Matched work high-intensity interval and continuous running induce similar increases in PGC-1α mRNA, AMPK, p38, and p53 phosphorylation in human skeletal muscle

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Submitted 16 August 2011; accepted in final form 12 January 2012

Bartlett JD, Hwa Joo C, Jeong TS, Louhelainen J, Cochran AJ, Gibala MJ, Gregson W, Close GL, Drust B, Morton JP. Matched work high-intensity interval and continuous running induce similar increases in PGC-1α mRNA, AMPK, p38, and p53 phosphorylation in human skeletal muscle. J Appl Physiol 112: 1135–1143, 2012. First published January 29, 2012; doi:10.1152/japplphysiol.01040.2011.—The aim of the present study was to test the hypothesis that acute high-intensity interval (HIT) running induces greater activation of signaling pathways associated with mitochondrial biogenesis compared with moderate-intensity continuous (CONT) running matched for exercise capacity. In a repeated-measures design, 10 active men performed two running protocols consisting of HIT (6 × 3-min at 90% maximal oxygen consumption [V̇O₂max]) interspersed with 3-min recovery periods at 50% V̇O₂max with a 7-min warm-up and cool-down period at 70% V̇O₂max) or CONT (50-min continuous running at 70% V̇O₂max). Both protocols were matched, therefore, for average intensity, duration, and distance run. Muscle biopsies (vastus lateralis) were obtained preexercise, postexercise, and 3 h postexercise. Muscle glycogen decreased (P < 0.05) similarly in HIT and CONT (116 ± 11 vs. 111 ± 17 mmol/kg dry wt, respectively). Phosphorylation (P-) of p38MAPKThr180/Tyr182 (1.9 ± 0.8-fold vs. 1.5 ± 0.2-fold) and AMPKThr172 (1.5 ± 0.3- vs. 1.5 ± 0.1-fold) increased immediately postexercise (P < 0.05) in HIT and CONT, respectively, and returned to basal levels at 3 h postexercise. P-p53Ser15 (HIT, 2.7 ± 0.8-fold; CONT, 2.1 ± 0.8-fold), P-AMPKThr172 (HIT, 4.2 ± 1.7-fold; CONT, 4.5 ± 0.9-fold) and HSF2 mRNA (HIT, 4.4 ± 2-fold; CONT, 3.5 ± 1-fold) increased 3 h postexercise (P < 0.05) although neither parameter increased (P > 0.05) immediately postexercise. There was no difference between trials for any of the above signaling or gene expression responses (P > 0.05). We provide novel data by demonstrating that acute HIT and CONT running (when matched for average intensity, duration, and work done) induces similar activation of molecular signaling pathways associated with regulation of mitochondrial biogenesis. Furthermore, this is the first report of contraction-induced p53 phosphorylation in human skeletal muscle, thus highlighting an additional pathway by which exercise may initiate mitochondrial biogenesis.

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Regular endurance exercise induces an increase in skeletal muscle mitochondrial density (29). At a molecular level, mitochondrial adaptations to endurance training are thought to be due to the cumulative effects of transient increases in mRNA transcripts encoding mitochondrial proteins that follow each acute training session (46). Upon the onset of contraction, homeostatic perturbations within skeletal muscle [e.g., increased AMP/ATP ratio, Ca2+, reactive oxygen species (ROS), lactate; reduced glycogen availability, etc.] result in the activation of a number of regulatory protein kinases that, in turn, phosphorylate downstream targets such as transcription factors or transcriptional coactivators (41). Two putative kinases that have emerged as key players inherent to the intracellular signaling cascades sensitive to muscle contraction are the adenosine monophosphate-activated protein kinase (AMPK) and the p38 mitogen-activated protein kinase (p38MAPK) (22, 38, 39). These kinases converge upon the regulation of peroxisome proliferator-activated receptor coactivator (PGC-1α), a transcriptional coactivator repeatedly cited as the “master regulator of mitochondrial biogenesis” (47). The importance of PGC-1α in regulating mitochondrial content and function is evident from rodent studies demonstrating that overexpression increases oxidative enzyme activity (37), improves insulin sensitivity (5), protects against sarcopenia (61), and also improves exercise capacity (11).

