Effect of eccentric exercise velocity on akt/mtor/p70^{s6k} signaling in human skeletal muscle

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Abstract: It has been suggested that muscle tension plays a major role in the activation of intracellular pathways for skeletal muscle hypertrophy via an increase in mechano growth factor (MGF) and other downstream targets. Eccentric exercise (EE) imposes a greater amount of tension on the active muscle. In particular, high-speed EE seems to exert an additional effect on muscle tension and, thus, on muscle hypertrophy. However, little is known about the effect of EE velocity on hypertrophy signaling. This study investigated the effect of acute EE-velocity manipulation on the Akt/mTORCI/p70^{S6K} hypertrophy pathway. Twenty subjects were assigned to either a slow (20°·s^{-1}; ES) or fast EE (210°·s^{-1}; EF) group. Biopsies were taken from vastus lateralis at baseline (B), immediately after (T1), and 2 h after (T2) the completion of 5 sets of 8 repetitions of eccentric knee extensions. Akt, mTOR, and p70^{S6K} total protein were similar between groups, and did not change postintervention. Further, Akt and p70^{S6K} protein phosphorylation were higher at T2 than at B for ES and EF. MGF messenger RNA was similar between groups, and only significantly higher at T2 than at B in ES. The acute manipulation of EE velocity does not seem to differently influence intracellular hypertrophy signaling through the Akt/mTORCI/p70S6K pathway.

Key words: muscle tension, molecular response, skeletal muscle hypertrophy.

Résumé : Il semble que la tension musculaire joue un rôle de premier plan dans l'activation des voies intracellulaires de l'hypertrophie du muscle squelettique, et ce, par l'augmentation du facteur mécanique de croissance (MGF) et d'autres cibles en aval. L'exercice pliométrique (EE) suscite une plus grande tension dans le muscle sollicité. Notamment, un EE accompli à haute vélocité exercerait un effet additionnel sur la tension musculaire et, de ce fait, sur l'hypertrophie du muscle. Néanmoins, on sait très peu de choses sur les effets de la vélocité d'un EE sur la signalisation de l'hypertrophie du muscle. Cette étude analyse l'effet d'une séance de variation de la vélocité d'un EE sur la voie Akt/mTORCI/p70^{S6K} de l'hypertrophie du muscle. On répartit 20 sujets dans deux groupes EE, l'un à basse vélocité (20 °·s^{-1}; ES) et l'autre, à haute vélocité (210 °·s^{-1}; EF). On prélève des biopsies du vaste externe avant la séance d'exercices (B), immédiatement après (T1) et 2 h après (T2) avoir fait 5 séries de 8 répétitions de l'extension du genou en mode pliométrique. On n'observe pas de différences entre les groupes en ce qui concerne les concentrations totales de protéines (Akt, mTOR et p70^{S6K}) ; on n'observe pas non plus de variations après la séance d'exercices. En outre, on observe une plus grande concentration des formes phosphorylées d'Akt et de p70^{S6K} en T2 comparativement à B tant chez ES que chez EF. L'expression de l'ARNm du MGF est du même ordre chez les deux groupes, mais elle est significativement plus grande en T2, comparativement au groupe ES en B. La variation de la vélocité d'un exercice pliométrique ne semble pas avoir d'effet sur la voie de signalisation Akt/mTORCI/p70S6K de l'hypertrophie du muscle.

Mots-clés : tension musculaire, réponse moléculaire, hypertrophie du muscle squelettique.

[Traduit par la Rédaction]

Introduction

Several authors have studied the effects of different exercise modes (i.e., concentric or eccentric exercise (EE)) on skeletal muscle fiber hypertrophy (Higbie et al. 1996) and strength gains (Hortobágyi et al. 1996; Seger et al. 1998). Training protocols consisting of only concentric exercise are thought to result in less hypertrophy than EE or a combination of concentric exercise and EE (Hather et al. 1991; Higbie et al. 1996).

Additionally, the movement velocity achieved during EE

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has been shown to affect the magnitude of muscle mass increment (Farthing and Chilibeck 2003*b*; Shepstone et al. 2005). For instance, Farthing and Chilibeck (2003*b*) reported greater hypertrophy of the elbow flexors after 8 weeks of fast EE training, compared with slow EE. In line with these findings, Shepstone et al. (2005) showed that fast, rather than slow, EE training promotes greater type IIa (~22% vs. 6%, respectively) and IIx (~28% vs. 10%, respectively) fiber hypertrophy of the elbow flexors. Recently, it has been suggested that acute changes in protein synthesis signaling are associated with the intracellular events leading to an increase in muscle mass following resistance training in humans (Terzis et al. 2008). However, the effects of EE velocity on the intracellular signaling mechanisms that regulate protein synthesis remain to be elucidated.

