Maximal lengthening contractions increase p70 S6 kinase phosphorylation in human skeletal muscle in the absence of nutritional supply

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Eliasson, Jörgen, Thibault Elfegoun, Johnny Nilsson, Rickard Kühnke, Björn Ekblom, and Eva Blomstrand. Maximal lengthening contractions increase p70 S6 kinase phosphorylation in human skeletal muscle in the absence of nutritional supply. Am J Physiol Endocrinol Metab 291: E1197–E1205, 2006.—The aim of this study was to compare the training stimuli of eccentric (lengthening) and concentric (shortening) contractions regarding the effect on signaling enzymes involved in protein synthesis. Ten male subjects performed 4 × 6 maximal eccentric contractions on one leg followed by 4 × 6 maximal concentric contractions on the other. Six additional subjects performed the same protocol, but with maximal concentric and submaximal eccentric exercise of equal force to that of the maximal concentric contractions. Muscle biopsy samples were taken from the vastus lateralis before, immediately after, and 1 and 2 h after exercise in both legs. The average peak force produced during the maximal eccentric exercise was 31% higher than during the maximal concentric exercise, 2,490 (±100) vs. 1,894 (±108) N (P < 0.05). The maximal eccentric contractions led to two- to eightfold increases in the phosphorylation of p70 S6 kinase (p70S6k) and the ribosomal protein S6 that persisted for 2 h into recovery but no significant changes in phosphorylation of Akt or mammalian target of rapamycin (mTOR). Maximal concentric and submaximal eccentric contractions did not induce any significant changes in Akt, mTOR, p70S6k, or S6 phosphorylation up to 2 h after the exercise. The results indicate that one session of maximal eccentric contractions activates p70S6k in human muscle via an Akt-independent pathway and suggest that maximal eccentric contractions are more effective than maximal concentric contractions in stimulating protein synthesis in the absence of a nutritional intake, an effect that may be mediated through a combination of greater tension and stretching of the muscle. Exercise in combination with nutritional supply during recovery after exercise (27). In contrast, other studies report the same increase in muscle cross-sectional area after eccentric and concentric exercise training (25) as well as the same increase in the rate of protein synthesis after exercise (13, 31).

Changes in the rate of protein synthesis are likely to be mediated through activation of enzymes controlling protein translation in muscle, including protein kinase B (or Akt), the mammalian target of rapamycin (mTOR), and p70 S6 kinase (p70S6k) (29). Activation of p70S6k increases the phosphorylation of the ribosomal protein S6 and thereby enhances the translation of 5′TOP mRNAs, i.e., mRNAs that encode for ribosomal proteins and elongation factors (23). A role for the Akt/mTOR/p70S6k pathway in controlling muscle growth was first reported by Bodine et al. (5), who showed that activation of this pathway was necessary for adaptive hypertrophy and recovery after atrophy in rat muscle. In two recent studies, resistance exercise in rats or high-frequency stimulation of rat muscle in vitro was also reported to activate this pathway, supporting the view that the Akt/mTOR/p70S6k pathway is involved in contraction-induced muscle growth (2, 6). Furthermore, results in experimental animals suggest that the mode of contraction is crucial for activating p70S6k phosphorylation in muscle. Stimulation of the muscle via the sciatic nerve led to maximal phosphorylation of p70S6k in muscles performing high-resistance lengthening contractions, whereas in muscles performing shortening contractions a smaller increase or no change was found (3). In addition, high-frequency electrical stimulation of the muscle in vitro led to increases in p70S6k phosphorylation, whereas low-frequency stimulation did not stimulate the kinase (2). In human subjects, Cuthbertson et al. (10) found the same increase in phosphorylation of Akt and p70S6k after dynamic lengthening and shortening exercise when nutrition was supplied to optimize the anabolic response. However, the effect of lengthening and shortening contractions on phosphorylation of Akt, mTOR, and p70S6k in human muscle without nutritional supply is not known.

