

Increase in S6K1 phosphorylation in human skeletal muscle following resistance exercise occurs mainly in type II muscle fibers

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Submitted 2 November 2005; accepted in final form 17 January 2006

Koopman, René, Antoine H. G. Zorenc, Rudy J. J. Gransier, David Cameron-Smith, and Luc J. C. van Loon. Increase in S6K1 phosphorylation in human skeletal muscle following resistance exercise occurs mainly in type II muscle fibers. *Am J Physiol Endocrinol Metab* 290: E1245–E1252, 2006. First published January 24, 2006; doi:10.1152/ajpendo.00530.2005.—To investigate the in vivo effects of resistance exercise on translational control in human skeletal muscle, we determined the phosphorylation of AMP-activated kinase (AMPK), eukaryotic initiation factor 4E-binding protein (4E-BP1), p70/p85-S6 protein kinase (S6K1), and ribosomal S6 protein (S6). Furthermore, we investigated whether changes in the phosphorylation of S6K1 are muscle fiber type specific. Eight male subjects performed a single high-intensity resistance exercise session. Muscle biopsies were collected before and immediately after exercise and after 30 and 120 min of postexercise recovery. The phosphorylation statuses of AMPK, 4E-BP1, S6K1, and S6 were determined by Western blotting with phospho-specific and *pan* antibodies. To determine fiber type-specific changes in the phosphorylation status of S6K1, immunofluorescence microscopy was applied. AMPK phosphorylation was increased approximately threefold immediately after resistance exercise, whereas 4E-BP1 phosphorylation was reduced to $27 \pm 6\%$ of preexercise values. Phosphorylation of S6K1 at Thr⁴²¹/Ser⁴²⁴ was increased 2- to 2.5-fold during recovery but did not induce a significant change in S6 phosphorylation. Phosphorylation of S6K1 was more pronounced in the type II vs. type I muscle fibers. Before exercise, phosphorylated S6K1 was predominantly located in the nuclei. After 2 h of postexercise recovery, phospho-S6K1 was primarily located in the cytosol of type II muscle fibers. We conclude that resistance exercise effectively increases the phosphorylation of S6K1 on Thr⁴²¹/Ser⁴²⁴, which is not associated with a substantial increase in S6 phosphorylation in a fasted state.

skeletal muscle; translation initiation; immunohistochemistry; human; AMP-activated protein kinase; p70/p85 S6 protein kinase

SKELETAL MUSCLE PROTEIN TURNOVER has been shown to be stimulated after an acute bout of resistance exercise (9, 28). The process of mRNA translation initiation forms an important regulatory site in the overall control of muscle protein synthesis (6). Recent evidence suggests that the phosphatidylinositol 3-kinase signaling pathway plays a key role in this process (19, 31). The activity of the signaling cascade controlling muscle protein synthesis is largely determined by the phosphorylation of the mammalian target of rapamycin (mTOR) and its subsequent activation of the p70/p85 S6 protein kinase (S6K1) and

the eukaryotic initiation factor 4E-binding protein (4E-BP1) (19).

Both S6K1 and 4E-BP1 can modulate translation initiation and control the binding of mRNA to the 40S ribosomal subunit (19). 4E-BP1 can bind to the initiation factor eIF4E, thereby acting as a translational repressor preventing the formation of the eIF4F scaffolding complex, which is necessary for efficient binding of the 40S ribosomal subunit to mRNA (1, 15). Activation of the mTOR pathway results in 4E-BP1 hyperphosphorylation and a subsequent dissociation of the 4E-BP1·eIF4E complex, allowing it to participate in the translation initiation process. Another mechanism regulating the binding of mRNA to the 40S ribosomal subunit involves the phosphorylation of ribosomal protein S6, which is controlled by the activity of S6K1 (1). S6K1 consists of two isoforms: a 70-kDa cytoplasmic isoform (p70^{S6K}) and a 85-kDa nuclear isoform (p85^{S6K}) (22). The activation of both S6K1 isoforms has been shown to phosphorylate S6 on the 40S subunit in close proximity to the eIFs and mRNA (22). Phosphorylation of S6 is considered to alter the interaction of the protein with these components, thereby promoting mRNA translation (7). Interestingly, in rodents, the phosphorylation status of S6K1 after resistance exercise has been reported to be an excellent marker for the long-term increase in skeletal muscle mass (3).

