Training intensity modulates changes in PGC-1α and p53 protein content and mitochondrial respiration, but not markers of mitochondrial content in human skeletal muscle

Cesare Granata,* Rodrigo S. F. Oliveira,* Jonathan P. Little,† Kathrin Renner,‡ and David J. Bishop*†

*Institute of Sport, Exercise and Active Living (ISEAL), College of Sport and Exercise Science, Victoria University, Melbourne, Victoria, Australia; †School of Health and Exercise Sciences, University of British Columbia, Kelowna, British Columbia, Canada; and ‡Department of Internal Medicine III, University Hospital of Regensburg, Regensburg, Germany

ABSTRACT Exercise training has been associated with increased mitochondrial content and respiration. However, no study to date has compared in parallel how training at different intensities affects mitochondrial respiration and markers of mitochondrial biogenesis. Twenty-nine healthy men performed 4 wk (12 cycling sessions) of either sprint interval training (SIT; 4–10 × 30-s all-out bouts at ∼200% of peak power output (W_{\text{peak}})), high-intensity interval training (HIIT; 4–7 × 4 min intervals at ∼90% W_{\text{peak}}), or sublactate threshold continuous training (STCT; 20–36 min at ∼65% W_{\text{peak}}). The STCT and HIIT groups were matched for total work. Resting biopsy samples (vastus lateralis) were obtained before and after training. The maximal mitochondrial respiration in permeabilized muscle fibers increased significantly only after SIT (25%). Similarly, the protein content of peroxisome proliferator-activated receptor γ coactivator (PGC)-1α, p53, and plant homeodomain finger–containing protein 20 (PHF20) increased only after SIT (60–90%). Conversely, citrate synthase activity, and the protein content of TFAM and subunits of the electron transport system complexes remained unchanged throughout. Our findings suggest that training intensity is an important factor that regulates training-induced changes in mitochondrial respiration and that there is an apparent dissociation between training-induced changes in mitochondrial respiration and mitochondrial content. Moreover, changes in the protein content of PGC-1α, p53, and PHF20 are more strongly associated with training-induced changes in mitochondrial respiration than mitochondrial content. — Granata, C., Oliveira, R. S. F., Little, J. P., Renner, K., Bishop, D. J. Training intensity modulates changes in PGC-1α and p53 protein content and mitochondrial respiration, but not markers of mitochondrial content in human skeletal muscle. FASEB J. 30, 959–970 (2016). www.fasebj.org

Key Words: mitochondrial biogenesis • exercise • PHF20 • TFAM • mitochondrial remodeling

Mitochondria are key components of skeletal muscles that provide the energy necessary for almost all cellular activities and play an important role in ageing and cell pathology. For example, mitochondria have been implicated in many age-related degenerative diseases, cardiomyopathies, atherosclerosis, and in a large variety of metabolic disorders (1, 2). Although early studies focused on changes in mitochondrial enzymes, such as citrate synthase (CS) activity, a common biomarker of mitochondrial content (3), subsequent studies have suggested that increasing mitochondrial respiratory function may contribute more to improvements in both health (2) and endurance performance (4).

Exercise is one factor that provides a powerful stimulus to increase both mitochondrial content (5) and respiratory function (6), yet little is known about the optimal exercise dose and whether mitochondrial content and respiratory function are altered by similar or different exercise prescriptions. Although many factors (e.g., participants’ characteristics, and training volume, frequency, and duration) may contribute, it has been suggested that training intensity is a key factor that modulates changes in mitochondrial respiratory function (7). However, only a handful of studies have been conducted to investigate training-induced changes in mitochondrial respiratory function in humans (7–9), and only 1 study (6) directly compared the effects of 2 different training intensities on mitochondrial respiration in permeabilized muscle fibers—the gold-standard technique for determination of mitochondrial respiratory function (10). That study showed that mitochondrial...
respiration increases after 8 wk of high-intensity interval training (HIIT), but not after work-matched continuous training at a moderate intensity. This result is in contrast with findings from an earlier study reporting an increase in mitochondrial ATP production rate after 6 wk of continuous training at a moderate intensity (11). Moreover, no study has measured changes in mitochondrial respiration after all-out sprint interval training (SIT), which has been reported to induce mitochondrial adaptations similar to those of continuous training at a moderate intensity (12). Therefore, further investigation of the effects of training protocols using different intervals and training intensities on mitochondrial content and respiration is warranted.

The oxidative capacity of mitochondria is determined by the abundance of mitochondrial proteins and their functional capacity to produce ATP. This capacity is regulated by transcriptional activity, a process requiring the concerted integration of signaling, transcription, and translation events taking place in both the nucleus and the mitochondrion, followed by the import and incorporation of the newly generated proteins into the mitochondrial reticulum (13). Monitoring changes in these events not only allows for an indirect assessment of mitochondrial biogenesis, but may also contribute to a better understanding of the possible underlying cellular mechanisms. Peroxisome proliferator–activated receptor γ coactivator (PGC)-1α is a transcriptional coactivator that has been shown to regulate mitochondrial content and respiration in human skeletal muscle (13) and also metabolic control in rat skeletal muscle (14). Studies in human skeletal muscle have shown that exercise training leads to an increase in PGC-1α protein (5) and suggest that modulation of PGC-1α mRNA is sensitive to training intensity (15). However, there has been no investigation of the effects of training at different intensities on changes in PGC-1α protein abundance in conjunction with changes in both mitochondrial content and respiration.