It has been suggested that fast EE might impose greater mechanical stress than slow EE on the active muscle fibers (Enoka 1996; Chapman et al. 2008). The magnitude of the mechanical stress imposed on the skeletal muscle appears to influence mechano growth factor (MGF) messenger (m)RNA expression (Tidball 2005), a muscle isoform of insulin-like growth factor-1. MGF has the ability to activate the PI3K/Akt/mTORCI/p70^{S6K} pathway, which is known to enhance protein synthesis (Schmelzle and Hall 2000; Nader et al. 2005). Previous studies have shown that exercise-induced acute increments in the p70^{S6K} phosphorylation are highly related to the training-mediated increases in muscle mass in both animals (Baar and Esser 1999; Bodine et al. 2001; Nader et al. 2005) and humans (Koopman et al. 2006; Terzis et al. 2008). Additionally, muscle tension has been shown to result in significant phosphorylation of mTOR and p70s6k proteins in an Akt-independent way (Hornberger et al. 2006*a*, 2006*b*), emphasizing the role of increased muscle tension in the activation of this specific hypertrophy signaling pathway.

Considering these findings, it seems reasonable to hypothesize that an acute bout of fast EE is able to produce higher muscle mechanical stress than slow-velocity EE, thereby inducing greater activation of the PI3K/Akt/mTORCI/p70^{S6K} pathway. Thus, the aim of this study was to investigate the effects of an acute bout of EE performed at high and low velocities on the expression and phosphorylation of the regulatory components of the PI3K/Akt/mTORCI/p70^{S6K} hypertrophy pathway (i.e., MGF, Akt, mTOR complex I (mTORCI), and p70^{S6K}).

Materials and methods

Participants

Twenty-four physically active males, not enrolled in any form of strength training for at least 6 months prior to the study and without any history of musculoskeletal disorders, took part in the study. All subjects were healthy and free of any drug or nutritional supplement ingestion. Due to personal reasons, 4 subjects did not complete the experimental protocol. Participants were assigned to experimental groups on the basis of their performance on a maximal isometric knee extensor torque test (MVIC). Based on their MVIC values, subjects were ranked in quartiles, and participants from each quartile were randomly assigned to either a slow 20°·s^{-1} (ES; n = 11, 77.2 ± 10.5 kg, 1.76 ± 0.06 m, and 25.36 ± 5.0 years) or a fast 210°·s^{-1} (EF; n = 9, 76.3 ± 9.6 kg, 1.77 ± 0.03 m, and 26.40 ± 4.3 years) eccentric knee extensor exercise group. A 2-sample t test assured similar MVIC values between groups ($p > 0.05$). The study was approved by the local ethics committee (School of Physical Education and Sport, University of São Paulo, São Paulo, Brazil), and all of the subjects were informed of the inherent risks and benefits before a written informed consent was obtained.

Experimental design

All of the subjects were familiarized with the procedures and equipment. Both groups performed an acute bout of EE at 2 different velocities (20°·s^{-1} and 210°·s^{-1}). Muscle samples from the vastus lateralis muscle were obtained prior to the EE (baseline; B), immediately after the EE (T1), and 2 h after the EE (T2). Participants fasted for 8 h before the experiment. The familiarization and experimental sessions were performed at the same time of day (i.e., between 0800 and 1100 hours).

Familiarization session

Before the experimental session, all of the subjects underwent a familiarization session with the MVIC test and with the EE, using an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, N.Y., USA). In the MVIC familiarization session, subjects were positioned on the dynamometer and performed five 5-s trials, with a 3-min interval between trials, with their nondominant leg. In the EE familiarization session on the isokinetic dynamometer, 2 sets of 10 repetitions at 120°·s^{-1} were performed with the nondominant leg. We chose to use a nonspecific velocity and the nondominant leg to avoid any velocity-specific residual effects on the hypertrophy signaling pathways. Subjects' positioning on the isokinetic dynamometer followed the same pattern in the familiarization session, the MVIC test, and the EE bout. In short, they were seated in the dynamometer's chair with a 90° hip flexion. The knee from the dominant leg was positioned near the lever arm on the apparatus, and the anatomical axis of rotation of this joint was aligned with the dynamometer's rotation axis. The lower edge of the contact pad of the lever arm was positioned at the nearest proximal site to the lateral malleolus. Individual settings on the dynamometer were recorded for future reference. Straps were used to minimize unwanted body movements, and the participants were instructed to keep their arms crossed at their chest.

