EBP1 (Cell Signaling, Danvers, MA) as well as PGC-1 (Millipore, Billerica, MA), all at concentrations of 1:500 or 1:1,000 in 1× TBST. After incubation, the membranes were washed and exposed to appropriate dilutions of anti-species horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Membranes were then rewashed before being exposed to a chemiluminescent liquid (Immuno-Star HRP substrate kit; Bio-Rad, Hercules, CA) for 2 min. Membranes where exposed using a Bio-Rad ChemiDoc system, and band densities were determined using image-analysis software. For phosphorylated AMPK blots, gels were run for 70 min at 180 V to ensure adequate separation of the α1- and α2-isoforms. Bands found at ~65 kDa were quantified as AMPKα1, whereas bands found at ~62 kDa were quantified as AMPKα2. Representative blots for all proteins quantified by Western blot are presented in Fig. 1.

Real-time RT-PCR. Total RNA was extracted from ~10 mg of muscle using the Aurum total RNA fatty and fibrous tissue kit (Bio-Rad). RNA was reverse transcribed to cDNA using the iScript kit (Bio-Rad). Forward and reverse primers complimentary to the human PGC-1α were designed by using OligoPerfect software (Invitrogen, Carlsbad, CA). The forward (5′-3′) primer sequence was CAA GCC AAA CCA ACA ACT TTA TCT CT, and the reverse (3′-5′) sequence was CAC ACT TAA GGT CGT GCG TTC AAT AGT C. Real-time RT-PCR was performed using an iCycler thermal cycler with IQ5 detection system and software with SYBR green chemistry (Bio-Rad). Changes in gene expression were normalized to the housekeeping gene cyclophilin, which did not change with exercise (data not shown), using the 2-ΔΔCT method (21). PCR products for both PGC-1α and cyclophilin amplified at equal efficiencies (>95%).

Statistical Analyses

PGC-1α gene expression and protein content data were analyzed using two-tailed paired *t*-tests, since comparisons were only made between rest and 3 h of recovery. Exercise performance, muscle metabolite, and signaling protein data were analyzed using a one-factor (time) repeated-measures analysis of variance (ANOVA). The level of statistical significance for all analyses was set at $P \leq 0.05$, and in the case of the ANOVA, a Tukey's honestly significant difference test was used to identify differences between time points. All data are means ± SE.

RESULTS

Exercise Performance

Peak power, mean power, and total work progressively decreased ($P \leq 0.05$) over the intermittent exercise session compared with *bout 1* (Table 1).

Muscle Metabolites

Muscle glycogen content was lower ($P \leq 0.05$) after exercise and recovery compared with rest (Table 2). Muscle lactate

