An acute bout of high-intensity interval training increases the nuclear abundance of PGC-1α and activates mitochondrial biogenesis in human skeletal muscle.

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Abstract

Low-volume, high-intensity interval training (HIT) increases skeletal muscle mitochondrial capacity yet little is known regarding potential mechanisms promoting this adaptive response. Our purpose was to examine molecular processes involved in mitochondrial biogenesis in human skeletal muscle in response to an acute bout of HIT. Eight healthy men performed 4 x 30-s bursts of all out maximal intensity cycling interspersed with 4 min of rest. Muscle biopsy samples (vastus lateralis) were obtained immediately before and after exercise, and after 3 and 24 h of recovery. At rest, the majority of peroxisome proliferator-activated receptor γ coactivator (PGC)-1α, a master regulator of mitochondrial biogenesis, was detected in cytosolic fractions. Exercise activated p38 mitogen activated protein kinase (MAPK) and 5′-AMP activated protein kinase (AMPK) in the cytosol. Nuclear PGC-1α protein increased 3 h into recovery from exercise, a time point that coincided with increased mRNA expression of mitochondrial genes. This was followed by an increase in mitochondrial protein content and enzyme activity after 24 h of recovery. These findings support the hypothesis that an acute bout of low-volume HIT activates mitochondrial biogenesis through a mechanism involving increased nuclear abundance of PGC-1α.

Key words: Exercise, mitochondria, peroxisome proliferator-activated receptor gamma co-activator 1
Abbreviations

ACC, acetyl-CoA carboxylase; AMPK, 5’-AMP activated protein kinase; COX, cytochrome c oxidase; CS, citrate synthase; LDH, lactate dehydrogenase; HIT, high-intensity interval training; p38 MAPK, p38 mitogen-activated protein kinase; PGC-1α, peroxisome proliferator-activated receptor γ co-activator 1α; VO₂peak, maximal oxygen uptake
Introduction

Skeletal muscle mitochondrial biogenesis is a classic adaptation to endurance exercise training (20, 21), as commonly demonstrated by increased expression, content and/or activity of mitochondrial proteins. An enhanced capacity for substrate transport and oxidation contributes to improved metabolic control during exercise at a given workload and enhanced functional capacity (21, 22). These adaptations also contribute to the beneficial effects of exercise in the prevention and treatment of chronic diseases including type 2 diabetes and cardiovascular disease (18, 19, 34). Understanding the molecular mechanisms that promote the muscle adaptive response to exercise therefore has relevance from a basic science, clinical and applied sport perspective (45).

It is now commonly accepted that acute exercise activates signaling pathways which convert various intra- and extra-cellular signals into changes in gene transcription (9, 23, 41). Ultimately, the phenotypic changes in skeletal muscle following training are the result of the cumulative effect of these transient changes in gene transcription. Peroxisome proliferator-activated receptor γ coactivator (PGC)-1α is a transcriptional co-activator which is recognized as a key regulator of mitochondrial biogenesis in muscle (28, 44). PGC-1α co-activates several transcription factors in order to activate an entire program of mitochondrial and metabolic adaptation (28, 44). Overexpression of PGC-1α in skeletal muscle recapitulates many aspects of endurance training adaptation, including increased mitochondrial content (3, 27, 29) and functional exercise capacity (7). As a result, PGC-1α is hypothesized to be a critical mediator of the
muscle adaptive response to training (7, 23, 36) and is regarded as a key molecular target for treatment of mitochondrial-related metabolic diseases (17, 30).