MVIC

The MVIC was assessed using the isokinetic dynamometer, with a knee angle of 60° (full extension = 0°). The participants performed three 5-s trials with a 3-min interval. The highest value was used for further analysis. Verbal encouragement was provided at every trial.

EE bout

The acute EE bout was comprised of 5 sets of 8 repetitions of eccentric knee extensions (0° to 90°) on the isokinetic dynamometer at either a slow (20°·s^{-1}) or fast (210°·s^{-1}) velocity, interspersed with a 3-min rest interval. After each repetition, the lever arm of the isokinetic dynamometer was passively returned to the initial position at a fixed velocity of 20°·s^{-1}. Peak torque, work, and impulse were calculated for

each repetition throughout the 5 sets of EE. Peak torque was considered to be the highest value of torque in each eccentric action. Work and impulse were calculated by integrating the area under the torque curves over the displacement and the time, respectively, using custom-made software.

Muscle biopsy

Muscle samples were taken from the midportion of the vastus lateralis of the participants' dominant legs, using the percutaneous biopsy technique with suction. Muscle specimens were dissected free from blood and connective tissue and were divided in half. The first half was immediately homogenized in ice-cold buffer for Western immunoblot analysis. The second half was frozen in liquid nitrogen and stored at –80 °C for real-time PCR analysis. Baseline muscle samples were obtained prior to the EE protocol. The T1 and T2 biopsies were performed immediately after the EE and 2 h after the completion of the EE protocol, respectively, and were obtained through an incision 3 cm above the previous biopsy point (Eliasson et al. 2006).

Western immunoblot analyses

Western immunoblots were assessed using a modified method of that described elsewhere (Eliasson et al. 2006). In short, muscle was rapidly removed and homogenized in a 1.5-mL extraction buffer (100 mmol·L^{-1} Trisma, 1% SDS, 100 mmol·L^{-1} sodium pyrophosphate, 100 mmol·L^{-1} sodium fluoride, 10 mmol·L^{-1} EDTA, and 10 mmol·L^{-1} sodium vanadate), and boiled for 10 min to denature proteins. The extracts were then centrifuged at 10 000*g* at 4 °C for 60 min to remove insoluble material. Protein determination in the supernatants was performed using the Bradford dye method, with the Bio-Rad reagent (Bio-Rad Laboratories, Richmond, Calif., USA). The proteins were treated according to Laemmli's method (Laemmli 1970), and boiled for 5 min before loading the proteins onto the SDS–PAGE. Samples containing 30 μg (mTOR, p70^{S6k}) or 40 μg (Akt) total protein were separated by the SDS–PAGE for 90 min (Akt) or 120 min (mTOR and p70S6k) at 100 V, using 4%–20% gels on the Criterion electrophoresis cell (Bio-Rad Laboratories). Proteins were then transferred to polyvinylidine fluoride membranes (Bio-Rad Laboratories) at 100 mA constant current for 1.5 h (Akt) or 3 h (mTOR, p70^{S6K}) on ice in a cold room (4 °C). Membranes were blocked in Tris-buffered saline (TBS; 10 mmol·L^{-1} Tris (pH 7.6), 100 mmol·L^{-1} NaCl), containing 5% of nonfat dry milk (mTOR) or StartingBlock blocking buffer (Akt and p70^{S6K}; Pierce Biotechnology, Rockford, Ill.), for 1 h, and then incubated overnight at 4 °C with commercially available primary phosphospecific polyclonal antibodies. Antibodies recognized phosphorylated Akt on Ser473, mTOR on Ser2448 (Cell Signaling Technology, Beverly, Mass., USA), and p70^{S6K} on Thr389 (Santa Cruz Biotechnology, Santa Cruz, Calif., USA). Antibodies were diluted at 1:1000 (Akt, mTOR) or 1:2000 (p70^{S6K}), either in TBS with 0.1% Tween-20 containing 2.5% of nonfat dry milk (mTOR) or StartingBlock blocking buffer containing 0.1% TBS with 0.1% Tween-20 (Akt and p70^{S6K}). The blots were subsequently incubated with a peroxidase-conjugated secondary antibody (1:10 000) for 1 h, and processed for enhanced chemiluminescence, according to the manufacturer's protocol, to visualize the autoradiogram. Band intensities were quantified with optical densitometry, using Scion Image software (Frederick, Md., USA), of the developed autoradiographs.

