Influence of aerobic exercise intensity on myofibrillar and mitochondrial protein synthesis in young men during early and late postexercise recovery

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Di Donato DM, West DW, Churchward-Venne TA, Breen L, Baker SK, Phillips SM. Influence of aerobic exercise intensity on myofibrillar and mitochondrial protein synthesis in young men during early and late postexercise recovery. Am J Physiol Endocrinol Metab 306: E1025–E1032, 2014. First published March 3, 2014; doi:10.1152/ajpendo.00487.2013.—Aerobic exercise is typically associated with expansion of the mitochondrial protein pool and improvements in muscle oxidative capacity. The impact of aerobic exercise intensity on the synthesis of specific skeletal muscle protein subfractions is not known. We aimed to study the effect of aerobic exercise intensity on rates of myofibrillar (MyoPS) and mitochondrial (MitoPS) protein synthesis over an early (0.5–4.5 h) and late (24–28 h) period during postexercise recovery. Using a within-subject crossover design, eight males (21 ± 1 yr, VO2peak 46.7 ± 2.0 ml·kg⁻¹·min⁻¹) performed two work-matched cycle ergometry exercise trials (LOW: 60 min at 30% Wmax; HIGH: 30 min at 60% Wmax) in the fasted state while undergoing a primed constant infusion of [ring-¹³C₆]phenylalanine. Muscle biopsies were obtained at rest and 0.5, 4.5, 24, and 28 h postexercise to determine both the “early” and “late” response of MyoPS and MitoPS and the phosphorylation status of selected proteins within both the Akt/mTOR and MAPK pathways. Over 24–28 h postexercise, MitoPS was significantly greater after the HIGH vs. LOW exercise trial (P < 0.05). Rates of MyoPS were increased equivalently over 0.5–4.5 h postexercise recovery (P < 0.05) but remained elevated at 24–28 h postexercise only following the HIGH trial. In conclusion, an acute bout of high- but not low-intensity aerobic exercise in the fasted state resulted in a sustained elevation of both MitoPS and MyoPS at 24–28 h postexercise recovery.

Aerobic exercise intensity: myofibrillar and mitochondrial protein synthesis

ADAPTATIONS TO AEROBIC-BASED EXERCISE include increases in mitochondrial protein content (both size and number of mitochondria) and subsequent improvements in muscle oxidative capacity and resistance to fatigue (20). Additionally, traditional aerobic exercise (16, 18) as well as high-intensity “sprint” training (19, 33) can also enhance skeletal muscle hypertrophy, an adaptation that would be contingent upon stimulation of myofibrillar protein synthesis (MyoPS) and expansion of the myofibrillar protein pool (31). Specific phenotypic outcomes (i.e., improved oxidative capacity and muscle hypertrophy) in response to divergent exercise stimuli must relate to changes in the synthesis of specific muscle protein subfractions and may be altered by the intensity of exercise (35). For example, work-matched performance of high-intensity resistance exercise results in greater rates of MyoPS than low-intensity resistance exercise (6, 22). An increase in the rate of mixed muscle protein synthesis has been reported after an acute bout of aerobic-based exercise (8, 17, 27); however, such measures preclude insight into the synthetic response of specific muscle protein subfractions, including mitochondrial and myofibrillar proteins. Whether manipulation of aerobic exercise intensity alters the synthesis of specific muscle protein subfractions is unknown.

Phosphorylation resulting in activation/deactivation of proteins in the Akt-mTOR pathway has been shown to be critical in the regulation of contraction-mediated increases in protein synthesis (12). Other contraction-dependent signaling pathways, such as the MAPK pathway, may also be involved in regulating contraction-mediated translational control (34, 36). Phosphorylation of p38 MAPK can affect transcription factors such as peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) (25, 34, 36). As a primary regulator of mitochondrial biogenesis, PGC-1α coordinates transcriptional activity and assists in coordinating the transcription of mitochondrial and nuclear DNA for mitochondrial biogenesis (13, 30). Higher-intensity aerobic exercise has been demonstrated to result in a greater increase in the mRNA abundance of PGC-1α compared with lower-intensity exercise (13), but whether aerobic exercise intensity alters rates of mitochondrial protein synthesis (MitoPS) is unknown.

