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Exercise Intensity and Rest Intervals Effects on Intracellular Signals and Anabolic Response of Skeletal Muscle to Resistance Training

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Abstract

Moro, T, Monaco, L, Naro, F, Reggiani, C, and Paoli, A. Exercise intensity and rest intervals effects on intracellular signals and anabolic response of skeletal muscle to resistance training. *J Strength Cond Res* 38(10): 1695–1703, 2024—Resistance training (RT) is one of the most important stimuli for muscle hypertrophy, and it may play also an important role on weight loss and fatty acids oxidation. Clearly, RT affects anabolic pathways, but the differences among various training techniques has been poorly investigated. We sought to compare the effect of 2 different intensities of training: high-intensity interval resistance training (HIIRT) and traditional resistance training (TRT), on muscle signaling pathway. Nine young healthy subjects performed HIIRT and TRT protocol on 2 different occasions and with different legs on leg extension. High-intensity interval resistance training technique consisted of 3 sets of 6 repetitions (reps) at 6 repetition maximum and then 20 seconds of rest and 2 or 3 repetitions (until exhaustion) repeated for 3 times with 2′30″ rest between sets, whereas TRT consisted of 3 sets of 15 reps with 75 seconds of rest between sets. Biopsies from the vastus lateralis were taken at baseline (pre), immediately (0 hours) at the end of training, and 6 hours (6 h) and 24 hours (24 h) after training. Western blot and real-time polymerase chain reaction messenger RNA (mRNA) analysis were performed to assess muscle signaling pathway activation. In both protocols, rpS6 phosphorylation significantly increased at 6 hours ($p < 0.05$). Traditional resistance training showed a significant increase at 24 hours of AMPK phosphorylation compared with HIIRT ($p < 0.05$), whereas no significant differences between groups were found for other proteins. mRNA analysis showed no differences between protocols except for striated muscle activator of Rho signaling. The manipulation of resistance training intensity through incomplete/short recovery does not induce different molecular anabolic and metabolic responses compared with a TRT method.
Trial Registration number: NCT04163120 retrospectively registered.

Key Words: strength training, recovery time, signaling, muscle hypertrophy

Introduction

Resistance training (RT) is one of the most efficient stimuli for skeletal muscle hypertrophy (17). However, RT can be performed with different modalities, for example, changing number of repetitions (reps), number of sets, rest time, speed of movement, and the like (2,33). Traditionally, it is recommended to perform heavy-load contractions (i.e., 70–85% 1 repetition maximum [RM]) and related medium-low number of repetitions (6–12 reps) to optimize skeletal muscle hypertrophy, whereas higher load and relative lower repetitions are suggested to implement muscle strength (2). These recommendations take their origin from a seminal article by DeLorme and Watkins (9) published in 1948, which fixed the standard for the number of repetitions and sets accepted for many years. However, the recent literature is still debating on this topic: some studies supports the efficacy of high load-low repetitions modalities to induce muscle hypertrophy (11,41), whereas other findings suggest that similar results can be achieved with low load and high number of repetitions (7,30).

The signaling network involved in skeletal muscle remodeling is multifaceted and complex (27), and it is influenced not only by the type of exercise (endurance or resistance) (17) but also by exercise modality. For example, a particular endurance training methodology, which consists in a sequence of high-intensity bouts with a very short recovery (HIT), was extensively investigated in the past years, mainly by Gibala et al. (14,25). Results showed that this kind of training stimulates mainly the signaling pathways (AMPK/PGC1α) involved in mitochondrial biogenesis and metabolic homeostasis without significant effects on the main anabolic axis (IGF-1/AKT/mTOR) (14). Resistance training is instead known to stimulate muscle protein synthesis by the activation of mTORC1 signaling pathway, which includes the phosphorylation of rpS6 and 4EBP1 (4). Removal of wasted and damaged proteins is another important process to guarantee a proper anabolic muscular response: autophagy has been indeed recognized as crucial for muscle protein turnover. Resistance training also stimulate muscle repair by specific transduction signals, such as STARS (striated muscle activator of Rho signaling), and mechano growth factor (MGF), which are sensitive to mechanical stimuli (23) and are important processes for muscular response to exercise. Even though recent studies in murine model proved that

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satellite cells are not necessary for muscle growth, their function on muscle repair is fundamental (19). Satellite cells are especially activated during intense exercise through different regulatory factors, such as myogenin, and also by MGF and interleukin-6 (IL-6).

In contrast to the growing interest in the manipulation of different RT variables and its effects on anabolic signaling (6), few data are available on the effects of RT resting period and intensity manipulation on mitochondrial biogenesis and metabolic homeostasis pathways (28). As a matter of fact, there are some evidences that RT acts on fat metabolism, helping body mass control (34). Interestingly, not only endurance training but also RT can activate AMPK, which is known to be an energy regulator and an inhibitor of mTOR pathway (5). In particular, AMPK is activated at highest intensity of exercise (8), when the AMP:ATP ratio is elevated. During HIT protocols, in response to a reduced glycogen concentration, AMPK activity and PGC1α messenger RNA (mRNA) expression drastically increase (13); it is plausible to hypothesize that a high-intensity resistance training protocol aimed at exhausting glycogen store could thus activate these same mitochondrial markers.

