

# Lengthening contractions differentially affect p70<sup>s6k</sup> phosphorylation compared to isometric contractions in rat skeletal muscle

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**Abstract** The purpose of this investigation was to determine if p70<sup>s6k</sup> phosphorylation is dependent on the mode of resistance exercise (e.g. isometric vs. lengthening). Two groups ( $n = 5$  each) of Female Sprague Dawley rats, ~12 weeks old, were tested. Rats were anesthetized and indwelling electrodes used to stimulate the right hind limb muscles via the sciatic nerve. The tibialis anterior (TA) muscle of Group 1 rats were exposed to three sets of ten isometric resistance contractions while the TA of Group 2 rats were exposed to three sets of ten resistance contractions that involved lengthening. Contralateral TA muscles served as non-exercised controls. The TA muscle was harvested 6 h post exercise and then the rat was euthanized. Muscle samples were processed to compare p70<sup>s6k</sup> phosphorylation between groups. A single bout of TA contractions that involved muscle lengthening resulted in significantly ( $p < 0.05$ ) higher levels of phospho-p70<sup>s6k</sup> six hours post exercise compared to controls and isometric contractions. The differences in total p70<sup>s6k</sup> six hours post exercise were not significantly different between groups. Results suggest that signal transduction pathways activated

by isometric exercise may differ (i.e., a non-p70<sup>s6k</sup> activation pathway) from that activated by lengthening exercise.

**Keywords** Eccentric · Isometric · Protein synthesis · Strength training

## Introduction

Skeletal muscle strength is essential for executing all forms of physical activity and considerable effort is being made to understand the stimuli and cellular mechanisms that lead to muscle hypertrophy and increased strength. Understanding these mechanisms will facilitate the development of effective strategies to increase muscle strength and enhance movement performance. Several studies have been directed at elucidating the stimuli and signal transduction pathways leading to translation initiation, protein synthesis, and adaptive hypertrophy in skeletal muscle (Baar and Esser 1999; Bodine et al. 2001; Broznick and Birnbaum 1998; Hernandez et al. 2000; Hornberger et al. 2004; Jefferies et al. 1994a, b; Nader and Esser 2001; Parkington et al. 2004; Sakamoto et al. 2002, 2003; Sakamoto and Goodyear 2002). A review by Bolster et al. (2004) illustrated that strength training may stimulate three different transduction pathways [phosphatidylinositol-3 kinase (PI-3 kinase), PDK1, and Akt/PKB mTOR] culminating in the initiation of mRNA translation. A common denominator of these transduction pathways appears to be mutual downstream regulation of p70<sup>s6k</sup> phosphorylation. The protein kinase p70<sup>s6k</sup> is a 70 kDa protein that contributes to the regulation of protein translation (Jefferies et al. 1994a) and phosphorylation of p70<sup>s6k</sup> may play a role in increasing the overall rate of protein synthesis (Baar and Esser 1999).

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Though several studies have shown an increase in the phosphorylation of the protein kinase  $p70^{s6k}$  following acute bouts of exercise in rats (Baar and Esser 1999; Bodine et al. 2001; Hernandez et al. 2000; Nader and Esser 2001), other studies in rats (Brozinick and Birnbaum 1998; Sherwood et al. 1999) have not found enhanced phosphorylation of the protein kinase  $p70^{s6k}$  with contraction, while one human study showed both responses depending on the mode of exercise (Eliasson et al. 2006). The objective of this study was to determine if resistance exercise results in  $p70^{s6k}$  phosphorylation independent of the mode of resistance exercise. To achieve this objective, we elected to compare  $p70^{s6k}$  phosphorylation responses in rat tibialis anterior (TA) muscles subjected to acute bouts of isometric and eccentric resistance exercises, as well as controls that did not experience an acute bout of exercise. We tested the hypothesis that resistance exercise results in  $p70^{s6k}$  phosphorylation independent of the specific mode of resistance exercise.