The purpose of the present study was to examine the effect of acute bouts of work-matched aerobic exercise of different intensities on rates of MyoPS, MitoPS, and the phosphorylation status of signaling molecules of the Akt-mTOR and MAPK pathways during early (4 h) and late (24 h) postexercise recovery. To examine the independent effects of exercise, we chose to study subjects in the fasted state. We hypothesized that high-intensity (HIGH) cycle ergometry would elicit greater increases in MyoPS and MitoPS than work-matched low-intensity (LOW) cycling exercise. Additionally, we hypothesized that signaling molecule phosphorylation would align with the intensity-dependent differences in protein synthesis with greater activation of the Akt/mTOR and MAPK pathways and increased nuclear PGC-1α accumulation following HIGH exercise.

METHODS

Participants

Eight healthy, recreationally active men (means ± SE; 21 ± 1 yr, 82.5 ± 3.8 kg, 181 ± 2 cm, VO2peak 46.7 ± 2.0 ml·kg⁻¹·min⁻¹) were...
recruited to participate in the study. Participants reported participating in unstructured moderate-intensity aerobic exercise 1–2 times per week. All participants were informed of the purpose of the study, experimental procedures, and associated risks prior to participation and exercise testing. All participants gave verbal and written consent to a protocol approved by the Hamilton Health Science Research Ethics Board, conforming to the standards for the use of human subjects in research as outlined in the Declaration of Helsinki and with current Canadian Tri-Council Research Agency guidelines for use of human participants in research (http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/).

**Experimental Design**

The study consisted of prestudy maximal aerobic capacity measures, a brief familiarization session to assess the aerobic exercise intensities for the trials, and finally two infusion trials for resting and postexercise metabolic investigation per exercise intensity (4 infusion trials total). Each participant completed both exercise intensity trials, making this study a within-subject crossover design.

**Maximal aerobic capacity measurements.** Two weeks prior to the first infusion trial, participants reported to the laboratory and completed a V̇O₂peak test on a cycle ergometer (Lode, Groningen, Netherlands) with continuous oxygen uptake measurements (Ergocard Professional; Medisoft, Sorinnes, Belgium). The test began at 50 W and increased 1 W every 2 s until voluntary fatigue. Fatigue was defined by a respiratory exchange ratio greater than 1.1 and the inability to maintain 60 rpm on the cycle ergometer despite vigorous verbal encouragement. Peak power output in watts (Wmax), maximum heart rate (HRmax), and average cadence were recorded. Participants were asked to maintain a constant cadence (within 5–10 rpm) between 70 and 100 rpm. The positions of the saddle and handlebars were recorded for each participant and were repositioned accordingly for each subsequent exercise bout. The Wmax for each participant was used to determine the workload for the relative high-(HIGH, 60% Wmax) and low-intensity (LOW, 30% Wmax) exercise trials.

**Familiarization trial.** A familiarization session (~15 min) was carried out with each participant with the exercise intensity that was performed on the days of metabolic investigation and also to confirm the relative intensity of the exercise based on heart rate (HR) and oxygen consumption (VO₂). One week prior to the first infusion trial, participants completed a short bout of exercise at LOW and then HIGH workouts. HR was measured throughout the familiarization, and VO₂ was also measured in the last 2 min of both HIGH and LOW. The participants were asked to maintain the same constant pedaling cadence that was comfortable for them during the maximum aerobic capacity test, which was also maintained during trials.