In the present study we investigated 2 different protocols of RT, characterized by equivalent training volume but different training intensity (load, number of repetition, and recovery periods). We compared a traditional resistance training (TRT) program conforming to ACSM recommendations (3 sets of 15 repetitions at 60% 1RM with 75-second rest periods) with an high-intensity interval resistance training (HIIRT: 3 sets of 6RM at 80% 1RM followed by 20″ of rest, repetitions to failure with the same weight, another 20″ of rest, repetitions to failure using the same weight again, and again 20″ of rest and the repetitions to failure. 2' 30″ rest between sets.) on both metabolic homeostasis and anabolic pathways. We have already demonstrated that HIIRT increase postexercise oxygen consumption at 72 hours after a single bout of exercise and can be beneficial in reducing fat body mass (35). Because training intensity is crucial for the anabolic and metabolic responses to exercise, the comparison of these 2 protocols allowed us to determine the role of skeletal muscle signaling pathway on these variables. We hypothesized that the 2 training modalities would promote similar activation of muscle protein synthesis signaling, although HIIRT would be more effective in activating skeletal muscle fatty acid metabolism and mitochondrial biogenesis.

Methods

Experimental Approach to the Problem

To determine the effect of resistance training intensity controlling resting periods and training loads, we designed a crossover study, during which each subject performed 2 different modalities of a single bout of resistance training in a random order: TRT and HIIRT. Exercises differed for load intensity and rest between sets, whereas they were similar for total number of repetitions and sets. Muscle biopsies were collected at rest, immediately after, and at 6 and 24 hours after the exercise to assess proteins and genes involved in the regulation of protein synthesis (Akt, RPS6, 4EB-P1, ERK 1/2, STARS, IGF-1, IGF-1ea, MGF, myogenin, IL-6, PGC-1a4) and mitochondrial biogenesis (AMPK, ACC, PGC-1a).

Subjects

Nine young healthy subjects (age, 22.0 ± 1.4 years; body mass index, 22.4 ± 2.2 $kg \cdot m^{-2}$) took part in the study. Subject characteristics are reported in Table 1. All subjects were moderately active and had experience with resistance training, but none of them practiced regularly strength training (<2 sessions high-intensity exercise per week). Exclusion criteria for the study included history of recent myocardial infarction, severe cardiac arrhythmia, unstable angina, poorly controlled hypertension, poorly controlled diabetes mellitus, and frequent or complex ventricular ectopy, which might interfere with one's ability to adhere to exercise protocols. Previous muscle-skeletal injuries were not assessed; however, all subjects were healthy at the time of the experiment. The study was approved by the University of Padova Italy Human Ethical Commission of the Department of Biomedical Sciences (HEC-DSB 01/2012), in accordance with Helsinki's declaration of 1995 as modified in 2000.

Procedures

After signing a written informed consent form, subjects came for a preliminary session to familiarize with the protocols. On a different day, 6RM test on leg extension exercise was performed to assess the proper load for the 2 different exercise protocols: TRT and HIIRT. The study was a crossover trail in which all subjects were tested randomly for both protocols. Randomization list was generated using GraphPad QuickCalcs Web site: http://www.graphpad.com/quickcalcs/ConfInterval1.cfm. One week before each study day, a baseline muscle biopsy was taken from vastus lateralis muscle (pre). On the study day, subjects came again to the laboratory, and after a proper warm-up consisting of pedaling for 10 minutes at 50 W on a cycloergometer, 1 of the 2 training protocols was performed; sequential biopsies samples immediately after the exercise (0 hours) and at 6 and 24 hours after the exercise were then collected as described in Figure 1.

The same procedure was repeated the following week on the opposite leg to test the other exercise protocol. Subjects were instructed to avoid any kind of exercise, smoking, or drinking alcohol for the 24 hours before each training session. Moreover, subjects had been fasting overnight before each session (Figure 2).

Acute Resistance Exercise Protocols. The HIIRT protocol is a modified rest-pause technique previously described from our group and others (22,35). The method consisted of performing 3 minisets: after the first 6 repetition to exhaustion (6RM), subjects were allowed to rest 20 seconds, and then, they were asked to perform how many repetitions were able to complete with the same load; the cycle of 20-second rest and repetition to failure was repeated other 2 times. Then, after 2′30″ minute of rest, subjects were asked to repeat other 2 times the same routine from the beginning (i.e., 6 reps, 20 s, 2 or 3 reps, 20 s, 2 or 3 reps, 20 s, 2 or 3 reps—2′30″ min—6 reps, 20 s, 2 or 3 reps, 20 s, 2 or 3 reps, 20 s, 2 or 3 reps—2′30″ min—6 reps, 20 s, 2 or 3 reps, 20 s, 2 or 3 reps, 20 s, 2 or 3 reps; Figure 2). On average, subjects performed 15.3 ± 2.4 reps each set. The sum of all the repetitions performed during each set was usually composed of 12–15 repetitions. Subjects were instructed to perform all minisets to failure with the given load previously calculated from the 6RM test (if subjects were able to perform more the 6 reps at the first attempt, load was increased for the next set to ensure the complete exhaustion). Traditional resistance training protocol performed 3 sets of 15 repetitions at 60% 1RM with 75″ of rest between the sets.