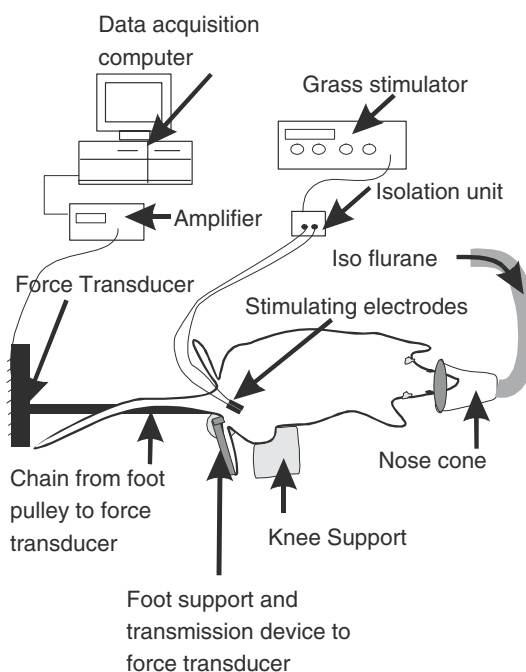
## Materials and methods

Female Sprague Dawley rats (Charles River Laboratories, Wilmington, MA, USA) ~12 weeks old were used to determine if  $p70^{s6k}$  phosphorylation is dependent on the mode of resistance exercise (e.g. isometric vs. lengthening). ‘‘Principles of laboratory animal care’’ (NIH publication No. 86–23, revised 1985) were followed. The Institutional Animal Care and Use Committee of the University of California, Davis, approved all experimental procedures. Rats were quarantined in a vivarium for up to 2 weeks to allow the rodents to adjust to their new environment and help reduce their stress level before intervention. Rats were randomly assigned to either an isometric resistance exercise group ( $n = 5$ ) or a lengthening resistance exercise group ( $n = 5$ ). Concentric resistance exercises were not included because of the experimental model used (see following paragraph). To reduce variability, measurements were obtained from the TA muscle because it is composed of predominately type IIx and IIb fibers (100% superficially and greater than 55% deep, Kanabara et al. 1997). Therefore, it is unlikely that any changes detected are due to fiber type differences.

All experiments were conducted using a custom setup and testing procedures, modeled from previous rodent exercise studies (Adams et al. 2004; Caiozzo et al. 1992; Wong and Booth 1988). The experimental setup consisted of an exercise platform designed to control ankle angle and transmit ankle torque to a custom force transducer, Grass stimulating electrodes (Astro-Med, Inc., West Warwick, RI, USA), Grass SIU5 stimulus isolation unit (Astro-Med, Inc., West Warwick, RI, USA), a Grass S88 stimulator (Astro-Med, Inc., West Warwick, RI, USA), and a data acquisition com-

puter (Windows NT running LabView software) (Fig. 1). Rats were sedated throughout the exercise training using a continual dose of isoflurane (2.5–3% isoflurane vaporized in 100% oxygen) via inhalation. Rats were placed on the platform with the right lower leg held stationary. The right foot was taped to a plate that could be held fixed or allowed to rotate through a set angle. The foot plate was connected via a gearing system to a force transducer that was used to quantify static ankle torque. Two platinum straight needle Grass stimulating electrodes were inserted into the sciatic notch of the rat’s right hind limb to stimulate the sciatic nerve, which activated all right hind limb muscles including the TA. The electrodes were connected to a Grass S88 stimulator via a Grass SIU5 stimulus isolation unit. The foot was positioned at  $\sim 60^\circ$  with respect to the tibia as this position is roughly in the middle of the foot’s range of motion.

The exercise protocol consisted of stimulating the sciatic nerve and controlling the ankle position to cause the TA muscle to either contract isometrically, or eccentrically followed by isometrically. The right leg’s TA muscle was stimulated to generate either a purely isometric contraction (group 1 rats) or a lengthening contraction followed by an isometric contraction (group 2 rats). For both contraction conditions, the muscles of the right hind



**Fig. 1** Schematic of testing setup used to provide lengthening and isometric resistance exercise to the tibialis anterior (TA) muscle of the rat right hind limb. Needle electrodes were used to stimulate the sciatic nerve. Special fixtures held the knee secure. The foot was connected to a pedal that was connected to a force transducer via a gearing system. A nose cone was used to supply anesthesia. The nerve was stimulated via a Grass Stimulator and the ankle torque recorded using a custom force transducer and a data acquisition computer

