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A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms

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High-intensity interval training (HIT) induces skeletal muscle metabolic and performance adaptations that resemble traditional endurance training despite a low total exercise volume. Most HIT studies have employed 'all out', variable-load exercise interventions (e.g. repeated Wingate tests) that may not be safe, practical and/or well tolerated by certain individuals. Our purpose was to determine the performance, metabolic and molecular adaptations to a more practical model of low-volume HIT. Seven men (21 ± 0.4 years, $\dot{V}_{O_2 peak} = 46 \pm 2$ ml kg^{-1} min^{-1}) performed six training sessions over 2 weeks. Each session consisted of 8–12 × 60 s intervals at $\sim$100% of peak power output elicited during a ramp $\dot{V}_{O_2}$ peak test (355 ± 10 W) separated by 75 s of recovery. Training increased exercise capacity, as assessed by significant improvements on both 50 kJ and 750 kJ cycling time trials ($P < 0.05$ for both). Skeletal muscle (vastus lateralis) biopsy samples obtained before and after training revealed increased maximal activity of citrate synthase (CS) and cytochrome *c* oxidase (COX) as well as total protein content of CS, COX subunits II and IV, and the mitochondrial transcription factor A (Tfam) ($P < 0.05$ for all). Nuclear abundance of peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) was $\sim$25% higher after training ($P < 0.05$), but total PGC-1α protein content remained unchanged. Total SIRT1 content, a proposed activator of PGC-1α and mitochondrial biogenesis, was increased by $\sim$56% following training ($P < 0.05$). Training also increased resting muscle glycogen and total GLUT4 protein content (both $P < 0.05$). This study demonstrates that a practical model of low volume HIT is a potent stimulus for increasing skeletal muscle mitochondrial capacity and improving exercise performance. The results also suggest that increases in SIRT1, nuclear PGC-1α, and Tfam may be involved in coordinating mitochondrial adaptations in response to HIT in human skeletal muscle.

Introduction

Endurance exercise training induces numerous morphological and metabolic adaptations in skeletal muscle, including mitochondrial biogenesis and an enhanced capacity to oxidize fuels such as glucose and fats (Holloszy, 1967; Holloszy & Booth, 1976). These adaptations to exercise training have significant scientific and clinical relevance, as increased physical activity is linked with improved metabolic health and reduced risk for many chronic disorders, including obesity, insulin resistance and type 2 diabetes (Warburton *et al.* 2006; Hawley, 2004). We (Burgomaster *et al.* 2005, 2006, 2007, 2008; Gibala *et al.* 2006) and others (Parra *et al.* 2000; Babraj *et al.* 2009) have demonstrated that high-intensity interval training (HIT) induces numerous physiological adaptations that resemble traditional endurance training, despite a low total exercise volume. For example, 2 weeks

of HIT was similar to 2 weeks of endurance training in leading to increases in exercise performance as well as the maximal activity and protein content of the mitochondrial enzyme cytochrome *c* oxidase (COX) (Gibala *et al.* 2006). Low-volume HIT has also been shown to promote improvements in markers of metabolic control and vascular endothelial function which are comparable to endurance training (Burgomaster *et al.* 2008; Rakobowchuk *et al.* 2008). What is most intriguing about these findings is that the volume of exercise and time spent training were ∼90% and ∼75% lower, respectively, with HIT compared to ET. This suggests that HIT is a potent and time-efficient strategy to induce skeletal muscle metabolic adaptations and improve functional exercise capacity. Given that 'lack of time' is the most commonly cited barrier to performing regular exercise in a variety of populations (Godin *et al.* 1994; Trost *et al.* 2002), low-volume HIT may represent an alternative to endurance training to improve metabolic health and reduce the risk for chronic diseases.

Our previous studies examining adaptations to low-volume HIT have used a training protocol that consists of repeated 'all out' maximal cycling efforts (i.e. repeated Wingate tests). This type of training is extremely demanding and requires a specialized cycle ergometer, and thus may not be safe or practical for some individuals. In light of this, our primary purpose in this study was to examine the exercise performance and muscle metabolic adaptations to a more practical model of HIT that is nonetheless still time efficient. Specifically, we kept total training time low in the present study (<30 min per session), decreased the absolute intensity of the intervals but increased interval duration, and reduced the recovery period between intervals. Despite these modifications, the training was still relatively 'time efficient' in that only ∼10–15 min of exercise was performed over a 20–30 min period during each training session. Other groups have shown that lower intensity HIT may be effective for inducing metabolic adaptations (Talanian *et al.* 2007; Perry *et al.* 2008), but the protocols involved interval training sessions lasting ≥ 60 min in duration. We hypothesized that our training model would provide a sufficient stimulus to improve muscle oxidative capacity and functional exercise performance, similar to that observed after our all-out Wingate-based training protocols (Burgomaster *et al.* 2005, 2006, 2007, 2008; Gibala *et al.* 2006). In addition, we also examined the effect of low-volume HIT on several proposed mediators of mitochondrial biogenesis and metabolic adaptation in skeletal muscle, including peroxisome proliferator-activated receptor γ co-activator (PGC)-1α, silent information regulator T1 (SIRT1), nuclear respiratory factor (NRF)-1, and mitochondrial transcription factor A (Tfam).

Methods

Subjects

Seven healthy young men (21 ± 1 years, 83 ± 4 kg) took part in the study. The subjects were recreationally active 2–3 times per week but none were engaged in a structured exercise training programme. The study was approved by the Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board and conformed to the *Declaration of Helsinki*. Following medical screening to rule out any conditions that might have precluded their participation, all subjects provided written informed consent.