High-intensity interval training (HIT) is characterized by brief repeated bursts of relatively intense exercise interspersed by periods of recovery. HIT has recently been shown to induce many adaptations normally associated with traditional endurance exercise training, including increased muscle mitochondrial capacity and improved endurance performance (5, 6, 13). Intriguingly, HIT induces these adaptations despite a much lower total exercise volume and reduced time commitment. Little is known regarding the molecular processes that regulate mitochondrial biogenesis in response to HIT, but evidence is accumulating to suggest that PGC-1α is involved. We recently showed that an acute bout of HIT, consisting of 4 x 30-s “all-out” cycling bouts separated by 4 min of rest, led to a robust increase in PGC-1α mRNA measured 3 h into recovery (15). Acute HIT also increased the activation of 5’AMP-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (MAPK), two kinases which can directly activate PGC-1α (10, 25, 26) and have been implicated in exercise-induced mitochondrial biogenesis (24, 35). These findings suggested that PGC-1α might be involved in the adaptive response to HIT, yet PGC-1α protein measured at the whole muscle level was unchanged immediately and 3 h after exercise (15). Based on initial findings in rodents (43), which were subsequently confirmed in human skeletal muscle (31), the initial adaptive response to endurance exercise may involve accumulation of PGC-1α in the
nucleus, where it can function to co-activate transcription factors and direct mitochondrial biogenesis (43). Thus, measuring PGC-1α in the nucleus may provide greater insight into its role in mitochondrial adaptations following exercise or other interventions.

In the current investigation we sought to examine potential mechanisms controlling the adaptive response to HIT by determining the effects of an acute bout of HIT on levels of nuclear PGC-1α. We also wanted to obtain a more comprehensive characterization of the response to an acute bout of HIT by examining signaling pathways linked to PGC-1α activation as well as changes in mRNA and protein expression of mitochondrial genes at selected time points throughout 24 hours of recovery. While the general approach was similar to our recent studies (15, 31), two important novel aspects of the current study included: 1) nuclear PGC-1α was measured in conjunction with mRNA expression of mitochondrial genes to provide a downstream indicator of activation; and 2) mRNA, protein, and activity of mitochondrial enzymes were measured up to 24-hr following a single session of low-volume HIT to provide a comprehensive time course of the human skeletal muscle adaptive response to this unique form of exercise. We hypothesized that an acute bout of HIT would increase nuclear PGC-1α and activate mitochondrial biogenesis as evidenced by increased mRNA expression, protein content, and maximal activity of selected mitochondrial enzymes.

Methods
Subjects

Eight young men (24±1 y, 81±3 kg) volunteered to take part in the study. Subjects were not specifically trained in any one sport or discipline but were habitually active several days per week. Subjects completed a brief medical history questionnaire and a physical activity readiness questionnaire (PAR-Q) prior to participation to rule out any contraindications to performing vigorous-intensity exercise. Details of exercise testing and risks associated with the experimental procedures were explained to subjects before they provided written informed consent. The project was approved by the Hamilton Health Sciences / Faculty of Health Sciences Research Ethics Board. Maximal oxygen uptake (VO₂peak), assessed during a ramp increase (30 W/min) cycling test to volitional exhaustion (13), was 45±4 ml/kg/min. Prior to the experimental trial, subjects also completed a familiarization session where they completed the acute HIT protocol (4 x 30-s all-out Wingate cycling tests separated by 4 min of rest).

Experimental protocol

At least one week following the familiarization session, subjects were asked to report to the laboratory in the morning approximately 2 h after ingesting their typical breakfast. The lateral aspect of one thigh was anesthetized (2% Lidocaine) and prepared for the extraction of muscle biopsy samples from the vastus lateralis using a Bergstrom needle adapted with suction (4). Four muscle biopsies were obtained during the experiment (two from each leg) and the relative arrangement was varied between subjects to avoid potential order bias. Each biopsy was obtained from a separate incision site spaced 2-3 cm apart.
Following a baseline resting biopsy, subjects completed a brief warm-up by 
cycling at 50 W for 5 min followed immediately by the acute HIT protocol. The 
HIT protocol consisted of 4 x 30-s all-out cycling at a constant load corresponding 
to 0.075 kg/kg body mass (i.e., Wingate tests) separated by 4 min of rest on an 
electronically-braked ergometer (Velotron, RacerMate Inc., Seattle, WA, USA). 
Heart rate was collected during exercise using telemetry (Polar A3, Polar 
Electro). Upon completion of the exercise test, subjects were immediately moved 
to an adjacent gurney for extraction of the second muscle biopsy sample which 
was taken within 2-3 min of the final 30-s sprint. A third biopsy was obtained 3 h 
after the post-exercise biopsy. During the 3-h period between the second and 
third biopsies, subjects remained in the laboratory where they rested or worked 
on a computer and were not allowed to ingest anything except for water. After the 
third biopsy, subjects were provided with a standardized lunch and were allowed 
to leave the laboratory. The following morning, at the same time and after 
consuming the same breakfast, subjects reported back to the laboratory for the 
fourth and final biopsy, which was obtained approximately 24 h following the 
initial HIT session. Following extraction of each biopsy, the muscle sample was 
quickly blotted to remove excess blood, sectioned into several pieces, and placed 
in separate vials before being frozen in liquid nitrogen.