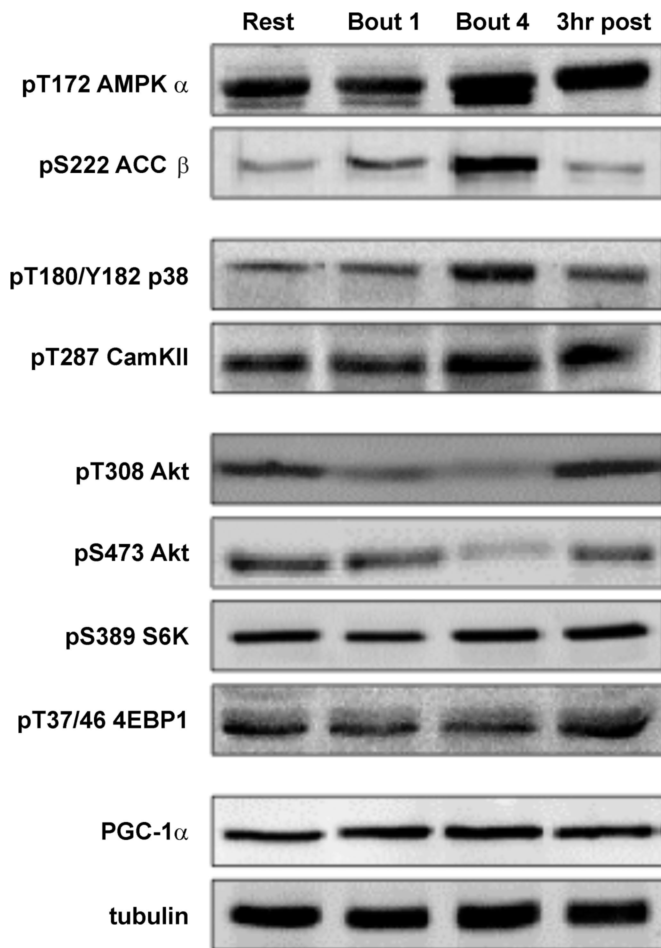

Fig. 1. Representative protein blots of protein expression measured before (rest) and immediately after 1 and 4 bouts of exercise, and after 3 h of recovery (3 h post). AMPK, AMP-activated protein kinase; ACC, acetyl-CoA carboxylase; p38, p38 MAPK; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase isoform II; 4E-BP1, 4E binding protein 1; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α.

and creatine were higher ($P \leq 0.05$) compared with rest after *bouts 1* and *4* but were not different after recovery (Table 2). Muscle phosphocreatine content was lower ($P \leq 0.05$) after *bouts 1* and *4* compared with rest, whereas muscle ATP was only lower ($P \leq 0.05$) after *bout 4* (Table 2).

Signaling Proteins

Phosphorylation of AMPK and the α1- and α2-subunits were higher ($P \leq 0.05$) after *bout 4* compared with all other time points (Fig. 2). ACC phosphorylation was higher after *bouts 1* and *4* compared with rest (Fig. 2). Phosphorylation of p38 MAPK ($P \leq 0.05$) was also higher after *bout 4* compared with rest, and although CaMKII phosphorylation followed a similar pattern, the change was not significant ($P = 0.18$; Fig. 3). Signaling through PKB/Akt, including phosphorylation of PKB-Thr^{308}, PKB-Ser^{473}, p70S6k, and 4E-BP1, was unchanged during exercise and recovery, although both sites on PKB tended to be lower after *bout 4* compared with preexercise ($P = 0.06$ and 0.08 for Thr^{308} and Ser^{473}, respectively; Fig. 4).

Table 1. *Peak power, mean power, and total work during exercise*

	Bout 1	*Bout 2*	*Bout 3*	*Bout 4*
Peak power, W	944±91	831±59*	796±34*	742±32*
Mean power, W	750±55	654±31*	603±23*	556±19*
Total work, kJ	22.5±1.6	19.6±0.9*	18.1±0.7*	16.7±0.6*

Bouts 1–4 refer to four 30-s bouts of all out cycle exercise. *$P \leq 0.05$ vs. *Bout 1*.

Table 2. *Skeletal muscle metabolites during exercise and recovery*

	Rest	*Bout 1*	*Bout 4*	Recovery
ATP	30±1	24±2	18±2*	25±3
Phosphocreatine	82±4	37±7*	22±6*	90±3
Creatine	53±6	98±8*	113±7*	45±4
Lactate	15±3	76±11*	104±9*	22±7
Glycogen	431±74	346±64*	300±78*	306±32*

Values are means ± SE expressed in mmol/kg dry wt. *Bouts 1 and 4* refer to immediately following the first and fourth 30-s bouts of all out cycle exercise; recovery refers to 3 h following the fourth bout of exercise. *$P \leq 0.05$ vs. rest.

PGC-1α

PGC-1α mRNA expression was increased approximately twofold above rest after 3 h of recovery ($P \leq 0.05$), but PGC-1α protein content was unchanged ($P = 0.84$; Fig. 5).

DISCUSSION

The present study examined changes in signaling proteins in human skeletal muscle after brief intense exercise, a stimulus traditionally linked to cell growth (3). Our interest was stimulated by work showing that training with short bursts of all out cycling, similar to the model used in the present study, leads to rapid increases in the maximal activities of mitochondrial enzymes (8, 14). Given its potency to induce a more oxidative phenotype, we hypothesized that an acute session of intense interval exercise would activate pathways proposed to regulate skeletal muscle remodeling after endurance exercise (18). The major novel finding from the present study was that four 30-s bursts of all out cycling stimulated signaling through AMPK and p38 MAPK, which are two important signaling cascades linked to PGC-1α and the regulation of mitochondrial biogenesis in skeletal muscle (19, 26).