Biopsy Protocol. The biopsies were taken approximately 1 cm proximal to each other using the thin needle procedure previously

Table 1
Protein and mRNA expression of some regulators of muscle mass.*†

	TRT			HIIRT		
	0h	6h	24h	0h	6h	24h
Akt (Ser473) protein	0.80 ± 0.06	0.73 ± 0.05	0.84 ± 0.07	1.30 ± 0.12	1.57 ± 0.16	1.34 ± 0.11
pERK1/2 (Thr202/Tyr204) protein	2.48 ± 0.26	2.21 ± 0.38	1.61 ± 0.16	2.40 ± 0.27	1.40 ± 0.17	1.05 ± 0.14
IGF-1 mRNA	1.78 ± 0.14	1.91 ± 0.23	1.44 ± 0.08	2.09 ± 0.13	1.27 ± 0.08	2.00 ± 0.21
IGF-1ea mRNA	1.90 ± 0.17	1.80 ± 0.23	1.30 ± 0.06	2.08 ± 0.11	1.27 ± 0.09	1.76 ± 0.18
Atrogin mRNA	0.79 ± 0.03	0.66 ± 0.07	1.66 ± 0.31	0.84 ± 0.06	0.42 ± 0.04	1.84 ± 0.23
Beclin mRNA	1.07 ± 0.06	1.32 ± 0.12	1.22 ± 0.04	1.07 ± 0.03	0.94 ± 0.04	1.58 ± 0.10
Myostatin mRNA	1.36 ± 0.16	0.62 ± 0.07	0.57 ± 0.10	2.00 ± 0.29	1.21 ± 0.17	0.54 ± 0.04

*TRT = traditional resistance training; HIIRT = high-intensity interval resistance training.
†All values are means ± *SE* expressed as fold change from basal.

described by Paoli et al. (36). Briefly, biopsy samples were collected from the vastus lateralis muscle approximately 15 cm from the superior edge of patella. Tru-Cut needles (PRECISA 1410 HS Hospital Service S.p.A, Latina, Italy) were used with a diameter of 14 G and an insertion cannula length of 100 mm. Subjects were asked to lie down, and the needle was inserted into the muscle after local anesthesia with 2 ml of xylocaine 2% injected subcutaneously and sterilization with Betadine. During each biopsy, 3 samples were collected in subsequent insertions of the inner notched rod of the needle. During each biopsy, an average of approximately 15 mg of muscle was collected. Samples were immediately frozen in liquid nitrogen and then stored at −80° C till the analysis.

Western Blot Analysis. Ten volumes of sample buffer were added to 6–8 mg of frozen tissue (50 mM Tris, pH 7.5, 1 mM EDTA, 15 mM NaCl, 1 mM $MgCl_2$, 10% vol/vol glycerol, 1% vol/vol Triton 1%,2% wt/vol SDS, and 0.1 mM DTT) plus phosphatase inhibitors (Complete 50X protease Inhibitor Cocktail Tablets e PhosSTOP 10x Phosphatase Inhibitor Cocktail Tables—Roche). The samples were then homogenized by applying mechanical forces through a plastic pestle and centrifuged for 10 minutes at 4° C at 13,000 rpm. Total protein content was determined by Folin-Lowry technique. Samples were diluted in Laemmli buffer and heated at 100° C for 5 minutes—30 mg of total protein were separated in 4–15% polyacrylamide gel (Bio-Rad Laboratories S.r.l) and let them running in Tris-glycine electrophoresis set at 16 mA for 75 minutes. Proteins were then transferred to a nitrocellulose membrane in semidry condition at 100 V for 60 minutes at 4° C. Membranes were blocked in 5% bovine serum albumin and then incubated overnight with the following primary antibody at 4° C: $pAMPK^{Thr172}$, acetyl-CoA carboxylase ($pACC^{Ser79}$), pERK $1/2^{Thr202/Tyr204}$, $pAkt^{Ser473}$, $pS6^{Ser235/236}$, and $p4EBP1^{Thr37/46}$ (Cell Signaling, Danvers, MA); antibody were diluted according to manufacturer's instructions. Blots were incubated with suitable secondary antibody for 1 hour at room temperature and exposed under a chemiluminescent solution (WesternSur PREMIUM Chemiluminescent Subs – Carlo Erba Reagentes S.r.l, Milano) for 5 minutes. Densitometric analysis and optical density measurements were obtained by a specific phospoimager software (Image Studio Licor - Version 4.0.21). The quantification was based on actin band as a reference for normalization.

Real-Time Polymerase Chain Reaction Analysis. High-quality RNA from muscle tissues was isolated using the RiboPure RNA Purification Kit (Ambion, Life Technologies) according to the manufacturer's instructions. The concentration and purity of RNA was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Aliquots of 300 ng of RNA were retrotranscribed in complementary DNA (cDNA). The isolated RNA was transcribed into cDNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Life Technologies, Waltham, MA). The cDNA was reverse transcribed for amplification with specific primers. The primer sets were designed on the basis of the nucleotide sequences of the