**Muscle Analyses**

**Total RNA isolation.** Approximately 20 mg of tissue was homogenized 
using an electronic homogenizer (Pro 250, Pro Scientific, Oxford, CT, USA) in 1 
ml of a commercially available TRIzol® reagent (Invitrogen), spun at 12000g for
10 min at 4°C to help remove insoluble material, and then separated into organic and aqueous phases using 200 μl chloroform according to manufacturer’s instructions. Total RNA was isolated from the aqueous phase following precipitation with 500 μl 70% ethanol using the RNeasy mini kit from Qiagen according to the method detailed by the manufacturer. On-column DNA digestion was performed. RNA concentration was measured by spectrophotometry (Nanodrop, ND1000) by measuring the absorbance at 260 nm (A260) and 280 nm (A280) with A260/A280 ratios above 1.8 indicating high quality RNA.

Real-time RT-PCR. First-strand cDNA synthesis from 100 ng of total RNA was performed with random oligo(dT) primers using a high capacity cDNA reverse transcription kit (Applied Biosystems Inc., Foster City, CA) according to manufacturer’s directions. The mRNA expression of PGC-1α, CS, COX subunit II, and COX subunit IV were quantified by quantitative real-time RT-PCR (iCycler real-time PCR system, Bio-Rad Laboratories, Hercules, CA) using SYBR Green chemistry (PerfeCTa SYBR® Green Supermix, iQ, Quanta BioSciences, Gaithersburg, MD), as described previously (1, 33). Forward and reverse primers (Table 1) for the aforementioned genes were designed based on sequences available in GenBank (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) using the MIT Primer 3 designer software (http://wi.mit.edu/cgi-bin/primer3/primer3_www.cgi), and were confirmed for specificity using the basic local alignment search tool (www.ncbi.nlm.nih.gov/BLAST/). Specificity of the amplified product was confirmed by melting point dissociation curves generated by the instrument. mRNA expression was quantified using the $2^{-ΔCt}$ method using
β-2 microglobulin, which did not change across time, as the house-keeping gene (33). All samples were run in duplicate simultaneously with negative controls which contained no cDNA.

**Preparation of whole muscle lysates.** Approximately 30-40 mg of frozen wet muscle was homogenized on ice in 25 volumes of buffer (70 mM sucrose, 220 mM mannitol, 10mM HEPES) supplemented with protease inhibitors (Complete Mini®, Roche Applied Science, Laval, PQ, Canada) using 50 strokes of a Kontes glass-on-glass homogenizer. Crude homogenates were centrifuged at 700g for 10 min at 4°C and the supernatant was extracted and taken as the whole muscle lysate for enzyme activity assays and Western blotting. Homogenates were subjected to three freeze-thaw cycles to help release mitochondrial proteins prior to enzyme activity measurements.