The membranes described above were incubated in a Restore Western blot stripping buffer (Pierce Biotechnology) for 30 min at 37 °C, and reprobed with the appropriate polyclonal antibodies for detection of the total expression levels of each protein. Protein expression of Akt, mTOR, and p70^{S6K} was determined by immunoblot analysis, as described above, using the respective polyclonal antibodies recognizing all forms of Akt, p70^{S6K}, and mTOR.

Gene expression analysis

Reverse transcription

Total cellular RNA was isolated from the muscle samples using TRIzol reagent (Invitrogen, Carlsbad, Calif., USA). Total RNA (1 μg) was typically used in a reaction containing oligo dT (500 μg·mL^{-1}), deoxynucleoside triphosphate (10 mmol·L^{-1} each), 5× first-strand buffer, dithiothreitol (0.1 mol·L^{-1}), and reverse transcriptase (200 U) (SuperScript II, Invitrogen). Reverse transcription was performed at 70 °C for 10 min, followed by 60 min at 42 °C and 10 min at 95 °C.

Primer design

Primer sets were designed by Primer Express software, version 2.0 (Applied Biosystems, Foster City, Calif., USA), using sequences accessed through GenBank, and were checked for specificity using the Nucleotide-Nucleotide Blast search (MGF forward, CGAAGTCTCAGAGAAGGAAAGG; MGF reverse, ACAGGTAACTCGTGCAGAGC).

Real-time PCR

All of the samples were analyzed in duplicate, and the reaction fluorescence was quantified with an ABI Prism 7300 sequence detector (Applied Biosystems), based on current methodology (Bustin 2002). The amplification analysis was performed with Applied Biosystems sequence detection software. Results were expressed using the comparative cycle threshold (Ct) method described in the manufacturer's User Bulletin No. 2 (Applied Biosystems). Ct represents the PCR cycle at which an increase in the gene's reporter fluorescence above a baseline signal can be detected. For each gene of interest, ΔCt values were calculated in all of the samples, as follows: Ct (gene of interest) – Ct (internal control gene). The ribosomal protein large P0 (*RPLP0*) gene was used as an internal control and, as expected, no change was observed (Westerbacka et al. 2008).

The calculation of the relative changes in the expression levels of 1 specific gene was performed by subtracting the ΔCt of the control group (used as the calibrator) from the corresponding ΔCts from the 2 experimental groups. The values and ranges given were determined as follows: $2^{-\Delta\Delta Ct}$ with ΔΔCt ± SE, where SE is the standard error of the mean ΔΔCt value (User Bulletin No. 2, Applied Biosystems). The final values for samples were reported as a fold difference relative to the expression of the control (calculated as $2^{-\Delta\Delta Ct}$), with the control arbitrarily set to 1.

Statistical analysis

A mixed model analysis was performed for protein and gene expression quantification, with groups (slow and fast

EE velocity) and time (B, T1, and T2) as fixed factors and subjects as a random factor (11). Additional mixed models, with groups (slow and fast EE velocity) and sets (first, second, third, fourth, and fifth) as fixed factors and subjects as a random factor, were performed to analyze torque, work, and impulse. Whenever a significant F value was obtained, a post hoc test with a Tukey's adjustment was performed for multiple comparison purposes. Significance level was set at $p < 0.05$.

Results

MVIC peak torque, work, and impulse

There were no differences in peak torque or total work values between groups ($p > 0.05$) (Figs. 1*a* and 1*b*). However, total impulse and impulse per set (main effect for EE velocity, 453% and 454% average difference, respectively) were significantly higher for the ES group (Figs. 1*c* and 1*d*).

MGF mRNA

MGF mRNA level was significantly increased in ES by 241% at T2 ($p < 0.05$). No significant differences were found from baseline values, despite a near 2-fold increase in MGF mRNA at T2 for EF. No differences were found at any time point between groups (Fig. 2).

Protein quantification

Total Akt did not present significant changes. Although phospho-Akt (pAkt) did not show significant differences between groups, it was significantly augmented for EE velocities at T1 and T2, compared with B (593% and 457% change, respectively; pooled data) (Figs. 3*a* and 3*b*). No changes were observed in total mTOR, but phospho-mTOR increased significantly at T1 for both ES and EF (189% change; pooled data) (Figs. 3*c* and 3*d*). Total $p70^{S6K1}$ was also not different between or within velocities. However, $p70^{S6K1}$ phosphorylation was enhanced at T1 and T2 for both groups (6176% and 3761% change, respectively; pooled data), compared with B values (Figs. 3*e* and 3*f*).