In addition to PGC-1α, the tumor suppressor protein, p53, has also emerged as a potential regulator of mitochondrial function, and several nuclear genes encoding mitochondrial proteins have p53 response elements in their promoter regions (42). Moreover, skeletal muscle from p53 knockout mice exhibit reduced PGC-1α (54) and mitochondrial transcription factor A protein (45) as well as total mitochondrial content (54) compared with wild-type animals. Acute contractile activity also increases phosphorylation of p53 in rodent muscle thus highlighting an additional pathway through which muscle contraction may induce mitochondrial biogenesis (54). Taken together, these studies suggest that p53 is an important modulator of mitochondrial content and function; however, no study to date has examined the exercise-induced response of p53 in human skeletal muscle.

Although the molecular mechanisms underpinning contraction-induced mitochondrial biogenesis are now beginning to be understood, the optimal exercise stimulus to induce mitochondrial adaptations remains to be determined. In this regard, several investigators have adopted high-intensity interval training (HIT) interventions (e.g., 4–6 × 30-s supramaximal cycling Wingate tests) as an alternative to more traditional continuous training [40–60 min cycling at 65% maximal oxygen consumption (V̇O₂max)] approaches. This model of HIT is a potent and time-efficient stimulus for increasing the oxidative capacity of human skeletal muscle as well as improving insulin sensitivity (3, 8, 9, 10, 21, 49, 62). In addition, an alternative form of HIT that is receiving increased research...
attention is running at high but submaximal intensities (e.g., 80–90% \( V_{\text{O}_2\text{max}} \)) for 4–5 repeated intervals of 2- to 4-min duration (28, 43, 44, 58, 63). Although this form of HIT is somewhat longer than the Wingate type protocols (a typical training session could last 30–60 min in total, as opposed to 15 min for Wingate-type sessions), this mode, duration, and relative exercise intensity are also applicable to clinical populations and has been well-tolerated by both cardiovascular (63) and metabolically diseased patients (58). Performing HIT in this way also induces greater feelings of perceived enjoyment compared with CONT (even when both protocols are matched for average intensity, duration, and work done), thereby demonstrating the potential for improved exercise adherence with this intervention (4). However, despite the apparent applicability and popularity of running among the general population, the acute signaling responses of human skeletal muscle to both HIT and CONT running are not well known.

With this in mind, the aim of the present study was to therefore characterize the acute signaling pathways activated in human skeletal muscle by acute HIT and CONT running exercise. Although both protocols were matched for average intensity, duration, and total work done (4), we hypothesized that HIT running would induce greater activation of signaling pathways associated with mitochondrial biogenesis compared with CONT, thereby increasing the efficiency of the training stimulus for a given exercise duration and quantity of work done. This hypothesis was based on the rationale that many of the contractile-induced stressors thought to signal kinase activation (e.g., increased AMP/ATP ratio, glycogen utilization, lactate, ROS, and Ca\(^{2+}\) production) would be expected to increase during brief periods of high-intensity exercise, to a greater extent compared with moderate-intensity exercise (24, 30, 52, 55). In this way, we postulated that performing repeated brief periods of high-intensity exercise (6 \( \times \) 3 min at 90% \( V_{\text{O}_2\text{max}} \) interspersed with 3-min recovery periods at 50% \( V_{\text{O}_2\text{max}} \)) would result in a greater summative metabolic stress and Ca\(^{2+}\) flux compared with sustained exercise at the same average intensity (i.e., 70% \( V_{\text{O}_2\text{max}} \)). Our chosen model of HIT has long-term physiological relevance as it has also been shown in our laboratory to induce training-induced oxidative adaptations (increased content of PGC-1\(\alpha\), COXIV, and SDH activity) of the vastus lateralis and gastrocnemius muscles (43).

