ORIGINAL ARTICLE

Resistance exercise-induced increase in muscle mass correlates with p70S6 kinase phosphorylation in human subjects

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Abstract The purpose of the present study was to investigate the possible relationship between a change in Thr^{389} phosphorylation of p70S6 kinase ($p70^{S6k}$) after a single resistance training session and an increase in skeletal muscle mass following short-term resistance training. Eight male subjects performed an initial resistance training session in leg press, six sets of 6RM with 2 min between sets. Muscle biopsies were obtained from the vastus lateralis before (T1) and 30 min after the initial training session (T2). Six of these subjects completed a 14-week resistance-training programme, three times per week (nine exercises, six sets, 6RM). A third muscle biopsy was obtained at the end of the 14-week training period (T3). One repetition maximum (1RM) squat, bench press and leg press strength as well as fat-free mass (FFM, with dual energy X-ray absorptiometry) were determined at T1 and T3. The results show that the increase in Thr^{389} phosphorylation of $p70^{S6k}$ after the initial training session was closely correlated with the percentage increase in whole body FFM ($r = 0.89$, $P < 0.01$), FFM_{leg} ($r = 0.81$, $P < 0.05$), 1RM squat ($r = 0.84$, $P < 0.05$), and type IIA muscle fibre cross sectional area ($r = 0.82$, $P < 0.05$) after 14 weeks of resistance training. These results may suggest that $p70^{S6k}$ phosphorylation is involved in the signalling events leading to an increase in protein accretion in human skeletal muscle following resistance training, at least during the initial training period.

Keywords Muscle hypertrophy · Protein translation · Resistance training · Skeletal muscle

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Introduction

Resistance training is a potent stimulus for the increase in human skeletal muscle mass (see e.g., MacDougall et al. 1980; Tesch 1988). However, the molecular mechanisms responsible for the training-induced muscle hypertrophy remain largely unresolved. Muscle hypertrophy can be induced by an increase in protein synthesis, a decrease in protein degradation or a combination of these two mechanisms. A single resistance exercise can increase the rate of protein synthesis up to 48 h after the exercise in human skeletal muscle (MacDougall et al. 1995; Phillips et al. 1997). This increase is likely to be mediated through changes in signal transduction, including activation of phosphatidylinositol 3-kinase (PI3K), Akt (or protein kinase B), mammalian target of rapamycin (mTOR) and

sequential activation of p70S6 kinase (p70^{S6k}) and the eukaryotic initiation factors (Bolster et al. 2003; Dreyer et al. 2006; Kubica et al. 2005). Previous reports have emphasised the importance of the Akt/mTOR/p70^{S6k} pathway in stimulating protein translation and muscle growth in animals (Atherton et al. 2005; Bolster et al. 2004; Nader 2005; Tidball 2005). A recent study in human subjects has also shown a stimulation of the Akt/mTOR/p70^{S6k} pathway in response to a single heavy resistance training stimulus (Dreyer et al. 2006). However, when subjects performed leg press exercise, a decrease in Akt phosphorylation, no change in mTOR phosphorylation and a partial phosphorylation of p70^{S6k} but no activation of the enzyme was found (Karlsson et al. 2004). Only in combination with intake of branched-chain amino acids an increase in Thr^{389} phosphorylation of p70^{S6k} and activation of the enzyme was found (Karlsson et al. 2004). This finding emphasises the role of nutritional status on the activation of the translational processes leading to the post-exercise increase in muscle protein synthesis. Furthermore, Eliasson et al. (2006) revealed that p70^{S6k} phosphorylation was significantly elevated after maximal eccentric resistance exercise but not after maximal concentric exercise. Collectively, these studies suggest that molecular signalling towards an increase in protein translation after resistance training is affected by the type of contraction and the exercise intensity and duration.