**Metabolic investigation and infusion protocol.** Participants underwent two experimental infusions on sequential days for both HIGH and LOW exercise (4 trials total) to study the response of both MyoPS and MitoPS during early (0.5–4.5 h) and later (24–28 h) postexercise investigation. At least 10 days separated the trials for the two intensities. Participants were asked to keep a diet record for the 48-h period preceding the first infusion protocol corresponding to each of the exercise trials (day 1 and day 3, respectively). A standardized meal representing 30% of each subject’s energy requirements (64% CHO, 17% PRO, 19% fat) was provided and consumed by 2000 on the evening before the first infusion trial for each condition. After an overnight fast and after refraining from physical activity for 2 h prior to the trial, participants reported to the laboratory at 0600 on day 1 (Fig. 1). A 20-gauge catheter was inserted into an antecubital vein of one arm, and a baseline blood sample was obtained. The catheter was kept patent with a 0.9% saline drip for repeated blood sampling. A second catheter was then inserted into the other arm for a primed constant infusion of L-[ring-13C6]phenylalanine (prime: 2 μmol/kg; infusion: 0.05 μmol/kg; Cambridge Isotope Laboratories, Cambridge, MA), which passed through a 0.2-μm filter. Participants rested on a bed until 3 h into the infusion, at which point a biopsy (~100–150 mg) was obtained from the vastus lateralis for fasted resting measurements. Muscle biopsies were obtained under local anesthesia (2% xylocaine) using a 5-mm Bergström needle modified for manual suction. Tissue obtained was blotted, freed of any visible connective tissue and fat, and immediately frozen in liquid N₂ and stored at −80°C until analysis. After the resting biopsy, participants began the exercise protocol on the same cycle ergometer that had been used in the V̇O₂peak test. Participants were randomized to complete the HIGH or LOW protocol during their first trial. The HIGH protocol consisted of 30 min at 60% Wmax, and the LOW protocol consisted of 60 min at 30% Wmax. By work-matching the protocols, we aimed to remove the influence of total energy expenditure during exercise as a potential confounding variable. Energy expenditure did not differ between the two exercise trials (Table 1). Measurements of HR were taken throughout the exercise bout, and VO₂ measurements were obtained three times during each ride. The participants returned to a bed to rest until 30 min after exercise, at which point the second biopsy was

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**Fig. 1. Schema of the experimental infusion study design. Asterisks represent blood draws; single arrows represent muscle biopsies.**
obtained. After another 4 h of tracer infusion, a third biopsy was obtained, and then the infusion was terminated. The diet was standardized for the infusion and subsequent day by providing participants with a meal immediately after the trial representing 50% of their daily caloric requirements and then providing a meal of identical macronutrient and caloric composition to consume in the evening before 2200. The next day, participants returned to the laboratory at 0700 after an overnight fast to undergo a second infusion (day 2; Fig. 1). The infusion protocol was carried out as on day 1, with biopsies obtained at 1.5 h and 5.5 h into the infusion to obtain 24- to 28-h postexercise measurements. Approximately 2 wk later, the participants returned to the laboratory to complete day 3 and day 4 (Fig. 1), performing the opposite exercise intensity to their first trial. The first biopsy was obtained 2 h into the infusion on both days, with exercise beginning at the appropriate time so that the biopsy was obtained 30 min postexercise.

Blood and Muscle Analysis

All blood samples were collected in heparinized evacuated containers and kept on ice until they were centrifuged to obtain plasma, which was subsequently aliquoted, frozen, and stored at −20°C until further analysis. Plasma [ring-13C6]phenylalanine enrichments were determined as previously described (6). Muscle intracellular (IC) free amino acids were extracted from a 10- to 15-mg piece of wet muscle obtained, and then the extract was centrifuged at 15,000 g for 10 min at 4°C. The resulting supernatant (nuclear extract) was transferred to an Eppendorf tube and a 100-μl 1:10 dilution was made for use in a BCA assay. Both the extract and the diluted supernatant were frozen at −80°C until further analysis.