limb were exposed to the same stimulation patterns (1 ms, 5.7 V pulse administered at a frequency of 100 Hz for a duration of 3 s). The stimulation produced 90–100% of the muscle's isometric maximum contraction, determined from pilot experiments. All exercised muscles were exposed to three sets of ten repetitions. Twenty seconds of rest were given between each muscle contraction and 5 min between each set. During stimulation of the group 1 rat muscles, the plantar flexor muscles produced greater torque than the dorsi flexor muscles causing a small rotation ( $<4^\circ$ ) of the 'fixed' foot pedal due to deflection of the beam used in the force transducer. The rat TA tendon acts across the ankle through a  $\sim 3$  mm lever arm (estimated from caliper measurements of dissected rat hind limbs). The muscle-tendon length change associated with a  $4^\circ$  rotation of the joint, for the tendon sliding over a bony surface located 3 mm from the joint center, corresponds to  $\sim 0.21$  mm. This is less than 0.7% of the total TA muscle-tendon length. This small stretch helped offset the muscle shortening that would have occurred as the active muscle developed force and stretched the tendon, thus creating very close to a pure isometric contraction of the TA muscle. During stimulation of the group 2 rat muscles, the foot was initially held in the same position as for the group 1 rats (i.e. at  $\sim 60^\circ$  with respect to the tibia). However, once force began to develop, the ankle was allowed to plantar flex  $60^\circ$  in approximately 100 ms, the natural rate achieved as the dorsi flexors yielded to the plantar flexors, causing the TA muscle to lengthen. At the end of the  $60^\circ$  ankle plantar flexion movement, the ankle was held fixed and the TA experienced an isometric contraction for the remainder of the stimulation. The goal was to have all exercised muscles experience a similar force magnitude and duration so that the primary difference between groups was a brief lengthening of the muscle in the group 2 rats. The contralateral TA muscle received no stimulation and served as a control for both groups.

Following the exercise bout, the rats remained under observation until the anesthetic effects diminished. They were then returned to their vivarium and allowed water but not food for the next 6 h.

Six hours post exercise rats were again anesthetized with the appropriate concentration of isoflurane and the TA muscles of both hind limbs were removed, weighed, and frozen, and the rat euthanized. The 6 h time point for muscle harvesting was selected based on work by Baar and Esser (1999). They showed that p70<sup>s6k</sup> phosphorylation peaked between 3 and 6 h following an acute exercise bout and that the 6 h peak had a high correlation ( $r = 0.998$ ) with long-term increases in muscle hypertrophy resulting from 6 weeks of training with two exercise bouts per week. The rat was euthanized by CO<sub>2</sub> inhalation following muscle harvesting.

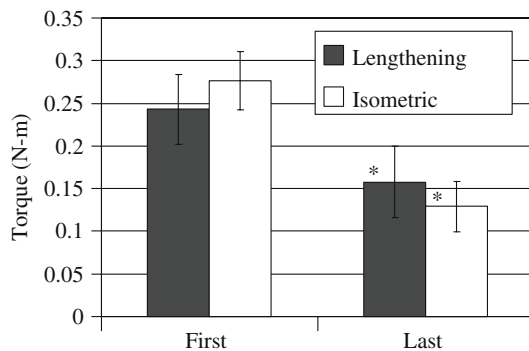
Tissue samples were processed to determine p70<sup>s6k</sup> phosphorylation. Samples were stored in a  $-80^\circ\text{C}$  freezer until time of analysis. At time of analysis a small piece ( $\sim 250$  mg) of the tissue sample was homogenized with a mechanical homogenizer (PowerGen 700, Fischer Scientific) in 1 ml of cold Mueller buffer [50 mM HEPES (pH 7.4), 4 mM EGTA, 10 mM EDTA, 15 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> · H<sub>2</sub>O, 100 mM  $\beta$ -glycerophosphate, 0.1% Triton X-100, 50  $\mu\text{g}/\text{ml}$  leupeptin, 33  $\mu\text{g}/\text{ml}$  aprotinin, 25 mM NaF, 5 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mM PMSF] (Spangenburg and McBride 2006). The piece of muscle was removed by cutting from the ventral to the dorsal aspect through the middle of the muscle, so all portions tested were removed from a very similar region of the muscle. Therefore, it is unlikely that any differences detected in p70<sup>s6k</sup> phosphorylation were due to fiber type composition particularly considering that the TA muscle is almost entirely fast glycolytic muscle fibers. Samples were then centrifuged (Eppendorf Centrifuge 5415R, Westbury, NY, USA) at  $4^\circ\text{C}$  and 13,000 rpm for 10 min. The supernatant of each sample was poured off into a separate tube and  $\sim 20$   $\mu\text{l}$  kept on ice (the rest was frozen and stored at  $-80^\circ\text{C}$  for future analysis) for protein concentration analysis. The protein concentration of the samples was determined in triplicate via the Bradford procedure (Bio-Rad protein assay, Hercules, CA, USA). Protein samples were solubilized in sample buffer (62.5 mM Tris-HCL, 2% SDS, 2.5%  $\beta$ -mercaptoethanol, 7.5% glycerol, 0.005% bromophenol blue) to a concentration of 5 mg/ml and stored on ice in sample tubes until they were boiled for 5 min. One-hundred microgram of total protein was loaded into the lanes of a 7.5% SDS-Page gel and run for 1 h at 150 V. After electrophoretic separation, proteins were transferred at  $4^\circ\text{C}$  to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) at 50 V for 1 h in transfer buffer (25 mM Tris-Base, 192 mM glycine, and 20% methanol) for Western blot assay. The PVDF membrane was then stained with Ponceau S to verify equal transfer of proteins (data not shown). Immunodetection was begun by blocking the PVDF membrane with blocking buffer [5% non-fat dry milk (NFDM) in Tris-base saline (TBS)-T (0.1% Tween 20)] for 1 h at room temperature on a rotator. After blocking, the membrane was serially rinsed with TBS-T to remove all blocking reagent. The membrane was incubated with rabbit anti-p70<sup>s6k</sup> primary antibody (Cell Signaling, MA, USA) in a 1:500 dilution buffer of 3% NFDM in TBS-T on a rocker over night at  $4^\circ\text{C}$ , after which it was washed three times for 5 min with TBS-T at room temperature. To determine phosphorylated p70<sup>s6k</sup> content (Thr-389, Cell Signaling, MA, USA), a duplicate membrane was exposed to mouse anti-phospho-p70<sup>s6k</sup> primary antibody (1:500). To determine the total p70<sup>s6k</sup> content, the membrane was incubated at room temperature with a goat anti-rabbit secondary antibody in a 1:2000 dilution buffer of 3% NFDM in TBS-T.