**MATERIALS AND METHODS**

**Subjects.** Ten recreationally active men volunteered to participate in the study (mean ± SD: age, 20 ± 1 yr; weight, 73 ± 8 kg; height, 1.77 ± 0.03 m; \( V_{\text{O}_2\text{max}} \), 52 ± 7 ml·kg\(^{-1}\)·min\(^{-1}\)). The experimental procedures and potential risks associated with the study were explained, and subjects gave written informed consent prior to participation. Subjects refrained from additional exercise outside of the study requirements as well as from alcohol and caffeine intake for at least 48 h before any of the testing sessions. None of the subjects had history of neurological disease or musculoskeletal abnormality, and none was under pharmacological treatment during the course of the study. The study was approved by the Ethics Committee of Liverpool John Moores University.

**Experimental design.** In a randomized crossover design (separated by 7 days) and after having initially been assessed for \( V_{\text{O}_2\text{max}} \), subjects attended the laboratory after an overnight fast and performed either high-intensity interval running (HIT) or moderate-intensity continuous running (CONT). Muscle biopsies were obtained from the vastus lateralis preexercise, postexercise, and 3 h after exercise and venous blood samples obtained pre- and postexercise. During the 3-h period between the postexercise and 3 h postexercise biopsies, subjects remained seated in the laboratory and performed light activities such as reading or working on a computer. Heart rate was measured continuously during exercise (Polar S610i, Kempele, Finland) and ratings of perceived exertion (RPE) were obtained at regular intervals during the protocol (7). Blood lactate was also assessed from fingertip capillary samples pre- and postexercise, as well as after 13, 16, 25, 28, 40, and 43 min of exercise (Lactate Pro, Arkay, Japan). In the 30 min preceding exercise, subjects consumed 5 ml/kg body mass of water only and were allowed to drink a further 3 ml/kg of water after 15 and 30 min of exercise (no other form of beverage such as energy containing or caffeinated drinks was permitted at any time). Subjects also consumed water ad libitum during the 3-h window between postexercise and 3-h biopsies, and intake of food during this period was prohibited. The pattern of fluid intake was recorded in the initial trial and repeated for the subsequent trial. Subjects completed a 3-day food diary preceding the first exercise trial and repeated the same energy intake in the 3 days before their second exercise trial. Subsequent dietary analysis was performed by the computer software program Microdiet (Downlee Systems, UK), and average daily macronutrient intake in the 72 h prior to exercise was 1,910 kcal: carbohydrate (CHO) 49 ± 8%, protein 26 ± 8%, and fat 28 ± 8%.

**Assessment of \( V_{\text{O}_2\text{max}} \).** All participants were initially assessed for \( V_{\text{O}_2\text{max}} \) using an incremental exercise test performed on a motorized treadmill (HP Cosmos, Germany). Oxygen uptake was measured continuously during exercise using an on-line gas analysis system (Metamax, Cortex, Germany). The test began with a 3-min stage at a treadmill speed of 10 km/h followed by 3-min stages at 12, 14, and 16 km/h. Upon completion of the 16-km/h stage, the treadmill inclined by 2% every 3 min thereafter until volitional exhaustion. The \( V_{\text{O}_2\text{max}} \) was stated as being achieved by the following end-point criteria: 1) heart rate within 10 beats/min of age-predicted maximum, 2) respiratory exchange ratio > 1.1, and 3) plateau of oxygen consumption despite increased workload (23).

**Exercise protocols.** Both exercise protocols were performed on a motorized treadmill (HP Cosmos, Germany). The HIT protocol commenced with a 7-min warm up at a running velocity corresponding to 70% \( V_{\text{O}_2\text{max}} \) followed by 6–3 min bouts at a running velocity corresponding to 90% \( V_{\text{O}_2\text{max}} \). The high-intensity–intervals were separated by 3-min active recovery periods at a running velocity corresponding to 50% \( V_{\text{O}_2\text{max}} \). Following the interval and recovery periods, participants then performed a 7-min cool down at a running velocity corresponding to 70% \( V_{\text{O}_2\text{max}} \). The exercise protocol gave a total of 18 min of high-intensity exercise and 18 min of active recovery time, thus giving a total interval exercise time of 36 min. When including the warm-up and cool-down times, the total duration of the exercise protocol was 50 min. The CONT protocol consisted of 50 min continuous running at a running velocity corresponding to 70% \( V_{\text{O}_2\text{max}} \). When completed in this way, we have previously observed that the HIT and CONT protocols are matched for average intensity, energy expenditure, duration, and distance run (4).