The myofibrillar enriched pellet was washed with H2O and centrifuged at 15,000 g for 5 min at 4°C. Myofibrillar proteins were further extracted and hydrolyzed as described previously (4–6). The free amino acids from the mitochondrial and myofibrillar enriched fractions were purified using cation exchange chromatography (Dowex 50WX8-200 resin; Sigma-Aldrich, St. Louis, MO) and converted to their N-acetyl-n-propyl ester derivatives for GC-MS analysis (29).


table 1. Characteristics of low- and high-intensity exercise trials

<table>
<thead>
<tr>
<th>Workload, W</th>
<th>Time, min</th>
<th>Average %VO2peak</th>
<th>Average %HRMAX</th>
<th>Work, kJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW</td>
<td>99 ± 4</td>
<td>48 ± 2</td>
<td>66 ± 2</td>
<td>367 ± 19</td>
</tr>
<tr>
<td>HIGH</td>
<td>198 ± 7</td>
<td>70 ± 3*</td>
<td>90 ± 1*</td>
<td>369 ± 19</td>
</tr>
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Values are means ± SE *Significantly different from LOW intensity, P < 0.001.

Immunoblot Analysis

Both saroplasmonic and nuclear extracts were used for immunoblot analysis for presence and/or phosphorylation of signaling molecules. The protein concentration of the extracts was determined using the BCA assay (Thermo Fisher Scientific, Rockford, IL). Samples were prepared to the same concentration by dilution with distilled deionized H2O and denatured with Laemmli sample buffer and heated to 95°C. On a 10% SDS-PAGE gel, 20–40 μg of protein (depending on the protein target) was loaded and run at 120 V for 1–1.5 h. Proteins were transferred onto a PVDF membrane using Fast Semi-Dry Transfer (Thermo Fisher Scientific). Membranes were blocked at room temperature (RT) for 1 h using 5% wt/vol milk or BSA in Tris-buffered saline with 0.1% Tween 20 (TBST). Membranes were incubated in primary antibody in TBST at 4°C overnight (Santa Cruz Biotechnology, Santa Cruz, CA; rabbit polyclonal phospho-p70 S6K1 [Thr189, 1:1,000 in TBST, #22171, Cell Signaling Technology, Danvers, MA]; rabbit polyclonal histone 2B [H2B; 0.1 μg/ml in TBST, #ab7190; Cell Signaling Technology, Danvers, MA]; rabbit polyclonal phospho-pTyr (Pep2148, 1:1,000 in TBST, #2971, rabbit monoclonal phospho-p38 MAPK [Thr180/Tyr182, 1:1,000 in TBST; #4511, rabbit polyclonal phospho-ERK1/2 (Thr202/Tyr204, 1:1,100 in TBST, #9101), and rabbit monoclonal α-tubulin (1:2,000 in TBST, #2125). Membranes were washed with TBST and then incubated with secondary antibody anti-rabbit HRP-linked antibody (1:10,000 in TBST; GE Healthcare Life Sciences, Baie D’Urfé, QC, Canada; NA934) at RT for 1 h. After a washing, membranes were visualized using chemiluminescence (Supersignal West Dura Extended Substrate, Thermo Fisher Scientific) and imaged using Fluorochem SP Imaging system (Alpha Innotech, Santa Clara, CA). Images were quantified using National Institutes of Health ImageJ software and normalized to the appropriate loading control. α-ublin and H2B were used as loading controls in saroplastic and nuclear protein extracts. Both α-ublin and H2B were demonstrated to be valid loading controls (i.e., representative of total protein loaded, determined by quantifying Ponceau staining) for saroplastic and nuclear protein extracts, respectively. The degree of enrichment of saroplastic and nuclear protein extracts was determined using Western blotting using MHC1, LDH, and COXIV as markers. Most important
to our analysis, the sarcoplasmic fraction was free of detectable COXIV (mitochondrial proteins), and the nuclear extract was free of detectable LDH (sarcoplasmic proteins).