This procedure was repeated for the phosphorylated p70<sup>s6k</sup> content membrane using a horse anti-mouse secondary antibody, which was diluted 1:2000 in 3% NFD in TBS-T. Following secondary antibody incubation, the membrane was serially rinsed with TBS-T, incubated for 5 min with enhanced chemiluminescent substrate (Pierce, Rockford, IL, USA), and exposed to Kodak-XAR5 autoradiographic film for the appropriate duration. Upon development of the film, the integrated optical density (IOD) of the protein bands was quantified using Image Quant 5.0 (Molecular Dynamics; renamed Amersham Biosciences, Sunnyvale, CA, USA).

A normality test of the optical density data, representing the phosphorylated p70<sup>s6k</sup> content, failed and a Kruskal–Wallis one-way analysis of variance on ranks (SigmaStat V3.1) was performed to test for significant differences between groups ( $p < 0.05$  was considered significant). The differences in the median values among the groups were greater than would be expected by chance and a Student–Newman–Keuls Method was used to perform a pairwise multiple comparison between all groups.

## Results

Torque data collected from the first exercise bout were not significantly different between the lengthening and isometric exercise groups, but the torque data from the last isometric contractions were significantly ( $p < 0.05$ ) lower than the first isometric contractions for both exercise modalities (Fig. 2). These data suggest the two exercise protocols resulted in similar torque outputs and declines in muscle performance over the duration of the exercise bout. This suggests that the exercised TA muscles from the two groups likely experienced similar peak forces and fatigue.

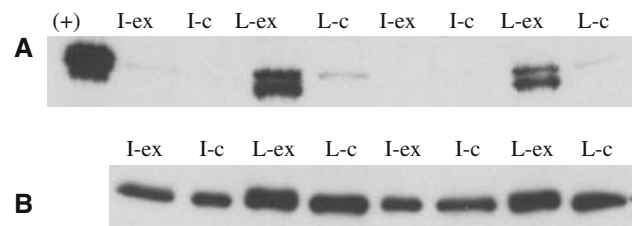


**Fig. 2** Average torque (N m) output of rat hind limb about the ankle during the first and last isometric contraction of the first exercise set and the first and last lengthening contraction of the first exercise set. Results are expressed as the average torque ( $n = 5$  for each group). Error bars represent  $\pm$ SE. The asterisk indicates that the torque output from the first contraction was significantly different than the torque in the last contraction ( $p < 0.05$ )