It is well known that different individuals respond differently to a certain resistance-training programme (MacDougall et al. 1980). Results from experimental animals show a close relationship between Thr^{389} phosphorylation of p70^{S6k} after a single high frequency electrical stimulation protocol and the increase in skeletal muscle mass following 6 weeks of electrical stimulation (Baar and Esser 1999). Similar studies in human subjects have not previously been carried out. We hypothesise that the individual response to a resistance training programme correlates with the acute activation of skeletal muscle p70^{S6k}. To test this hypothesis, we have investigated the correlation between the change in phosphorylation of p70^{S6k} in muscle biopsies taken before and after a single resistance training session with the increase in skeletal muscle mass following a 14-week period of resistance-training in healthy men. In addition, changes in Akt and mTOR phosphorylation in muscle biopsies taken before and after the exercise, were measured.

Methods

Subjects

Twelve male physical education students (mean ± SD; age 21 ± 1 year, height 181 ± 1 cm, weight 83 ± 1 kg)

participated in the study. All of the subjects were healthy with no neuromuscular problems and did not participate in resistance training programmes during the year before the initiation of the study. After a thorough oral and written description of the procedure they gave their written consent. Subjects were randomly divided into two groups: Training group (T, $n = 8$) and Control group (C, $n = 4$). Two of the subjects from the T group did not complete the 14-week training programme. However, they participated in the initial training session and the biopsy procedure at T1 and T2 (see below). All procedures were performed according to the principles outlined in the Declaration of Helsinki and were approved by the local ethical committee.

Study design

The T group of subjects followed a 14-week resistance-training programme while the C group maintained their normal everyday activities but did not participate in any training. Both groups were evaluated before the initiation of the training period (T1) as well as after the completion of the 14-week training period (T3). The evaluation included maximal strength tests (1RM squat, leg press and bench press), as well as an estimation of fat-free mass with dual energy X-ray absorptiometry (DXA) of the whole body (FFM$_{WB}$) and of the legs (FFM$_{leg}$). Muscle biopsies from vastus lateralis were obtained at the beginning (T1) and the end (T3) of the training period. An additional, muscle biopsy from vastus lateralis was obtained from the T group only, 30 min after the initial resistance exercise stimulus (T2).

Strength testing

Strength testing was performed before (T1) and after (T3) the 14-week training period. At T1, 1RM was performed twice with an interval of 1 week between the two tests, and the better of the two measurements was used in further analysis. In addition, at T1, the 6RM load for leg press was also defined. Assessment of maximal muscle strength (1RM) on the squat, bench press (free weights) and leg press (machine) was performed according to previous reports (Beachle et al. 2000). Briefly, after a short warm up on a stationary bicycle and stretching exercises, subjects performed incremental submaximal efforts, until they were unable to lift a heavier weight. Maximal strength was determined for all of the three exercises in the same day in random order with a rest period of 30 min between each exercise. In all cases, two of the authors were present and vocally encouraged each trial of each subject. Strength testing at T1 was performed at least 1 week before the initial resistance exercise session, while at T3 it was performed 3–4 days after the last training session.

Dual X-ray absorptiometry (DXA)

DXA measurements were made during the last week before the initiation of the training period (T1) as well as during the first week after the completion of the 14-week training programme (T3). A total body scan was performed (DXA model DPX-L, LUNAR Radiation, Madison, WI, USA) and analysed using the LUNAR Radiation body composition programme. Fat mass, lean mass and bone mineral content were determined for the total body and for arm, leg and trunk regions. All analyses were performed by two different investigators and the mean value was calculated and used in further analyses.

Initial resistance training session

Two hours before reporting to the laboratory, the subjects had a light meal that consisted of 0.5 l skimmed milk and a cheese sandwich (480 kcal, 52% carbohydrates, 29% fat, and 19% protein), in order to stimulate their exercise performance (American College of Sports Medicine 2000). Thirty minutes after reporting to the laboratory, a muscle biopsy was taken from the lateral part of the quadriceps muscle, vastus lateralis, using the needle biopsy technique (Bergstrom 1962). The biopsy was taken approximately 20 cm away from mid patella of the right (dominant) leg. The training session was initiated 30 min after the first biopsy. After a standard warm up with light loads on the leg press machine (45° inclination), the subjects performed six sets of 6RM with an interval of 2 min between the sets. Each repetition was completed in 2.5–3 s. Subjects were vocally encouraged to perform their best. The total time of the resistance exercise session was approximately 12 min. After executing the last repetition of the last set, they rested quietly in a chair until the time of the second biopsy, which was performed 30 min after the last repetition. The muscle biopsy at T2 was obtained at least 5 cm proximal to the T1 incision point.