Experimental protocol

The experimental protocol consisted of familiarization procedures, baseline testing, a 2 week exercise training intervention, and post-training measurements.

Familiarization. Subjects initially performed an incremental cycling test to volitional fatigue on an electronically braked cycle ergometer (Lode Excalibur v2.0, Groningen, the Netherlands) to determine peak oxygen uptake ($\dot{V}_{O_2peak}$) using an online gas collection system (Moxus modular oxygen uptake system, AEI technologies, Pittsburgh, PA, USA). Subjects began pedalling at 50 W for 2 min and the workload was increased by 1 W every 2 s thereafter until volitional fatigue. Mean $\dot{V}_{O_2peak}$, based on the highest value averaged over 30 s for each subject, was 46 ± 2 ml kg^{-1} min^{-1}. On two separate days, subjects performed 50 kJ and 750 kJ cycling time trials on an electronically braked ergometer to become familiar with these performance tests. For these familiarization tests, conditions were identical to baseline and post-testing time trials. The ergometer was set to linear mode so that resistance increased proportional to cadence and force. Subjects were instructed to complete the tests as quickly as possible with no verbal, temporal or physiological feedback as previously described (Gibala *et al.* 2006). The only feedback provided was work completed, which was presented to subjects as 'distance covered' on a computer monitor. Feedback was presented in units of distance rather than work completed (i.e. 50 kJ was equated to 2 km, and 750 kJ was equated to 30 km). Exercise duration and average power were recorded upon completion of each test.

Baseline testing. Prior to training, a resting needle muscle biopsy sample was obtained from the *vastus lateralis* of one thigh under local anaesthesia (1% xylocaine) as previously described (Gibala *et al.* 2006). The muscle sample was immediately frozen in liquid nitrogen and stored at $-80°$C

until further analyses. One hour following the biopsy, subjects performed a 50 kJ cycling time trial, followed ~48 h later by a 750 kJ time trial.

Training. Three days following the 750 kJ time trial, subjects initiated the training protocol, which consisted of six sessions over 2 weeks (Monday, Wednesday, Friday). Each training session consisted of repeated 60 s efforts of high-intensity cycling at a workload that corresponded to the peak power achieved at the end of the ramp $\dot{V}_{O_2 peak}$ test (355 ± 10 W). These intervals were interspersed by 75 s of cycling at a low intensity (30 W) for recovery. Subjects completed eight high-intensity intervals during the first two training sessions, 10 intervals during the third and fourth sessions, and 12 intervals on the final two sessions. A 3 min warm-up at 30 W was performed each day prior to training. The total time commitment during each training session therefore ranged from ~20 to 29 min, including warm-up and recovery, for a total commitment of 2 h and 25 min of exercise over 2 weeks. All subjects completed all training sessions without complications. We did not specifically evaluate psychosocial variables of exercise tolerance. However, the modified HIT protocol was generally well tolerated and subjects did not report any feelings of dizziness, light-headedness, or nausea that is occasionally experienced by subjects after Wingate-based training.

Post-training measurements. Approximately 72 h following the last training session, a resting muscle biopsy was obtained from the same leg as used for the first biopsy, separated by 2–3 cm from the original incision site. Subjects also performed 50 and 750 kJ time trials, the nature and timing of which were identical in all respects to baseline testing.

Muscle analyses

The biopsy samples were initially sectioned under liquid nitrogen into several pieces for analyses of enzyme activity and protein content as detailed below. One piece was freeze-dried, powdered and dissected free of all non-muscle elements for analyses of muscle glycogen (see below).

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, 10 mM Hepes) supplemented with protease inhibitors (Complete Mini, Roche Applied Science, Laval, PQ, Canada) using 50 strokes of a glass-on-glass homogenizer. Homogenates were centrifuged at 700 g for 10 min and the supernatant was taken as the whole muscle lysate for enzyme activity assays and Western blotting. Homogenates were subjected to two freeze–thaw cycles to help

release mitochondrial proteins prior to enzyme activity measurement.

Preparation of nuclear extracts. Nuclear fractions were prepared from 30–40 mg of wet muscle using a commercially available nuclear extraction kit (NE-PER no. 78833, Pierce, Rockford, IL, USA). Briefly, samples were homogenized in CER-I buffer containing protease inhibitors using an electronic homogenizer (Pro 250, Pro Scientific, Oxford, CT, USA). Pellets containing nuclei were obtained by centrifugation at 16,000 g for 10 min at 4°C and were subsequently washed four times in PBS to remove cytosolic contaminating proteins. Nuclear proteins were extracted in nuclear extraction reagent (NER) supplemented with protease inhibitors according to the manufacturer's instructions. Enrichment and purity of nuclear fractions was confirmed by the abundance of nuclear matrix protein p84 and absence of the cytosolic protein lactate dehydrogenase (LDH) in Western blot analyses (Wright *et al.* 2007) (Supplementary Fig. 1).

Mitochondrial enzyme activity. The maximal activities of citrate synthase (CS) and cytochrome *c* oxidase (COX) were determined in whole muscle lysates using a spectrophotometer (Cary Bio-300, Varion, Inc., Palo Alto, CA, USA) as we have previously described (Gianni *et al.* 2004). Enzyme activities were expressed in mmol (kg protein)$^{-1}$ h^{-1} wet weight (w.w.).