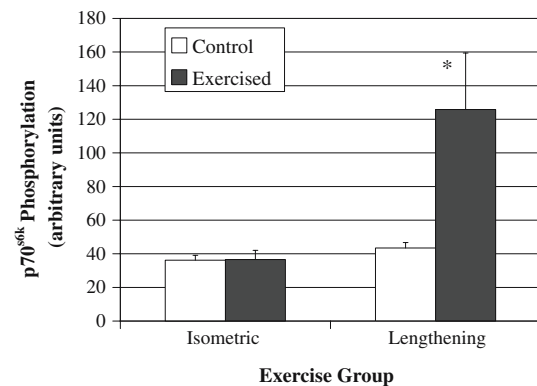
Western blot analyses (Fig. 3a, b), followed by optical density analyses (Fig. 4), indicated that three sets of ten contractions involving lengthening induced significant ( $p < 0.05$ ) increases in p70<sup>s6k</sup> phosphorylation while three sets of ten isometric contractions failed to increase p70<sup>s6k</sup> phosphorylation (Fig. 3a). Six hours post exercise, p70<sup>s6k</sup> phosphorylation was nearly three times greater in the exercised muscles that involved lengthening contractions compared to the contralateral muscles that received no stimulation, however no significant differences were detected in the exercise versus contralateral muscles from the isometric group (Fig. 4). There were no differences in total p70<sup>s6k</sup> expression 6 h post exercise between exercised and contralateral control groups (Figs. 3b, 4).

## Discussion

The data presented here demonstrate that p70<sup>s6k</sup> phosphorylation (Thr-389) is not equally sensitive to all types of mus-



**Fig. 3** Western blots showing **a** p70<sup>s6k</sup> phosphorylation and **b** total p70<sup>s6k</sup>. In **a** dark bands represent p70<sup>s6k</sup> phosphorylation 6 h post acute exercise for positive control (+), both exercised (*ex*) and contralateral (*c*) TAs of two rats trained with isometric model (I) and two rats trained with lengthening model (L). (+) control are L6 myotubes exposed to 10 nM recombinant insulin for 20 min. In **b** the total p70<sup>s6k</sup> present 6 h post exercise in muscles obtained from four rats is illustrated



**Fig. 4** Relative levels of p70<sup>s6k</sup> phosphorylation in the TA muscle 6 h following an acute exercise bout. Results are expressed in arbitrary units for the median ( $n = 5$ ) optical density. Error bars represent the 95% confidence interval. The asterisk indicates that the lengthening exercised group was significantly greater than the isometric exercised, and the contralateral control for both groups ( $p < 0.05$ )

cle contractions that are capable of inducing muscle hypertrophy. It has generally been accepted that p70<sup>s6k</sup> phosphorylation increases with exercise bouts that result in muscle hypertrophy, however, our data suggest that this mechanism is not necessarily conserved as previously suggested. We found that repeated bouts of contractions involving lengthening (three sets of ten maximum contractions) can increase p70<sup>s6k</sup> phosphorylation by nearly three-fold, while repeated isometric contractions (three sets of ten maximum contractions) fail to induce any detectable increase in p70<sup>s6k</sup> phosphorylation compared to non-exercised contralateral controls.

Understanding the signal transduction mechanisms responsible for maintaining and increasing muscle strength is fundamental for identifying strategies to enhance muscle strength and movement performance. Acute weight training exercise bouts in humans and rats have been shown to result in a net increase in muscle protein synthesis and associated ribosomal mechanisms [Baar and Esser 1999 (rat); Chesley et al. 1992 (human); Wong and Booth 1990a (rat); Wong and Booth 1990b (rat)] and chronic weight training (acute bouts of weight training performed on regular intervals over the course of several weeks) has been shown to cause muscle hypertrophy [Adams et al. 2004 (rat); Caiizzo et al. 1992 (rat); Ikai and Fukunaga 1970 (human)] and strength gains [Garfinkel and Cafarelli 1992 (human); Ikai and Fukunaga 1970 (human)]. Taken together these results demonstrate that chronic weight training regimens can cause a net increase in muscle protein synthesis resulting in functional hypertrophy (i.e., an increase in muscle cross-sectional area not attributed to edema) of the exercised muscle.