Discussion

In this study, we compared the effects of an acute bout of EE performed at different velocities on the expression and phosphorylation of proteins related to the Akt/mTORCI/$p70^{S6K1}$ hypertrophy pathway. Our main findings were that slow EE promoted a significant increase in the MGF mRNA level after 2 h, compared with slow EE resting values ($p < 0.05$), and that slow and fast EE produced similar increments in Akt, mTOR, and $p70^{S6K1}$ phosphorylation ($p < 0.05$). Thus, the initial hypothesis that acute fast EE induces greater MGF mRNA expression and activation of the PI3K/Akt/mTORCI/$p70^{S6K}$ hypetrophy pathway than slow EE was not confirmed.

The mTORCI/$p70^{S6K}$ is one of the most important pathways involved in the acute and chronic enhancement of muscle protein synthesis leading to skeletal muscle fiber hypertrophy (Bolster et al. 2003; Kubica et al. 2005; Baar et al. 2006; Bodine 2006). Accordingly, Kubica et al. (2005) found that rapamycin, an mTOR inhibitor, impaired muscle protein synthesis after an acute exercise bout in rats. In the absence of rapamycin, mTOR has been shown to be upregulated after an acute exercise bout in both animals and humans (Bolster et al. 2003; Terzis et al. 2008). Other studies corroborate the hypothesis that mTORCI/$p70^{S6K}$ pathway activation is involved in increasing protein synthesis after a resistance training bout in humans (Baar and Esser 1999; Eliasson et al. 2006; Koopman et al. 2006). For instance, Terzis et al. (2008) showed an increase in $p70^{S6K}$ phosphorylation after a single bout of resistance exercise. In addition, the increased $p70^{S6K}$ phosphorylation was highly correlated with the increase in free fat mass ($r = 0.81$) and in type IIa fiber cross-sectional area ($r = 0.82$), emphasizing the relationship between the acute increments in the pathway activation and the long-term exercise-induced increase in muscle mass.

Muscle contraction mode acutely affects the Akt/mTORCI/$p70^{S6K}$ pathway activation. Eliasson et al. (2006) compared the effect of different contraction modes (concentric vs. eccentric) on Akt/mTORCI/$p70^{S6K}$ pathway activation, and reported that an EE protocol resulted in greater activation of this pathway than concentric exercise. It is known that EE results in greater force per active fiber, increasing mechanical stimulus to the muscle fiber (Enoka 1996; Chapman et al. 2008), which is known to activate the Akt/mTORCI/$p70^{S6K}$ pathway (Schmelzle and Hall 2000; Nader et al. 2005; Tidball 2005). However, there is little information regarding the effect of contraction velocity on this signaling pathway.

Our results demonstrated that both EE (slow and fast) protocols successfully activated the Akt/mTORCI/$p70^{S6K}$ signaling pathway. Therefore, velocity seems to have no effect on the transient activation of this pathway. A possible explanation for the greater hypertrophy responses observed in previous studies using high-velocity EE (Farthing and Chilibeck 2003*b*; Shepstone et al. 2005) is that the Akt/mTORCI/$p70^{S6K}$ pathway might be differently modulated during long-term training periods; we only investigated the acute response to EE. It is also possible that alternative intracellular pathways, other than the Akt/mTORCI/$p70^{S6K}$ pathway, might be differentially activated in response to this exercise mode. In fact, EE has been shown to decrease the expression of muscle-atrophy–related factors (e.g., MAFbx, atrogin-1, and MuRF) (Okada et al. 2008), which allows for speculation about the diminished expression of such factors when modulating EE velocity. Alternatively, Leloup et al. (2006) proposed that growth factor (i.e., insulin-like growth factor) treatment, in an in vivo model, increased myoblast migration through increased calpain activity and expression, constituting another possible mechanism of interest. Altogether, these data suggest that Akt/mTOR-related signaling differences cannot account for the previously observed greater skeletal muscle hypertrophy response after fast EE (Farthing and Chilibeck 2003*b*; Shepstone et al. 2005). It is also plausible that other intracellular mechanisms may respond differently to the intervention. Further research is required to provide additional insight on the effect of EE velocity on skeletal muscle hypertrophy.