**Calculations**

The fractional synthetic rates (FSR) of myofibrillar and mitochondrial proteins were calculated using the precursor-product equation

\[
\text{FSR} \left( \% / \text{h} \right) = \frac{(E_{2b} - E_{1b})}{(E_p \times 1/t)} \times 100
\]

where \(E_{2b}\) and \(E_{1b}\) are the bound protein enrichments at times 2 and 1, respectively, and \(E_p\) is the average enrichment of the precursor, intracellular phenylalanine, during steady state. Since participants were "tracer naïve", the baseline preinfusion blood sample enrichment represents the naturally abundant \(^{13}\text{C}\) enrichments and was used for \(E_p\) to determine resting FSR. In this calculation, we used an incorporation time from 30 min after the start of the infusion to the time of the biopsy, which has been previously validated (3).

**Statistical Analysis**

Aerobic exercise trial data were analyzed using two-tailed paired sample Student’s \(t\)-test. Data within an exercise trial (\%HR\text{max}, \%VO\text{2peak}, and plasma enrichment) were analyzed using a two-way repeated-measures ANOVA. To isolate differences between means for which there was not a resting value in each condition, immunoblot and FSR data were analyzed using a one-way ANOVA, with structured contrasts to determine time- and condition-dependent differences. When appropriate, post hoc analysis was performed with a Student-Newman-Keuls test to isolate significant pairwise differences. Correlations were two-tailed Pearson correlations. All statistical analyses were performed using SPSS Statistics (v. 19; IBM, Armonk, NY, USA). All data are presented as means ± SE. Statistical significance was accepted at \(P < 0.05\).

**RESULTS**

**Aerobic Exercise Trial**

All participants completed the exercise at the prescribed intensity. The average \%VO\text{2peak} and \%HR\text{max} were significantly higher in the HIGH trial than in the LOW trial (\%VO\text{2peak} 76 ± 3 vs. 48 ± 1, \%HR\text{max} 90 ± 1 vs. 66 ± 2, \(P < 0.001\); Table 1). During the HIGH trial, \%VO\text{2peak} was higher in the final 5 min of the bout than at 10 min into the exercise bout (\(P < 0.05\)). The same was also observed for \%HR\text{max} during the HIGH exercise trial (\(P < 0.05\)). No change in \%VO\text{2peak} was observed during the LOW exercise bout. Total work was not different between HIGH and LOW trials (Table 1; \(P = 0.46\)).

**Plasma and Intracellular Enrichments**

The free plasma tracer enrichment was not different between the 0.5- to 4.5- and 24- to 28-h postexercise incorporation times.

**Protein Synthesis**

Myofibrillar FSR was increased in early recovery (0.5–4.5 h) compared with rest in both exercise trials (\(P < 0.05\); Fig. 2A). In late recovery (24–28 h), myofibrillar FSR returned to rest in the LOW trial, but remained elevated with HIGH exercise (\(P = 0.05\)). Mitochondrial FSR was significantly different between HIGH and LOW conditions in late recovery (\(P < 0.05\); Fig. 2B). Western blot images of MHCI (shown in green), LDH (shown in blue), and COXIV (shown in red) in the myofibrillar, sarcoplasmic, nuclear, and mitochondrial preparations are shown in Fig. 3.

**Cell Signaling**

There was a significant difference at 0.5 h postexercise between the HIGH and LOW trials for phospho-mTOR\text{Ser2448} (\(P < 0.005\); Fig. 4A). This effect was no longer present at 4.5 h postexercise. At 24 and 28 h postexercise, phospho-mTOR\text{Ser2448} was not different from resting levels in either
condition. There was no effect of the exercise on phospho-p70 S6K1 Thr389 at the time points measured (Fig. 4B). Myofibrillar FSR in early and late recovery after HIGH exercise was positively correlated with phospho-mTORSer2448 at 0.5 h postexercise ($r = 0.953$, $P < 0.001$ and $r = 0.866$, $P < 0.01$, respectively). No relationship was observed between phospho-p70 S6K1 Thr389 and myofibrillar FSR (data not shown). Aerobic exercise did not result in an increase in the phosphorylation of ERK1/2 Thr202/Tyr204 (Fig. 4C). There was a main effect of time for the phosphorylation status of p38Thr180/Tyr182 ($P < 0.005$; Fig. 4D), where phosphorylation was significantly higher at 4.5 h postexercise compared with rest and 0.5, 24, and 28 h postexercise ($P < 0.05$). There was no effect of aerobic exercise on nuclear PGC-1α content at the time points examined (Fig. 4E). Representative blot images are shown in Fig. 5.