Our limited understanding of the interaction between the type of mechanical stimulus and p70<sup>s6k</sup> phosphorylation was the motivation for testing the hypothesis that resistance exercise results in p70<sup>s6k</sup> phosphorylation independent of the specific mode of resistance exercise. This hypothesis is consistent with the idea presented by Baar and Esser (1999) that since activation of p70<sup>s6k</sup> is critical for increased protein synthesis in response to a hypertrophic stimulus in cardiac myocytes and because p70<sup>s6k</sup> is phosphorylated following high-resistance lengthening contractions in skeletal muscle, the possibility exists that cardiac and skeletal muscles undergo hypertrophy via a conservative mechanism. Based on their data, Baar and Esser (1999) suggested that p70<sup>s6k</sup> phosphorylation may play a critical role in skeletal muscle growth and also demonstrate promise as a marker for the phenotypic changes that characterize this growth. Our data suggest that p70<sup>s6k</sup> phosphorylation is either dependent upon the type of contraction or the time point at which the muscle is analyzed. More specifically, since we found no increases in p70<sup>s6k</sup> activation with isometric contractions, either this form of contraction does not

elicit p70<sup>s6k</sup> phosphorylation (based on residue Thr-389) or the time point at which we removed the muscle was not appropriate for detecting changes in phosphorylation of p70<sup>s6k</sup> with isometric contraction. Our results are consistent with a recent study by Eliasson et al. (2006) involving human muscle. Eliasson et al. (2006) reported that maximal eccentric, but not maximal concentric or submaximal eccentric contractions led to increases in the Thr-389 phosphorylation of p70<sup>s6k</sup> and phosphorylation of the ribosomal protein S6 via an Akt independent pathway. Results from our study and the Eliasson et al. study suggest that p70<sup>s6k</sup> phosphorylation may be dependent on both resistance mode and intensity. In addition, it is also equally plausible that isometric contraction may induce phosphorylation of p70<sup>s6k</sup> at multiple different amino acid residues, this point must be considered since it is estimated that p70<sup>s6k</sup> can be phosphorylated at various different points within the structure. Since, we only measured phosphorylation status of residue Thr-389, our findings are specific to this residue of the protein. As such, these data provide important evidence that needs to be considered when measuring protein phosphorylation of various signaling proteins during exercise. The difference in the p70<sup>s6k</sup> phosphorylation response between the isometric and lengthened TA muscles may result from different sensitivities in signaling pathways to the type of mechanical stimuli, and it is very possible that the predominate protein synthesis pathway activated by isometric exercise may be a non-p70<sup>s6k</sup> activation pathway.

Contraction type may differentially affect p70<sup>s6k</sup> phosphorylation by differentially affecting the upstream mechanisms that are responsible for activating p70<sup>s6k</sup>. Clearly, data have shown that mechanical load and eccentric contraction can induce activation of p70<sup>s6k</sup>, however, the necessary upstream mechanism that mediates the activation of p70<sup>s6k</sup> remains elusive. It has been speculated that contraction-induced increases in muscle specific levels of IGF-I may mediate the increase in p70<sup>s6k</sup> phosphorylation. However, Garma et al. (2007) recently found that isometric, concentric and eccentric contractions all induced IGF-I mRNA levels to the same extent in the active skeletal muscle. Thus, if IGF-I levels are elevated for all types of contraction and p70<sup>s6k</sup> phosphorylation is not elevated following isometric contraction, then it is unclear what is upstream of p70<sup>s6k</sup> phosphorylation. It is possible to speculate that it maybe necessary to simultaneously activate a parallel mechanism that is necessary for complete phosphorylation of p70<sup>s6k</sup>. For example, recent data have suggested that the lipid second messenger, phosphatidic acid, is increased by contraction and can induce mTOR leading to p70<sup>s6k</sup> phosphorylation (Hornberger et al. 2006). Thus, it is possible that this is a parallel major mechanism that muscle contraction is utilizing to induce p70<sup>s6k</sup> phosphorylation.



It is not readily apparent why the different contractions are not equally effective in inducing p70<sup>S6k</sup> phosphorylation, particularly since Adams et al. (2004) recently found that eccentric, isometric, and concentric contractions are all equally effective at inducing skeletal muscle hypertrophy. Further, the authors also found that the increases in anabolic response to all three types of contraction were quite similar. However, it is equally possible that p70<sup>S6k</sup> is not the only mechanism that is capable of inducing increases in protein synthesis and further it is even more likely that there are multiple redundant mechanisms in a muscle cell capable of increasing protein synthesis in response to contraction. In fact, this has been previously suggested by Eliasson et al. (2006).