Western blotting. Protein concentrations of whole muscle lysates and nuclear fractions were determined using a commercial assay (BCA Protein Assay, Pierce, Rockford, IL, USA). 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 h at 100 V. Proteins were transferred to nitrocellulose membranes for 1 h at 100 V. Ponceau S staining was performed following the transfer and was used to control for equal loading and transfer between lanes. In order to verify the efficacy of this method we performed preliminary experiments which demonstrated a linear proportional relationship between protein loaded (5–20 μg) and whole lane Ponceau S quantification using NIH Image J software ($R^2 = 0.99$, $n = 6$, data not shown). Ponceau S quantification also did not differ between pre- and post-training samples ($P = 0.88$, data not shown). Membranes were blocked at room temperature (RT) by incubating in 5% fat-free milk Tris buffered saline 0.1% Tween-20 (TBS-T). Blots were then incubated with primary antibodies overnight at 4°C in 3% fat-free milk or fatty acid-free BSA. A rabbit monoclonal antibody from Cell Signaling Technology (Danvers, MA, USA) was used to detect PGC-1α. Polyclonal antibodies directed towards the C-terminus of SIRT1 and GLUT4 were from Millipore (Billerica, MA, USA). Tfam, NRF-1 and TATA box

binding protein antibodies were from Abcam (Cambridge, MA, USA). 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). After incubation in appropriate secondary antibody for 1 h at RT, proteins were detected by chemiluminescence (Supersignal West Dura, Pierce) and quantified by spot densitometry using FluorChem SP Imaging system and software (Alpha Innotech Corp., San Leandro, CA, USA). TATA box binding protein was used to control for nuclear protein yield between pre- and post-training samples.

Muscle glycogen. Briefly, ∼2 mg of freeze-dried muscle was incubated in 500 μl 2.0 N HCl and heated for 2 h at 100°C to hydrolyse the glycogen to glucosyl units. The solution was subsequently neutralized with an equal volume of 2.0 N NaOH and analysed for glucose using an enzymatic assay adapted for fluorometry (Passoneau & Lowry, 1993).

Physical activity and nutritional controls

Subjects were instructed to maintain normal dietary and physical activity practices throughout the study. For 2 days prior to the biopsy procedures and exercise performance tests, subjects were asked to refrain from any exercise except for activities of daily living. In order to minimize variability in muscle metabolism attributable to diet, subjects were instructed to consume the same types and quantities of food for 2 days prior to the resting muscle biopsy and time trial tests. Subjects completed food diaries prior to baseline testing and these were collected, photocopied, and returned to subjects before post-training procedures so that individual diets could be replicated.

Statistical analyses

All data were analysed using Student's *t* test for paired data with significance set at $P \leq 0.05$ (Sigma Stat v3.10; Systat Software Inc., San Jose, CA, USA). All data is presented as mean ± S.D. unless otherwise indicated.

Results

Exercise performance

Time to complete the 50 kJ and 750 kJ time trials improved by 11% and 9%, respectively, after training ($P = 0.04$ and $P = 0.005$; Fig. 1). These changes were associated with significant increases in mean power output from 397 ± 27 to 436 ± 22 W in the 50 kJ test ($P = 0.01$) and 200 ± 7 to 221 ± 8 W in the 750 kJ test ($P = 0.005$). In order to confirm the effects of training, we also compared time trial performance on familiarization tests compared to baseline. Performance on the familiarization tests (∼14–16 days prior to training) were not different from pre-training values (50 kJ test: 129 ± 8 s *vs.* 130 ± 9 s, $P = 0.89$; 750 kJ test: 64 ± 3 min *vs.* 63 ± 2 min, $P = 0.48$).

Mitochondrial enzymes

The maximal activity of COX increased by 29% after training ($P = 0.02$; Fig. 2A), which was similar to the 35% and 38% increase in protein content of COX subunits II ($P = 0.01$; Fig. 2B) and IV ($P = 0.002$; Fig. 2C), respectively. Training also increased the maximal activity and protein content of CS by ∼16% and 20%, respectively (both $P = 0.01$; Fig. 3).

Regulators of mitochondrial biogenesis

The protein content of PGC-1α measured in nuclear fractions was elevated by ∼24% post-training ($P = 0.02$; Fig. 4A), but whole muscle PGC-1α protein was unchanged ($P = 0.25$; Fig. 4B). Total SIRT1 content increased by ∼56% following training ($P = 0.03$; Fig. 5). Tfam total protein content increased by ∼37% with training ($P = 0.002$; Fig. 6) whereas NRF-1 protein content remained unchanged ($P = 0.52$; data not shown).
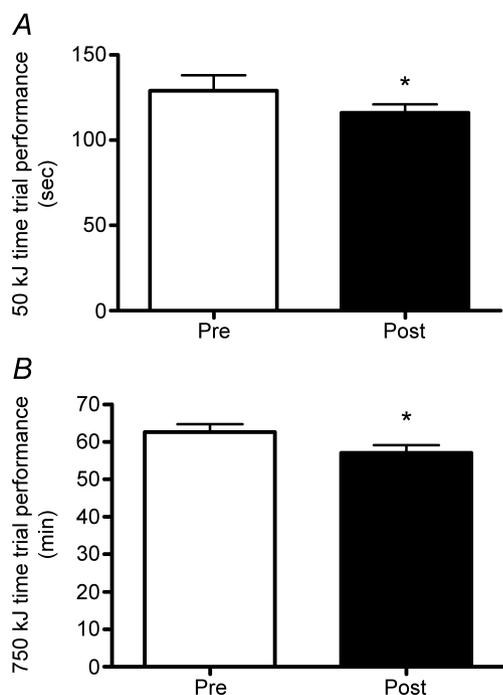

Figure 1. Two weeks of high-intensity interval training improves cycling time trial performance
A, time to complete 50 kJ cycling time trial before (pre) and after (post) training. B, time to complete 750 kJ cycling time trial before (pre) and after (post) training. *$P < 0.05$ *vs.* pre-training. Values are means ± S.E.M.