Fourteen-week training protocol

Six of the eight subjects completed the 14-week training programme. The exercise programme aimed at strengthening the whole body, with an emphasis on the quadriceps muscle group. The following nine resistance exercises were used at all times by all subjects: (1) free weight exercises (squat until 90° of the knees, bench press, arm curls, overhead press leg press), (b) exercises on machines (leg press at 45° inclination, elbow extensions on a pulley, seated rowing, sit-ups, back extensions [Supersport, Athens]). During the first 2 weeks, subjects performed two sets of 8-10RM twice a week. For the rest of the period, they performed three sets of 6RM for each exercise, three times per week. The load was increased daily, in order to meet the

6RM-goal. The interval between sets was 2–3 min. Approximately 95% of the training sessions were followed strictly. The rest of the sessions were partly completed, because of small injury problems.

Tissue processing

The muscle specimens obtained at T1 were cut in two pieces; one was placed immediately in liquid nitrogen for baseline measurements of the phosphorylation state of Akt, mTOR and p70^{S6k}. The other piece was aligned and placed in embedding compound and frozen in isopentane pre-cooled to its freezing point for histochemical analysis of fibre type composition and fibre CSA. The biopsies obtained at T2 were quickly removed from the needle and placed immediately in liquid nitrogen and samples taken at T3 were placed in embedding compound and frozen in isopentane pre-cooled to its freezing point. All samples were kept at −80°C until the day of analysis. The biopsies at T1 and T3 were obtained at the same time of the day.

ATPase histochemistry

Serial cross-sections, 10-μm thick, from the embedded samples obtained at T1 and T3 were cut at −20°C and stained for myofibrillar ATPase after pre-incubation at pH 4.3, 4.6 and 10.3 (Brooke and Kaiser 1970a, b). Samples from each subject obtained at T1 and T3 were incubated at the same time in the same jar. An average of 470 ± 48 muscle fibres were classified as type I, IIA or IIX from each sample. The CSA of at least 300 fibres from each sample was measured with an image analysis system (ImagePro, Media Cybernetics Inc, Silver Spring, MD, USA) at a known and calibrated magnification. The percentage type II fibre area was calculated as the percentage type II fibres multiplied by the mean CSA of type II fibres.

Western blot analyses

Muscle biopsies were freeze-dried and dissected free from blood, connective and adipose tissue. Approximately 2 mg of sample was homogenised in ice-cold buffer (20 mM Hepes pH 7.4, 1 mM EDTA, 5 mM EGTA, 10 mM $MgCl_2$, 50 mM β-glycerophosphate, 1 mM Na_3VO_4, 2 mM DTT, 1% Triton X-100, 20 μg/ml leupeptin, 50 μg/ml aprotinin, 40 μg/ml PMSF, 1% phosphatase inhibitor cocktail [Sigma P2850]) at a dilution of 80 μl/mg of dry-weight muscle. Homogenates were rotated for 60 min at 4°C and centrifuged at 10,000g for 10 min at 4°C to remove cell debris. Protein concentration was determined in aliquots of the supernatant using a BCA protein assay (Pierce Biotechnology, Rockford, IL).

Aliquots of muscle lysate were mixed with Laemmli sample buffer and heated to denature proteins. Samples