The tumor suppressor p53 is also an important regulator of mitochondrial biogenesis (16). Studies in cells and mice indicate that ablation of p53 leads to a reduction in mitochondrial respiration and content, and endurance performance (17, 18). p53 is also up-regulated after a single bout of exercise in human skeletal muscle (19) and, similar to PGC-1α, has been shown to regulate the mitochondrial transcription machinery via modulation of mitochondrial transcription factor A (TFAM) (20, 21). Furthermore, by regulation of downstream targets, p53 can control mitochondrial respiration by modulating the balance between glycolytic and oxidative pathways (17) and has been linked with mitochondrial dynamics and the maintenance of mitochondrial morphology (16). Although the signaling events upstream of p53 are not yet fully known, plant homeodomain finger-containing protein 20 (PHF20) has been shown to up-regulate p53 transcription (22) and to stabilize p53 protein (23), emphasizing the role of PHF20 as a regulator of p53. Despite evidence demonstrating the importance of the p53 pathway in the regulation of mitochondrial biogenesis, to our knowledge there has been no examination of the effects of a period of exercise training at different intensities on p53 and upstream regulators and downstream targets.

The purpose of this research was to compare the effects of 12 sessions (4 wk) of sublactate threshold continuous training (STCT), HIIT, and SIT on markers of mitochondrial biogenesis in the skeletal muscle of young moderately-trained men. Given that an increase in mitochondrial respiration (8, 9), markers of mitochondrial biogenesis (5), and endurance performance (8) has been reported after 6 to 10 training sessions, we hypothesized that 12 training sessions would result in positive physiological and mitochondrial adaptations. We further hypothesized that higher training intensities would produce greater improvements in mitochondrial respiration and that these changes would be associated with increased protein content of the transcription factors known to regulate mitochondrial biogenesis.

MATERIALS AND METHODS

Participants and ethics approval

Thirty-one healthy men volunteered to take part in this study. Only participants who were 1) healthy men aged 18–35 yr, 2) free of medications before and during the study, 3) nonsmokers, 4) not regularly engaged in a structured training program, 5) moderately trained (i.e., whose aerobic activity in the 6 mo before commencement of the study was limited to a maximum of 3 h/wk of light, unstructured aerobic activity), and 6) not regularly engaged in cycling-based sports (e.g., cycling, mountain biking, and triathlon) were allowed to take part in the research. After medical screening to rule out any conditions that may have precluded their participation (e.g., cardiovascular, metabolic, or musculoskeletal problems), the participants were informed of the study requirements, benefits, and risks, before giving written informed consent. Approval of the study’s procedures, which conformed to the standards set by the latest revision of the Declaration of Helsinki, was granted by the Victoria University Human Research Ethics Committee. After initial screening and testing, participants were matched by the power (in watts) attained at the lactate threshold (WLT) and randomly assigned to the STCT (n = 10), HIIT (n = 11), or SIT (n = 10) group. Twenty-nine participants completed the study, whereas 2, one each from the STCT and SIT groups, withdrew because of time constraints. Baseline physiological parameters for each group are described in Table 1.

Study design

The experimental protocol consisted of, in the following order, a 20-km cycling time trial (20k TT), a graded exercise test (GXT) (participants were previously familiarized with both tests), and a resting muscle biopsy. All tests were separated by a minimum of

(continued from previous page)

graded exercise test; HIIT, high-intensity interval training; invR, inverse respiratory control ratio; LCR, leak control ratio; oxphos, oxidative phosphorylation; PCR, phosphorylation control ratio; PGC, peroxisome proliferator-activated receptor γ coactivator; PHF20, plant homeodomain finger-containing protein 20; SCO2, synthesis of cytochrome c oxidase 2; SCR, substrate control ratio; SIT, sprint interval training; STCT, sublactate threshold continuous training; SUIT, substrate-uncoupler–inhibitor titration protocol; TBST, Tris-buffered saline-Tween; TFAM, mitochondrial transcription factor A; W02Peak, peak oxygen uptake; WLT, power at the lactate threshold; Wpeak, peak power output.
TABLE 1. Baseline characteristics of the participants

| Measurement                      | STCT group (n=9) | HIIT group (n=11) | SIT group (n=9) |
|----------------------------------|------------------|-------------------|----------------
| Age (yr)                         | 21 ± 2           | 21 ± 1            | 21 ± 3         |
| Height (cm)                      | 179.3 ± 6.3      | 180.0 ± 10.7      | 180.6 ± 7.3    |
| $V_{02\text{peak}}$ (ml·min$^{-1}$·kg$^{-1}$) | 46.7 ± 3.8      | 45.1 ± 7.2        | 47.1 ± 7.8     |

All values are means ± sd.

48 h (72 h for the biopsy trial). During the training phase, the participants trained 3 times a week for 4 wk, with the STCT and HIIT groups matched for total work. Because of the nature of sprint training, it was not possible to match the SIT group for total work with the other 2 groups. Seventy-two hours after the last training session a resting muscle biopsy was performed, followed by the 20k TT and GXT, separated by 48 h. The study duration inclusive of pre- and post-training testing was ~7 wk.

**Testing procedures**

Participants were required to refrain from any strenuous physical activity for the 48 h before each performance test (72 h for the biopsy trial), from alcohol and any exercise for 24 h before testing, and from food and caffeine consumption for the 2 h before each test. Each type of test was performed at the same time of the day during the course of the entire study with the purpose of avoiding variations caused by changes in circadian rhythm.