To identify potential post-translational modifications which might promote or sustain the increase in nuclear PGC-1α, we attempted to immunoprecipitate PGC-1α and measure phosphorylation and/or acetylation status. Unfortunately, these attempts were unsuccessful using up to 150 μg of nuclear protein with both the Cell Signaling Technology antibody (no. 2178) and the H-300 antibody from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Immunoprecipitation of PGC-1α may require greater amounts of protein (Canto *et al.* 2009), which was not possible to obtain from nuclear fractions prepared from human muscle biopsy samples in the present study.
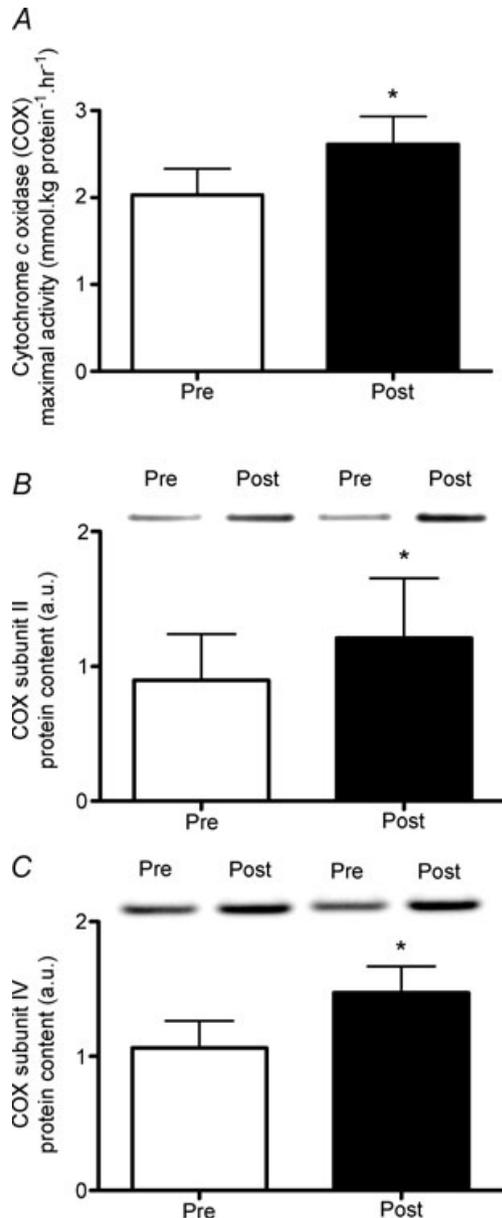
GLUT4 and muscle glycogen

Protein content of GLUT4 increased by 119% ($P = 0.04$; Fig. 7) and resting muscle glycogen increased by 17% following training (from 476.96 ± 29.28 to 558 ± 19 mmol (kg dry weight)$^{-1}$, $P = 0.05$).

Discussion

The present study demonstrates that 2 weeks of low-volume, constant-load interval exercise is a practical, time-efficient strategy to induce mitochondrial biogenesis
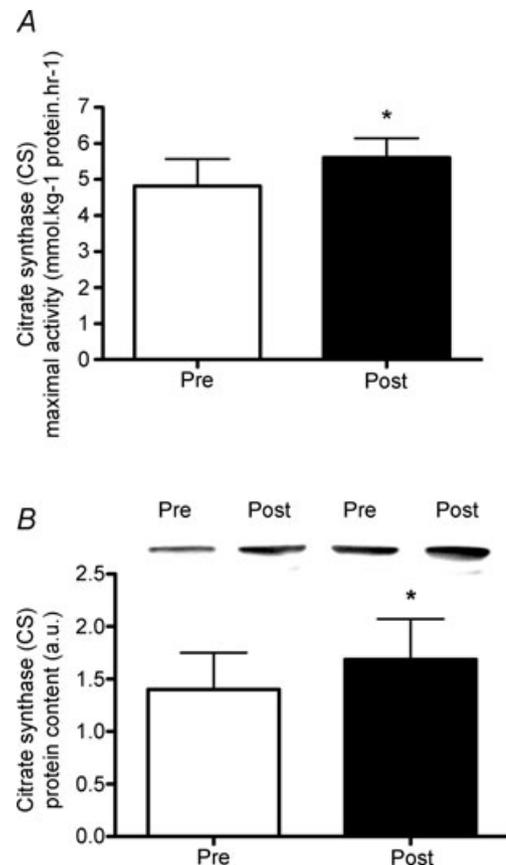

Figure 2. High-intensity interval training increases activity and content of the mitochondrial enzyme cytochrome c oxidase
A, maximal activity (mmol (kg protein)$^{-1}$ h^{-1} w.w.) of cytochrome *c* oxidase (COX) measured in whole muscle homogenates prepared from muscle biopsy samples (v. lateralis) obtained before (pre) and after (post) training. *B*, protein content of COX subunit II before (pre) and after (post) training. *C*, protein content of COX subunit IV before (pre) and after (post) training. *P < 0.05 vs.* pre-training. Values are means ± s.e.m. Representative Western blots from two subjects are shown.