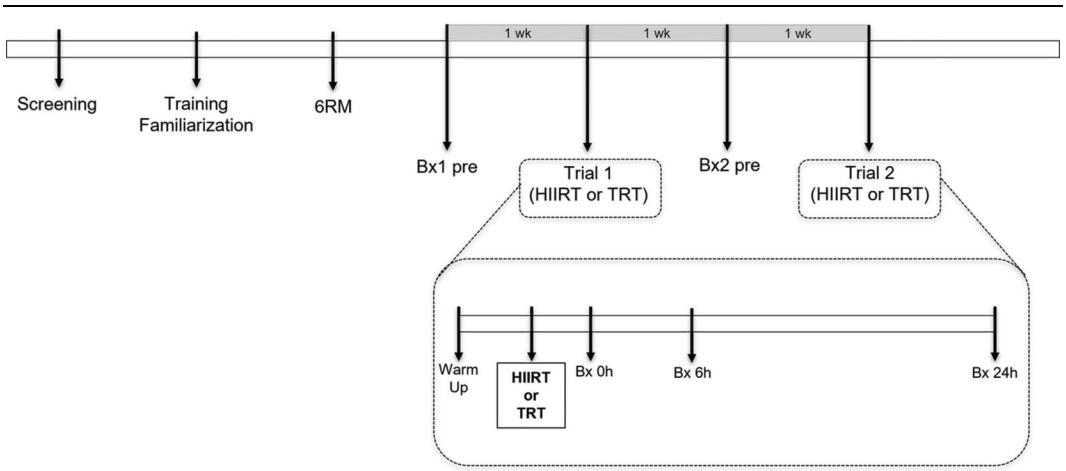

Figure 1. Study design. Bx = biopsy; 1RM = 1 repetition maximum test.

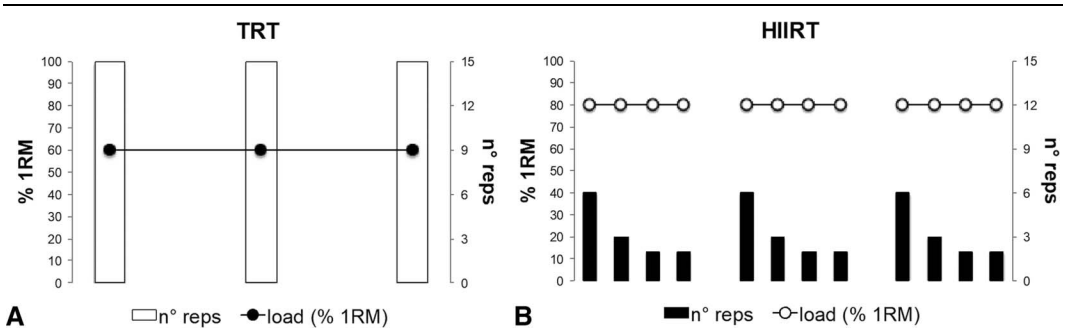

Figure 2. Exercise training protocols.

analyzed genes retrieved from databases and were synthesized by Eurofins Genomics (Ebersberg, Germany); primer sequences are reported in Supplementary Material (http://links.lww.com/JSCR/A299). Quantitative polymerase chain reaction (qPCR) was performed using the SYBR Green PCR Master mix (Applied Biosystems, Life Technologies) according to the protocol for use in the Applied Biosystems 7,500 Real-Time PCR System. For the quantification analysis, the comparative threshold cycle (Ct) method was used. The Ct values of each gene were normalized to the Ct value of GAPDH in the same RNA sample. Primer-blast (NCBI) was used to design primers. Melt curves from qPCR revealed a single peak indicative of a single amplicon. Primer efficiency was performed by analyzing the slope of the amplification curves from a dilution series experiment. The gene expression levels were evaluated by fold change using the equation 2^{-ddCt}. The mRNA levels in the samples from the different training protocols were expressed as the fold change compared with the "Pre" or "baseline" samples.

Statistical Analyses

Data are expressed as means ± *SE*. Statistical analysis was performed with GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA). Sample size and power were calculated for mTORC1 pathway; preliminary data from our laboratory indicate that the *SD* for phosphorylation of mTORC1 signaling proteins is typically 0.02 arbitrary units, which allowed us to detect a power of 80% with 8 subjects.

Through the Shapiro-Wilk W test, the normality between the groups was assessed. An independent samples t-test was used to test baseline differences between the groups. In-group effects were analyzed using 1-way Analysis of variance (ANOVA). The effects of training were analyzed using a 2-way ANOVA (time x treatment); in case of significant main effects or interactions, Bonferroni *post hoc* test was performed. Significance was set at $p \leq 0.05$.

Results

No significant differences were detected through 2-way ANOVA between training groups in any of the investigated pathways except for STARS expression and 5′ AMP-activated protein kinase (AMPK).

Promoters of Muscle Hypertrophy (Akt, RPS6, 4EB-P1, ERK 1/2, STARS, IGF-1, IGF-1ea, MGF)

The phosphorylation status of Akt and ERK 1/2 was unchanged from baseline and was similar between the groups (Table 1). rpS6 phosphorylation was significantly higher at 6 hours after exercise (time effect $p < 0.001$), and its activation holds true in both exercise (TRT $p = 0.001$; HIIRT $p = 0.009$; Figure 3). During TRT protocol, 4E-BP1 phosphorylation was significantly lower ($p = 0.01$) immediately after exercise (0h) and then returned to baseline levels in the following time points; no significant changes were detected during HIIRT (Figure 3).

IGF-1 and IGF-1ea mRNA expression were not altered after exercise (Table 1). Expression of MGF mRNA was elevated 24 hours after TRT, whereas after HIIRT, its expression had a tendency ($p = 0.06$) to stay elevated during all time points with a significant ($p = 0.02$) peak of expression at 6 hours after exercise (Figure 3).