The purpose of the present study was to compare 1) maximal concentric and maximal eccentric contractions and 2) maximal concentric and submaximal eccentric contractions (of equal force to that of the maximal concentric contractions) regarding the stimulatory effect on signaling proteins in skeletal muscle in the absence of a nutritional intake. The subjects performed one-leg concentric and eccentric exercise with alternating legs. Skeletal muscle biopsies were taken before and repeatedly
during the recovery period, and the phosphorylation of Akt, mTOR, p70S6K, and S6 was determined by immunoblot analyses using phosphospecific antibodies.

**METHODS**

**Subjects**

Sixteen healthy male subjects participated in the study after being fully informed orally and in writing of its purpose and the possible risks involved before providing informed consent. The subjects did not perform endurance or resistance exercise on a regular basis. The subjects did not perform endurance or resistance exercise on a regular basis. The risks involved before providing informed consent. The subjects did not perform endurance or resistance exercise on a regular basis. The Ethics Committee of the Karolinska Institute approved the study protocol.

**Preparatory Tests**

Prior to the experiment, subjects participated in two preparatory tests. The tests were carried out on a modified leg press machine equipped with a force transducer (Nobel Elektronik KPG-4 T10; Vishay Nobel, Karlshoga, Sweden). Force was sampled and amplified using an analog device (Nobel Elektronik BKI-5; Vishay Nobel). Force data were A/D converted, transferred to a computer, and analyzed regarding peak force amplitude. The preparatory tests were designed to determine each subject’s one-leg maximum force and to familiarize the subjects with the exercise procedure. The individual settings of the machine were documented for each subject. Specific attention was given to preventing the subjects from performing the movement at a knee angle of <90° and to avoiding extension at a knee angle >180°.

Electromyographic activity (EMG) was recorded from the right limb (vastus lateralis, ~15 cm proximal to the patella and on the mid-muscle belly of the hamstrings) in four subjects. The skin above the recorded muscles was shaved and cleansed with alcohol. Disposable bipolar surface electrodes (Blue Sensor M-00-S; Medicotest, Oelstykke, Denmark) with a 35-mm inter-electrode distance were used. The recording surface of each electrode was 6 × 3 mm. The electrodes were aligned longitudinally in the muscle fiber direction. Raw EMG signals were preamplified 600 times and filtered through a band-pass filter with low and high cutoff frequencies of 6 and 1,500 Hz, respectively. The filtered signal was converted to a root mean square of the raw signal, was then sampled at 100 Hz using an AD536 circuit (Analog Devices, Norwood, MA) with an average constant of 100 ms. The converted signal, the root mean square of the raw signal, was then sampled at 100 Hz using a MuscleLab system (Model 4000e; Ergotest Technology, Langesund, Norway). The maximal isometric voluntary contractions (MVC) in the vastus lateralis and hamstrings were performed with the hip and knee joint of the right limb in a fixed position. The mean EMG activity in the vastus lateralis and hamstrings muscles during the concentric and eccentric force production phases and MVC were calculated. The angular displacement of the right knee joint was recorded by means of a lightweight electrogoniometer taped over the joint. The angular velocity at the right knee joint was calculated to be ~40°/s⁻¹ (or 0.8 rad/s⁻¹) during the shortening and lengthening contractions.

**Vo2max**

Vo2max was determined by use of an online system (Amis 2001 Automated Metabolic Cart; Innovision, Odense, Denmark) while running on a treadmill. The speed and incline of the treadmill was gradually increased until exhaustion (1).

**Experimental Protocol**

**Part 1: maximal concentric vs. maximal eccentric contractions.**

Ten subjects participated in this first part of the study. They were told not to engage in any intensive physical activity for 2 days prior to the experiment. On the day of the experiment, the subjects arrived at the laboratory in the morning after fasting overnight. A catheter was inserted in the antecubital vein, and the subjects rested for 30 min in a supine position. A resting blood sample was taken and after local anesthesia had been induced; a muscle biopsy sample was taken from the lateral part of the quadriceps muscle (vastus lateralis) of the leg selected for exercise. The subject was seated on the leg press machine and warmed up with 10 eccentric or concentric contractions (same contraction type as the following exercise) at 50% of the one-leg maximum, rested for 2 min, and then the subject performed four sets of six maximal eccentric or concentric contractions with a 5-min rest between sets. After 1 h of rest in a supine position, the subject repeated the same procedure with the opposite leg and the other contraction type, i.e., maximal concentric contractions when maximal eccentric contractions were performed with the previous leg and vice versa. Each repetition was done at a set pace so that each eccentric and concentric movement phase took 2.4 s. The eccentric movement was performed from straight leg (180° knee angle) to bent leg (90°), and the concentric movement was performed from bent leg (90°) to straight leg (180°). Six of 10 subjects started the exercise protocol with maximal one-leg eccentric exercise followed by maximal concentric exercise with the opposite leg, and four subjects started with concentric exercise followed by eccentric exercise. Figure 1 shows a schematic presentation of the experimental protocol.