Figure 3. High-intensity interval training increases activity and content of the mitochondrial enzyme citrate synthase
A, maximal activity (mmol (kg protein)$^{-1}$ h^{-1} w.w.) of citrate synthase (CS) measured in whole muscle homogenates prepared from muscle biopsy samples (v. lateralis) obtained before (pre) and after (post) training. *B*, protein content of CS before (pre) and after (post) training. *P < 0.05 vs.* pre-training. Values are means ± s.e.m. Representative Western blots from two subjects are shown.
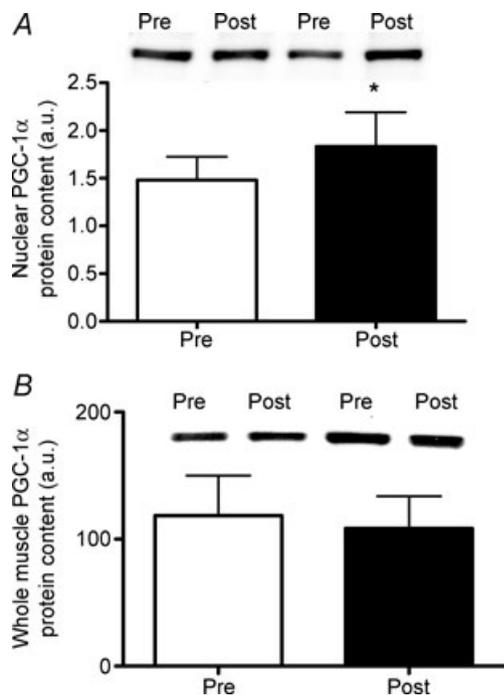
Figure 4. High-intensity interval training increases nuclear but not whole muscle PGC-1α

A, protein content of peroxisome proliferator-activated receptor γ co-activator (PGC)-1α measured in nuclear fractions prepared from muscle biopsy samples (v. lateralis) obtained before (pre) and after (post) training. *B*, protein content of PGC-1α measured in whole muscle homogenates before (pre) and after (post) training. *P < 0.05 *vs.* pre-training. Values are means ± s.e.m. PGC-1α antibody specificity is demonstrated in Supplementary Fig. 2. Representative Western blots from two subjects are shown.

in skeletal muscle and improve functional exercise capacity. The changes we observed in maximal enzyme activity, exercise performance, as well as resting muscle glycogen were comparable to changes we have previously
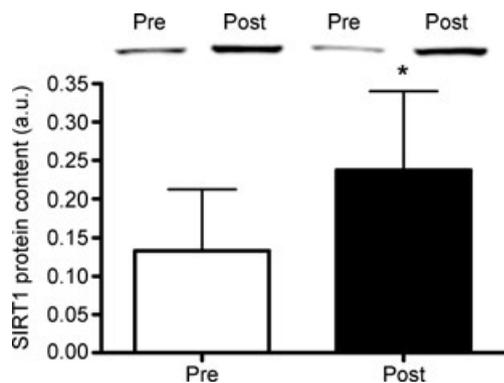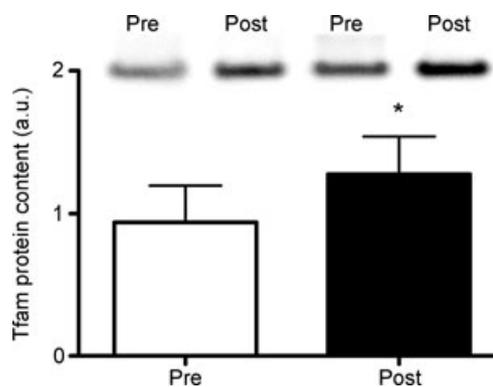

Figure 6. High-intensity interval training increases Tfam protein content

Protein content of mitochondrial transcription factor A (Tfam) measured in whole muscle homogenates prepared from muscle biopsy samples (v. lateralis) obtained before (pre) and after (post) training. *P < 0.05 *vs.* pre-training. Values are means ± s.e.m. Representative Western blots from two subjects are shown.

measured after 2 weeks of 'all out' Wingate-type training, as well as a much larger volume of traditional endurance training (Burgomaster *et al.* 2005, 2006; Gibala *et al.* 2006). The present study also extends our previous work by providing mechanistic insight into the molecular events that potentially mediate the skeletal muscle adaptive response to HIT. To our knowledge, for the first time in humans we show that the nuclear abundance of PGC-1α was increased after exercise training. The protein content of SIRT1, an NAD$^+$-dependent deacetylase which has been shown to deacetylate and activate PGC-1α (Cantó *et al.* 2009; Gerhart-Hines *et al.* 2007) was also elevated post-training, as was content of Tfam, a mitochondrial transcription factor which is induced by PGC-1α (Wu *et al.* 1999).

Figure 5. High-intensity interval training increases SIRT1 protein content

Protein content of SIRT1 measured in whole muscle homogenates prepared from muscle biopsy samples (v. lateralis) obtained before (pre) and after (post) training. *P < 0.05 *vs.* pre-training. Values are means ± s.e.m. Representative Western blots from two subjects are shown.
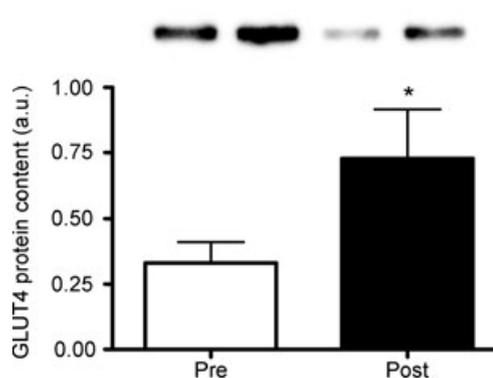

Figure 7. High-intensity interval training increases GLUT4 protein content

Protein content of glucose transporter 4 (GLUT4) measured in whole muscle homogenates prepared from muscle biopsy samples (v. lateralis) obtained before (pre) and after (post) training. *P < 0.05 *vs.* pre-training. Values are means ± s.e.m. Representative Western blots from two subjects are shown.