**DISCUSSION**

We report here that both high- and low-intensity aerobic exercise stimulate increases in MyoPS during early (0.5–4.5 h) recovery, whereas in late recovery (24–28 h) a sustained elevation in MyoPS was observed only in the HIGH trial (Fig. 2A). We observed an increase in the phosphorylation status of mTOR Ser2448 only after the HIGH trial, the extent of which was correlated with rates of MyoPS during early and late recovery. This observation is interesting in light of the knowledge that aerobic exercise can serve as a stimulus to promote muscle hypertrophy under certain conditions (16, 18). Thus, it is possible that higher-intensity aerobic exercise may, over time, induce a degree of muscle hypertrophy not seen with low-intensity aerobic exercise. Alternatively, the sustained elevation in MyoPS rates at 24 h postexercise following the HIGH trial may reflect an increase in muscle protein turnover to assist in remodeling and protein renewal. Our findings demonstrate that aerobic exercise intensity influenced the synthesis of specific muscle protein fractions, which has bearing on the interpretation of findings from studies of mixed muscle protein synthesis following endurance exercise (17, 27). It has previously been reported that in the fed state, rates of MyoPS are increased above resting values 24 h after both resistance exercise (4, 6) and high-intensity aerobic exercise (11, 28). Thus, loading appears to “sensitize” the muscle to protein provision late into the postexercise recovery period, which also appears true even with low-intensity endurance exercise (15). In support of this notion, we observed intensity-dependent differences in the phosphorylation of mTOR Ser2448 at 0.5 h following the HIGH, but not the LOW, exercise trial (Fig. 4A).
An increase in the phosphorylation of mTOR$^{\text{Ser2448}}$ after cycling exercise has been observed previously (7, 9, 10, 26, 27, 35), but the response appears transient with aerobic compared with resistance exercise (7). To our knowledge, ours is the first report of an aerobic exercise intensity-dependent effect on mTOR$^{\text{Ser2448}}$ phosphorylation. Interestingly, mTOR$^{\text{Ser2448}}$ phosphorylation at 0.5 h was correlated to rates of MyoPS in early and late recovery from the HIGH exercise trial. However, the phosphorylation of p70 S6K1$^{\text{Thr389}}$, which is often taken as a proxy of mTOR activity and is a known regulator of mRNA translation initiation and elongation (21), was not elevated. This finding is in agreement with previous work of Mascher et al. (2007), who reported that a 1-h bout of cycle ergometer exercise at 75% $\text{VO}_2\text{max}$ increased the phosphorylation status of mTOR$^{\text{Ser2448}}$ at 0.5 h but had no effect on p70 S6K1$^{\text{Thr389}}$ (26). The lack of change in p70 S6K1$^{\text{Thr389}}$ phosphorylation also corroborates results from previous studies of aerobic exercise in which biopsies were taken at similar time points (7, 9, 10). It should, however, be acknowledged that a single phosphorylation site (Ser$^{2448}$) of mTOR was measured; other phosphorylation sites that regulate mTOR activity were not measured. It is not clear what role, if any, the early divergent response of mTOR$^{\text{Ser2448}}$ phosphorylation may have played in mediating the divergent protein synthesis responses observed in late recovery. More research is required to determine the mechanisms determining the sustained increases in MyoPS during late (e.g., 24 h) postexercise recovery.