Additionally, one may speculate that the differences in mechanical output (i.e., total impulse) between the EE protocols could have accounted for these findings. In our study, the ES produced greater total impulse throughout the exercise sets. However, peak torque and total work values did not differ between groups. It is known that impulse is dependent on

Fig. 1. Mechanical output during EE. (*a*) Peak eccentric torque over 5 sets of 8 eccentric contractions. (*b*) Total work performed over 5 sets of 8 eccentric contractions. (*c*) Total impulse over 5 sets of 8 eccentric contractions. (*d*) Impulse in each of the 5 sets. *, $p < 0.05$ compared with EF. EE, eccentric exercise; ES, slow EE; EF, fast EE.

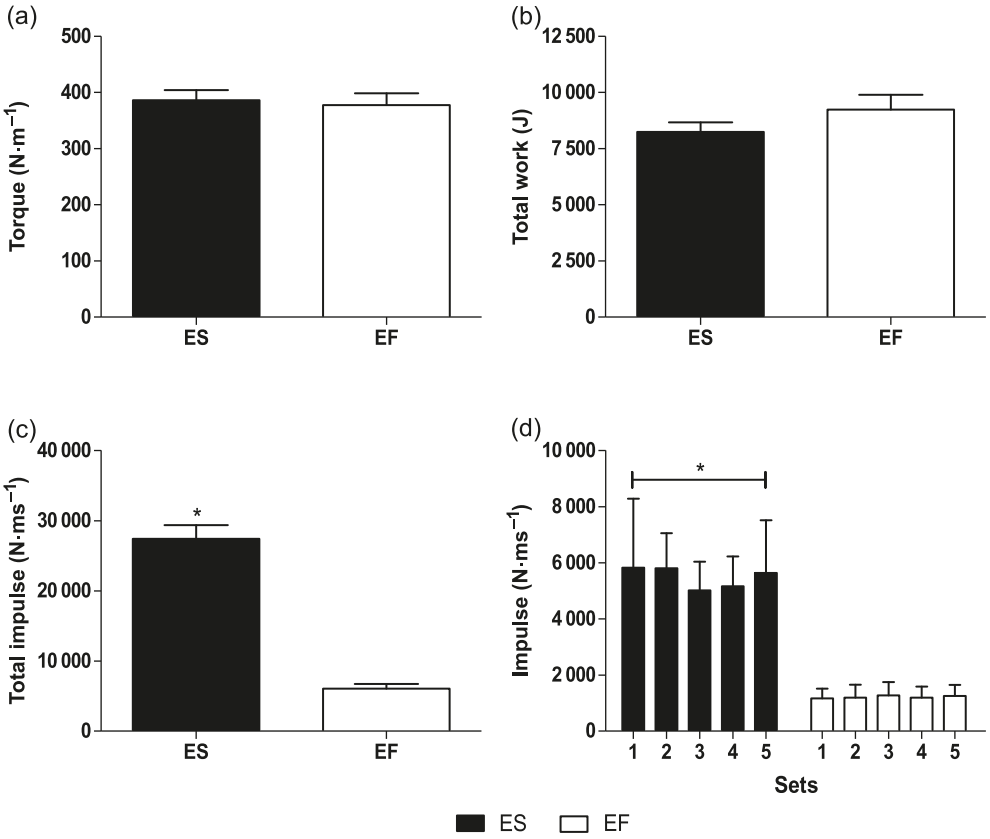
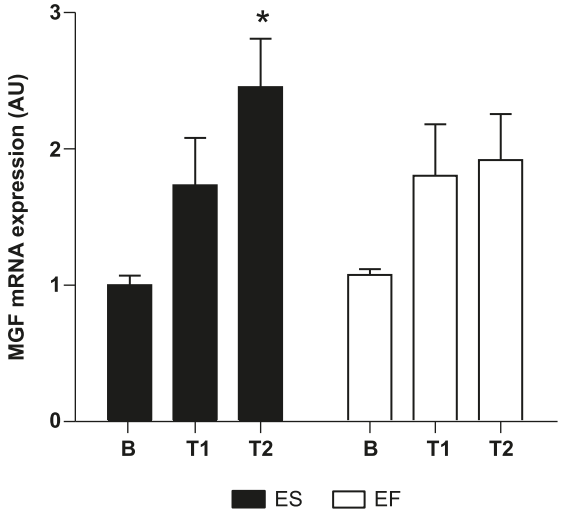


Fig. 2. Mechano growth factor (MGF) mRNA expression in ES and EF. EE, eccentric exercise; ES, slow EE; EF, fast EE; B, baseline; T1, immediately after EE; and T2, 2 h after EE. *, $p < 0.05$ compared with B.