**Muscle biopsies.** Muscle biopsies were obtained from separate incision sites (2–3 cm apart) from the lateral portion of the vastus lateralis muscle pre-, pos-t, and 3 h after the exercise protocol using a Bard Monopty Disposable Core Biopsy Instrument 12 gauge \( \times \) 10 cm length (Bard Biopsy Systems, Tempe, AZ). Samples were obtained (~60 mg) under local anesthesia (0.5% marcaine) and immediately frozen in liquid nitrogen and stored at −80°C for later analysis. Samples were analyzed for muscle glycogen, AMPKThr172, p38MAPKThr180/Tyr182, p53ser15, and PGC-1\(\alpha\), HSP72, and MusSD mRNA content.

**Muscle analysis.** Approximately 3–6 mg of freeze dried sample was powdered, disected free of all visible nonmuscle tissue, and subsequently hydrolyzed by incubation in 500 \( \mu \)l of 1 M HCl for 3–4 h at 100°C. After cooling to room temperature, samples were neutralized by the addition of 250 \( \mu \)l 0.12 mol/l Tris-2.1 mol/l KOH
membranes were washed for a further 3 manufacturer’s protocol. RNA quality and quantity were determined using Trizol reagent (Invitrogen), according to the manufacturer’s protocol. Gene-specific expression data were obtained using probes selected from Human Universal Probe Library (Roche Diagnostics) with compatible oligonucleotide primers (MWG Eurofins). The primers and corresponding probes are shown in Table 1. One microtiter of each sample was analyzed in triplicate with negative controls using AB 7500 Real-Time Quantitative PCR instrument (Applied Biosystems) and Agilent Brilliant II qPCR Master Mix with Low ROX (Agilent Technologies). One microtiter of cDNA, 500 nM of primer, and 200 nM of probe were used for each 20-μl reaction (Table 1). The following cycling parameters were used: 50°C for 2 min, initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/elongation at 60°C for 1 min. Data were collected and analyzed using AB SDS 1.43 Software (Applied Biosystems, Foster City, CA). Changes in mRNA content were calculated according to the 2^−ΔΔCt method where GAPDH was used as the housekeeping gene.

Blood analyses. Blood samples were drawn pre- and postexercise from a superficial vein in the antecubital crease of the forearm using standard venepuncture techniques (Vacutainers Systems, Becton-Dickinson). Samples were collected into vacutainers containing EDTA or lithium heparin and stored on ice until centrifugation at 1,500 g for 15 min at 4°C. Following centrifugation, aliquots of plasma were stored at 80°C for later analysis. Samples were analyzed for plasma glucose, nonesterified fatty acids (NEFA), and glycerol concentration using commercially available kits (Randox Laboratories, Antrim, UK). Each sample was analyzed in duplicate.

Statistical analysis. Statistical analysis was conducted using the Statistical Package for Social Sciences software program (version 15). Data were analyzed using a two-way repeated-measures General Linear Model where the within factor was time and the between factor was exercise condition (HIT vs. CONT). Where there were significant main effects, Bonferroni post hoc tests were used to locate the differences. All data in text, figures, and tables are presented as mean (SE) with P values ≤ 0.05 indicating statistical significance.