Blood samples were taken at rest, during exercise, immediately after exercise, and repeatedly in the recovery period (after 15, 30, and 60 min) for the first exercise. After the second exercise, additional blood samples were taken in the recovery period after 90 and 120 min. Eight biopsy samples were obtained from the lateral part of quadriceps femoris, vastus lateralis, from each subject: four samples from the right leg and four from the left leg, using a Weil-Blackesley conchotome (AB Wisex, Mölndal, Sweden), as previously described (17). The first biopsy sample was taken ~15 cm above the patella and the following samples 3 cm above the previous biopsy point. The muscle biopsy samples were taken at rest and immediately after the exercise as well as during recovery at 1 and 2 h after exercise. All biopsy samples were immediately (within 10 s) frozen in liquid nitrogen and stored at ~80°C for later analysis. The choice of time points for biopsy sampling was based on our previous data in human subjects (26) and on observations in experimental animals (6) showing a rapid and sometimes temporary increase in kinase phosphorylation after resistance exercise.

**Part 2: maximal concentric vs. submaximal eccentric contractions**

Six additional subjects participated in the second part of the study. The same experimental protocol as described above was applied except that, instead of maximal eccentric contractions, the subjects performed four sets of six submaximal eccentric contractions with the...
same force as for the maximal shortening contractions. Three of the subjects started the exercise test with maximal concentric exercise followed by submaximal eccentric exercise, and three subjects started with the eccentric exercise followed by the concentric exercise.

Blood Analyses

Blood samples were drawn in heparinized tubes and centrifuged at 9,000 g for 3 min, and the plasma was stored at −80°C. Plasma glucose and lactate concentrations were analyzed according to Bergmeyer (4). Plasma creatine kinase activity was measured using a Reflectron reflectance photometer (Roche Diagnostics, Basel, Switzerland).

Tissue Processing

Muscle biopsy specimens were freeze-dried, dissected free from blood and connective tissue, and homogenized in ice-cold buffer (2 mM HEPES, pH 7.4, 1 mM EDTA, 5 mM EGTA, 10 mM MgCl₂, 50 mM β-glycerophosphate, 1 mM Na₃VO₄, 2 mM DTT, 1% Triton X-100, 2 μg/ml leupeptin, 5 μg/ml aprotinin, 40 μg/ml PMSF) at a dilution of 80 μl/mg of dry weight muscle. The inclusion of an additional phosphatase inhibitor cocktail (P 2850; Sigma) to the homogenizing buffer did not affect Ser/Thr phosphorylation of the measured kinases. Homogenates were rotated for 60 min at 4°C, centrifuged at 10,000 g for 10 min at 4°C to remove cell debris, and stored at −80°C. Protein was determined in aliquots of the supernatant diluted 1:10 in distilled water using a bicinchoninic acid protein assay (Pierce Biotechnology, Rockford, IL).