**20k TT**

Cycling time trials were performed on an electronically braked cycle ergometer (VeloTron; RacerMate, Seattle, WA, USA) after a warm-up involving cycling for 4 min at 66% of $W_{1\text{LT}}$, followed by 2 min at $W_{1\text{LT}}$, and 2 min of rest. During the time trial, the participants were allowed access to only cadence and completed distance. Heart rate was monitored (Polar-Electro, Kempele, Finland) during all exercise trials and training sessions.

**GXT**

A discontinuous graded exercise test was performed on an electronically braked cycle ergometer (Excalibur, v2.0; Lode, Groningen, The Netherlands) to determine peak oxygen uptake ($V_{02\text{peak}}$), peak power output ($P_{02\text{peak}}$), $W_{1\text{LT}}$ [by the modified $D_{\text{Max}}$ method (24)], and training intensities for the STCT and HIIT groups. The test consisted of 4-min stages at a constant power output, interspersed with 30 s of rest. The test began at 60, 90, or 120 W, depending on the participant's fitness level, and was subsequently increased by 30 W every 4 min. Before the test and during the 30 s rest, capillary blood samples were taken from the fingertip for measurement of blood lactate concentration ([La$^{-}$]). The participant was instructed to keep a cadence >60 rpm and was allowed to view only the cadence and elapsed time. The test was stopped when the participant reached volitional exhaustion or cadence dropped below 60 rpm. The $P_{02\text{peak}}$ was determined as the power of the last completed stage plus 7.5 W for every additional minute completed.

**Gas analysis during the GXT**

During the GXT, expired air was continuously analyzed for $O_2$ and $CO_2$ concentrations via a gas analyzer (Moxus 2010; AEI Technologies, Pittsburgh, PA, USA), which was calibrated immediately before each test. The ventilator was calibrated with a 3-L syringe (Hans Rudolph, Shawnee, KS, USA). $V_{O2}$ was recorded every 15 s and the average of the 2 highest consecutive 15-s readings was recorded as a participant’s $V_{02\text{Peak}}$.

**Capillary blood sampling**

Glass capillary tubes were used to collect ~50 μL of blood at the various time points during the GXT. Capillary blood [La$^{-}$] was determined with a blood lactate analyzer (2300 STAT Plus; YSI, Yellow Spring, OH, USA), which was regularly calibrated using precision standards.

**Muscle biopsies**

Before and after training, resting muscle biopsies were taken from the vastus lateralis muscle with a biopsy needle with suction, under local anesthesia of the skin and fascia (1% lidocaine). Muscle samples were cleaned of excess blood, fat, and connective tissue; 1 portion (10–20 mg) was immediately immersed in ~3 ml of ice-cold biopsy preservation solution (BioPS; see below) for in situ measurement of mitochondrial respiration. The remaining portion was immediately frozen in liquid nitrogen and stored at ~80°C for subsequent analyses.

**Training intervention**

All training sessions were performed on an electronically braked cycle ergometer (VeloTron; RacerMate) and were preceded by an 8 min warm-up as described in the 20k TT section.

**STCT**

Training sessions consisted of continuous cycling at a fixed power equivalent to 90, 92.5, 95, and 97.5% of baseline $W_{1\text{LT}}$ for wk 1–4, respectively (Fig. 1). The workload was matched to that completed by the HIIT group.
HIIT

Training sessions consisted of 4-min cycling intervals interspersed with a 2-min recovery at 60 W. Training intensities were defined as \( W_{LT} + x(W_{peak} - W_{LT}) \), where \( x = 0.35, 0.50, 0.65, \) and 0.75 for wk 1–4, respectively (Fig. 1). For both STCT and HIIT, training intensity was set relative to \( W_{LT} \) rather than \( W_{peak} \), as metabolic and cardiac stresses are similar when individuals of differing fitness levels exercise at a percentage of the \( W_{LT} \), but can vary significantly when they train at a percentage of \( W_{peak} \) (25).

SIT

Training sessions consisted of 30-s bouts of all-out cycling against resistance set at 0.075 kg/kg body mass (BM) interspersed with a 4-min rest (Fig. 1). During the last 15 s of the rest period the participants were instructed to reach a cadence of 80–100 rpm against no resistance, and, in the last 2 s, they began pedaling as fast as possible, before the load was applied by the software (Velotron Wingate; RacerMate).

Physical activity and nutritional control

The participants were instructed to maintain a normal dietary pattern and their routine physical activity throughout the study. To minimize the variability in muscle metabolism attributable to diet, they were provided a standardized dinner (55 kJ/kg BM, providing 2.1 g carbohydrate/kg BM, 0.5 g fat/kg BM, and 0.6 g protein/kg BM) and breakfast (41 kJ/kg BM, providing 1.8 g carbohydrate/kg BM, 0.2 g fat/kg BM, and 0.3 g protein/kg BM), to be consumed 15 and 3 h before the biopsy trials, respectively. Participants were also required to keep a nutrition diary in which they recorded the last 3 meals before each performance test undertaken during baseline testing, and they were asked to replicate the nutritional intake thereafter before the same type of test.