The expression of STARS mRNA presented a significant time × exercise interaction ($p = 0.01$). Post hoc analysis showed that STARS mRNA significantly increased at 6 hours only in the TRT group and had a trend ($p = 0.08$) to remain elevated at 24 hours; moreover, the difference between the 2 groups was statistically significant at 6 hours after exercise ($p = 0.004$; Figure 3).

Transcriptional Regulation of Muscle Hypertrophy (Myogenin, IL-6, PGC-1a4)

Myogenin mRNA expression revealed a different trend between exercise modalities, even without reaching statistical difference: TRT increased myogenin levels only after 6 hours from exercise, whereas HIIRT presented a higher activation since the very immediate moment after exercise (Figure 4).

IL-6 mRNA levels drastically increased after 6 hours from both exercise modalities (TRT $p = 0.01$; HIIRT $p = 0.002$) but only HIIRT maintained its expression elevated ($p = 0.07$) at 24 hours after exercise (Figure 4). The expression of PGC1a4 mRNA, a transcriptional coregulator of muscle hypertrophy, had a similar trend in both protocols with any significant activation at any time point (Figure 4).

Protein Degradation (Atrogin, Beclin)

We investigate 2 regulators of the autophagy-lysosome system: atrogin 1 and beclin. No significant differences were observed between exercise groups. Atrogin mRNA levels decreased at 6 hours after both exercise ($p > 0.05$), and the elevated expression showed in both group at 24 hours was not statistically significant probably because of the high intersubject variability. Beclin mRNA expression slightly increased during the 24 hours after exercise; only the TRT group presented a trend to significance ($p = 0.06$) at 24 hours after the exercise bout (Table 1).

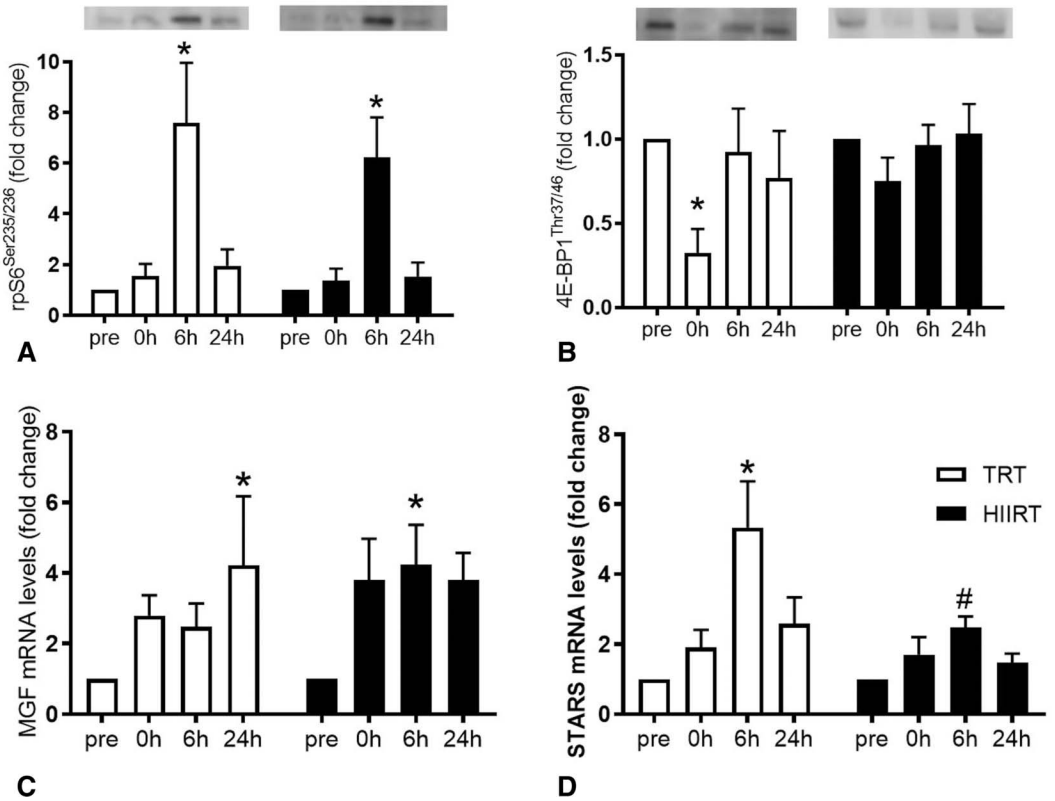

Figure 3. Changes in protein and messenger RNA levels of marker related to muscle hypertrophy compared with basal level after exercise. Data are mean ± *SE*. *Significantly different from pretraining ($p < 0.05$); #Significantly different from TRT ($p < 0.05$). TRT = traditional resistance training.

Muscle Hypertrophy Inhibition

Myostatin is a negative regulator of muscle hypertrophy. No significant changes were observed in myostatin expression after exercise; the levels of mRNA slightly increased immediately after both conditions (0 hours) and returned to lower levels after 24 hours from training with significant differences compared to baseline (Table 1).

Mitochondrial Pathway (AMPK, ACC, PGC-1a)

AMPK, ACC, and PGC-1a are linked to the cellular energy and mitochondrial biogenesis regulation. We observed a significant exercise effect ($p = 0.03$) for AMPK with the 2 exercises intensity showing a significant difference ($p = 0.01$) at 24 hours after exercise. Overall, after TRT exercise, AMPK phosphorylation tended to be higher compared with baseline at all time points, reaching a peak ($p = 0.04$) at 24 hours, whereas no alteration was observed after HIIRT (Figure 5).