containing 20 μg of total protein were separated by SDS-PAGE. Following electrophoresis, proteins were transferred to polyvinylidine fluoride membranes (Bio-Rad Laboratories, Richmond, CA). Membranes were blocked in TBS (Tris Buffered Saline; 10 mM Tris pH 7.6, 100 mM NaCl), containing 5% non-fat dry milk (mTOR and p70^{S6k} on Ser^{424}/Thr^{421}) or in StartingBlock blocking buffer (Akt and p70S6 kinase on Thr^{389}) (Pierce Biotechnology) for 1 h and then incubated overnight at 4°C with commercially available primary phosphospecific antibodies. Antibodies recognised phosphorylated Akt on Ser^{473}, mTOR on Ser^{2448} or Ser^{2481}, p70^{S6k} on Ser^{424}/Thr^{421} (Cell Signaling Technology Inc., Beverly, MA) or p70^{S6k} on Thr^{389} (Santa Cruz Biotechnology, Santa Cruz, CA). Membranes were then washed in TBS with 0.1% Tween 20 (TBST) or TBST containing 2.5% non-fat dry milk and incubated with appropriate secondary antibody for 1 h at room temperature. Phosphorylated proteins were visualised by enhanced chemiluminescence and the optical density was quantified by densitometric scanning using a Gel Doc 2000 in combination with Quantity One version 4.4.0 (Bio-Rad Laboratories).

The membranes were incubated in Restore™ Western blot stripping buffer (Pierce Biotechnology) and re-probed with appropriate antibodies for detection of the total expression levels of each protein. Total protein expression was determined by immunoblot analysis as described above, using the respective antibodies recognising all forms of Akt, p70^{S6k} (Cell Signaling Technology Inc.) and mTOR (Santa Cruz Biotechnology). Optical density of the phosphorylated protein was adjusted to the optical density of the respective total protein band. A detailed description of the Western blot analysis has been given previously (Eliasson et al. 2006).

Statistics

Data are presented as means and standard deviation (±SD). Pearson's r product moment correlation coefficient was used to explore the relationship between different variables. Student's t test for paired observations was used to evaluate differences in phosphorylation of Akt, mTOR and p70^{S6k} between T1 and T2. A two-way factorial ANOVA with repeated measures was employed to evaluate physiological, anthropometrical and performance changes before and after exercise, between the T and C groups. $P < 0.05$ was chosen as a two-tail level of significance.

Results

Impact of the 14-week resistance training

Table 1 presents the physiological, anthropometrical and performance changes after the 14-week period of resistance training. There was a significant increase in 1RM leg press (30%, $P < 0.01$), 1RM squat (31%, $P < 0.01$), 1RM bench press (24%, $P < 0.01$), whole body FFM (2.3%, $P < 0.05$), and mean fibre CSA (19%, $P < 0.01$). The percentage of type IIX fibres decreased from $16 \pm 5\%$ before training to $9 \pm 3\%$ after the training period ($P < 0.01$).

Phosphorylation of Akt, mTOR and p70^{S6k} after the initial training stimulus

Ser^{473} phosphorylation of Akt was decreased by $57 \pm 30\%$ ($P < 0.05$) 30 min after the initial training stimulus (Figs. 1, 2). Phosphorylation of mTOR at Ser^{2448} was increased by

Table 1 Resistance-training induced responses after 14 weeks. Values are mean ± SD

Parameter	Training group ($n = 6$)		Control group ($n = 4$)	
	Before	After	Before	After
Body weight (kg)	83.4 ± 2.7	84.0 ± 2.7	81.1 ± 2.2	81.2 ± 2.2
1RM leg press (kg)	244 ± 39.2	$326^{**} \pm 51.4$	228 ± 36	237 ± 38
1RM squat (kg)	104 ± 24.5	$139^{**} \pm 29.4$	102 ± 18	104 ± 20
1RM bench press (kg)	79.8 ± 39.9	$101^{**} \pm 32.8$	80.2 ± 22	81.4 ± 22
Whole body FFM (kg)	65.0 ± 3.4	$65.4^{*} \pm 3.4$	64.1 ± 3	64.0 ± 3.8
Legs FFM (kg)	23.2 ± 1	$23.9^{*} \pm 1.2$	23.0 ± 1.2	23.0 ± 1
Arms FFM (kg)	7.92 ± 0.5	$8.16^{*} \pm 0.5$	8.10 ± 0.6	8.10 ± 0.8
BMD (g/cm^2)	1.41 ± 0.05	1.42 ± 0.05	1.40 ± 0.04	1.40 ± 0.04
Body fat (%)	17.4 ± 3.9	17.6 ± 2.4	17.0 ± 1.8	17.1 ± 1.6
Type I (%)	46.1 ± 8.3	48.4 ± 9.3	51.3 ± 7.8	56.8 ± 8.2
Type IIA (%)	37.6 ± 7.8	42.5 ± 6.8	30.2 ± 8.8	25.7 ± 9.4
Type IIX (%)	16.4 ± 4.9	$9.1^{**} \pm 3.9$	18.5 ± 8.8	17.5 ± 6.4
Type I CSA (μm^2)	$5,271 \pm 549$	$6,003^{**} \pm 705$	$5,012 \pm 650$	$4,980 \pm 498$
Type IIA CSA (μm^2)	$5,445 \pm 897$	$6,671^{**} \pm 762$	$5,289 \pm 838$	$5,198 \pm 974$
Type IIX CSA (μm^2)	$5,190 \pm 877$	$5,522^{**} \pm 1038$	$4,990 \pm 1028$	$4,868 \pm 822$