Skeletal muscle analyses

Fiber preparation and high-resolution respirometry

Muscle fibers were mechanically separated with pointed forceps in ice-cold BioPS containing (in mM) 2.77 CaK2EGTA, 7.25 K2EGTA, 5.77 Na2ATP, 6.56 MgCl2, 20 taurine, 50 2-(N-morpholino)ethanesulfonic acid (MES), 15 Na2-phosphocreatine, 20 imidazole, and 0.5 DTT adjusted to pH 7.1 (26). The plasma membrane was permeabilized by gentle agitation for 30 min at 4°C in BioPS containing 50 μg/ml saponin and was followed by 3 washes in MiR05, a respiration medium containing (in mM, unless specified) 0.5 EGTA, 3 MgCl2, 60 potassium-lactobionate, 20 taurine, 10 KH2PO4, 20 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 110 sucrose, and 1 mM bovine serum albumin (BSA), essentially fatty acid–free (pH 7.1) (26). Mitochondrial respiration was measured in duplicate (from 3–4 mg wet weight of muscle fibers) in MiR05 at 37°C by using the high-resolution Oxygraph-2k (Oroboros, Innsbruck, Austria). Oxygen concentration (in nanomoles per milliliter) and flux (in picomoles per second per milligram) were recorded with DatLab software (Oroboros). Reoxygenation by direct syringe injection of \( O_2 \) was necessary to maintain \( O_2 \) levels between 275 and 450 nmol/ml and to avoid potential oxygen diffusion limitation.

Mitochondrial respiration protocol

A substrate–uncoupler–inhibitor titration (SUIT) protocol was used (26), and the SUIT sequence was as follows: pyruvate (5 mM) and malate (2 mM) in the absence of adenylates were added for measurement of leak respiration (\( L \)) through complex I (CI) (\( CI_1 \)). ADP (5 mM) was added for measurement of maximum oxidative phosphorylation (oxphos) capacity (\( P \)) through CI (\( CI_1 \)), followed by addition of succinate (10 mM) for measurement of \( P \) through CI-II combined (\( CI-II_1 \)). Cytochrome \( c \) (10 μM) was then added to test for outer mitochondrial membrane integrity, followed by a series of stepwise carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) titrations (0.75–1.5 μM), for measurement of electron transport system (ETS) capacity (\( E \)) through CI-II (\( CI-II_1 \)). Rotenone (0.5 μM), an inhibitor of CI, was added to determine \( E \) through CI (\( CI_1 \)), whereas addition of antimycin A (2.5 μM), an inhibitor of CIII, allowed measurement and correction of residual oxygen consumption (ROX), indicative of nonmitochondrial oxygen consumption. Ascorbate (2 mM) and N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD; 0.5 mM), artificial electron donors for CIV, were then added to measure \( E \) through CIV (CIV(\( E \))). Respiratory flux control ratios (FCRs) were calculated. In brief, the leak control ratio (LCR) is the quotient of \( CI_1 \) over \( CI-II_1 \); the phosphorylation control ratio (PCR) is the quotient of \( CI-II_1 \) over \( CI-I_1 \); the coupling control ratio is the quotient of \( CI_1 \) over \( CI-II_1 \) and is equivalent to the inverse respiratory control ratio (inv-RCR); the substrate control ratio (SCR) at constant \( P \) is the quotient of \( CI_1 \) over \( CI-II_1 \); the reserve capacity of CIV (CIV(\( R \))res) was determined as the quotient of \( CI-II_1 \) over CIV(\( E \)).

Preparation of whole-muscle lysates

Frozen muscle (~10–20 mg) was homogenized in an ice-cold lysis buffer (1:20 w/v) containing 50 mM Tris, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, a protease/phosphatase inhibitor cocktail (Cell Signaling Technology (CST), Danvers, MA, USA), and 1 mM PMSF, adjusted to pH 7.4. Muscle homogenates were rotated at 4°C for 60 min and centrifuged at 15,000 g at 4°C for 20 min, and the supernatant was used for immunoblot analysis and enzyme activity assay. Protein concentration was determined in triplicate with a commercial colorimetric assay (Protein Assay kit-II; Bio-Rad, Gladstone, NSW, Australia).

Immunoblot analysis

Muscle lysates (10–25 μg) were separated by electrophoresis on SDS polyacrylamide gels (8–12%) and transferred to PVDF membranes, before being blocked for 1 h in 5% skim milk in Tris-buffered saline with 0.1% Tween-20 (TBST). Membranes were incubated overnight at 4°C with the following primary antibodies: apotosis-inducing factor (AIF), dynamin-related protein 1 (DRP1), mitofusin 2, p53, and PHF20 (all from CST); PGC-1α (EMS-Millipore, Billerica, MA, USA); synthesis of cytochrome \( c \) oxidase 2 (SCO2) (Santa Cruz Biotechnology, Santa Cruz, CA, USA); and TFAM and total oxphos (Abcam, Cambridge, UK). The membranes were then incubated at room temperature with the appropriate host species–specific secondary antibody for 90 min, before being exposed to a chemiluminescence solution. Three TBST washes were performed between each step. Protein bands were visualized with a Bio-Rad Versa-Doc imaging system, and bands were quantified with Bio-Rad Quantity One software (Bio-Rad). An internal standard was loaded on each gel, and each lane was normalized to that value, to reduce gel-to-gel variability. Coomassie blue staining was used to qualitatively verify correct loading and equal transfer between lanes (27). Statistical analysis was performed on the raw density data (Supplemental Table S1). For graphical purposes, each
participant’s time point value was normalized to baseline; therefore, immunoblot data are presented as fold change vs. baseline.