If p70<sup>S6k</sup> phosphorylation is required to stimulate muscle hypertrophy, then the isometric exercise protocol (three sets of ten contractions) used in this study may be incapable of stimulating an increase in muscle mass if performed chronically over several weeks. We did not chronically stimulate the TA muscles in this study, however, other studies (Adams et al. 2004; Garfinkel and Cafarelli 1992; Ikai and Fukunaga 1970) have used similar isometric strength training protocols and found an increase in both muscle strength and size. Therefore, we expect that if these muscles were repeatedly stimulated to produce maximal isometric contractions over several weeks, then the TA muscles from the two exercised groups would have experienced similar size and strength gains.

Although the p70<sup>S6k</sup> phosphorylation (Thr-389) has been implicated as an excellent candidate for predicting the anabolic response of skeletal muscle to mechanical stimuli (i.e., exercise), it is not the only possible route in which cellular stress can be translated into cellular growth. Some of these alternate routes include eukaryotic initiation factor 2B (eIF2B) and eIF4E binding protein 1 (4E-BP1) that globally increases rates of protein synthesis or preferentially increases the translation of specific mRNAs, respectively (Bolster et al. 2003a, b, 2004). Deldicque et al. (2005) conclude in a recent review, that interpretation of results from investigations focusing on the role exercise has on translational control of protein synthesis “is complex, since the type, the frequency and the intensity of muscle contractions, and also the type of muscle studied and the training status of the subjects represent factors that may lead to diverse responses.” Results from the current study provide additional evidence of the complex interactions that exist between exercise and translational control of protein synthesis. Current evidence would suggest that multiple signal transduction pathways play a role in stimulating skeletal muscle hypertrophy and it is reasonable to suggest that these pathways are preferentially activated depending on the mechanical stimuli (e.g. lengthening, isometric) (Rennie et al. 2004). In practice, these pathways are probably

simultaneously activated to different degrees during common resistance training programs that involve a combination of isometric, shortening and lengthening muscle actions.

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**Conflict of Interest** The authors have no relationships that present a conflict of interest regarding the worked presented in this article.

## References

- Adams GR, Cheng DC, Haddad F, Baldwin KM (2004) Skeletal muscle hypertrophy in response to isometric, lengthening, and shortening training bouts of equivalent duration. *J Appl Physiol* 96(5):1613–1618
- Baar K, Esser K (1999) Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol* 276(1 Pt 1):C120–C127
- Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, Zlotchenko E, Scrimgeour A, Lawrence JC, Glass DJ, Yancopoulos GD (2001) Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* 3(11):1014–1019
- Bolster DR, Kimball SR, Jefferson LS (2003a) Translational control mechanisms modulate skeletal muscle gene expression during hypertrophy. *Exerc Sport Sci Rev* 31(3):111–116
- Bolster DR, Kubica N, Crozier SJ, Williamson DL, Farrell PA, Kimball SR, Jefferson LS, (2003b) Immediate response of mammalian target of rapamycin (mTOR)-mediated signaling following acute resistance exercise in rat skeletal muscle. *J Physiol* 15;553(Pt 1):213–220
- Bolster DR, Jefferson LS, Kimball SR (2004) Regulation of protein synthesis associated with skeletal muscle hypertrophy by insulin-, amino acid- and exercise-induced signaling. *Proc Nutr Soc* 63(2):351–356
- Broznick JT Jr, Birnbaum MJ (1998) Insulin, but not contraction, activates Akt/PKB in isolated rat skeletal muscle. *J Biol Chem* 273(24):14679–14682
- Caiozzo VJ, Ma E, McCue SA, Smith E, Herrick RE, Baldwin KM (1992) A new animal model for modulating myosin isoform expression by altered mechanical activity. *J Appl Physiol* 73(4):1432–1440
- Chesley A, MacDougall JD, Tarnopolsky MA, Atkinson SA, Smith K (1992) Changes in human muscle protein synthesis after resistance exercise. *J Appl Physiol* 73(4):1383–1388
- Deldicque L, Theisen D, Francaux M (2005) Regulation of mTOR by amino acids and resistance exercise in skeletal muscle. *Eur J Appl Physiol* 94:1–10
- Eliasson J, Elfegoun T, Nilsson J, Köhnke R, Ekblom B, Blomstrand E (2006) Maximal lengthening contractions increase p70S6 kinase phosphorylation in human skeletal muscle in the absence of nutritional supply. *Am J Physiol Endocrinol Metab* 291(6):E1197–E1205
- Garfinkel S, Cafarelli E (1992) Relative changes in maximal force, EMG, and muscle cross-sectional area after isometric training. *Med Sci Sports Exerc* 24(11):1220–1227