FFM fat-free mass, *1RM* maximum muscle strength, *CSA* cross sectional area, *BMD* bone mineral density

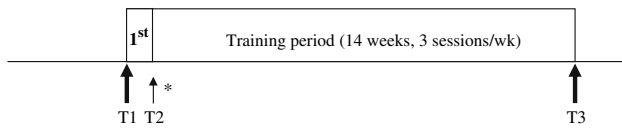*$P < 0.05$, **$P < 0.01$ before versus after the training period

Fig. 1 Overview of the study design. T1 and T3: muscle biopsy, *1RM* strength and *DXA*. T2: muscle biopsy 30 min after the initial training stimulus (*1st*: first training session)

$25 \pm 14\%$ ($P < 0.05$), whereas Ser^{2481} phosphorylation did not change significantly (data not shown). Phosphorylation of $p70^{S6k}$ at both Ser^{424}/Thr^{421} and Thr^{389} was enhanced severalfold 30 min after the initial training stimulus (Fig. 2). No significant correlation was observed between the percentage type II fibre area of vastus lateralis at T1 and the change in phosphorylation of $p70^{S6k}$ ($r = 0.1$) after the initial training stimulus.

Phosphorylation of Akt, mTOR and $p70^{S6k}$ in relation to the 14-week training response

The change in Ser^{424}/Thr^{421} phosphorylation of $p70^{S6k}$ was significantly correlated with the percentage increase in FFM_{WB} ($r = 0.82$, $P < 0.05$), FFM_{leg} ($r = 0.81$, $P < 0.05$), 1RM leg press ($r = 0.88$, $P < 0.05$) and 1RM squat ($r = 0.83$, $P < 0.05$). Moreover, the change in Thr^{389}

phosphorylation of $p70^{S6k}$ was significantly correlated with the percentage increase in FFM_{WB} ($r = 0.89$, $P < 0.01$; Fig. 3), FFM_{leg} ($r = 0.81$, $P < 0.05$; Fig. 3), 1RM squat ($r = 0.84$, $P < 0.05$; Fig. 3) and type IIA fibre CSA ($r = 0.82$, $P < 0.05$; Fig. 3). Also the percentage increase in mean fibre CSA tended to relate to the change in Thr^{389} phosphorylation of $p70^{S6k}$ ($r = 0.74$, ns). The change in the phosphorylation state of Akt at Ser^{473}, mTOR at Ser^{2448} and Ser^{2481} after the initial training stimulus, was not significantly correlated to any of the changes in performance, physiological or anthropometrical parameters.