**CS activity assay**

CS activity was determined in triplicate on a microtiter plate by adding 5 μl of a 2 mg/ml muscle homogenate, 40 μl of 3 mM acetyl CoA, and 25 μl of 1 mM 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB) in Tris buffer to 165 μl 100 mM Tris buffer (pH 8.3) kept at 30°C. After addition of 15 μl of 10 mM oxaloacetic acid, the plate was immediately placed in an xMark-Microplate spectrophotometer (Bio-Rad) at 30°C, and after 30 s of linear agitation, absorbance at 412 nm was recorded every 15 s for 3 min. CS activity is reported as moles per hour per kilogram protein.

**TABLE 2. Participants’ parameters before and after 4 wk of training**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>STCT (n = 9)</th>
<th>HIIT (n = 11)</th>
<th>SIT (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM (kg)</td>
<td>Pre 77.4 ± 10.6</td>
<td>80.2 ± 13.8</td>
<td>84.5 ± 19.4</td>
</tr>
<tr>
<td></td>
<td>Post 76.7 ± 10.8</td>
<td>80.0 ± 13.3</td>
<td>84.8 ± 18.1</td>
</tr>
<tr>
<td>W_{LT} (W)</td>
<td>Pre 194.9 ± 46.1</td>
<td>198.1 ± 27.4</td>
<td>204.4 ± 39.7</td>
</tr>
<tr>
<td></td>
<td>Post 208.9 ± 50.0</td>
<td>214.7 ± 29.7</td>
<td>222.3 ± 45.4</td>
</tr>
<tr>
<td>W_{peak} (W)</td>
<td>Pre 275.6 ± 54.6</td>
<td>264.1 ± 37.4</td>
<td>280.8 ± 48.2</td>
</tr>
<tr>
<td></td>
<td>Post 284.4 ± 62.5</td>
<td>293.2 ± 34.3</td>
<td>293.3 ± 51.5</td>
</tr>
<tr>
<td>20k TT time (s)</td>
<td>Pre 2216.7 ± 183.8</td>
<td>2247.7 ± 147.5</td>
<td>2162.3 ± 143.1</td>
</tr>
<tr>
<td></td>
<td>Post 2130.9 ± 176.0</td>
<td>2138.1 ± 90.7</td>
<td>2131.9 ± 165.1</td>
</tr>
<tr>
<td>CS activity (mol · h⁻¹ · kg protein⁻¹)</td>
<td>Pre 9.3 ± 1.6</td>
<td>8.0 ± 2.2</td>
<td>9.4 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>Post 9.8 ± 0.8</td>
<td>8.6 ± 1.8</td>
<td>9.6 ± 1.9</td>
</tr>
</tbody>
</table>

Pre, before training; post, after training. All values are means ± sd. *P < 0.05, main effect of time; #P < 0.05 vs. values before training (pre) of the same group.

**TABLE 3. Respiration values**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>STCT (n = 9)</th>
<th>HIIT (n = 11)</th>
<th>SIT (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI-L</td>
<td>Pre 9.0 ± 2.8</td>
<td>4.9 ± 2.2*#</td>
<td>8.1 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Post 8.3 ± 3.1</td>
<td>5.3 ± 1.6*#</td>
<td>10.0 ± 1.8</td>
</tr>
<tr>
<td>CI-P</td>
<td>Pre 63.2 ± 15.3</td>
<td>45.2 ± 8.4*#</td>
<td>59.1 ± 8.0</td>
</tr>
<tr>
<td></td>
<td>Post 60.1 ± 10.6</td>
<td>45.1 ± 6.2</td>
<td>70.8 ± 13.8</td>
</tr>
<tr>
<td>CI+II-P</td>
<td>Pre 88.6 ± 19.3</td>
<td>68.1 ± 11.6*#</td>
<td>85.6 ± 12.1</td>
</tr>
<tr>
<td></td>
<td>Post 89.5 ± 15.5*#</td>
<td>65.6 ± 7.7</td>
<td>106.9 ± 19.0*#</td>
</tr>
<tr>
<td>CI+II-E</td>
<td>Pre 111.4 ± 24.1</td>
<td>87.4 ± 16.2</td>
<td>107.4 ± 12.0</td>
</tr>
<tr>
<td></td>
<td>Post 110.7 ± 21.1</td>
<td>87.9 ± 15.9</td>
<td>139.6 ± 36.7*#</td>
</tr>
<tr>
<td>CI-E</td>
<td>Pre 44.2 ± 11.9</td>
<td>41.7 ± 7.7</td>
<td>47.0 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>Post 46.9 ± 13.7</td>
<td>41.1 ± 4.5</td>
<td>60.9 ± 17.5*#</td>
</tr>
<tr>
<td>CI-V-E</td>
<td>Pre 132.6 ± 30.9</td>
<td>124.0 ± 17.9</td>
<td>123.2 ± 22.3</td>
</tr>
<tr>
<td></td>
<td>Post 128.5 ± 36.6</td>
<td>125.1 ± 14.0</td>
<td>160.9 ± 49.2</td>
</tr>
</tbody>
</table>

Pre, before training; post, after training. All values are means ± sd. *P < 0.05, main effect of time; †P < 0.05, main effect of training; ‡P < 0.05 vs. pre of the same group; †P < 0.05 vs. pre-STCT and pre-SIT; #P < 0.05 vs. pre of the same group; *P < 0.05 vs. post-HIIT; †P < 0.05 vs. post-SIT; ‡P < 0.05 vs. post-SIT.