The phosphorylation of ACC was similar in both groups, with a notable but not significant increase immediately after exercise and returned to base level at 6 hours after exercise (Figure 5). PGC-1a mRNA expression remained unchanged after both exercise; an interesting trend ($p = 0.09$) to decrease was observed at 6 hours after TRT bout (Figure 5).

Discussion

It has been widely demonstrated that changes in RT's intensity and volume modulate the hormonal response (1), but even though some authors do not support the view that acute response to exercise could be translated in long-term adaptation (29), this kind of study can give important information on the mechanisms that could lead to such adaptations.

The aim of this study was to compare the acute effects of 2 different protocols of resistance exercise on the activation of both anabolic (IGF-1/AKT/mTOR) and mitochondrial (AMPK/PGC1α) homeostasis signaling pathways in skeletal muscle. The main finding of our study is that resistance exercise intensity, modulated via different loads (% of 1RM) and rest between sets, resulted in similar stimulation of the anabolic and myogenic pathways. Interestingly, the effect of exercise modalities was significant in 2 different signaling factors: STARS, an index of regeneration and repair activated by mechanical stimulus (23), and AMPK, a cellular energy sensor responsible for inhibiting anabolic processes and stimulating mitochondrial metabolism (16).

To our knowledge, this is one of the few studies that compare the molecular mechanisms activated by a session of resistance exercise with different intensity and pause duration but similar training volume. A recent study by McKendry et al. (28) demonstrated that 4 sets of 8–14 repetitions (±5) interspersed by 1-minute rest result in a blunted anabolic signaling response compared with the same protocol with a longer rest interval (5 minutes). However, in the study by McKendry et al., the load and the number of repetitions are similar in both 1-minute and 5-minute rest groups, and it is hard to reconcile these data with an exercise performed until real muscular failure (40). Actually, HIIRT technique is not a simple reduction of rest intervals between sets, it is instead a modified rest-pause method, which is characterized by short pause between repetitions within a

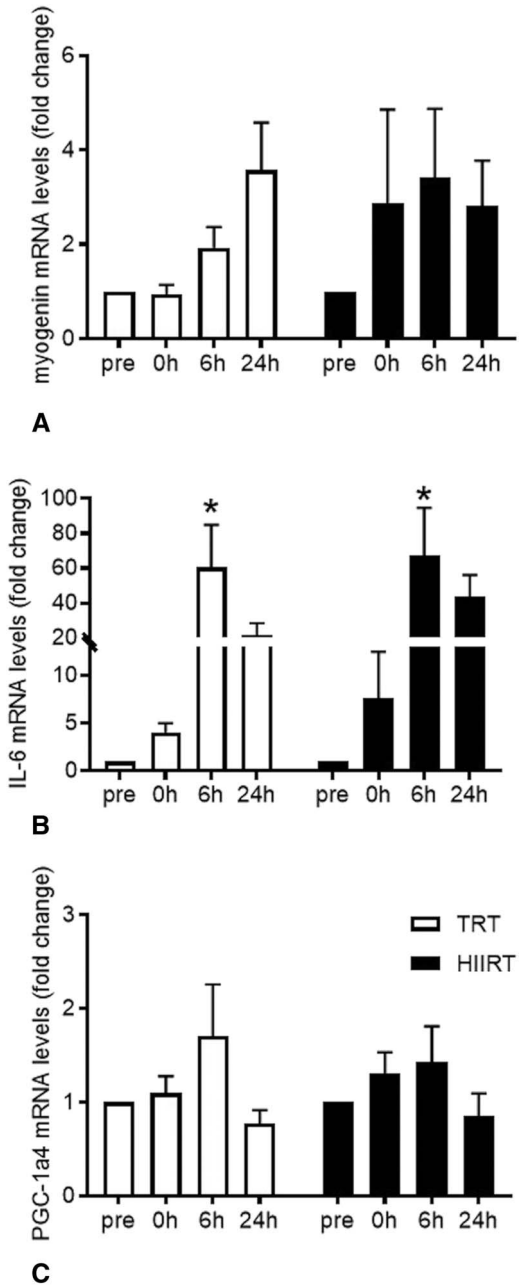

Figure 4. Changes in messenger RNA levels of proteins related to the transcriptional regulation of muscle hypertrophy compared with basal level after exercise. Data are mean ± *SE*. *Significantly different from pretraining ($p < 0.05$).