Western Immunoblot Analyses

Aliquots of muscle lysate were solubilized in Laemmli sample buffer and heated to denature proteins. Samples containing 30 (mTOR, p70S6K, and S6) or 40 μg (Akt) total protein were separated by SDS-PAGE for 90 min (Akt) or 120 min (mTOR, p70S6K, and S6) at 100 V using 4–20% gels on Criterion electrophoresis cell (Bio-Rad Laboratories, Richmond, CA). All eight samples from one subject were run on the same gel. After electrophoresis, gels were incubated in transfer buffer (25 mM Tris·HCl, 192 mM glycine, and 20% methanol) for 30 min to equilibrate gels for optimal transfer. Proteins were then transferred to polyvinylidene fluoride membranes (Bio-Rad Laboratories) at 100 mA constant current for 1.5 h (Akt) or 3 h (mTOR, p70S6K, and S6) on ice in a cold room at 4°C. Membranes were blocked in Tris-buffered saline (TBS; 10 mM Tris, pH 7.6, 100 mM NaCl) containing 5% nonfat dry milk (mTOR, p70S6K on Ser2448/Ser2481, p70S6K on Ser420/Ser421, and S6 on Ser235/Ser236 (Cell Signaling Technology, Beverly, MA) or p70S6K on Thr389 (Santa Cruz Biotechnology, Santa Cruz, CA). Antibodies were diluted 1:1,000 (Akt, mTOR, p70S6K on Ser420/Ser421, and S6) or 1:2,000 (p70S6K on Thr389) either in TBS with 0.1% Tween-20 (TBS-T) containing 2.5% nonfat dry milk (mTOR, p70S6K on Ser2448/Ser2481, and S6) or 0.1% TBS-T (Akt and p70S6K on Thr389). Membranes were then washed in TBS-T or TBS-T containing 2.5% nonfat dry milk, incubated with secondary antibody (horseradish peroxidase-linked anti-rabbit IgG; Cell Signaling Technology), and diluted 1:10,000 (Akt, p70S6K, and S6) or 1:5,000 (mTOR) in their respective primary antibody solutions for 1 h followed by washing in TBS-T (Akt and p70S6K on Thr389) or TBS-T containing 2.5% nonfat dry milk (mTOR, p70S6K on Ser2448/Ser2481, and S6). Phosphorylated proteins were visualized by enhanced chemiluminescence reagents according to the manufacturer’s protocol (SuperSignal west femto maximum sensitivity substrate; Pierce Biotechnology) and quantified by densitometric scanning using a Gel Doc 2000 in combination with Quantity One version 4.4.0 (Bio-Rad Laboratories).

The membranes described above were incubated in Restore Western blot stripping buffer (Pierce Biotechnology) for 30 min at 37°C and reprobed with appropriate polyclonal antibodies for detection of the total expression levels of each protein. Protein expression of Akt, mTOR, p70S6K, and S6 was determined by immunoblot analysis as described above, using the respective polyclonal antibodies recognizing all forms of Akt, p70S6K, and S6 (Cell Signaling Technology) and mTOR (Santa Cruz Biotechnology).

Statistics

Data are presented as means ± SE of the mean. A two-factorial (time, mode of contraction) repeated-measures ANOVA was employed to analyze changes in the phosphorylation state of the different kinases in muscle after exercise and for changes in plasma concentration of glucose and lactate. When a significant overall effect was indicated, a Fisher least significant difference post hoc test was performed. Student’s t-test for paired observations was employed to compare plasma creatine kinase activities before and 1 h after exercise. Significance was accepted at P < 0.05.

RESULTS

The maximal voluntary eccentric force was, on average, 31% higher than the maximal voluntary concentric force: 2,490 (±100) and 1,894 (±108) N (P < 0.05), respectively (Fig. 2).

Fig. 2. A: force developed during 4 sets of 6 maximal concentric or eccentric contractions. For each subject, an average of 6 contractions in 1 set were calculated. Subsequently, means ± SE for the 1st to 4th sets were calculated. Values are means ± SE of the mean for 9 subjects. Force data from 1 subject was not recorded due to technical problems. B: force developed during maximal concentric and submaximal eccentric contractions. Values are means ± SE of the mean for 6 subjects. #P < 0.05 for eccentric vs. concentric contractions.

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Muscle force for the second part of the study is also presented in Fig. 2; the maximal concentric and submaximal eccentric forces were 1,889 ± 114 and 1,836 ± 92 N, respectively. EMG activity recorded in four subjects during the pretests revealed high activation of the vastus lateralis muscle (see below) and low activation of the hamstring muscle (15% of MVC) during maximal concentric contractions and both types of eccentric muscle action. The EMG activation level of vastus lateralis during maximal concentric, submaximal, and maximal eccentric contractions vs. MVC was 146, 74, and 109%, respectively. The mean vastus lateralis EMG activation level was higher in all four subjects during maximal concentric contractions compared with both submaximal as well as maximal eccentric contractions.