**Preparation of nuclear and cytosolic extracts.** Nuclear and cytosolic fractions were prepared from 30-40 mg of wet muscle using a commercially available nuclear extraction kit with slight modifications (Pierce NE-PER® #78833, Rockford, IL, USA). Muscle samples were homogenized in CER-I buffer containing protease and phosphatase (PhosSTOP®, Roche Applied Science, Laval, PQ, Canada) inhibitors with 4 x 5-s pulses using the aforementioned electronic homogenizer. Homogenates were centrifuged at 16000g for 10 min at 4 °C and the supernatant taken as the crude cytosolic fraction. Pellets containing nuclei were subsequently washed four times in PBS to remove cytosolic contaminating proteins. Pellets were rehomogenized in NER buffer supplemented with 0.1% Igepal ® and inhibitors using a Teflon pestle and
sonicated briefly on ice. Following 40 minutes of incubation, samples were re-
centrifuged at 16000g and the supernatant was taken as the soluble nuclear
fraction. Enrichment and purity of nuclear fractions was confirmed by the
abundance of nuclear matrix protein p84 and histone H3 and the absence of the
cytosolic protein lactate dehydrogenase (LDH) in Western blot analyses
(Supplementary Figure 1).

Western blotting. Protein concentrations of whole muscle lysates and
nuclear fractions were determined using a commercial assay (BCA Protein
Assay, Pierce, Rockford, IL, USA). Samples were diluted to the same
concentration with milli-Q water and denatured by mixing with 4X Laemmli’s
buffer and heating at 95°C for 5 min. Equal amounts of protein (5-20 μg,
depending on the protein of interest) were loaded onto 7.5-12.5% SDS-PAGE
gels and separated by electrophoresis for 2-2.5 hours at 100 V. Proteins were
transferred to nitrocellulose membranes for 1 hr at 100 V. Ponceau S staining
was performed following the transfer to confirm equal loading and transfer
between lanes. Membranes were blocked at room temperature (RT) by
incubating in 5% fat-free milk TBS-T. Blots were then incubated with primary
antibodies overnight at 4 °C in 3% fat-free milk or BSA. Membranes were then
washed for 3 x 5 min and incubated in appropriate species-specific secondary
antibody for 1 hour at RT in 3% fat-free milk TBS-T. Membranes were rewashed
for 3 x 15 min and protein bands were detected by chemiluminescence
(Supersignal® West Dura, Pierce), captured using Fluorchem SP Imaging
system and software (Alpha Innotech Corp., San Leandro, CA, USA), and
quantified using NIH ImageJ software. A rabbit monoclonal antibody from Cell
Signaling Technology was used to detect PGC-1α (Beverly, MA, USA).
Phosphorylated p38 MAPK (threonine-180/tyrosine-182), total p38 MAPK, and
phosphorylated ACC (serine-79) antibodies were from Cell Signaling Technology.
COX subunit -II and -IV mouse monoclonal antibodies were from MitoSciences
(Eugene, OR, USA). CS rabbit polyclonal antibody was a kind gift from Dr. Brian
Robinson (The Hospital for Sick Children, Toronto, ON, Canada). Alpha-tubulin
(Cell Signaling Technology) was used as a loading control in whole muscle
analyses and TATA box binding protein (Abcam, Cambridge, MA, USA) was
used to control for nuclear protein yield. Specificity of the PGC-1α antibody has
been demonstrated previously (30).

Mitochondrial enzyme activity. The maximal activities of citrate synthase
(CS) and cytochrome c oxidase (COX) were determined in whole muscle lysates
at baseline and 24 h recovery using a spectrophotometer (Cary Bio-300, Varion,
Inc., Palo Alto, CA) as we have previously described (12). Enzyme activities were
expressed in mmol·kg of protein⁻¹·hr⁻¹ wet weight (w.w.).

Statistical Analyses
Mitochondrial enzyme activity data were analyzed using a Student’s paired
t-test since only baseline and 24 h data were compared. All other variables were
analyzed using a one-way repeated measures ANOVA. Significant main effects
were further analyzed using a Tukey’s HSD post-hoc test. Significance was set at
p≤0.05.
1 **Results**

2 **Exercise performance and heart rate**

3 Mean power output averaged over the four intervals was 575±63 W or 177±14% of peak power achieved during the VO\textsubscript{2}peak test. Peak heart rate elicited during the intervals corresponded to 93±1% of maximal heart rate.