RESULTS

Physiological and metabolic responses to HIT and CONT. Heart rate, blood lactate, and RPE data during exercise are shown in Table 2. In accordance with the differences in activity profiles between trials, the exercise-induced increases (P < 0.01) in heart rate, RPE, and blood lactate was significantly greater (P < 0.01) in HIT compared with CONT. Muscle glycogen decreased (P < 0.001) by ~30% in both conditions with no difference (P = 0.618) between exercise protocols (Table 3). Similarly, both exercise protocols increased plasma glucose concentration (P = 0.002) with no difference between conditions. However, the exercise-induced increases in plasma NEFA (P < 0.001) and glycerol (P < 0.001) were significantly greater in the CONT trial compared with the HIT protocol (P = 0.04 and P = 0.02, respectively) (Table 3). Exercise-induced kinase activation. Representative Western blots are shown in Fig. 1. Phosphorylation of AMPKThr172 increased 1.5-fold postexercise (P = 0.04) with no difference.

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>GCTCTCTGCTCTCCCTGTTCTC</td>
<td>ACGACCAAATCCGTTGACTC</td>
<td>60</td>
</tr>
<tr>
<td>MsSOD</td>
<td>CTCGGACAAACCTGAGCTTTA</td>
<td>TGATTGCTCTCCGAGAATCT</td>
<td>22</td>
</tr>
<tr>
<td>HSP72</td>
<td>ACGAAGGAGGACGCGAGATTC</td>
<td>GCGCTTGTTAGACCTGATCA</td>
<td>70</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>CAAGGCAAAATCGGACGTTTATCCT</td>
<td>GACACTTTAGGCGGCTTGAATAGTC</td>
<td>13</td>
</tr>
</tbody>
</table>
At this time point, p53 phosphorylation was increased immediately postexercise (Fig. 2). Similarly, phosphorylation of p38MAPKThr180/Tyr182 increased 1.9- and 1.5-fold immediately following exercise for HIT and CONT, respectively (P < 0.001), although there was no significant difference between exercise protocols (P = 0.47) (Fig. 3).

PGC-1α mRNA and protein content. Muscle PGC-1α mRNA content increased fourfold at 3 h following exercise (P = 0.01) with no difference between conditions (P = 0.80; Fig. 4A). There were no changes in total PGC-1α protein content at any time during the HIT or CONT trials (P = 0.20; Fig. 4B).

p53 phosphorylation. p53Ser15 phosphorylation showed a tendency to increase immediately postexercise (P = 0.07) but did not reach statistical significance until 3 h following exercise. At this time point, p53 phosphorylation was increased 2.7-fold and 2.1-fold in HIT and CONT trials, respectively (P = 0.01). There was no difference in p53Ser15 phosphorylation between exercise protocols (P = 0.91; Fig. 5).

HSP72 and MnSOD mRNA. Although there was a 3- to 4-fold increase in HSP72 mRNA immediately postexercise, this did not reach statistical significance (P = 0.10). At 3 h postexercise, however, there was an approximate 4-fold increase in both trials (P = 0.04) with no difference (P = 0.87) between conditions (Fig. 6A). In contrast, neither exercise protocol increased MnSOD mRNA content at any time point postexercise (P = 0.44) (Fig. 6B).

**DISCUSSION**

The aim of the present study was to characterize the acute signaling responses of human skeletal muscle to HIT and CONT running exercise protocols when matched for average intensity, duration, and distance run. We provide novel data by demonstrating that both HIT and CONT running induces comparable AMPK and p38MAPK phosphorylation immediately postexercise with similarly increased PGC-1α mRNA at 3 h postexercise. Additionally, this is the first report of exercise-induced p53 phosphorylation in human skeletal muscle. However, contrary to our hypothesis, we observed no differences in activation of the above signaling cascades between HIT and CONT running protocols that are known to be matched for work done (4).