A practical model of HIT is a time-efficient training stimulus

We have previously shown that low-volume HIT consisting of 4–6 repeated 30 s 'all-out' Wingate cycling tests with 4 min recovery is an effective stimulus for improving mitochondrial enzyme capacity and exercise performance (Burgomaster *et al.* 2005, 2006, 2007, 2008; Gibala *et al.* 2006). Similar to the present study, Wingate-based HIT also leads to elevated levels of resting muscle glycogen (Burgomaster *et al.* 2005, 2006, 2008; Gibala *et al.* 2006). Although Wingate-based HIT is a very potent and time-efficient training strategy (Burgomaster *et al.* 2005, 2006, 2007, 2008; Gibala *et al.* 2006), training necessitates an all-out effort on a specialized cycle ergometer against a high resistance. This requires a high level of subject motivation and can result in feelings of nausea and discomfort due to the extreme physical exertion. For these reasons, Wingate-based HIT may not be practical or suitable for the general population (Coyle, 2005), especially obese individuals and older adults who may benefit most from the adaptations to interval exercise training.

The training protocol in the current study was designed to involve lower-intensity intervals at a constant workload without the need for specialized equipment. Interval intensity, duration and training volume were based on several *a priori* assumptions. In our previously published HIT studies, recreationally active males typically achieve a maximal aerobic power of ∼350 W during a ramp $\dot{V}_{O_2peak}$ test and produce a mean power output of ∼700 W over each 30 s sprint interval (Burgomaster *et al.* 2005; Gibala *et al.* 2006). Thus, to closely match the amount of external work completed in a 30 s Wingate, intervals were set at 100% maximal aerobic power (i.e. ∼350 W) for 60 s. However, the relationship between training intensity and duration is not linear (Dudley *et al.* 1982). That is, simply matching work using a lower training intensity may not have the same training effect (Dudley *et al.* 1982). Therefore, the prescribed number of intervals per session was doubled compared to Wingate-based HIT (Burgomaster *et al.* 2005, 2006, 2007, 2008; Gibala *et al.* 2006) such that subjects performed 8–12 intervals at 100% maximal aerobic power, resulting in a concomitant doubling of total external energy expenditure to ∼210 kJ per training session. This volume of exercise is still considerably lower than traditional endurance-type training (Gibala *et al.* 2006; Burgomaster *et al.* 2008). In addition to a low training volume, one of the most attractive features of HIT is that metabolic and performance adaptations are achieved with a very low time commitment (Coyle, 2005; Gibala *et al.* 2006). In keeping with the time efficiency of HIT, total training time commitment was kept low in the present study. Training sessions involved only 8–12 min of exercise spread out over a ∼18–26 min training session. Therefore, total weekly time commitment averaged only ∼60 min. Given that lack of time is the number one perceived barrier to performing regular exercise (Godin *et al.* 1994; Trost *et al.* 2002), a low-volume HIT program similar to the one employed in this study may be a potent, practical, and time-efficient exercise strategy for increasing muscle mitochondrial content and functional exercise capacity. Since increases in skeletal muscle mitochondrial capacity and physical fitness are associated with improved metabolic health (Bruce *et al.* 2003; Booth & Roberts, 2008) low-volume HIT may represent an alternative exercise strategy for promoting important health benefits. Although we did not include a control group in the current investigation, there were clearly no differences when comparing exercise performance on tests conducted ∼2 weeks apart prior to the training intervention. In addition, we have previously established that the muscle metabolic and performance adaptations after short-term HIT are attributable to the training *per se*, since controls subjects show no change when tested 2 weeks apart with no exercise intervention (Burgomaster *et al.* 2005, 2006, 2007). While the current investigation was conducted in young, healthy male subjects, there is growing appreciation for the benefits of interval-based exercise training in several chronic disease states. For example, higher volume interval training at a similar intensity (4×4 min at 90% maximal heart rate interspersed with 3 min rest periods) has been shown to be more effective than traditional continuous endurance-type training for improving muscle metabolic parameters and clinical outcomes in patients with obesity (Schjerve *et al.* 2008), metabolic syndrome (Tjønna *et al.* 2008), and heart failure (Wisløff *et al.* 2007). Our findings indicate that the HIT protocol utilized in this study may lead to muscle and performance adaptations which are linked with improved metabolic health despite a lower volume and time commitment. It will be interesting to evaluate whether the current low-volume HIT protocol leads to health benefits in conditions of metabolic disease and we are currently conducting studies in this regard.