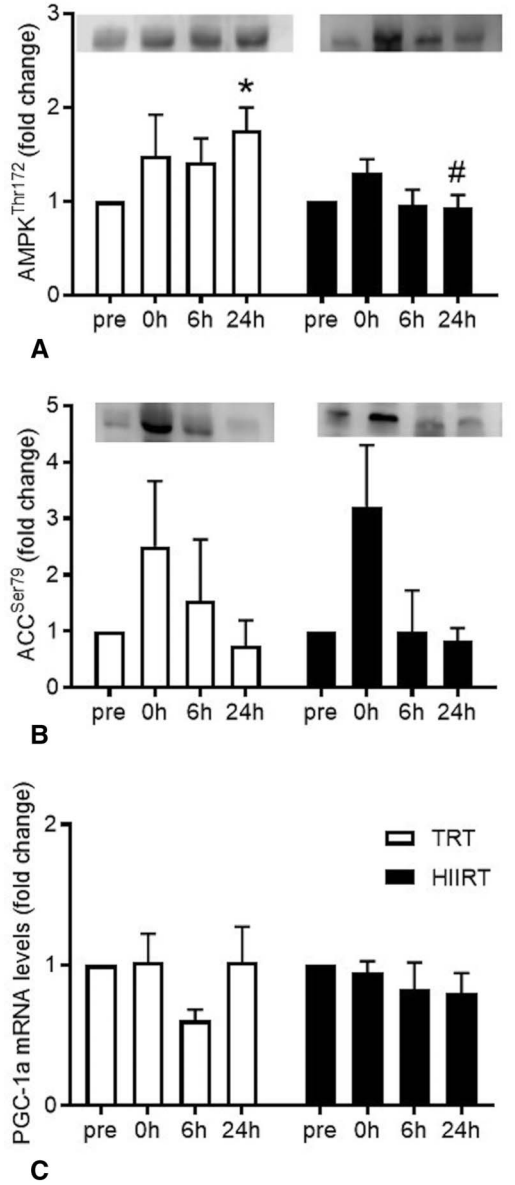

Figure 5. Changes in protein and messenger RNA levels of marker related to metabolic pathway and mitochondrial biogenesis compared with basal level after exercise. Data are mean ± *SE*. *Significantly different from pretraining ($p < 0.05$); #Significantly different from TRT ($p < 0.05$). TRT = traditional resistance training.

single set (22). The goal of the technique is to deplete all intramuscular creatine phosphate and maximize the chance to complete each set to fatigue. Literature presents different modality to apply this concept (22,35); in the present study, HIIRT protocol was built up to match the training volume of a typical resistance exercise protocol aimed at hypertrophy. Our results showed that the 2 protocols induce similar phosphorylation of rpS6 and 4E-BP1, which are the 2 most important downstream substrates of mTOR pathway. These results suggested that changing the intensity of a single bouts of resistance exercise may lead to the same ultimate increase in protein synthesis.

Additionally, beside the IGF-1/AKT/mTOR axis, skeletal muscle hypertrophic growth profits the activation of other transduction signals, such as ERK 1/2, STARS, and MGF. ERK 1/2 is a kinase with a direct impact on muscle growth, by the activation of factors involved in translation, such as rpS6 and eukaryotic initiation factor 4E (eIF4E). Previous studies (6,43) already observed that ERK 1/2 is activated when a great number of repetitions is performed. Our results agreed with this finding, indeed, TRT protocol was composed of a greater number of consecutive repetitions compared with HIIRT, and this might explain the greater, even though not statistically significant, activation of ERK 1/2 observed along the 24 hours post exercise. The phosphorylation of ERK 1/2 was indeed similar immediately after the exercise bout but drastically reduced at 6 and 24 hours after HIIRT, although it was maintained elevated after TRT (Table 1).

We also observed an increase in STARS mRNA level at 6 hours after exercise only in the TRT group. STARS gene expression is activated after a mechanical stimulus, and it is an index of regeneration and repair. It has been shown that eccentric exercise has a greater effect on STARS activation compared with concentric type (23). Interestingly, in our study, the activation was greatest after TRT protocol, which has a longest time under 305 on the muscle fiber, but a lower load stress compared with HIIRT. These results seem to suggest that high-intensity resistance training, which involves interruption between repetitions and thus with less continuous cycle of contractions to complete the task, are less effective in stimulating mechanical stress and thus activating the component of hypertrophic response mediated by STARS activation.

Over the past decade, evidence suggested that mechanical overload may promote the activation of a splice variant of the peroxisome proliferator-activated receptor *g* coactivator 1a (PGC-1a) known as PGC-1a4 (42). Some authors suggested that PGC-1a4 upregulates the expression of IGF-1 and suppresses myostatin, which is an inhibitor of myoblast proliferation and differentiation (38). However, other authors did not support the hypothesis that PGC-1a4 may regulate exercise-induced hypertrophy and, on the contrary, suggested that acute exercise modulate momentarily all PGC-1α isoforms (26). Our results do not seem to confirm any of the hypothesis mentioned above because we have not shown any differences in the activation of this protein between the 2 modes of exercise.

Mechano growth factor is another splice variant isoform of IGF-1 with important function in muscle repair and activation of quiescent satellite cells (18), which is also upregulated in response to mechanical load (15). It was demonstrated that MGF expression increases at 2.5 hours after 10 x 6 reps at 80% 1RM (15), and it was elevated at 24 hours after a 3×8-12 reps training (21). We observed similar results; HIIRT presented elevated levels of MGF since the very first moment after exercise with a peak at 6 hours, whereas TRT presents greater expression only after 24 hours. We also observed higher levels of IGF-1 mRNA after HIIRT compared with TRT immediately after exercise (0 hours) and at 24 hours; however, these differences were not statistically significant.

On the other side, we did not observe any significant changes in myostatin expression after exercise. IL-6 was identified as an important regulator of satellite cells activation (39). Indeed, in vitro experiment showed that in the absence of IL-6 mRNA, the myogenic differentiation is blunted, whereas it is enhanced when IL-6 is overexpressed (3). Despite its proinflammatory nature, IL-6 can also exert anti-inflammatory effects during exercise and directly affect muscle growth and exercise capacity; moreover, its release seems to be strictly correlated to exercise intensity and energy availability (20). In our study, we observed an overall increase in IL-6 mRNA activation at 6 and 24 hours after exercise. However, after HIIRT protocol, the levels were higher compared with TRT, strengthening the hypothesis that HIIRT may stimulate satellite cells expression in a greater extent than a traditional lower-intensity training. Even though it has been well established that satellite cells are not fundamental for muscle hypertrophy (24), their activation and differentiation can contribute long term to muscle growth.