Discussion

The main finding of the present study is that the increase in $p70^{S6k}$ phosphorylation after a single resistance exercise stimulus relates well to the training-induced increase in muscle mass and performance. Specifically, $p70^{S6k}$ phosphorylation at Ser^{424}/Thr^{421} and Thr^{389} after the initial resistance training bout is closely associated with the increase in whole body FFM, leg FFM, 1RM leg press strength, 1RM squat strength and type IIA muscle fibre CSA, after a short-term (14 weeks) resistance-training programme in healthy young individuals. Despite the limitations related to the

Fig. 2 Phosphorylation of Akt, mTOR, and $p70^{S6k}$ in skeletal muscle before and 30 min after a single resistance training session. Values in the graph are arbitrary units (mean $\pm$ SD of the mean for eight subjects). Representative immunoblots from two subjects are shown above each graph. *$P < 0.05$ versus before exercise

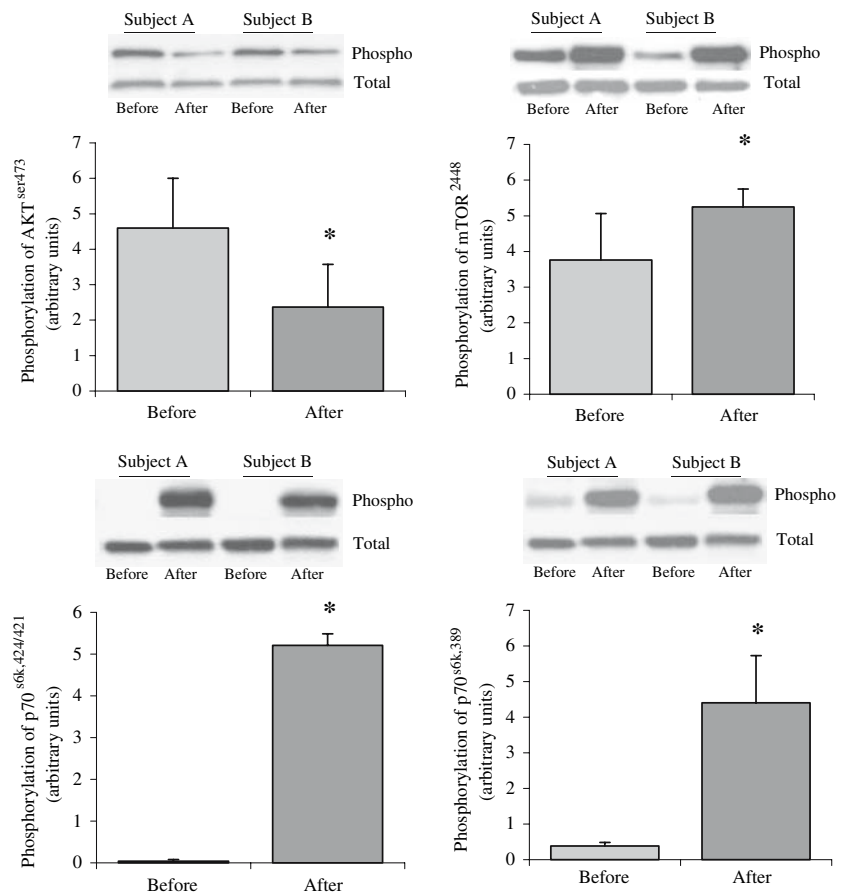**Fig. 3** Correlations between the increase in Thr[389] phosphorylation of p70[S6k] in skeletal muscle during a single resistance training session and the percentage change in (**a**) 1RM squat strength, (**b**) whole body fat-free mass (**c**) leg fat-free mass and (**d**) type IIA muscle fibre cross sectional area after 14 weeks of resistance training. All correlations were statistically significant (*n* = 6). *1RM* maximum strength, *FFM* fat-free mass, *CSA* cross-sectional area
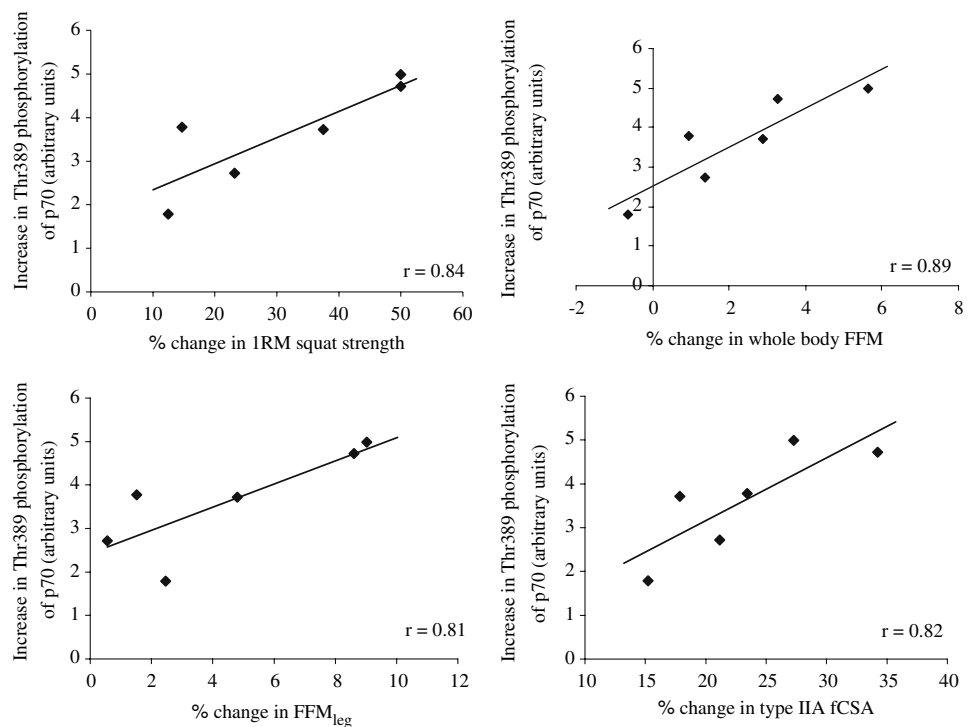
small number of subjects, these results suggest that p70[S6k] phosphorylation (which represents well its activity) is associated with the signalling events leading to an increase in skeletal muscle protein synthesis following resistance exercise in human subjects. A recent work by Fujita et al. (2007) also supports the importance of the mTOR/p70[S6k] phosphorylation towards an acute increase in muscle protein synthesis after resistance exercise.