- Garma T, Kobayashi C, Haddad F, Adams GR, Bodell PW, Baldwin KM (2007) Similar acute molecular responses to equivalent volumes of isometric, lengthening, or shortening mode resistance exercise. *J Appl Physiol* 102(1):135–143
- Hernandez JM, Fedele MJ, Farrell PA (2000) Time course evaluation of protein synthesis and glucose uptake after acute resistance exercise in rats. *J Appl Physiol* 88(3):1142–1149
- Hornberger TA, Stuppard R, Conley KE, Fedele MJ, Fiorotto ML, Chin ER, Esser KA (2004) Mechanical stimuli regulate rapamycin-sensitive signalling by a phosphoinositide 3-kinase-, protein kinase B- and growth factor-independent mechanism. *Biochem J* 15:380(Pt 3):795–804
- Hornberger TA, Chu WK, Mak YW, Hsiung JW, Huang SA, Chien S (2006) The role of phospholipase D and phosphatidic acid in the mechanical activation of mTOR signaling in skeletal muscle. *Proc Natl Acad Sci USA* 21;103(12):4741–4746
- Ikai M, Fukunaga T (1970) A study on training effect on strength per unit cross-sectional area of muscle by means of ultrasonic measurement. *Int Z Angew Physiol* 28(3):173–180
- Jefferies HBJ, Reinhard C, Kozma SC, Thomas G (1994a) Rapamycin selectively represses translation of the “polypyrimidine tract” mRNA family. *Proc Natl Acad Sci USA* 10;91(10):4441–4445
- Jefferies HBJ, Thomas G, Thomas G (1994b) Elongation factor-1 alpha mRNA is selectively translated following mitogenic stimulation. *J Biol Chem* 11;269(6):4367–4372
- Kanabara K, Sakai A, Watanabe M, Furuya E, Shimada M (1997) Distribution of fiber types determined by in situ hybridization of myosin heavy chain mRNA and enzyme histochemistry in rat skeletal muscles. *Cell Mol Biol* 43(3):319–327
- Nader GA, Esser KA (2001) Intracellular signaling specificity in skeletal muscle in response to different modes of exercise. *J Appl Physiol* 90(5):1936–1942
- Parkington JD, LeBrasseur NK, Siebert AP, Fielding RA (2004) Contraction-mediated mTOR, p70S6k, and ERK1/2 phosphorylation in aged skeletal muscle. *J Appl Physiol* 97(1):243–248
- Rennie MJ, Wackerhage H, Spangenburg EE, Booth FW (2004) Control of the size of the human muscle mass. *Annu Rev Physiol* 66:799–828
- Sakamoto K, Goodyear LJ (2002) Invited review: intracellular signaling in contracting skeletal muscle. *J Appl Physiol* 93(1):369–383
- Sakamoto K, Hirshman MF, Aschenbach WG, Goodyear LJ (2002) Contraction regulation of Akt in rat skeletal muscle. *J Biol Chem* 277(14):11910–11917
- Sakamoto K, Aschenbach WG, Hirshman MF, Goodyear LJ (2003) Akt signaling in skeletal muscle: regulation by exercise and passive stretch. *Am J Physiol Endocrinol Metab* 285(5):E1081–E1088
- Sherwood DJ, Dufresne SD, Markuns JF, Cheatham B et al (1999) Differential regulation of MAP kinase, p70(S6K), and Akt by contraction and insulin in rat skeletal muscle. *Am J Physiol* 276(5 Pt 1):E870–E878
- Spangenburg EE, McBride T (2006) Inhibition of stretch-activated channels during eccentric muscle contraction attenuates p70S6K activation. *J Appl Physiol* 100(1):129–135
- Wong TS, Booth FW (1988) Skeletal muscle enlargement with weightlifting exercise by rats. *J Appl Physiol* 65(2):950–954
- Wong TS, Booth FW (1990a) Protein metabolism in rat gastrocnemius muscle after stimulated chronic concentric exercise. *J Appl Physiol* 69(5):1709–1717
- Wong TS, Booth FW (1990b) Protein metabolism in rat tibialis anterior muscle after stimulated chronic eccentric exercise. *J Appl Physiol* 69(5):1718–1724