Molecular clues regarding the adaptive response to HIT

In order to gain a better understanding of the mechanisms by which low-volume HIT promotes the muscle adaptive response, we examined several regulators of mitochondrial biogenesis in resting muscle biopsy samples obtained before and after training. We first examined the total protein content of PGC-1α, a transcriptional co-activator which plays a crucial role in co-ordinating mitochondrial gene transcription (Lin *et al.* 2005; Hood & Saleem, 2007; Wright *et al.* 2007). A role for PGC-1α in the adaptive response to exercise training is highlighted by the findings

that acute endurance (Pilegaard *et al.* 2003) and interval (Gibala *et al.* 2009) exercise increase skeletal muscle PGC-1α mRNA and that muscle-specific over-expression of PGC-1α in mice results in increased mitochondrial content and prolonged endurance exercise capacity (Calvo *et al.* 2008). Perhaps somewhat surprisingly, and in contrast to longer term endurance and/or interval training (Russell *et al.* 2003; Burgomaster *et al.* 2008; Morton *et al.* 2009), PGC-1α measured at the whole muscle level was unchanged following training. However, total PGC-1α protein content may not be entirely indicative of PGC-1α activation; rather PGC-1α activity may be primarily determined by its subcellular location (Rim *et al.* 2004; Wright *et al.* 2007; Cowell *et al.* 2007; Sano *et al.* 2007; Anderson *et al.* 2008; Olson *et al.* 2008) and several post-translational modifications (Knutti *et al.* 2001; Jäger *et al.* 2007; Cantó *et al.* 2009). Therefore, we prepared nuclear fractions from skeletal muscle biopsy samples and examined the nuclear abundance of PGC-1α to further examine the potential role of PGC-1α in the adaptive response. Results indicated that training resulted in a significant increase in nuclear PGC-1α protein. Thus, our novel findings suggest that the total amount of PGC-1α may not be affected by short-term training but that its subcellular location, an indicator of PGC-1α activation (Wright *et al.* 2007), may be altered such that more PGC-1α protein is present in the nucleus in the initial adaptive response to training. Greater nuclear PGC-1α would presumably be more conducive for promoting or maintaining an increase in mitochondrial biogenesis via increased co-activation of transcription factors linked to mitochondrial gene expression. Accordingly, we found increases in several classical markers of mitochondrial biogenesis, including the maximal activity and protein contents of CS and COX. Alternatively, measuring PGC-1α in nuclear fractions may be a more sensitive technique than measuring the content of this protein at the whole muscle level, increasing the ability to detect changes following training. Regardless, our findings suggest that subcellular location of PGC-1α may be an important consideration when examining the role of this critical regulator of mitochondrial biogenesis in response to exercise training.

We have recently demonstrated that acute Wingate-based HIT leads to activation of AMPK and p38 MAPK as well as increased mRNA expression of PGC-1α (Gibala *et al.* 2009). These responses are comparable to the effects of acute endurance exercise (Yu *et al.* 2001; Pilegaard *et al.* 2003; Lee-Young *et al.* 2008) and suggest that both training methods may act through similar pathways to induce mitochondrial biogenesis. The low-volume HIT protocol in the current study may therefore have promoted adaptation partly through repeated activation of signalling pathways which activate PGC-1α and mitochondrial biogenesis. Additional cellular factors promoting the increase in nuclear PGC-1α

and mitochondrial biogenesis cannot be fully elucidated in the present study, but the training-induced increase in SIRT1 protein content may be involved. SIRT1 is a NAD$^+$-dependent type III deacetylase which, in exercising mouse muscle and C2C12 myotubes, deacetylates PGC-1α and appears critical for PGC-1α activation (Cantó *et al.* 2009). In rats, it was recently shown that exercise training increases in SIRT1 protein in some muscles in the absence of increases in total PGC-1α (Suwa *et al.* 2008). These authors (Suwa *et al.* 2008) suggested that the increase in SIRT1 may serve to increase PGC-1α activation, circumventing a need to increase total PGC-1α content to drive mitochondrial biogenesis. Similarly, Canto and colleagues (2009) have recently shown that acute exercise activates PGC-1α and mitochondrial gene transcription via a pathway involving SIRT1-mediated deacetylation of PGC-1α. Data from human skeletal muscle is scarce, but SIRT1 and PGC-1α mRNA expression have been shown to increase in parallel following caloric restriction and caloric restriction combined with exercise, two interventions conclusively demonstrated to improve mitochondrial function (Civitarese *et al.* 2007). Our results seem to agree with these studies and may extend previous findings to suggest that an increase in SIRT1 protein, in human skeletal muscle, coincides with an increase in the nuclear abundance of PGC-1α. Together, our findings indicate that 2 weeks of low-volume HIT may induce skeletal muscle mitochondrial biogenesis by a mechanism involving co-ordinated induction and/or activation of SIRT1 and PGC-1α. The exact nature of SIRT1-mediated mitochondrial biogenesis has not been fully elucidated, but deacetylation of PGC-1α by SIRT1 may increase PGC-1α nuclear abundance, sequester or stabilize PGC-1α in the nucleus, or influence formation of different nuclear PGC-1α protein complexes (Rodgers *et al.* 2008; Cantó *et al.* 2009; Cantó & Auwerx, 2009). However, it must be noted that total SIRT1 protein content may not be entirely reflective of its deacetylase activity; rather SIRT1 activity may be primarily regulated by metabolic factors such as the NAD$^+$:NADH ratio, intracellular nicotinamide levels, and several post-translational modifications (Cantó & Auwerx, 2009). Nonetheless, SIRT1 activity and protein content are highly correlated (Gurd *et al.* 2009) and an increase in total SIRT1 protein would theoretically increase the potential for interaction with its targets, such as PGC-1α.