4 **PGC-1\textalpha protein content**

5 At rest, the majority of PGC-1\textalpha was detected in the cytosol compared to the nucleus (Figure 1A). The protein content of PGC-1\textalpha measured in nuclear fractions was unchanged immediately after exercise, increased by ~66% at 3 h recovery, and was not significantly different from baseline at 24 h recovery (p=0.027, Figure 1B). PGC-1\textalpha protein content measured in whole muscle lysates was unchanged immediately and 3 h after exercise but was increased by ~57% at 24 h recovery (p=0.042, Figure 1C).

6 **mRNA expression**

7 The mRNA expression of PGC-1\textalpha was unchanged immediately after exercise, increased ~750% at 3 h recovery and returned to basal levels at 24 h (p<0.001, Figure 2A). Mitochondrial enzymes followed a similar pattern as CS (77%, p=0.017, Figure 2B), COX II (83%, p=0.007, Figure 2C), and COX IV (76%, p=0.009, Figure 2D) mRNA expression were all increased at 3 h of recovery but were not different from baseline at any other time point.

8 **Signaling proteins**

9 Phosphorylation status of p38 MAPK measured in cytosolic fractions was higher immediately after exercise, returned to basal levels at 3 h recovery, and was
again significantly elevated at 24 h recovery (p=0.004, Figure 3A). Cytosolic ACC phosphorylation, a marker of AMPK activation (25), was increased immediately after exercise (p=0.008, Figure 3B).

Mitochondrial enzyme protein content

The protein content of CS was unchanged immediately and 3 h after exercise, but was increased by ~30% following 24 h of recovery when compared to baseline (p=0.04, Figure 4A). Similarly, COX II protein content was increased by ~29% at 24 h recovery (p=0.032, Figure 4B). COX IV protein was increased by ~43% at 3 h recovery and ~30% at 24 h recovery (p<0.001, Figure 4C).

Mitochondrial enzyme activities

The maximal activity of CS was increased by 14% when measured 24 h following exercise (p=0.024, Figure 5A). The maximal activity of COX tended to be higher 24 h following exercise (~19%), but this did not reach statistical significance (p=0.10, Figure 5B).

Discussion

One of the most established adaptations to traditional high-volume endurance exercise training is an increase in skeletal muscle mitochondrial content (20-22, 43). Recent studies have shown that low-volume HIT is also a potent stimulus for mitochondrial biogenesis, despite a substantially lower total exercise and training time commitment (5, 6, 13, 14). Here, we characterize for the first time a potential mechanism promoting the adaptive response to HIT.
involving nuclear accumulation and activation of the transcriptional co-activator PGC-1α.

At rest, the majority of PGC-1α was found in the cytosol compared to the nucleus. In response to an acute bout of HIT, consisting of 4 x 30 s of “all-out” cycling with 4 min rest in between, there was an increase in nuclear PGC-1α protein content which coincided with elevated mRNA expression of several mitochondrial genes. This mechanism of activation of mitochondrial biogenesis is consistent with recent reports in rodents (43) and humans following 90-120 min of continuous endurance exercise (31). This suggests that the comparable mitochondrial adaptations observed after low-volume HIT and traditional endurance training (5, 13) may be mediated by some of the same mechanisms.

The present results also shed light on the rapidity of improvements in muscle oxidative capacity following low-volume HIT. The elevation in mitochondrial gene mRNA expression was followed by increased mitochondrial protein content and maximal enzyme activity within 24 hours of a single session of training. To our knowledge, this is the first study to report an increase in markers of mitochondrial content following a single session of low-volume HIT, involving a total of only 2 min of maximal effort cycling exercise. This further highlights the potency of low-volume HIT for inducing a functional increase in skeletal muscle mitochondrial capacity in humans (5, 6, 13) and highlights the ability of human skeletal muscle to rapidly increase metabolic capacity in response to the unique stimulus of HIT.