CI-L, leak respiration through CI; CI-P, maximum coupled mitochondrial respiration through CI; CI+II-P, maximum noncoupled mitochondrial respiration through CI+II; CI+II-E, maximum noncoupled mitochondrial respiration through CI+II; CI-E, maximum noncoupled mitochondrial respiration through CI; and CI-V-E, maximum noncoupled mitochondrial respiration through CI-V. Pre, before training; post, after training. All values are means ± sd. *P < 0.05, main effect of time; †P < 0.05, main effect of training; ‡P < 0.05 vs. pre of the same group; †P < 0.05 vs. post-HIIT; †P < 0.05 vs. post-SIT.
RESULTS

Training and performance measurements

Participants were matched for age, height, VO_{peak}, BM, W_{LT}, W_{peak}, and 20k TT time (Table 2) (all P > 0.05). Participants in the SIT group completed a significantly lower amount of total work (~35%) compared with those in the HIIT and STCT groups (1.3 ± 0.2, 3.6 ± 0.6, and 3.8 ± 0.7 MJ for the SIT, HIIT, and STCT groups respectively, P < 0.001). There were no changes in BM after training (main effect of time, P = 0.594; Table 2). W_{LT} increased after training in all 3 training interventions (all P < 0.001), with no difference between conditions (P = 0.754; Table 2). W_{peak} increased significantly after SIT (P = 0.011) and HIIT (P < 0.001), and there was a similar trend after STCT, although it failed to reach significance (P = 0.05; Table 2). 20k TT time improved significantly after STCT and HIIT (both P < 0.001), but did not change after SIT (P = 0.177; Table 2).

Mass-specific mitochondrial respiration

Absolute mass-specific mitochondrial respiration values are presented in Table 3, and changes are shown in Fig. 2A. SIT was the only group that increased maximum coupled (CI+IIP) and noncoupled (CI+IIE) respiration (interaction, P = 0.008 and 0.028 respectively). CI+IIP increased significantly after SIT (P < 0.001) and did not change after HIIT or STCT (both P > 0.05). Similarly, CI+IIE increased significantly after SIT (P = 0.001) and did not change after HIIT or STCT (both P > 0.05). CIV_E also increased significantly after SIT (P = 0.002) and did not change after HIIT or STCT (both P > 0.05; interaction, P = 0.027). Although it failed to reach statistical significance, the same trend was observed for CI_F (interaction, P = 0.055) and CIV_E (interaction, P = 0.061), whereas CI_L did not change (main effect of time, P = 0.312). The data presented in this study showed no effect of the addition of cytochrome c as a control for outer mitochondrial membrane integrity. There were no significant differences in any of the FCRs measured (all P > 0.05; Table 4).

Mitochondrial-specific respiration

CS activity did not change significantly after any of the training interventions (main effect of time, P = 0.227; statistically not significant). Despite not reaching statistical significance, the same trend was observed for CI_F and CIV_E (interaction, P = 0.045 and 0.045, respectively). Specifically, CI+IIP/CS increased significantly after SIT (P = 0.014) and did not change after HIIT or STCT (both P > 0.05). Similarly, CI+IIE/CS increased significantly after SIT (P = 0.013) and did not change after HIIT or STCT (both P > 0.05; interaction, P = 0.013). Despite not reaching statistical significance, the same trend was observed for CI_L/CS (interaction, P = 0.107) and CIV_E/CS (P = 0.072). CI_L/CS did not change (main effect of time, P = 0.931).

Statistical analysis

All values are reported as means ± SD, unless specified otherwise. A 1-way ANOVA was used to assess differences between the 3 groups at baseline and for total training workload. To investigate the influence of treatment and time and the interaction between both of these variables, 2-way ANOVA for repeated measures were used. Significant interactions and main effects were further analyzed with a Tukey’s honestly significant difference post hoc test. The least-squares linear regression analysis was used to calculate correlation coefficients between variables, according to Pearson’s product moment (r). SigmaStat software (Jandel Scientific, San Rafael, CA, USA) was used for all statistical analyses. The level of statistical significance was set at P < 0.05.
Protein content of transcription factors

PGC-1α protein content increased by 61 ± 58% after SIT (P = 0.011), but did not change significantly after HIIT or STCT (both P > 0.05; Fig. 3A). p53 protein content increased by 93 ± 77% after SIT (P = 0.004), but did not change significantly after HIIT or STCT (both P > 0.05; Fig. 3B). The protein content of PHF20 increased by 84 ± 76% after SIT (P = 0.003), but did not change significantly after HIIT or STCT (both P > 0.05; Fig. 3C). TFAM protein content did not change after training (main effect of time, P = 0.400; Fig. 3D).

Protein content of subunits from the 5 ETS complexes and of mitofusin-2, DRP1, SCO2, and AIF

The protein content of subunits from the 5 ETS complexes did not change significantly after training (all P > 0.05; Fig. 4). Protein content of mitofusin 2 increased after training, with no significant difference between groups (main effect of time, P = 0.047; Fig. 5A). DRP1 protein content showed a trend toward increasing after training, with no difference between groups (main effect of time, P = 0.063; Fig. 5B). The protein content of SCO2 and AIF did not change with training (main effect of time, both P > 0.05; Fig. 5C, D, respectively).

Correlation between mass-specific mitochondrial respiration and performance measurements

There was a significant linear correlation between CI+IIP and 20k TT performance before training (r = -0.65; P < 0.001; Fig. 6A). No correlation was found between changes in CI+IIP and 20k TT performance with training (r = 0.21; P = 0.279; Fig. 6B), and the initial correlation was no longer significant after training (r = -0.26; P = 0.188). Likewise, CI+IIIP correlated significantly with \( W_{\text{peak}} \) and \( W_{\text{peak}} \) before training (r = 0.60 and r = 0.67, respectively; both P < 0.001), but there was no correlation between their changes (r = 0.08 and r = -0.35, respectively; both P > 0.05) or after training (r = 0.27 and r = 0.16, respectively; both P > 0.05).