Exercise-induced phosphorylation of AMPK is well documented in human skeletal muscle following cycling-based protocols (17, 22, 38, 39). To the authors’ knowledge, however, this is the first report of AMPK phosphorylation in response to running exercise. Given that exercise increases AMPK phosphorylation in an intensity-dependent manner (18, 51), we hypothesized that HIT would augment AMPK signaling to a greater extent compared with CONT. Despite these reports, our data demonstrate no differences in exercise-induced AMPK phosphorylation between HIT and CONT running. Our chosen model of HIT consisted of 6 × 3-min periods at 90% V̇O₂max interspersed with 3-min active recovery periods at 50% V̇O₂max, as well as a combined 14-min period of warm up and cool down at 70% V̇O₂max. In this way, the majority of exercise in the HIT protocol (32 min) was performed at intensities equal to or greater than 70% V̇O₂max. However, it appears that the repeated 3-min intervals at 90% V̇O₂max within this time scale (i.e., 18 min) offer no additional augmentation with regard to AMPK phosphorylation compared with that of 50 min of CONT running at 70% V̇O₂max. Support for this hypothesis stems from the data of Howlett et al. (30) where it can be estimated that an important allosteric regulator of AMPK signaling, AMP concentration, only differs by ~2 µmol/kg dw when

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**Table 2. Heart rate, RPE, and blood lactate during the HIT and CONT protocols**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Heart rate, beats/min*‡</th>
<th>RPE, AU*‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>89 ± 7</td>
<td>1136</td>
</tr>
<tr>
<td>10</td>
<td>184 ± 9</td>
<td>2008</td>
</tr>
<tr>
<td>13</td>
<td>163 ± 15</td>
<td>1135</td>
</tr>
<tr>
<td>22</td>
<td>188 ± 8</td>
<td>2008</td>
</tr>
<tr>
<td>25</td>
<td>163 ± 13</td>
<td>1135</td>
</tr>
<tr>
<td>40</td>
<td>189 ± 8</td>
<td>2008</td>
</tr>
<tr>
<td>43</td>
<td>163 ± 12</td>
<td>1135</td>
</tr>
<tr>
<td>50</td>
<td>178 ± 9</td>
<td>2008</td>
</tr>
</tbody>
</table>

**Table 3. Muscle glycogen and plasma glucose, NEFA, and glycerol before and after completion of the HIT and CONT protocols**

<table>
<thead>
<tr>
<th></th>
<th>HIT Preexercise</th>
<th>HIT Postexercise</th>
<th>CONT Preexercise</th>
<th>CONT Postexercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle glycogen, mmol/kg dry wt</td>
<td>307 ± 54</td>
<td>191 ± 62*</td>
<td>301 ± 59</td>
<td>190 ± 69*</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.4 ± 0.4</td>
<td>6.5 ± 0.6*</td>
<td>5.7 ± 0.7</td>
<td>6.7 ± 0.7*</td>
</tr>
<tr>
<td>NEFA, mmol/l</td>
<td>0.37 ± 0.22</td>
<td>0.64 ± 0.28*</td>
<td>0.44 ± 0.19</td>
<td>1.1 ± 0.43*‡</td>
</tr>
<tr>
<td>Glycerol, µmol/l</td>
<td>54 ± 20</td>
<td>179 ± 59*</td>
<td>49 ± 25</td>
<td>246 ± 60*‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. NEFA, nonesterified fatty acids. *Significant main effect of exercise, †significant main effect of condition, ‡significant interaction: P < 0.05.
comparing 3 min of exercise at 90% vs. 70% $\dot{V}O_{2\max}$. It is possible, therefore, that a difference in relative exercise intensity of 20% $\dot{V}O_{2\max}$ between exercise protocols that are already relatively intense may not be sufficient to cause further metabolic signaling responses. Furthermore, the 6 × 3-min recovery periods at 50% $\dot{V}O_{2\max}$ may not offer a metabolic stress severe enough to induce AMPK signaling given that exercise at comparable intensities does not induce AMPK phosphorylation (18). In contrast, CONT running for 50 min at 70% $\dot{V}O_{2\max}$ likely offers a sustained stimulus for AMPK phosphorylation as opposed to the transient periods of stress induced by the intermittent bursts of activity inherent to HIT running.