*Nuclear accumulation of PGC-1α in response to low-volume HIT*
PGC-1α has emerged as a key regulator of mitochondrial biogenesis by virtue of its ability to co-ordinate and amplify the expression of networks of genes by co-activating several transcription factors which bind to promoter regions of mitochondrial and metabolic genes (28, 44). PGC-1α is hypothesized to mediate exercise-induced mitochondrial biogenesis (11, 23), but the molecular mechanisms by which PGC-1α regulates this response are only beginning to be unraveled. If PGC-1α is involved in the initial adaptive response to exercise, there must exist a mechanism for activating this important transcriptional co-activator that circumvents the need to increase its total content, as total PGC-1α does not appear to increase in response to an acute exercise stimulus (15, 31, 43).

Based on experiments in rats, it has been hypothesized that the redistribution of PGC-1α from the cytosol to the nucleus might be an important molecular mechanism of adaptation to prolonged exercise in rodent skeletal muscle (43). Our findings demonstrate, for the first time, that such a mechanism seems to operate in human skeletal muscle in response to low-volume HIT. At rest, the majority of PGC-1α in human skeletal muscle was present in the cytosol, but in response to acute HIT there was an increase in nuclear PGC-1α, with a coinciding increase in mRNA expression of several mitochondrial genes whose expression is controlled by PGC-1α. PGC-1α protein is known to co-activate its own promoter through an auto-regulatory loop involving the MEF2 transcription factor (16) and/or Sirt1/MyoD (2). Therefore, the increase in PGC-1α mRNA
expression at 3 h recovery provides further evidence of PGC-1α activation. Whole muscle PGC-1α remained unchanged at 3 h recovery, supporting redistribution from the cytosol to the nucleus as a mechanism of activation for this transcriptional co-activator. The subsequent increase in total PGC-1α at 24 h recovery may serve to sustain the increase in mitochondrial content (43) and/or play an adaptive role by increasing sensitivity to a subsequent metabolic stressor, such as exercise.

Potential signaling mechanisms involved in adaptation to low-volume HIT

Although endurance exercise has long been known to induce mitochondrial biogenesis in skeletal muscle, the potential signals promoting this response have only recently begun to be identified. It is now generally accepted that acute alterations in intramuscular homeostasis act as signals to activate signal transduction pathways that converge to increase mitochondrial gene transcription (9, 23, 41). Over the course of training, the cumulative effect of the repeated response to these acute signals results in an increase in mitochondrial protein content and increased oxidative capacity. However, there have been very few studies in humans that explicitly demonstrate this response. Furthermore, the time-course for mitochondrial adaptation to exercise in human skeletal muscle is not well studied.

Our findings demonstrate a coordinated response whereby activation of signaling proteins immediately following exercise is followed by nuclear
accumulation of PGC-1α protein and increased mitochondrial gene transcription measured at 3 h of recovery, and then a subsequent increase in mitochondrial protein content and enzyme activity measured at 24 h of recovery. HIT led to increased phosphorylation of p38 MAPK and ACC, a marker of AMPK activation (25), immediately following exercise. Both p38 MAPK and AMPK are activated in response to metabolic stress, and studies in vitro and in cell culture have demonstrated that both kinases can directly phosphorylate and activate PGC-1α (10, 25, 26). Given that we found the majority of PGC-1α residing in the cytosol at rest and that both of these kinases were activated in the cytosol in response to exercise, it is tempting to speculate that exercise-induced activation of p38 MAPK and AMPK may be involved in activating PGC-1α. However, further evidence is needed to confirm if p38 MAPK and AMPK interact with and phosphorylate PGC-1α in vivo in human skeletal muscle. Unexpectedly, p38 MAPK phosphorylation was also higher after 24 hours recovery, a finding that may be related to the fact that p38 MAPK is involved in other cellular responses during recovery from high-intensity exercise. Indeed, p38 MAPK is known to be involved in the response to inflammation, reactive oxygen species, growth factors, and various cellular stressors (42).