DISCUSSION

In the present study, 4 wk of SIT increased mitochondrial respiration in young moderately-trained men, whereas 4 wk of training at lower intensities (STCT, HIIT) induced no change, despite much higher training volumes. In addition, the results show for the first time that 4 wk of SIT increased the protein content of p53. This result, along with changes in PGC-1α protein content may help to explain the greater changes in mitochondrial respiration after SIT. Furthermore, the protein content of PHF20, a transcription factor upstream of p53, also increased after SIT, providing further insights into the cellular events that may contribute to the adaptive response to exercise training.

Mass-specific mitochondrial respiration

A novel aspect of this research was to assess the effects of different training intensities on training-induced changes in mitochondrial respiration. Our results show that CI+IIP increased by 25% after 4 wk of SIT, whereas neither HIIT nor STCT was associated with an increase in mass-specific respiration. These findings highlight the role of training intensity as an important determinant of changes in mitochondrial respiration. Although caution is advised, because of various methodological differences between studies, such as training volume and duration, or the differences in age and training status of the populations examined, it is relevant to compare our results with those in the literature that assessed training-induced changes in mitochondrial respiration, and CIVRes, CIV reserve capacity (CI+IIP/CIVE). FCRs were calculated from mass-specific respiration measurements in permeabilized muscle fibers (vastus lateralis), obtained from muscle biopsies taken before and after 4 wk of training at each of the 3 training intensities. Pre, before training. Post, after training. All values are means ± s.
STCT groups (89 and 66% of W_{Peak}, respectively) did not improve mass-specific mitochondrial respiration, and the results are in agreement with those for the continuous training group (71% of W_{Peak}) in Daussin et al. (6).

It has been suggested that workload and oxygen-uptake fluctuations, rather than training volume or intensity, are key determinants of training-induced improvements in mitochondrial respiration (6). However, although workload and oxygen uptake fluctuations are hallmarks of both SIT and HIIT, only SIT, which elicits a much higher training intensity, increased mass-specific mitochondrial respiration in our study. This finding further strengthens the notion that training intensity is an important factor for training-induced improvements in mitochondrial respiration. Similar to Daussin et al. (6), our research suggests that training volume is less important in determining changes in mitochondrial respiration, as neither STCT nor HIIT improved mass-specific mitochondrial respiration after 4 wk of training, despite a training volume almost 3 times greater than SIT. This result may be explained by findings from previous reports that showed muscle fiber activation increases with exercise intensity (28) and that SIT elicits greater reliance on oxidative phosphorylation within each bout and with each subsequent bout (29). Therefore,
Although further research is needed, the higher training intensity associated with SIT may have improved mitochondrial respiration by maximally stressing the oxidative phosphorylation system and by recruiting a wider fiber pool than HIIT and STCT.

Mitochondrial qualitative and quantitative changes

Our results showed that no training intervention significantly altered CS activity, a commonly used biomarker of mitochondrial content (3). Although many studies have reported increased CS activity after training at various intensities, the literature also provides evidence of unaltered or even decreased CS activity after training (30). The reasons for these discrepancies are not readily apparent, but may relate to methodological differences in CS activity assay (30), the participants’ fitness level or training status, and differences in training duration or study design. To separate qualitative and quantitative changes, we determined mitochondrial-specific respiration, by normalizing mass-specific respiration to CS activity. Figure 2B shows that changes in mitochondrial-specific respiration mirrored the changes in mass-specific respiration. This finding indicates that SIT resulted in increased mitochondrial respiration relative to mitochondrial content. Although cross-sectional research has reported that differences in the maximal aerobic capacity of individuals of various fitness levels are accompanied by modifications in mitochondrial-specific respiration (31), our results are the first to show that these modifications can be achieved in as little as 4 wk of SIT in moderately-trained individuals. In addition, the lack of change in the protein content of subunits from the 5 ETS complexes (similar to CS activity) provides further evidence that changes in mitochondrial respiration can take place before, or independent of, changes in mitochondrial content. These findings also highlight a dissociation between mitochondrial respiration and mitochondrial content, as was demonstrated in mice (32). Although further research is needed, the lack of change in mitochondrial content may be the result of matched changes in protein synthesis and degradation, such that replacement of damaged proteins with newly synthesized ones may contribute to the increase in mitochondrial respiration (33). Finally, analysis of the FCRs indicates that there was no change in the index of uncoupling (LCR), the limitation of oxphos capacity by the phosphorylation system (PCR), the degree of coupling between oxidation and phosphorylation (inv-RCR), and substrate control (SCR and CIVRes). These results suggest that increased oxidative capacity after SIT is mediated by an improvement in mitochondrial-specific respiration without changes in the coupling and substrate control of the respiratory system. Further investigation of the possible underlying mechanisms is warranted.

Mitochondrial proteins and transcription factors

To investigate the cellular events associated with the adaptive response to training, we measured the protein content of key regulators of mitochondrial biogenesis. PGC-1α protein content was significantly increased only after SIT, in agreement with a report of increased PGC-1α in whole-muscle fractions of male participants after 3 wk of SIT (34). Although separate studies have shown that PGC-1α can be increased after SIT, HIIT, or continuous training (5, 12, 34), no study to date has reported changes in PGC-1α protein content in response to exercise training at 3 different intensities within 1 study. Research has indicated that, after a single bout of exercise, PGC-1α mRNA is regulated in an intensity-dependent manner (15), but that this intensity-dependent regulation is limited to exercise intensities below peak power (35). However, our findings indicate that the intensity-dependent regulation of PGC-1α protein may extend to intensities above peak power. The importance of training intensity is strengthened further when considering that, despite much greater training volumes, neither STCT nor HIIT increased PGC-1α protein content.