The maximal eccentric contractions led to a fourfold increase in p70S6k phosphorylation on Ser^{235/236} and a two-fold increase in phosphorylation on Thr^{389} directly after exercise that remained 1 and 2 h after exercise, whereas the maximal concentric contractions did not cause any significant changes in p70S6k phosphorylation (Fig. 3, A and B). Also, the phosphorylation of the ribosomal protein S6 on Ser^{235/236} was increased severalfold after the maximal eccentric contractions, fourfold directly after and eightfold 1 and 2 h after exercise (Fig. 3C). After the maximal eccentric exercise, a tendency for increased mTOR phosphorylation on Ser^{2448} was found; however, the two-way ANOVA revealed no significant main effect of time or mode of contraction (Fig. 3D). The phosphorylation of mTOR on Ser^{2481} (data not shown) and Akt on Ser^{473} was not influenced by any of the maximal exercises (Fig. 3E). When the subjects performed submaximal eccentric exercise (same force as for maximal concentric contractions), no significant changes were found in the phosphorylation of p70S6k on Ser^{235/236} or Thr^{389}, S6 on Ser^{235/236}, mTOR on Ser^{2448}, or Akt on Ser^{473} after exercise (Fig. 4).

There was no change in plasma lactate concentration during any of the exercises (data not shown). The increase in plasma lactate concentration was similar during the maximal eccentric and maximal concentric exercises, whereas the level was higher in the maximal concentric exercise than in the submaximal eccentric exercise (Fig. 5). By the start of the second exercise, the plasma lactate concentration was back to the same level as before the first exercise (Fig. 5). Plasma creatine kinase activity was not significantly changed after any of the maximal exercises; however, the activity after the eccentric exercise was 39% higher than before exercise (elevated in 5 of the 6 subjects). The mean activity was 143 ± 52 U/l before and 145 ± 52 U/l (n = 4) 1 h after the maximal concentric exercise and 143 ± 36 U/l before and 199 ± 48 U/l (n = 6) 1 h after the maximal eccentric exercise. Assays were performed only in samples from the maximal experiments, where four subjects started the exercise with concentric and six subjects started with eccentric contractions. Furthermore, creatine kinase activities are given for the first exercise only, since the activity may continue to increase, particularly after the maximal eccentric exercise, and hence, influence the level after the second exercise.

There was no measurable effect of the previous exercise on the state of phosphorylation of any of the measured kinases in the biopsy sample from the contralateral leg taken before the second exercise.

DISCUSSION

In the present study, we report that maximal isokinetic lengthening contractions caused a significant increase in p70S6k phosphorylation on Ser^{235/236} and Thr^{389} in human skeletal muscle that persisted for 2 h into recovery. The enzyme p70S6k is phosphorylated on several Ser/Thr residues, including Ser^{124}/Thr^{241}, which is necessary to facilitate phosphorylation on Thr^{389} that is required to fully activate the enzyme (32). In addition, phosphorylation of the ribosomal protein S6, the substrate of p70S6k, was markedly elevated after the maximal lengthening contractions, further supporting the notion that p70S6k is activated. In a previous study, we (26) found that regular resistance exercise, consisting of both concentric and eccentric movements, only partially phosphorylated p70S6k but did not activate the enzyme, as evidenced by the lack of change in phosphorylation of the ribosomal protein S6. Phosphorylation on Thr^{389} was increased only in combination with branched-chain amino acid intake. The present results suggest that four sets of six maximal eccentric contractions, a relatively minor muscular effort, can activate p70S6k and thus stimulate translation initiation in the absence of a nutritional intake. This effect was not observed after the maximal concentric or submaximal eccentric exercises with the specific protocol employed. It can also be speculated whether maximal concentric contractions with a different exercise protocol, for example, increased number of repetitions and/or different speed of contraction, can activate p70S6k. However, the present data suggest that maximal eccentric contractions are a more potent stimulator of translation initiation than maximal concentric contractions in the absence of an immediate nutritional intake. The rapid response in p70S6k and S6 phosphorylation after exercise agrees with results from animal studies (6) and seems to occur earlier than changes in myogenic and metabolic gene expression (36). The mRNA levels of myogenic and metabolic genes were increased from 2 to 12 h after resistance exercise with the highest values 4–8 h after exercise (36).