Animal studies have shown increased AMPK activation and PGC-1α mRNA expression in skeletal muscle after prolonged low-intensity swimming (29) and increased AMPK activity and increased PGC-1α protein expression after electrical stimulation designed to mimic endurance exercise (1). These observations have been replicated in humans (18, 29), although the role of exercise intensity in AMPK activation and the minimum "dose" of exercise necessary to simulate an increase in PGC-1 mRNA are equivocal. Exercise or contraction in rodents is generally associated with activation of both α1- and α2-AMPK catalytic subunits, whereas α2 appears more sensitive to exercise in humans (17). This may be related to both fiber type recruitment and relative work intensity, with higher workloads associated with more pronounced changes in muscle phosphorylation potential, as evidenced by the marked decrease in ATP content in the present study. Similar to the work

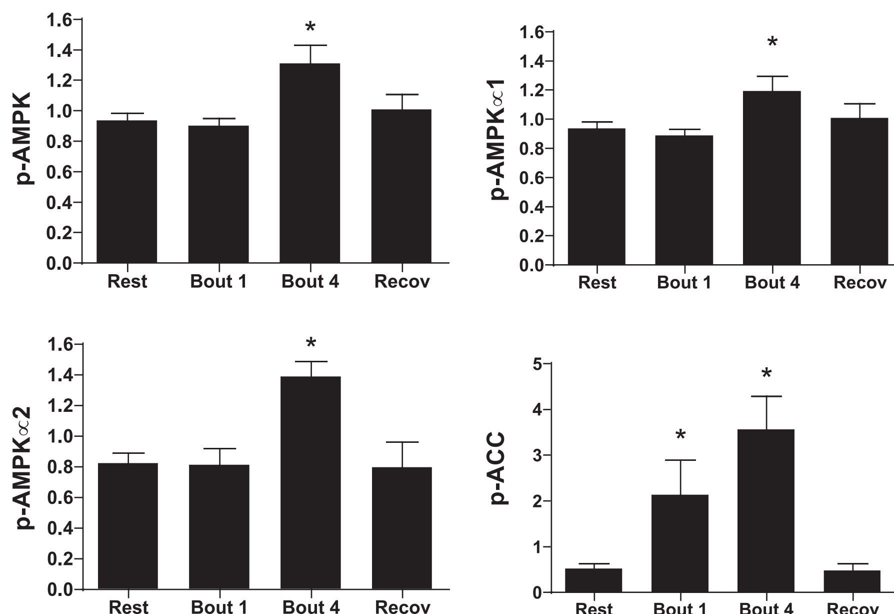

Fig. 2. Phosphorylated (p) AMPK, AMPKα1, AMPKα2, and ACC at rest, after 1 and 4 bouts of 30-s all out cycling exercise, and after 3 h of recovery (recov). $*P \leq 0.05$ vs. rest.

of Chen et al. (9), who reported greater activity of AMPKα1 and -α2 after a bout of all out cycling, we found an exercise-induced increase in the phosphorylation state of both AMPK subunits. However, recent evidence suggests that α2/β2/γ3-AMPK heterotrimers are preferentially activated during short, intense exercise and that the preferential activation of this heterotrimer is not reflected by an increase in total AMPKα phosphorylation and activity (4). Furthermore, activation of the α2/β2/γ3-AMPK heterotrimer correlates with ACC phosphorylation (4). Given that we saw a significant increase in ACC phosphorylation after the first exercise bout, it is possible that α2/β2/γ3-AMPK was immediately activated in the current exercise protocol.

Most studies of acute PGC-1α regulation in humans have used very prolonged exercise interventions (23, 33), and it has been suggested that sustained contractile activity lasting >1 h may be required before an increase in PGC-1α mRNA is observed (28). However, we have shown presently that a surprisingly small dose of very intense exercise (equal to only 2 min of all out cycling) was sufficient to increase PGC-1α mRNA during recovery. To our knowledge, only one previous study has examined the effect of interval-type exercise on PGC-1α mRNA in humans (12); however, the volume of exercise was much greater than in the present study. De Filippis et al. (12) had subjects cycle for 8 min at moderate intensity (70% maximum heart rate), then for 2 min at a higher intensity (90%), followed by 2 min with no resistance, and this was repeated four times. The relative increase in PGC-1α mRNA reported in that study (8-fold change from rest measured after 5 h of recovery) was greater than in our study (2-fold increase after 3 h of recovery). However, total exercise time in the present study was only 1/20th the protocol used by De Filippis et al. (12), and total training time (including recovery periods between intervals) was less than one-third.