Muscle glycogen availability and utilization, another regulator of AMPK (65), were also not different between trials, which may have contributed to the similar AMPK phosphorylation observed in our study. Both resting glycogen concentration (>300 mmol/kg dw) and utilization (>100 mmol/kg dw) were similar to that observed by us (43) and others (1, 59) while using similar average exercise intensities and subject populations to that studied here. The similar decreases in muscle glycogen content may have been due to the alternating bouts of exercise in HIT between 90% $\dot{V}O_{2\max}$ (thus heavily reliant on CHO oxidation) and 50% $\dot{V}O_{2\max}$ (more reliant on lipid oxidation), as opposed to 50 min of CONT running at 70% $\dot{V}O_{2\max}$, which likely induces sustained glycogen utilization. It is likely, however, that if our HIT protocol had incorporated intermittent periods of supramaximal exercise similar to Wingate-type HIT protocols (i.e., >100% $\dot{V}O_{2\max}$), then much greater rates of glycogen utilization would have occurred, even at the expense of reduced exercise durations (24). Such rapid rates of glycogen depletion may therefore be one of the contributing mechanisms contributing to increased AMPK phosphorylation that is apparent even after just 4 × 30-s bouts of supramaximal exercise (22). Clearly, future studies examining acute signaling responses to running exercise of supramaximal exercise intensity would also be warranted.

p38MAPK is a stress-activated kinase that has repeatedly shown to be phosphorylated following acute cycling (17, 22, 38, 39) and marathon running (6, 66). Consistent with these data, we also observed increased p38MAPK phosphorylation following more practically applicable running exercise protocols, although the magnitude of phosphorylation was not different between HIT and CONT. This finding is similar to recent data demonstrating that p38MAPK phosphorylation is not affected by exercise intensity, even when a large difference between exercise protocols exists such as 80% vs. 40% $\dot{V}O_{2\max}$ (18). Similar
to AMPK (27, 32, 65), p38MAPK phosphorylation is also sensitive to cytosolic calcium (64), glycogen availability (13), and ROS (25). Given the difficulties in directly assessing ROS and also due to the limited tissue obtained by our chosen biopsy technique, we attempted to indirectly ascertain the degree of redox signaling induced by our exercise protocols by examining changes in expression of two redox-sensitive genes, HSP72 (19, 34) and MnSOD (25). Although we observed no changes in MnSOD mRNA at any time point, we observed similar fourfold increases in HSP72 expression after HIT and CONT at 3 h postexercise. This magnitude and time course of adaptation is in agreement with previous literature in which similar subject populations and CONT exercise protocols have been studied (48). These are the first data, however, to observe increased expression of members of the heat shock family in response to HIT running.

In accordance with the role of AMPK and p38MAPK as upstream signaling kinases regulating PGC-1α expression (12, 64), we observed a similar fourfold increase in PGC-1α mRNA at 3 h postexercise in both exercise protocols with no concomitant changes in PGC-1α protein content. This time course of PGC-1α expression is well documented in human muscle following cycling protocols (22, 38, 39). In contrast, it appears that changes in PGC-1α protein are typically only observed within days of exercise (2, 46). To the authors’ knowledge, only one study has measured PGC-1α mRNA changes in response to acute running (45 min of CONT running at 75% VO_{2\text{max}}) where fivefold and fourfold changes (albeit not statistically significant) were observed in the soleus and vastus lateralis muscle, respectively, at 4 h postexercise (26). Our data extend these findings by confirming increased muscle PGC-1α expression in response to running exercise while also demonstrating that the magnitude of response is comparable between HIT and CONT when matched for work done.