It has been speculated that the anabolic response to resistance exercise is muscle fiber type specific (24, 37). It has been shown that human soleus as opposed to vastus lateralis muscle is less responsive to resistance exercise (37). In accordance, in rats, the phosphorylations of PKB (or Akt), mTOR, and S6K1 after resistance exercise have been reported to be more pronounced in muscle tissue containing a greater proportion type II muscle fibers (3, 27, 31), as opposed to those muscle groups containing more type I fibers (27). Moreover, with the use of immunohistochemistry in rat tibialis muscle, it was recently shown that mTOR phosphorylation is selectively increased in type IIa fibers for up to several hours after resistance exercise (27). Data on the activation of the translation initiation machinery in human skeletal muscle remain scarce (10, 17). Information on the potential muscle fiber type-specific changes in the phosphorylation status of proteins involved in translation initiation response to exercise in humans is still lacking.

Both S6K1 and mTOR have been reported to be localized in the cytosol (41). However, recent studies have reported these proteins to be localized both in the cytosol and in the nuclei of

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human embryonic kidney (HEK)-293 cells (18). The latter implies that subcellular localization of mTOR and/or S6K1 could play a functional role. In HEK-293 cells, nuclear import of mTOR has been shown to enhance 4E-BP1 phosphorylation and S6K1 activation, whereas nuclear export of mTOR attenuated the phosphorylation/activation, suggesting that both activation of S6K1 and phosphorylation of 4E-BP1 require nuclear mTOR (18). Similar nuclear transport has also been reported for S6K1 (18). These observations suggest that the intracellular cytosolic and/or nuclear localization of these key regulatory proteins represent a level of control in the regulation of skeletal muscle protein synthesis. So far, the subcellular localization of S6K1 and the subsequent changes that occur in response to resistance exercise have not been studied in human muscle.

The purpose of the present study was to investigate the mode of activation of the mRNA translation initiation machinery downstream of mTOR in skeletal muscle tissue after a single bout of resistance exercise *in vivo* in humans. Besides measuring the phosphorylation state of S6K1, 4E-BP1, and S6, we aimed to investigate whether changes in S6K1 phosphorylation are specific for muscle fiber type and/or subcellular localization.

METHODS

Subjects

Eight healthy male volunteers with no history of participation in any regular exercise program were recruited to participate in the present study. Subjects' characteristics are shown in Table 1. All subjects were informed of the nature and possible risks of the experimental procedures before their written informed consent was obtained. This study was approved by the Medical Ethics Committee of the Academic Hospital Maastricht.

Pretesting

We assessed body composition using the hydrostatic weighing method in the morning after an overnight fast. Residual lung volume was measured by the helium dilution technique by use of a spirometer (Volugraph 2000; Mijnhart, Bunnik, The Netherlands). Body weight was measured with a digital balance that has an accuracy of 0.001 kg (E1200; August Sauter, Albstadt, Germany). Body fat percentage was calculated with Siri's equation (33). Fat-free mass was calculated by subtracting fat mass from total body mass.

To familiarize subjects with the resistance exercise protocol and equipment, a familiarization trial was performed. Proper lifting technique was demonstrated and practiced for each of the two lower-limb exercises (leg press and leg extension) and for the three upper-body exercises (chest press, shoulder press, and lat-pulldown). Thereafter,

maximum strength was estimated with the multiple repetitions testing procedure (23).

In an additional exercise session, at least 1 wk before the experimental trial, the subjects' 1-repetition maximum (1RM) was determined (21). After a warm-up, the load was set at 90–95% of the estimated 1RM and increased after each successful lift until failure. A 5-min resting period between subsequent attempts was allowed. A repetition was valid if the subject was able to complete the entire lift in a controlled manner without assistance.

Standardization Diet and Activity Before Testing

All subjects received a strict standardization diet for 2 days before the resistance exercise test trial, which was performed in the morning after an overnight fast. Subjects were provided with a measured amount of food products, beverages, and instant meals and were allowed to drink water *ad libitum*. Subjects were instructed to take all main meals (breakfast, lunch, and dinner) and between-meal snacks at predetermined time intervals during each day. The standardization diet provided 0.15 MJ·kg⁻¹·day⁻¹ containing 15 energy percentages (En%) of protein, 30 En% of fat, and 55 En% of carbohydrate. All volunteers were instructed to refrain from any sort of heavy physical exercise during the 2 days before the test trial.