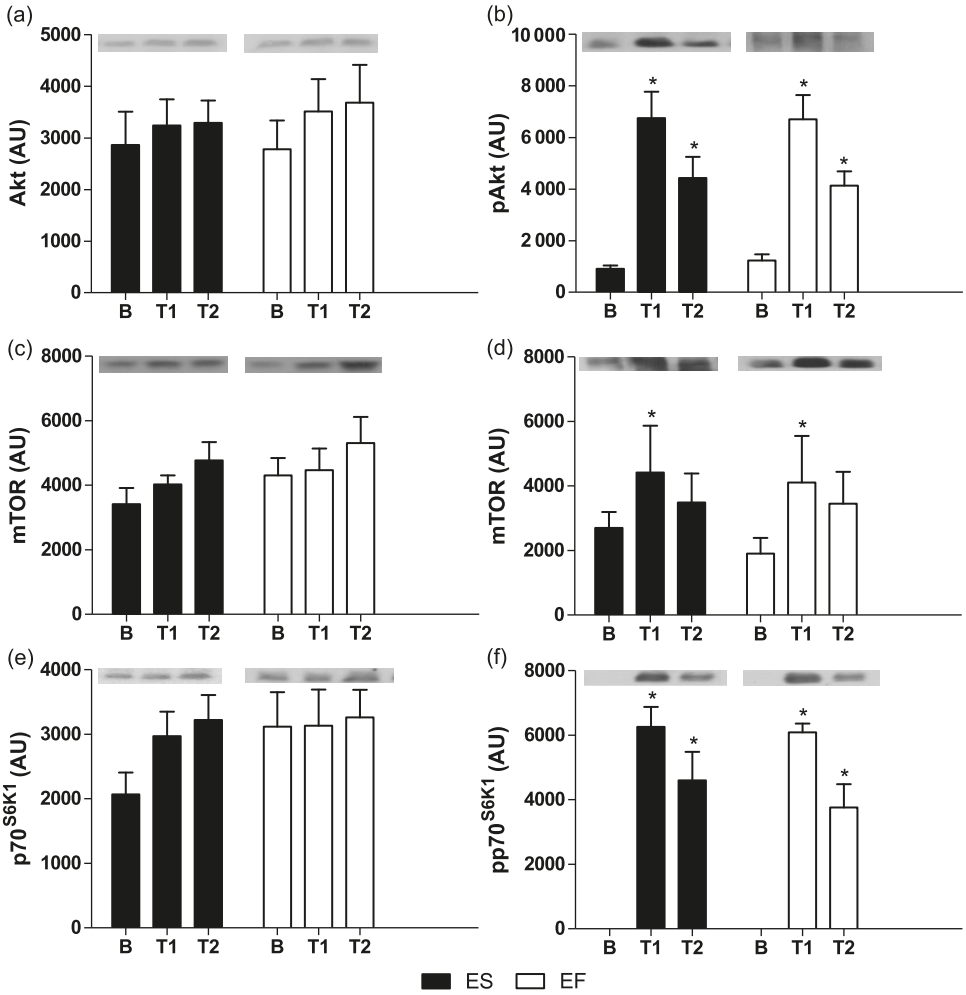


torque production through the range of motion and time under tension (Crewther et al. 2005). Therefore, contraction velocity has a direct effect on time under tension, accounting for the significant differences observed for total impulse values. Indeed, time under tension in the ES group was 10-fold that of the EF. While the ES group performed the EE at $20°·s^{-1}$, resulting in a 4.5-s (90° range of motion) time under tension, the fast velocity of the EF group allowed only 0.43 s under tension because of the high movement speed ($210°·s^{-1}$).

Despite the lesser time under tension in the EF group, it is possible that fast EE may have induced a higher mechanical overload than slow EE (Enoka 1996; Chapman et al. 2008). In fact, it is known that resistance-exercise–induced mechanical overload increases MGF mRNA expression (Rommel et al. 2001; Goldspink 2005; Heinemeier et al. 2007; Liu et al. 2008), which in turn is related to Akt/mTORCI/$p70^{S6K}$ pathway activation (Kimball et al. 2002; Sartorelli and Fulco 2004).