Although SIRT1 has been primarily recognized as a positive regulator of mitochondrial biogenesis (Rodgers *et al.* 2005; Civitarese *et al.* 2007; Canto *et al.* 2009; Gerhart-Hines *et al.* 2007; Suwa *et al.* 2008), recent data from Gurd and colleagues (2009) has challenged this view. Electrotransfection of the SIRT1 gene into an isolated hindlimb muscle of rats increased SIRT1 protein content by ∼250% and was accompanied by a decrease in PGC-1α and mitochondrial protein content (Gurd *et al.*

2009). Thus, SIRT1 may not always be associated with increased mitochondrial biogenesis. It is possible that the large increase in SIRT1 protein following electro-transfection produces different effects from the more modest increase in SIRT1 protein following training. Furthermore, although the electrotransfection technique is a powerful approach for increasing SIRT1 protein in an isolated muscle, it may not be representative of the *in vivo* metabolic environment which promotes the adaptive response to exercise training. Clearly, more research is required to clarify the effects of exercise training and other manipulations on SIRT1 protein and mitochondrial biogenesis in skeletal muscle *in vivo*.

It is well established that exercise training results in an increase in mitochondrial content in skeletal muscle (Holloszy, 1967; Holloszy & Booth, 1976), yet the under-lying mechanisms directing this adaptive response are not completely understood. A functional increase in mitochondrial content requires complex co-ordination of genes encoded by nuclear and mitochondrial DNA (Scarpulla, 2006, 2008). PGC-1α and Tfam appear to play integral roles regulating transcription of mitochondrial genes in the nucleus and mitochondria, respectively (Hood *et al.* 2006; Scarpulla, 2006, 2008). Our results suggest that an increase in nuclear PGC-1α and total Tfam protein content following training may play integral roles in co-ordinating the expression of mitochondrial proteins to drive an increase in mitochondrial biogenesis. Indeed, we found that COX subunit IV (nuclear encoded) and COX subunit II (mitochondrial encoded) were both increased following training, as was COX maximal activity. Further supporting a role for a co-ordinated increase in mitochondrial biogenesis was the finding that the protein content and maximal activity of the TCA cycle enzyme CS was increased with training. The training-induced increase in muscle oxidative capacity is likely to have played a role in improving exercise capacity as it would presumably allow subjects to exercise at a higher metabolic rate during the cycling time trials following training. Given the link between muscle oxidative capacity and insulin sensitivity (Bruce *et al.* 2003) and the important role that skeletal muscle mitochondria play in regulating whole body metabolism (Handschin *et al.* 2007), the adaptations promoted by low-volume HIT may also have important health implications. Interestingly, Babraj *et al.* (2009) have recently shown that 2 weeks of Wingate-based HIT improves insulin sensitivity in a sample of healthy young male subjects similar to the present study.

Despite the fact that we did not directly measure insulin sensitivity, the increase in muscle glycogen and GLUT4 following training provides some evidence that muscle insulin sensitivity may have been improved. Increases in resting muscle glycogen following training are common and have been linked with increased insulin sensitivity (Perseghin *et al.* 1996; Greiwe *et al.* 1999) and/or increased glucose transport capacity as a result of increased GLUT4 content (McCoy *et al.* 1996). Although less studied than its role in mitochondrial biogenesis, PGC-1α appears to be involved in regulating skeletal muscle glycogen content (Wende *et al.* 2007; Calvo *et al.* 2008). Over-expression of PGC-1α in mice increases resting muscle glycogen content (Calvo *et al.* 2008) and PGC-1α deficient animals have impaired muscle glycogen resynthesis following exercise (Wende *et al.* 2007). The regulation of skeletal muscle glycogen content by PGC-1α is not completely under-stood, but may involve PGC-1α mediated up-regulation of GLUT4 (Michael *et al.* 2001) and/or modulation of enzymes involved in glycogen synthesis and degradation (Wende *et al.* 2007). Therefore, the training induced increase in nuclear PGC-1α may have played a role in mediating the increase in GLUT4 and muscle glycogen.

Conclusions and significance

Previous low-volume HIT studies (Parra *et al.* 2000; Burgomaster *et al.* 2005, 2006, 2007, 2008; Gibala *et al.* 2006; Babraj *et al.* 2009) have utilized all-out exercise intervals on a specialized cycle ergometer and our findings are unique in that the HIT protocol was designed to be more practical and attainable for the general population. Six sessions of eight to twelve 60 s constant load intervals, completed in ~18–26 min per session and requiring ~1 h of total exercise time commitment per week resulted in significant improvements in functional exercise performance and skeletal muscle mitochondrial biogenesis. Although the molecular mechanisms promoting the muscle adaptive response could not be fully elucidated, we found changes in several proposed regulators of mitochondrial biogenesis following training. Resting levels of SIRT1 and Tfam were higher after training, as was the nuclear abundance of PGC-1α. Together, the results demonstrate that a practical low-volume HIT programme is effective for improving muscle metabolic capacity and functional performance and shed light on potential mechanisms by which exercise training promotes mitochondrial adaptations in skeletal muscle. Future research should examine whether practical low-volume HIT can improve markers of metabolic health in healthy individuals and those at risk of developing chronic inactivity-related diseases to determine whether this type of training is an effective health-enhancing exercise strategy.

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Author contributions

M.J.G., J.P.L., G.P.W. and M.A.T. conceived the study design. G.P.W. ran the exercise testing and supervised the training sessions. M.A.T. performed all the medical procedures. J.P.L. and A.S. performed the laboratory experiments and analysed the data. J.P.L., A.S. and M.J.G. interpreted the data and wrote the manuscript with input from all authors. Testing and experiments were performed in the laboratories of M.J.G. and M.A.T at McMaster University.

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