Experimental Trials

On the day of the test, subjects arrived at the laboratory by car or public transportation at 8:00 AM after an overnight fast. After 30 min of supine rest, a basal blood sample was collected, and a muscle biopsy was taken from the vastus lateralis muscle. Thereafter, subjects performed a 5-min low-intensity warm up on a cycle ergometer, followed by three sets of 10 repetitions on three resistance exercise machines targeting upper-body muscle groups (chest press, shoulder press and lat-pulldown; Jimsa Benelux, Rotterdam, The Netherlands). The latter exercises were included to provide a whole-body warm up to reduce the risk of injury. Thereafter, subjects performed eight sets of 10 repetitions on the horizontal leg press machine (Technogym, Rotterdam, The Netherlands) and eight sets of 10 repetitions on the leg extension machine (Technogym). Both exercises were performed at 75% of the individual 1RM with 2-min rest intervals between sets and required ~45 min to complete. All subjects were verbally encouraged during the exercise session to complete the entire protocol. Immediately after cessation of exercise, a second muscle biopsy sample was taken, after which the subjects rested supine for 2 h. After 30 min and 2 h of postexercise recovery, additional muscle biopsies were taken.

Muscle Biopsies

Muscle biopsy samples were collected from both legs. The first two biopsies were taken from the same incision in one leg; the last two were taken from the same incision in the contralateral leg. When biopsy samples were taken from the same incision, the first sample was taken from a different region (distal of the incision, with the needle pointing inward) than the second (proximal with the needle pointing outward). Muscle biopsies were obtained from the middle region of the vastus lateralis muscle (15 cm above the patella) and ~3 cm below entry through the fascia using the percutaneous needle biopsy technique (4).

Muscle samples were freed from any visible nonmuscle material and rapidly frozen in liquid nitrogen. Muscle samples (~40 mg) for Western blotting analyses were freeze dried, and collagen, blood, and other nonmuscle fiber material were removed from the muscle fibers under a light microscope. The isolated muscle fiber mass (6–10 mg) was weighed, and eight volumes (8× dry weight of isolated muscle fibers × wet-to-dry ratio) of ice-cold buffer (in mmol/l: 20 HEPES, pH 7.4, 100 KCl, 50 β-glycerophosphate, 50 NaF, 1 dithiothreitol, 0.5 Na₃VO₄, 0.2 EDTA, 0.1 PMSF, and 1 benzamide) were added (5).

Table 1. *Subjects' characteristics*

Age, yr	22.5 ± 0.9
Weight, kg	74.9 ± 2.8
Height, m	1.80 ± 0.01
BMI, kg/m ²	23.3 ± 0.7
%Body fat, %	17.8 ± 2.4
Fat-free mass, kg	61.3 ± 2.1
Fat mass, kg	13.6 ± 2.1
Hb A _{1c} , %	5.3 ± 0.1
1RM leg press, kg	198 ± 7
1RM leg extension, kg	105 ± 3

Values are means ± SE. BMI, body mass index; Hb, hemoglobin; 1RM, one-repetition maximum.

The tissue was then homogenized, after which homogenates were centrifuged for 5 min at 1,000 *g* and 4°C. Thereafter, the supernatant was centrifuged at 10,000 *g* at 4°C for 10 min, resolved in SDS buffer, and boiled for 5 min at 100°C.

About 20 mg of each muscle sample were frozen in liquid nitrogen-cooled isopentane and embedded in Tissue-Tek for immunohistochemical analysis (Sakura Finetek, Zoeterwoude, The Netherlands).

Muscle Sample Analysis

Antibodies. Polyclonal primary phospho-specific antibodies [anti-phospho-S6K1 (Thr⁴²¹/Ser⁴²⁴), anti-phospho-S6 (Ser²³⁵/Ser²³⁶), anti-phospho-4E-BP1 (Thr³⁷), and anti-phospho-AMP-activated kinase (AMPK) α 1/2 (Thr¹⁷²)] and anti-S6K1, anti-S6, anti-4E-BP1, and anti-AMPK α 1/2 antibodies were purchased from Cell Signaling Technologies (Beverly, MA).