Despite the increase PGC-1α mRNA, PGC-1α protein content was unchanged after exercise in the present study. This finding is consistent with two other human studies that showed no change in PGC-1α protein despite increased mRNA expression after an acute bout of moderate intensity cycle exercise lasting 1 (10) or 3 h (33). In contrast, the recent study by De Filippis et al. (12) reported that PGC-1α protein content was increased by 20 and 40%, respectively, when measured 30 and 300 min after exercise. Both high-intensity intermittent and low-intensity prolonged exercise have been reported to acutely increase PGC-1α protein content in rodent muscle (31). It is possible that despite the acute increase in PGC-1α mRNA, more than one dose of intense intermittent exercise is necessary to increase PGC-1 α protein content in human muscle. Performing repeated bouts of cycle exercise similar to the protocol used in the present study has been shown to increase PGC-1α protein content after several weeks of training (7). Additional work is necessary to resolve the early time course of molecular events leading to mitochondrial biogenesis in response to intense interval exercise.

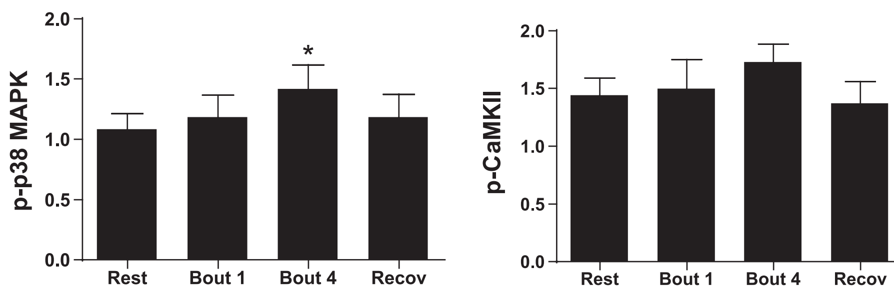

Fig. 3. Phosphorylation of the p38 MAPK and CaMK II at rest, after 1 and 4 bouts of 30-s all out cycling exercise, and after 3 h of recovery. $*P \leq 0.05$ vs. rest.

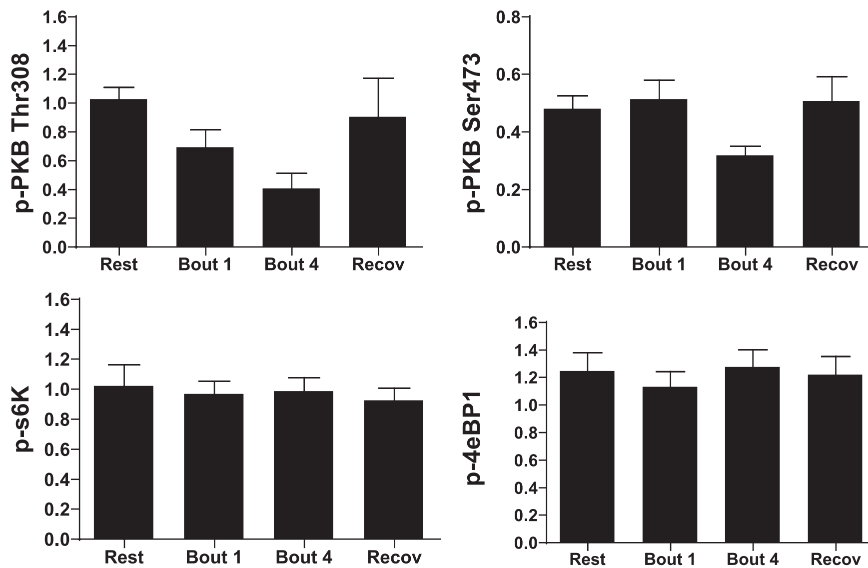

Fig. 4. Phosphorylation of PKB/Akt [Thr[308] and Ser[473] sites and downstream targets p70 ribosomal S6 kinase (p70S6k) and 4E-BP1] at rest, after 1 and 4 bouts of 30-s all out cycling exercise, and after 3 h of recovery.