Myogenin is one of the myogenic regulatory factors that stimulate muscle hypertrophy promoting myoblast differentiation (12), and it is activated from 6 to 12 hours after a bout of high-intensity resistance training (70–80% 1RM) (44,45). We observed a gradual increase of myogenin mRNA expression until a significant peak was reached at 24 hours post exercise; however, HIIRT seems to elevate myogenin immediately after exercise, whereas lower-exercise intensity presented a delayed effect on its activation.

Beside the activation of the IGF-1/AKT/mTOR axis, muscle mass can be regulated by the modulation of catabolic processes. Autophagy is a key component of muscle protein turnover because guarantee the removal of wasted and damaged proteins. The role of autophagy during resistance training in humans is not completely clear, thus neither the effect of training intensity on its regulation. We investigated the role of 2 components of the protein degradation mechanism: atrogin 1, which stimulates proteins polyubiquitination and transfers them for the definitive degradation process in the proteasomes, and Beclin 1, which is essential for the formation of the autophagosome. We did not observe any significant differences between exercise groups. Data showed that both mRNA levels decreased after exercise, and they were elevated at 24 hours post exercise probably because of the repair process after muscle contraction. Atrogin-1 expression was found to be significantly ($p < 0.05$) suppressed 6 hours after exercise when those were considered together, and this suppression seems to be driven by TRT as no change was observed after HIIRT exercise, confirming that atrogin-1 expression is more downregulated when the muscle contraction is longer compare with a short time to complete the number of repetition, as suggested by other authors (32).

The second specific aim of this study was to investigate the effect of these 2 different exercise intensities on the signaling pathways regulating energy metabolism. In a previous study (35), we found a greater increase in resting energy expenditure 24 hours after a single bout of high-intensity interval resistance training compared with a traditional low-intensity training. Based on this finding, we hypothesized that HIIRT could have a greater influence on fatty acid oxidation, and this could be achieved by the activation of the AMP kinases/acetyl CoA carboxylase (AMPK/ACC) pathway. Another study (14) showed that intense exercise increases the AMP:ATP ratio and consequently AMPK activity, which phosphorylates ACC, resulting in a reduced synthesis of malonylCoA and stimulation of fatty acid oxidation. Moreover, IL-6 mRNA expression was higher after HIIRT protocol compare with TRT. Also, IL-6 is a proinflammatory cytokine, to which has been attributed a metabolic role related to glycogen levels depletion (10,37). The rationale behind the use of HIIRT technique for fat loss is its hypothesized capacity to deplete muscle glycogen and stimulate lipolysis. Interestingly, our results contrast this hypothesis, showing a greater activation of AMPK/ACC after TRT compared with HIIRT, in which the activation seems to be temporary and limited to a few hours after exercise. AMPK pathway is related to peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), which promotes mitochondrial gene transcription. Several studies (14,25) demonstrated that high-intensity endurance training regulates PGC-1α gene expression.

Our results are not consistent with those observed by Gibala et al. during high-intensity endurance trials (14) because PGC-1α expression was not activated and did not differ between the 2 exercise protocols in our present study. One possible explanation for this discrepancy could be the timing of measurements: samples were taken 3 hours after exercise in the study by Gibala et al., and thus the effect could be already blunted because in this study the biopsy was performed at 6 and 24 hours.

Moreover, our results seem to contrast some studies that had observed that the concomitant activation of PGC-1α and AMPK

could inhibit mTOR and thus protein synthesis (5,31). As mentioned above, the choice of the timing for the biopsy sample represents a limitation of the present study. In particular, the lack of sampling perfomed during the elapsed time between the very end of the exercise session and 6 hours after the end could made impossible to detect greatest and significant differences between these 2 protocols of exercise. Actually, other studies have shown that the greatest activation of Akt signaling and/or AMPK pathway occurs just after 1 or 3 hours after the acute exercise session. Another limitation is the reduced sample size and the difficulty to perfectly equate the load between exercises. It is, moreover, important to recall that recent studies demonstrate that the acute response to exercise is not always predictive of long-term adaptations (29); thus, our results would need to be further supplemented with longer-time intervals to test the real effectiveness of the 2 protocols in inducing muscle growth or activation of the catabolic response.

Practical Applications

To the best of our knowledge, this is the first time that HIIRT is studied to determine how short-term rest between sets can influences skeletal muscle signaling pathway. Resistance exercise intensity is one of the most important factors to promote muscle metabolism and a clear function of all its multifaceted need to be deeply investigated. Our study suggests that the molecular response to high-intensity interval exercise is comparable to lower-intensity exercise, inducing similar protein synthesis activation and possibly translating in similar muscle growth responses. HIIRT, which requires a shorter exercise time commitment (approximately 30% less then TRT) is a modified rest pause technique that requires high loads (6RM) and allows short recovery time (20 seconds) between sets. Compared with TRT, which is a classical training method (3 sets of 15 repetitions at 60%1RM), HIIRT grants for complete muscle exhaustion and can be a valid alternative to practitioners to reduce the time of training.

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