Anti-caveolin-3 was from BD Biosciences (San Jose, CA), and the monoclonal antibody raised against adult human slow myosin heavy chain or A4.951 was from Developmental Studies Hybridoma Bank, developed by Dr. Blau. Appropriate secondary conjugated antibodies (GARIGALexa555 and GAMIG₁Alexa488) were purchased from Molecular Probes (Leiden, The Netherlands).

Western blotting. Equal amounts of protein (40 μ g/lane) were run on either 10% (S6K1 and S6) or 12% (AMPK and 4E-BP1) SDS-polyacrylamide (200 V, miniprotein 3 cell; Bio-Rad, Hercules, CA), and proteins were transferred (2 h, 250 mA, Criterion blotter; Bio-Rad) to 0.45-mm nitocellulose membranes. After Ponceau S staining and destaining, membranes were blocked in 5% nonfat dry milk power (NFD; Bio-Rad) in Tris-buffered saline containing 0.1% Tween 20 (TBST) for 1 h. Thereafter, a 1:1,000 dilution of the primary (phospho)-specific antibody (all from Cell Signaling Technology) in 5% NFD-TBST was added and incubated overnight at 4°C on a shaker. After the membranes were washed four times for 5 min in 15 ml 5% NFD-TBST, the membranes were incubated with a 1:10,000 dilution of the horseradish peroxidase-conjugated secondary antibody (Pierce) in 5% NFD-TBST. Thereafter, the membranes were washed in 25 ml of TBST for 5, 15, 5, and 5 min. Light-sensitive film (CL-Xposure; Pierce) was used to detect immunoreactive bands using chemiluminescent substrate (SuperSignal CL; Pierce). Autoradiographic film was scanned densitometrically, and quantification was performed with the program Quantity One version 4.2.1 (Bio-Rad). α -Actin was used to standardize for the amount of protein loaded. Phosphorylation of AMPK, 4E-BP1, S6K1, and S6 was expressed relative to the total amount of each protein. Data were analyzed as the percent change in phosphorylation state from preexercise values for each subject.

Immunohistochemistry. Multiple serial sections (5 μ m) from biopsy samples collected before, immediately after, and 30 and 120 min after exercise were thaw-mounted together on uncoated, precleaned glass slides for each subject, carefully aligned for cross-sectional analysis. Sections were fixed for 5 min in methanol, followed by 1 min in acetone. Slides were then incubated overnight at 4°C with anti-caveolin-3 and anti-phospho-S6K1 antibodies (1:200 and 1:50 in PBS, respectively). Slides were rinsed for 3 \times 5 min with PBS and then incubated for 45 min with appropriate secondary antibodies diluted together with 5 μ g/ml 4',6-diamidino-2-phenylindole (DAPI; to visualize nuclei) in PBS. After several washes with PBS, stained sections were embedded in Mowiol and covered with a coverslip. All muscle cross sections were stained and prepared within a single batch, using the same antibody preparation to minimize variability in staining efficiency. Serial sections were stained for slow myosin heavy chain with the monoclonal A4.951 antibody to determine the proportion and cross-sectional area of types I and II muscle fibers, which was measured as previously described (38).

After 24 h, glass slides were examined with a Nikon E800 fluorescence microscope (Uvikon, Bunnik, The Netherlands) coupled to a Basler A113 C progressive scan color charge-coupled device camera,

with a Bayer color filter. Epifluorescence signal was recorded with a Texas red excitation filter (540–580 nm) for S6K1, a fluorescein isothiocyanate excitation filter (465–495 nm) for caveolin-3, and a DAPI UV excitation filter (340–380 nm) for the nuclei.