In experimental animals, Baar and Esser (3) reported a different response in p70S6k phosphorylation in muscles after lengthening vs. shortening contractions, similar to the present results in human muscle. The increase in p70S6k was maximal (similar to insulin-stimulated increase) in the muscles performing lengthening contractions, whereas a smaller or no increase was found in muscles after shortening contractions (3). Nader and Esser (28) reported that a prolonged increase in p70S6k phosphorylation in the tibialis anterior muscle was observed only in response to high-frequency electrical stimulation, causing eccentric contractions of the muscle, suggesting that the lengthening contractions were more effective in activating p70S6k. The prolonged effect on p70S6k differs from the present results, where p70S6k phosphorylation peaked at 1 h after exercise and already tended to decrease again at 2 h recovery (Fig. 3). This difference may be explained, at least in part, by the electrical stimulation protocol, leading to an activation of all fibers in the rat muscle, which may not be the case during maximal voluntary contraction by human subjects. Furthermore, the animals were allowed free access of food in the period after contraction, which may also have contributed to prolong the effect on p70S6k. In contrast, Parkington et al. (30) reported that both concentric and eccentric contractions caused elevated phosphorylation of p70S6k in the fast-twitch muscles.
Fig. 3. Phosphorylation of p70 S6 kinase (p70S6k) on Ser\textsuperscript{424}/Thr\textsuperscript{421} (A), Thr\textsuperscript{389} (B), S6 on Ser\textsuperscript{235}/Ser\textsuperscript{236} (C), mammalian target of rapamycin (mTOR) on Ser\textsuperscript{2448} (D), and Akt on Ser\textsuperscript{473} (E) in skeletal muscle before and after maximal concentric and eccentric contractions. Representative immunoblots are shown above each graph. Values in the graphs are arbitrary units (means ± SE of the mean for 10 subjects). *P < 0.05 vs. before exercise; #P < 0.05 for eccentric vs. concentric contractions.
of the rat but no effect in the slow-twitch soleus muscle, suggesting a fiber-type specific effect rather than an effect of the contraction mode. Future studies that investigate whether the observed effect of lengthening contractions on p70S6k activity in human skeletal muscle (Fig. 3, A and B) also involves a fiber type-specific effect are necessary.

The rate of protein synthesis was not measured in the present study, but recent data demonstrate increases in both p70S6k phosphorylation and rates of protein synthesis in young subjects after infusion of insulin and amino acids or after exercise (10, 15), supporting the notion that an increase in p70S6k activity is important for stimulating protein synthesis. In the exercise study (10), similar increases in p70S6k phosphorylation were reported after dynamic shortening and lengthening contractions (stepping up with one leg onto a knee-high box and stepping down with the other carrying 25% of body
In the present study, Akt phosphorylation was not affected by either eccentric or concentric exercise, suggesting that p70S6k is phosphorylated via an alternative signaling pathway that is independent of Akt in human muscle. This conforms with results in two recently published studies (8, 12) where Akt phosphorylation was unaffected by maximal leg extension exercise in endurance-trained subjects. In the latter study, both Ser473 and Thr308 phosphorylation of Akt were assessed and reported to be unchanged after resistance exercise (12). Furthermore, in the study by Coffey et al. (8), Akt was unaltered in both endurance- and strength-trained subjects 3 h after exercise, and p70S6k phosphorylation was increased immediately after exercise in the endurance-trained but not in the strength-trained subjects. Results from another study (9) indicate that changes in Akt phosphorylation are dependent on the mode of contraction, with results in two recently published studies (8, 12) where Akt phosphorylation was unaffected by maximal leg extension exercise in endurance-trained subjects. In the latter study, both Ser473 and Thr308 phosphorylation of Akt were assessed and reported to be unchanged after resistance exercise (12). Furthermore, in the study by Coffey et al. (8), Akt was unaltered in both endurance- and strength-trained subjects 3 h after exercise, and p70S6k phosphorylation was increased immediately after exercise in the endurance-trained but not in the strength-trained subjects. Results from another study (9) indicate that changes in Akt phosphorylation are dependent on the mode of contraction, with results in two recently published studies (8, 12) where Akt phosphorylation was unaffected by maximal leg extension exercise in endurance-trained subjects. In the latter study, both Ser473 and Thr308 phosphorylation of Akt were assessed and reported to be unchanged after resistance exercise (12).