The results of the present study are in concert with those of Baar and Esser (1999) who reported a very close relationship (*r* = 0.998) between the increase in Thr[389] phosphorylation of p70[S6k] after a single bout of high-frequency electrical stimulation of rat muscle and the increase in muscle mass after 6 weeks of electrical stimulation. In the present study, the correlation coefficients between the increase in p70[S6k] phosphorylation during the initial resistance training stimulus and the increase in physiological and performance parameters were rather high, considering the number of parameters that might influence the training outcome in human subjects (effort applied during training, nutritional habits, small injuries, daily psychological variation, etc.). Moreover, this close coupling was not affected by the subjects' initial training state (e.g. muscular strength) or their physiological characteristics (e.g. body composition). Rather, it seems that a single resistance training stimulus, with a standard training intensity and load, is sensed differently by different individuals and induces a different molecular and overall adaptation response.

Three phosphorylation sites have been discovered for mTOR: Ser[2481], which is thought of as an autophosphorylation site, Ser[2448] which is thought to be stimulated by Akt, and Thr[2446], which is proposed to be stimulated by AMP activated protein kinase (AMPK) (Deldicque et al. 2005; Cheng et al. 2004). Ser[2481] phosphorylation of mTOR was not altered significantly after the initial resistance-training stimulus, whereas Ser[2448] phosphorylation of mTOR was increased. The increase in Ser[2448] phosphorylation is consistent with results on human subject performing heavy resistance exercise (Dreyer et al. 2006) but in contrast to others in which the exercise was less intense (Creer et al. 2005; Blomstrand et al. 2006). Moreover, a lack of effect on Ser[2481] phosphorylation has previously been reported in human subjects and in rodent muscle subjected to mechanical stimulation in vitro (Eliasson et al. 2006; Hornberger and Chien 2006). However, despite the fact that Ser[2448] phosphorylation of mTOR was increased after the initial training stimulus, this increase was not related with the performance or muscle mass increase after the 14-week training period.