Digitally captured images (\times 240 magnification) with five fields of view per muscle cross section (12 ± 1 fibers per field of view) were processed and analyzed with Lucia 4.81 software (Nikon, Düsseldorf, Germany). The phospho-S6K1-specific fluorescence signal was quantified for each muscle fiber, resulting in a total of 60 ± 2 muscle fibers analyzed for each muscle cross section (32 ± 2 type I and 27 ± 2 type II muscle fibers) and for each muscle nuclei, resulting in a total of 117 ± 7 muscle fiber nuclei analyzed for each muscle cross section (58 ± 5 nuclei of type I and 59 ± 5 nuclei of type II muscle fibers). Muscle nuclei were selected based on the DAPI staining. An intensity threshold representing minimal intensity values corresponding to nuclear phospho-S6K1 was set manually and was uniformly used for all images. Phospho-S6K1 content of muscle nuclei was expressed as the percentage of the nuclear area that was stained. Intracellular phospho-S6K1 content was expressed as mean staining intensity.

Statistics

All data are expressed as means \pm SE. ANOVA for repeated measures were applied to determine differences in phosphorylation status in the protein of interest over time. In case of a significant *F*-ratio, a Scheffé's post hoc test was applied to locate the differences. AMPK phosphorylation statuses before and after exercise were compared with a paired *t*-test. Statistical significance was set at $P < 0.05$.

RESULTS

Resistance Exercise

Mean 1RM measured during the pretest was 198 ± 7 kg on the horizontal leg press and 105 ± 3 kg on the leg extension exercise. Therefore, average weight lifted during the resistance exercise was set at 148 ± 5 and 79 ± 3 kg for the leg press and leg extension, respectively. All subjects completed eight sets with 10 repetitions on the leg press. One subject was unable to finish 10 repetitions during the sixth set, after which resistance was reduced to 65% of the individual 1RM. All subjects completed eight sets of 10 repetitions on the leg extension machine.

Western Blotting Results

Western blotting with phospho-specific antibodies showed that AMPK phosphorylation was increased threefold after resistance exercise ($P < 0.05$). Thr¹⁷² phosphorylation of AMPK assessed with Western blotting is shown in Fig. 1. Thr³⁷ phosphorylation of 4E-BP1 assessed with Western blotting is presented in Fig. 2. 4E-BP1 phosphorylation was reduced immediately after resistance exercise ($P < 0.05$), after which levels returned to baseline values after 30 min of recovery. After 120 min of postexercise recovery, 4E-BP1 phosphorylation was significantly reduced compared with resting values ($P < 0.05$). Thr⁴²¹/Ser⁴²⁴ phosphorylation of S6K1 assessed with Western blotting is shown in Fig. 3. After resistance exercise, S6K1 phosphorylation was increased and was significantly higher 30 min postexercise compared with basal resting values ($P < 0.05$). Ser²³⁵/Ser²³⁶ phosphorylation of S6 assessed with Western blotting is presented in Fig. 4. No significant differences in S6 phosphorylation were observed after exercise.

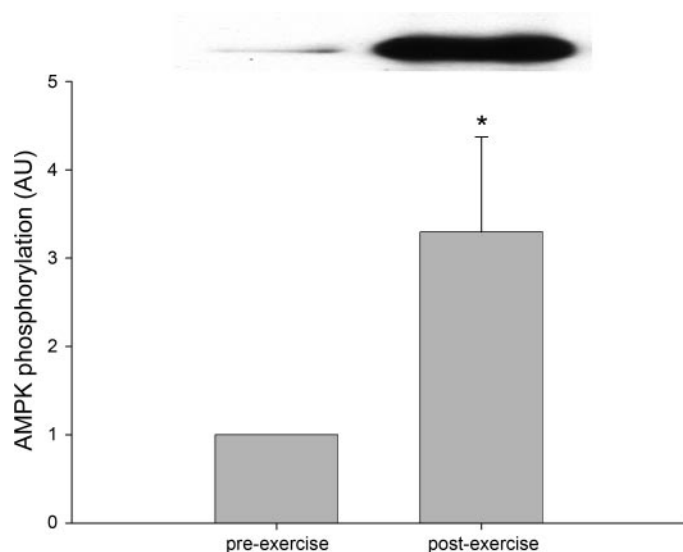


Fig. 1. AMP-activated protein kinase (AMPK) phosphorylation at Thr¹⁷² in vastus lateralis muscle at rest, immediately postexercise, and after 30 and 120 min of postexercise recovery. *Top*: representative immunoblots. Values are means + SE. AU, arbitrary unit. *Significantly different from resting values.