In the present study, only SIT increased p53 protein content, indicating a possible intensity-dependent regulation similar to that of PGC-1α. This increase, coupled with greater mitochondrial respiration after SIT, is consistent with the role of p53 as a metabolic regulator and its ability to modulate the switch from glycolytic to oxidative energy.
provision (17). In mouse liver mitochondria, this modulation is mediated downstream of p53 by SCO2 (17). However, subsequent research has failed to provide evidence of p53 control of SCO2 in both mouse (20) and human (36) skeletal muscle. The lack of change in SCO2 protein content after 4 wk of training seems consistent with these findings. Another means of p53’s control of mitochondrial respiration is through transcriptional regulation of AIF, a protein that assists in the assembly of CI (16). Our results indicate that AIF protein content did not change after training, consistent with the findings of past research in humans (37). p53 has also been linked with mitochondrial dynamics (16). In the present study, the protein content of mitofusin 2 increased after training, with DRP1 showing a similar trend ($P=0.063$), but there were no significant differences between groups for either protein. p53 can also regulate mitochondrial transcription by interacting with TFAM (20, 21), a nuclear-encoded gene that controls mtDNA transcription and maintenance (13). Our results indicate that TFAM protein content was unchanged after training, in agreement with prior findings (5). This result is also consistent with the hypothesis that mitochondrial adaptations and mtDNA transcription depend on the activation of existing TFAM (5) and its interaction

| Figure 5. Fold change compared to baseline of the protein content of mitofusin-2 (A), DRP1 (B), SCO2 (C), and AIF (D) in whole-muscle lysates prepared from skeletal muscle biopsy samples (vastus lateralis). Biopsies were obtained at rest at baseline (dotted line) and after 4 wk of training at each of the 3 training intensities STCT, HIIT, and SIT. Representative immunoblots (from left to right: pre-STCT, post-STCT, pre-HIIT, post-HIIT, pre-SIT, post-SIT) are presented above each target protein. All values are means ± SD. $^\dagger$ $P<0.05$, main effect of time. $P$ value next to a label indicates a trend toward a main effect of time. |
with p53 inside the mitochondria (21), rather than the abundance of TFAM itself.

Similar to p53 protein content and mass-specific respiration, PHF20 protein content increased only after SIT. PHF20 is a transcription factor that binds directly to the p53 promoter and transcriptionally upregulates (22) and stabilizes (23) p53. Therefore, a training-intensity-dependent increase in PHF20 protein may help to explain the increase in p53 protein only after SIT and suggests that 4 wk of SIT improve mitochondrial respiration via a mechanism involving the coordinated up-regulation of the PHF20–p53 pathway. Future investigations of these hypotheses are warranted.

Mitochondrial respiration and endurance performance

Mitochondrial respiration has been reported to be the strongest determinant of TT performance in highly-trained athletes (4). Consistent with these findings, our results revealed a significant linear correlation between pretraining CI+IIP and 20k TT performance across the 29 participants who completed the study. However, despite being the only training intervention to increase mitochondrial respiration, SIT did not improve 20k TT performance after training. This discrepancy between changes in mitochondrial respiration and endurance performance is further supported by the lack of correlation between changes in CI+IIP and 20k TT time, and the subsequent loss of correlation between these 2 parameters after training. This finding indicates that mitochondrial respiration was not the key mediator of training-induced changes in endurance performance for the moderately-trained population in our study. Nonetheless, SIT may represent an effective way to boost the oxidative capacity of moderately-trained men, which, when coupled with other training at lower intensity, could contribute to improved endurance performance.

CONCLUSIONS

Our results demonstrate that, in young moderately-trained men who complete a 4-wk training protocol, training intensity is an important factor that determines changes in mitochondrial respiration. We provide evidence that as little as 4 wk of SIT was sufficient to induce improvements in mitochondrial quality, such as increased mitochondrial-specific respiration. These results suggest that SIT promotes greater and faster mitochondrial adaptations in skeletal muscle of young moderately-trained men than does HIIT and STCT; however, our results and the literature suggest that physiological and mitochondrial adaptations can occur with longer STCT- or HIIT-like protocols. We also showed dissociation between changes in mitochondrial content and mitochondrial respiration. Our results indicate that changes in the protein content of PGC-1α, p53, and PHF20 may be more strongly associated with training-induced changes in mitochondrial respiration rather than mitochondrial content, and suggest that these changes may be mediated by different molecular pathways or time courses. The present study also highlights the potential role of PHF20 in the regulation of mitochondrial respiration in humans via the coordinated upregulation of the PHF20–p53 pathway.

The authors thank Ms. E. Brentnall and Mr. A. Ronacher for valuable help in data collection. This study was funded by a grant from the ANZ-MASON foundation (to D.J.B.) and Natural Sciences and Engineering Research Council of Canada Discovery Grant RGPIN 435807-13 (to J.P.L.). The authors declare no conflicts of interest.

REFERENCES


TRAINING-INDUCED MITOCHONDRIAL ADAPTATIONS


Received for publication July 6, 2015. Accepted for publication October 28, 2015.