In the present study, aerobic exercise, irrespective of intensity, did not elicit a significant increase from rest in fasted-state measures of MitoPS during early or late recovery, although there was a trend for such a response (Fig. 2B; $P = 0.18$). We did however, observe divergent responses in late recovery, whereby MitoPS rates were higher after the HIGH vs. LOW exercise trial. These results are in contrast with our previous findings of a stimulation of MitoPS following aerobic exercise (35); however, our previous study was performed under conditions of sustained hyperinsulinemia and hyperaminoacidemia, which may explain the divergent findings. Others have reported that protein provision before high-intensity repeated sprinting (9) and protein plus carbohydrate ingestion after prolonged higher-intensity aerobic exercise (2) did not affect the synthesis of mitochondrial proteins vs. nonprotein control.

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In the present study, aerobic exercise, irrespective of intensity, did not elicit a significant increase from rest in fasted-state measures of MitoPS during early or late recovery, although there was a trend for such a response (Fig. 2B; $P = 0.18$). We did however, observe divergent responses in late recovery, whereby MitoPS rates were higher after the HIGH vs. LOW exercise trial. These results are in contrast with our previous findings of a stimulation of MitoPS following aerobic exercise (35); however, our previous study was performed under conditions of sustained hyperinsulinemia and hyperaminoacidemia, which may explain the divergent findings. Others have reported that protein provision before high-intensity repeated sprinting (9) and protein plus carbohydrate ingestion after prolonged higher-intensity aerobic exercise (2) did not affect the synthesis of mitochondrial proteins vs. nonprotein control.
conditions. However, in neither of these previous studies (2, 9) was a resting rate of MitoPS reported, and so it is not possible to ascertain whether exercise per se, regardless of the nutrition provided, resulted in a stimulation of MitoPS. Sustained hyperinsulinemia and hyperaminoacidemia support increased MitoPS at rest (32), an effect that appears to require amino acids (1). Whether there is a necessity for protein provision to robustly stimulate MitoPS, as there is with MyoPS (29), is currently unknown. Given the relative size of the skeletal muscle mitochondrial protein pool (4–8%) vs. the myofibrillar protein pool (60–70%), it would seem that amino acids would not likely be rate limiting for MitoPS. The physiological relevance of the difference in MitoPS between HIGH and LOW exercise trials in late recovery (i.e., 24 h) is unclear from our acute measurements; however, our results may have implications for phenotypic adaptations following a period of chronic training. Further investigations are required to determine whether amino acid provision alters the exercise-mediated mitochondrial response, particularly in the late phase of recovery, and whether chronic performance of higher vs. lower intensities of aerobic exercise (even if energy matched) result in divergent increases in mitochondrial content.

An important regulator of mitochondrial biogenesis, PGC-1α, is thought to coordinate both nuclear and mitochondrial gene expression to induce mitochondrial biogenesis (14). In contrast to previous studies (23, 24), and in line with the absence of an early postexercise increase in MitoPS, we found that nuclear PGC-1α content was not increased after exercise (Fig. 5). The exercise models used in previous studies (23, 24) were of higher intensity than that used in the present study, which may explain the discrepant findings. While exercise intensity-dependent PGC-1α nuclear localization has not been reported, the response of PGC-1α mRNA postexercise is intensity dependent, with higher-intensity exercise inducing a 2.5-fold greater increase in mRNA 3 h postexercise than lower-intensity exercise (13). Another factor that may have contributed to the apparent lack of change in PGC-1α nuclear abundance is the timing of biopsy sampling. Nuclear localization of PGC-1α has been observed immediately and 3 h after high-intensity interval exercise (23, 24), which leads to increases in mitochondrial protein content at 24 h postexercise (23).

In conclusion, we present data demonstrating that MyoPS is elevated early during postexercise recovery following both HIGH and LOW bouts of aerobic exercise performed in the fasted state; however, only HIGH exercise extended the duration of the elevated MyoPS response. We did not observe an increase in rates of MitoPS or a change in PGC-1α nuclear localization after exercise performed in the fasted state. The greater rates of MitoPS after HIGH compared with LOW exercise during late postexercise recovery may serve to enhance the mitochondrial protein pool following chronic training.

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AUTHOR CONTRIBUTIONS

REFERENCES