It is difficult to conclusively identify molecular mechanisms responsible for muscle adaptation in human studies. Nonetheless, our findings provide evidence that the redistribution of PGC-1α from the cytosol to the nucleus in response to acute exercise may be involved in promoting mitochondrial adaptations to HIT. In resting human skeletal muscle, the majority of PGC-1α resided in the cytosol and
in response to an acute bout of HIT, nuclear PGC-1α increased yet whole muscle PGC-1α remained unaltered. Although these findings provide evidence for a change in PGC-1α sub-cellular localization, it cannot be ruled out that increased stability of PGC-1α may have also contributed to the ability to detect an increase in nuclear PGC-1α following acute HIT. PGC-1α is thought to be a rapidly degraded protein (37, 46) and therefore any factor that increased PGC-1α stability could enhance nuclear accumulation and promote mitochondrial gene transcription. The potential signals that presumably might target PGC-1α for the nucleus and/or increase stability to mediate this response cannot be ascertained with certainty in the present study, but may involve activation of p38 MAPK and AMPK. Both kinases were activated in the cytosol immediately after exercise, preceding the increase in nuclear PGC-1α. PGC-1α is regulated by numerous post-translational modifications and recently it has been shown in rodents that exercise might activate PGC-1α by a mechanism involving SIRT1-mediated deacetylation (8). Given that SIRT1 is an NAD+-dependent deacetylase (8), it is possible that changes in muscular NAD+/NADH and activation of SIRT1 might be involved in increasing nuclear PGC-1α protein content in response to HIT. Unfortunately, we could not address this potential mechanism in the current study.

A single session of low-volume HIT increases mitochondrial capacity

The finding that mitochondrial protein content and enzyme activity were increased after only 24 h of recovery from a single session of low-volume HIT
highlights the potency of this type of exercise for inducing mitochondrial biogenesis. We have previously shown increases in CS activity as well as COX II and IV protein despite no changes in steady-state mRNA following 2 wk of HIT or endurance training (13, 32). The present findings suggest that the increase in COX subunit protein content occurs rapidly after only one session of training. It is interesting to note that COX IV (nuclear-encoded) appeared to increase sooner than COX II (mitochondrial-encoded), perhaps reflecting a slight lag in the increase in protein expression for mitochondrial DNA-encoded ETC subunits. Further research is required to examine the relationship between nuclear- and mitochondrial-encoded ETC subunits and complex assembly in response to HIT.

**Perspectives and Significance**

In conclusion, this investigation demonstrates that a single session of low-volume HIT can increase mitochondrial capacity in human skeletal muscle and provides important mechanistic insight into this adaptive process. HIT appears to be a potent stimulus to activate PGC-1α and rapidly induce mitochondrial biogenesis. Given the potential benefits of increased skeletal muscle PGC-1α for improving insulin sensitivity (3), preventing age-related sarcopenia and metabolic decline (40), and improving mitochondrial myopathy phenotypes (38, 39), HIT may represent an effective strategy for improving metabolic health in a variety of populations. If nuclear PGC-1α is indicative of its activation, then studying the influence of different exercise stimuli on the nuclear accumulation of PGC-1α may be a valuable tool for evaluating the mechanisms and potential benefits of
different types of exercise. Furthermore, delineating the molecular mechanisms that increase nuclear PGC-1α would appear to be important if this transcriptional regulator is to be targeted for the treatment or prevention of disease.
Acknowledgements