Akt phosphorylation 30 min after the initial training stimulus was significantly decreased in the present study. In previous studies, Akt phosphorylation was either reduced or unchanged directly after a single session of resistance training in non-strength-trained individuals (Blomstrand et al. 2006; Dreyer et al. 2006; Eliasson et al. 2006) or endurance-trained subjects (Coffey et al. 2005; Deshmukh et al. 2006). In contrast, Creer et al. (2005) found an increase in Ser[473] phosphorylation of Akt 10 min after low intensity resistance exercise when the pre-exercise muscle glycogen content was high. The reason for these discrepancies

is unclear; however, the intensity and duration of the training session as well as the subjects' training background and sampling times are factors, which may influence the signalling response. Furthermore, the decrease in Akt and the concomitant increase in mTOR and p70[S6k] phosphorylation, suggests that these molecules are not necessarily sequentially activated. A possible explanation for the decrease in Akt phosphorylation after resistance exercise and the nonsignificant coupling between Akt and p70[S6k] phosphorylation could be an increase in AMPK activity, which remains elevated at least for an hour after a single bout of resistance exercise (Dreyer et al. 2006). Low [ATP] after resistance exercise might stimulate AMPK, which subsequently might inhibit Akt phosphorylation. However, it should be noted that in rats, increased AMPK activity decreases the phosphorylation of both Akt and mTOR as well as p70[S6k] (Bolster et al. 2004), which is inconsistent with the increase in mTOR and p70[S6k] phosphorylation found in the present study.

The increase in p70[S6k] phosphorylation during the initial resistance training stimulus tended to correlate to the percentage increase in mean fibre area (significantly correlated to the increase in type IIA fibre CSA) after 14 weeks of resistance training. This suggests a link between p70[S6k] and type II muscle fibres. In concert with this finding, a recent work reported that Ser[424]/Thr[421] phosphorylation of p70[S6k] was more pronounced in type II than in type I muscle fibres, after a single resistance exercise session (Koopman et al. 2006). In addition, a number of studies (e.g. Hather et al. 1991; MacDougall et al. 1980; Tesch 1988) including the present one, have shown that type II muscle fibres hypertrophy more than type I fibres in response to resistance training. Thus, we speculate that an individual with an enhanced p70[S6k] phosphorylation response after a single resistance training stimulus, holds a greater potential for resistance-training-induced muscle hypertrophy (therefore muscular strength) which is partly based upon an enhanced potential for an increase in type II fibre CSA.

The percentage increase in maximal muscle strength after the 14-week training period was strongly correlated to the increase in p70[S6k] phosphorylation during the initial training session. Resistance training-induced muscle strength relies not only on the increase in muscle mass but also on neural adaptations, especially during the first week of training (Narici et al. 1996). However, the subjects who participated in the present study, although refrained from resistance exercise at least for a year before, were familiar with such exercise paradigms through their university courses. This suggests that most of the improvement in muscle strength can be attributed to the increase in muscle mass. The finding that the percentage increase in 1RM squat was significantly correlated with the percentage increase in leg fat-free mass ($r = 0.92$, $P < 0.01$), supports this notion.

Fourteen weeks of resistance training can induce a significant increase in both strength and muscle mass (e.g. muscle fibre CSA) in relatively untrained individuals (Aagaard et al. 2001). During this early phase of muscle hypertrophy, the association between p70[S6k] phosphorylation after the initial training stimulus and the overall increase in fat-free mass, maximal muscle strength and type IIA fibre CSA, seems to be close. However, muscle hypertrophy due to an increase in protein synthesis might not rely on this specific mechanism for longer periods of training. Recently, Coffey et al. (2005) revealed that p70[S6k] phosphorylation was not altered after a single intense resistance-training stimulus in strength-trained athletes.

In conclusion, the present study reveals a close relationship between the increase in p70[S6k] phosphorylation at Ser[424]/Thr[421] and Thr[389] during an initial resistance training bout and the increase in whole body and leg FFM, 1RM leg press and squat strength, and type IIA muscle fibre CSA, as a response to a short-term resistance-training programme in healthy young individuals. Although, the number of subjects in the present study was limited, the results suggest that p70[S6k] phosphorylation is associated with the signalling events leading to an increase in protein accretion following resistance exercise in human subjects.

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