MAPK (19) and CaMK (34) signaling also have been implicated in the regulation of PGC-1α and mitochondrial biogenesis in skeletal muscle. We measured p38 MAPK and CaMKII on the basis that the activity and/or phosphorylation state of both enzymes has been shown to increase after prolonged moderate-intensity exercise in humans (27, 37). Recent evidence from studies on isolated muscle preparations (35) suggests that p38 MAPK is downstream of CaMKII in a signaling pathway by which increases in cytosolic calcium lead to increases in PGC-1, and inhibition of p38 MAPK prevents the calcium-induced increase in mitochondrial biogenesis. This is the first study to show that an acute bout of intense interval exercise stimulates signaling through p38 MAPK; however, we did not detect a significant change in the phosphorylation state of CaMKII. This could be due to temporal factors related to the timing of the muscle biopsies, insufficient statistical power due to the relatively small number of subjects, or the possibility that p38 MAPK activation following repeated sprint exercise is mediated by pathways other than CaMKII. For example, mitogen-activated protein kinase kinase (MKK)3 and MKK6 are classic activators of p38 MAPK in response to numerous stress stimuli (20). Although it was not possible to measure MKK3/6 activation in the present study, it should be noted that AMPK has been suggested to be an activator of MKK3 and p38 (36).

Finally, we assessed signaling through PKB, which has been implicated in muscle adaptation to various forms of exercise training (10) but especially the regulation of growth (5). We found no change in the phosphorylation of PKB (Thr[308] and Ser[473]). Although activation of Akt in response to moderate intensity exercise is equivocal, few studies have examined Akt activation with exercise regimes similar to that employed in the current study. Indeed, little change in Akt phosphorylation has been observed in response to an all out 30-s sprint on a cycle ergometer (32). Furthermore, we found no change in the phosphorylation status of specific downstream targets of Akt and mammalian target of rapamycin (mTOR) that are linked to protein synthesis, including p70S6k and 4E-BP1. The phosphorylation of S6K at T389 by mTOR and other kinases is required to relieve pseudosubstrate suppression of S6K activity (24). It most closely correlates with S6K activity and is required for activation of translation (25). Phosphorylation of 4EBP1 at T37/46 by mTOR is required to prevent inhibition of cap-dependent translation (6). These data suggest that the exercise protocol had no effect on skeletal muscle growth signaling at the time points measured. Our data do not allow us to directly evaluate the AMPK-PKB switch hypothesis (1); however, given the potency of interval training to stimulate the signaling pathways that lead to mitochondrial biogenesis, but not those responsible for muscle growth, it appears that such training may be closer to endurance type training that resistance training in terms of activation of signaling pathways.

In summary, the present study showed that four 30-s bouts of all out cycling increased phosphorylation of AMPKα1, AMPKα2, and p38 MAPK immediately following exercise and the mRNA expression of PGC-1α after 3 h of recovery. Specific signaling through AMPK and p38 MAPK to PGC-1α may therefore explain in part the metabolic remodeling induced by intense interval exercise training, including mito-

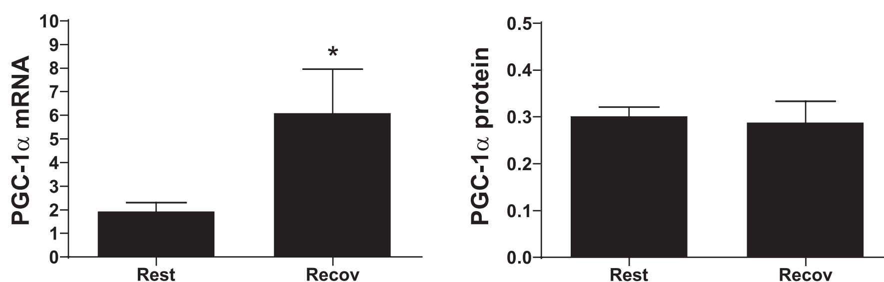

Fig. 5. mRNA expression and protein content of PGC-1α at rest and 3 h following an acute session of high-intensity interval exercise. $*P \leq 0.05$ vs. rest.

chondrial biogenesis and an increased capacity for glucose and fatty acid oxidation.

GRANTS

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