Interestingly, although only the ES protocol induced a significant increase in MGF mRNA expression from B to T2, these values were not different between the groups, and greater Akt/mTORCI/$p70^{S6K}$ activation, compared with EF, was not observed. Again, it may be that the greater muscle tension imposed during EF accounted for an increased mechanotransduction, resulting in similar downstream signaling activation (Burkholder 2007). In fact, it has been proposed that the skeletal muscle hypertrophy following EF is related to *Z* band streaming (Shepstone et al. 2005). *Z* bands are critical sites for mechanotransduction, because of the presence of phospholipase D (Hornberger et al. 2006*a*). The increased muscle tension during EF may lead to greater activation of phospholipase D; it has been suggested that this mediates stretch-induced signaling, activating mTORCI/$p70^{S6K}$

Fig. 3. Changes in total protein and protein phosphorylation. (*a*) Akt. (*b*) Akt phosphorylation. (*c*) mTOR. (*d*) mTOR phosphorylation. (*e*) $p70^{S6k1}$. (*f*) $p70^{S6k1}$ phosphorylation. EE, eccentric exercise; ES, slow EE; EF, fast EE; B, baseline; T1, immediately after EE; T2, 2 h after EE. *, $p < 0.05$ compared with B.



in an Akt-independent way (Hornberger et al. 2006*a*, 2006*b*). Additionally, it has been demonstrated that phosphatidic acid (resulting from increased phospholipase D activity) may activate $p70^{S6K}$ in an mTOR-independent fashion (Lehman et al. 2007). Thus, it is possible that muscle tension plays a role in velocity-specific hypertrophy pathway activation, which may have compensated for the time-under-tension difference observed between EF and ES.

Regardless of the interesting findings herein, caution should be exercised when interpreting and extrapolating these data. Given the inherent heterogeneity in the molecular responses after resistance training, a within-subject and (or) within-leg design would be optimal. However, it is important to note that even though such a design would be the most efficient in minimizing data variability, it would add great bias to our findings. EE is known to induce muscle damage, which may increase protein synthesis, activating the pathways we assessed in this study. Conversely, a single bout of EE is known to produce the repeated-bout effect, which is characterized by a strong minimization of EE-induced muscle damage (Nosaka and Clarkson 1995; Barroso et al. 2010) and activation of the Akt/mTORCI/$p70^{S6K}$ pathway (V. Tricoli, A. Blazevich, C. Ugrinowitsch, M.S. Aoki, and K. Nosaka 2010, unpublished data). The repeated-bout effect induced by a within-leg design could negatively affect our results, masking the effect of exercise velocity. Furthermore, EE is known to cause the greatest cross-education among muscle actions (Hortobágyi et al. 1997; Farthing and Chilibeck 2003*a*), affecting peak torque and total work between testing sessions. Finally, there is evidence of a contralateral repeated-bout effect (Howatson and Van Someren 2007), hampering the adoption of a within-subject design. In addition, one may argue that the EE was not matched for time under tension (TUT). However, matching TUT would require a far greater number of contractions in the EF group. Exercise volume has been demonstrated to play a role in the magnitude of exercise-induced muscle damage (Nosaka et al. 2001, 2002; Chapman et al. 2006, 2008; Chen and Nosaka 2006), affecting protein phosphorylation. Despite the importance of understanding the molecular responses to EE in TUT-matched conditions, previous studies have shown greater muscle hypertrophy with fast EE when total work, but not TUT, was matched (Farthing and Chilibeck 2003*b*; Shepstone et al. 2005). Last, it has been demonstrated that type II fibers contribute to a larger degree to the increase in the phosphorylation of $p70^{S6K}$ after EE (Tannerstedt et al.

2009). In this study, the fiber-type composition in the vastus lateralis muscle was unknown, which prevents conclusions regarding individual variation in muscle fiber-type composition.

In summary, our findings indicate that an acute bout of either low- or high-velocity EE similarly activated the Akt/mTORCI/$p70^{S6K}$ pathway. Despite previous data reporting greater muscle hypertrophy responses after higher-velocity EE, we observed no differences in acute Akt/mTORCI/$p70^{S6K}$ pathway activation between velocities. Probably other intracellular pathways related to the muscle hypertrophy/atrophy apparatus might be selectively activated in response to an EF training protocol. In the future, the observation of mechanotransduction-related proteins and the evaluation of other signaling pathways could possibly provide a new approach to the problem. Additionally, our work leaves room for future studies comparing Akt/mTORCI/$p70^{S6K}$ activation under similar TUT conditions across EE velocities.

Conflict of interest

The authors declare that they have no conflict of interest.

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