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Table 1. Primers used for real-time RT-PCR analyses of mRNA expression.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>PGC-1α</td>
<td>TTGCTAAACGACTCCGAGAA</td>
<td>TGCAAAGTTCCCTCTCTGCT</td>
</tr>
<tr>
<td>COX II</td>
<td>CGACTACGCGGACTAATCT</td>
<td>TCGATTGTCAACGTCAAGGA</td>
</tr>
<tr>
<td>COX IV</td>
<td>CGAGCAATTTCCACCTCTGT</td>
<td>GGTCACGCGATCCATATAAA</td>
</tr>
<tr>
<td>CS</td>
<td>GAGCAGGGTAAAGCCAAGAAT</td>
<td>CCCAACAGGACCTGTAGT</td>
</tr>
<tr>
<td>β-2</td>
<td>GGCTATCCAGCGTACTCCAA</td>
<td>GATGAAACCCAGACACATAGCA</td>
</tr>
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Author contributions

JPL, AS, MAT, and MJG contributed to the conceptual design. JPL, DB and AS performed experiments and analyzed data. JL recruited subjects and ran experimental trials. MAT performed medical procedures. JPL and MJG wrote the manuscript with input from all authors. Exercise trials were run in the laboratory of MAT and experiments performed in the laboratories of MJG and MAT.
Figure legends

Figure 1. (A) At rest, the majority of peroxisome proliferator-activated receptor γ coactivator (PGC)-1α was detected in cytosolic fractions prepared from human skeletal muscle biopsy samples. (B) PGC-1α protein content measured in nuclear fractions prepared from skeletal muscle biopsy samples taken before (Pre), after (Post), and at 3 (3 hr Rec) and 24 (24 hr Rec) hours of recovery from acute high-intensity interval training. (C) PGC-1α protein content measured in whole muscle fractions before (Pre), after (Post), and following 3 (3 hr Rec) and 24 (24 hr Rec) of recovery from an acute bout of high-intensity interval training. * P<0.05 vs. Pre.

Figure 2. Quantitative real-time RT-PCR analyses of mRNA expression of (A) peroxisome proliferator-activated receptor γ coactivator (PGC)-1α, (B) citrate synthase (CS), (C) cytochrome c oxidase (COX) II, and (D) COX IV in skeletal muscle biopsy samples taken before (Pre), after (Post), and at 3 (3 hr Rec) and 24 (24 hr Rec) of recovery from acute high-intensity interval training. β-2-microglobulin was used as a housekeeping gene. *P<0.05 vs. Pre.

Figure 3. Levels of (A) phosphorylated p38 mitogen activated protein kinase (p-p38 MAPK) and (B) phosphorylated acetyl CoA carboxylase (p-ACC) in cytosolic fractions prepared from skeletal muscle biopsy samples taken before (Pre), after (Post), and at 3 (3 hr Rec) and 24 (24 hr Rec) hours of recovery from acute high-
intensity interval training. Total p38 MAPK (p38) was used to control for equal
loading in (A). * P<0.05 vs. Pre.

**Figure 4.** Protein content of (A) citrate synthase (CS), (B) cytochrome c oxidase
(COX) II, and (C) COX IV in whole-muscle fractions prepared from skeletal
muscle biopsy samples taken before (Pre), after (Post), and at 3 (3 hr Rec) and
24 (24 hr Rec) hours of from acute high-intensity interval training. *P<0.05 vs.
Pre. Representative Western blots for each protein are shown in (D).

**Figure 5.** Maximal enzyme activity of (A) citrate synthase (CS) and (B)
cytochrome c oxidase (COX) measured in skeletal muscle biopsy samples taken
before (Pre) and at 24 hours of recovery (24 hr Rec) from acute high-intensity
interval training. *P<0.05.
A

Cyt Nuc Cyt Nuc

PGC-1α

B

Nuclear PGC-1α: TATA (a.u.)

Pre Post 3hr Rec 24 hr Rec

C

Whole muscle PGC-1α: Tubulin (a.u.)

Pre Post 3hr Rec 24 hr Rec
A

CS activity (mmol/kg protein/hr)

Pre 24 hr Rec

*  

B

COX activity (mmol/kg protein/hr)

Pre 24 hr Rec

p=0.10