The tumor suppressor protein, p53, has recently emerged as a regulator of mitochondrial function (35, 42, 45). p53 has been shown to modulate the activity of nuclear-encoded synthesis of cytochrome-c oxidase (SCO2), which is critical for the proper assembly of the cytochrome-c oxidase (COX) enzyme complex in the electron transport chain (42). Furthermore, PGC-1α also contains a p53 binding site in its promoter region (31) and skeletal muscles from p53 knockout mice display reduced PGC-1α protein content (54) and mitochondrial content (45, 54) compared with wild-type animals. Findings from a variety of cell types have shown that AMPK (33) and p38MAPK (56) phosphorylate p53 on serine 15, and acute contractile activity has been demonstrated to increase phosphorylation on this amino acid residue in rodent muscle (54). The present data confirm and extend these findings by demonstrating that p53^{Ser15} phosphorylation also increases in human skeletal muscle at 3 h postexercise. Based on the time course of this response and similar fold changes, it is tempting to speculate that AMPK and p38MAPK signaling during exercise leads to a temporal and coordinated downstream p53 phosphorylation followed by p53-mediated transcription of PGC-1α in the hours following exercise. Future studies are required, however, to
confirm the presence and physiological relevance of this signaling cascade. Furthermore, p53 has also been shown to regulate mitochondrial transcription factor A (45), and as such, the subcellular location of p53 following muscle contraction also warrants investigation.

In relation to our physiological data, it is noteworthy that CONT exercise was associated with greater elevations in plasma glycerol and NEFA compared with HIT. This finding may be due to a lactate-induced inhibition of lipolysis (40), given that lactate was consistently higher during HIT, and/or a reduction in adipose tissue blood flow during the repeated 3-min high-intensity intervals (50), thereby leading to reesterification (20). Although there have been some suggestions that NEFAs can act as signals to modify contractile-induced gene expression (14, 16), the present data confirm previous findings using either pharmacological (60) or carbohydrate-induced manipulation of NEFA availability (17) to collectively demonstrate the NEFA availability does not regulate exercise-induced PGC-1α expression.

The observation of increased lactate concentrations during HIT does not appear in accordance with similar muscle glycogen utilization between HIT and CONT running. It is important to note, however, that all muscle-related data were quantified in whole muscle homogenates as opposed to specific fiber types. Indeed, it is likely that our CONT protocol would predominantly recruit type I fiber (59) whereas our HIT protocol would stimulate both type II (during the interval periods) and type I fiber recruitment (during the active recovery periods). It is therefore possible that glycolytic flux (and hence muscle glycogen utilization) was particularly high in type II fiber during the 3-min interval periods, thereby contributing to high blood lactate levels at the completion of each interval (see Table 2, minutes 10, 22, and 40). It would appear, however, that the intensity and duration of the active recovery periods were not sufficient for lactate removal. In this regard, blood lactate remains elevated during the active recovery period even though glycogenolysis and glycolytic flux would likely be reduced given the reduced relative exercise intensity of 50% VO2max.

In addition to muscle glycogen utilization, it is possible that the comparable signaling responses observed here may have also arisen as a function of specific responses in individual fiber types that are reflective of recruitment patterns, but which ultimately result in comparable responses in mixed muscle biopsy samples. This is especially true considering that the contractile-induced phosphorylation of AMPK (36) and p38MAPK (57) as well as training-induced increases in PGC-1α protein content (53) are particularly evident in type II fiber when the intensity of contraction is >65% VO2max. Future studies investigating the effects of manipulations of exercise-related variables (e.g., duration, intensity, work-rest ratio, etc.) on cellular and molecular adaptations to exercise would clearly benefit from the inclusion of histochemical techniques staining for muscle glycogen content so as to evaluate the degree of specific muscle fiber recruitment patterns.

In summary, the present study characterizes, for the first time, the responses of the acute molecular signaling pathways thought to initiate mitochondrial biogenesis in human skeletal muscle in response to two physiologically relevant and practically applicable running exercise protocols. We demonstrate that acute HIT and CONT running (when matched for average intensity, duration, and work done) induce comparable increases in AMPK and p38MAPK phosphorylation as well as PGC-1α mRNA content in human skeletal muscle. Furthermore, this is the first report of exercise-induced p53 phosphorylation in humans, consistent with the notion that this protein may be involved in regulation of contraction-induced mitochondrial biogenesis.

ACKNOWLEDGMENTS

We thank all the subjects who took part in the study for their efforts during demanding exercise protocols.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