The tendency for an increase in mTOR phosphorylation on Ser2448 after the maximal eccentric exercise in the present study (P < 0.1 for main effect of time and for mode of contraction, respectively) may suggest that mTOR can activate p70S6k in human muscle or that p70S6k through a feedback mechanism phosphorylates mTOR, which was recently reported to occur in cell cultures (7, 19). However, the two- to threefold increase in p70S6k phosphorylation after lengthening contractions would have been expected to induce a clearer effect on mTOR than was found (Fig. 3), assuming there is the same feedback effect of p70S6k on mTOR in human muscle as there is in the investigated cell cultures.

Plasma creatine kinase activity was measured in the present study as a marker for skeletal muscle injury. The maximal eccentric contractions induced an increase in creatine kinase activity in five of six subjects, suggesting some degree of muscle damage. However, it should be pointed out that measurements of creatine kinase activity were made 1 h after exercise, and the activity is likely to increase further during recovery. This makes comparisons with earlier studies difficult, since assessments of muscle damage have often been made several hours or days after high-intensity eccentric resistance exercise (13, 35).

The higher force produced in the maximal eccentric vs. maximal concentric exercise, as well as the lower electrical activity, is consistent with earlier findings in human subjects (1, 33). During eccentric exercise there is an elongation and thus stretching of the muscle, which may have induced the observed increase in p70S6k phosphorylation. Force generation and stretch are factors, which can both activate protein synthesis, and they seem to have an additive effect on protein synthesis in rabbit muscle (14). An increase in p70S6k activation may thus be caused by a combined effect of muscle force/tension and mode of contraction. An effect of the eccentric muscle action per se would have induced an effect during the submaximal eccentric exercise as well, which was not noted. A higher neural activation level, expressed as the EMG amplitude, can also be ruled out as a possible stimulator of the kinases, as EMG recordings showed a lower activation level during maximal eccentric muscle action than during maximal concentric contractions.

Results in experimental animals have recently demonstrated that mechanical stimulation by passive stretching of the muscle.

**Fig. 5.** A: changes in plasma lactate concentration during and after maximal concentric and maximal eccentric contractions. B: maximal concentric and submaximal eccentric contractions. Values are means ± SE of the mean for 10 subjects in A and 6 subjects in B. *P < 0.05 vs. before exercise; #P < 0.05 for eccentric vs. concentric contractions.
in vitro increased phosphorylation of p70S6k on Ser^{124}/Thr^{421} as well as Thr^{389} in an Akt-independent way (20). The changes in p70S6k phosphorylation on Ser^{124}/Thr^{421} occurred in parallel with increases in p38 mitogen-activated protein kinase (MAPK) phosphorylation, and neither of these was blocked by rapamycin, suggesting no involvement of mTOR in phosphorylating p70S6k on these sites (20). In a previous study, a transient increase in p38 MAPK phosphorylation was found in the muscle after resistance exercise; however, the increase was completely suppressed 1 h after exercise when phosphorylation of p70S6k on Ser^{424}/Thr^{421} was still elevated, making a role for rapamycin, suggesting no involvement of mTOR in phosphorylating p70S6k less likely (26).

In summary, maximal eccentric but not maximal concentric or submaximal eccentric contractions led to increases in the Thr^{389} phosphorylation of p70S6k and phosphorylation of the ribosomal protein S6 via an Akt-independent pathway. This suggests that maximal eccentric exercise can fully activate p70S6k in human muscle and thus stimulate translation of specific proteins in the absence of an immediate nutritional intake, an effect that may be mediated through greater muscle tension and stretching of the muscle during the eccentric muscle action. These results can have important implications for training recommendations after muscle injury and for recreational athletes.

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