Immunohistological Analyses

Western blotting with phospho-specific antibodies showed that Thr⁴²¹/Ser⁴²⁴ phosphorylation of S6K1 was increased after resistance exercise and was significantly higher 30 min post-exercise compared with the observed resting values. Using immunohistochemical techniques, we were able to quantify changes in localization and phosphorylation of S6K1 in a fiber type-specific manner. To determine the cellular localization of S6K1 in muscle fibers, we used immunofluorescence staining of the muscle membrane, nuclei, and S6K1 (Thr⁴²¹/Ser⁴²⁴). As presented in Fig. 5, staining for phosphospecific S6K1 in the

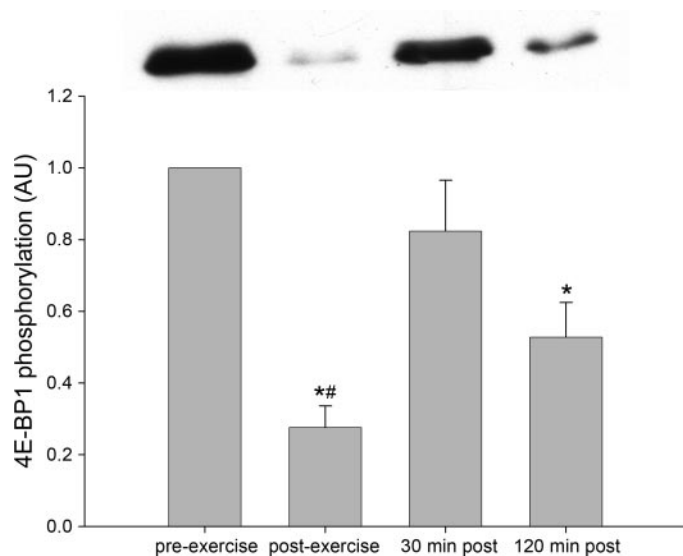


Fig. 2. Eukaryotic initiation factor 4E-binding protein (4E-BP1) phosphorylation at Thr³⁷ in vastus lateralis muscle at rest, immediately postexercise, and after 30 and 120 min of postexercise recovery. *Top*: representative immunoblots. Values are means + SE. *Significantly different from resting values; #significantly different from values observed after 30 min of postexercise recovery.

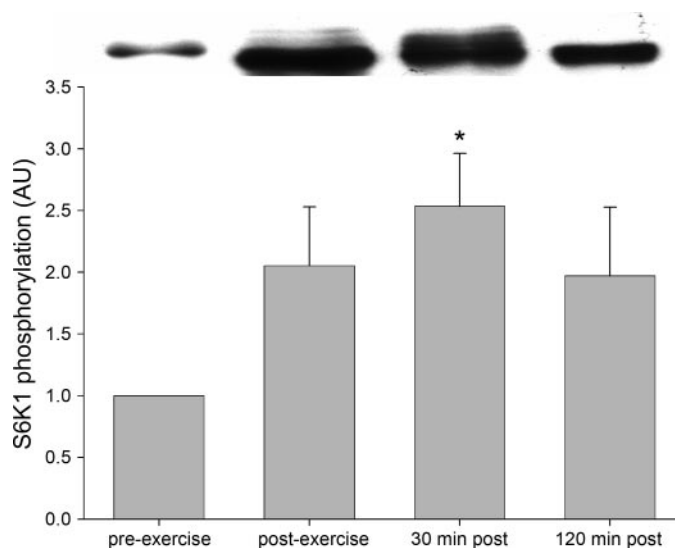


Fig. 3. p70/p85-S6 protein kinase (S6K1) phosphorylation at Thr⁴²¹/Ser⁴²⁴ in vastus lateralis muscle at rest, immediately postexercise, and after 30 and 120 min of postexercise recovery. *Top*: representative immunoblots. Values are means + SE. *Significantly different from resting values ($P < 0.05$).

preexercise muscle biopsies shows a predominant localization of phospho-S6K1 in the nuclei. However, after 2 h of postexercise recovery, we show a marked increase in phosphorylated S6K1 present in the cytosol (Fig. 6). In Fig. 6, images of representative cross sections of vastus lateralis muscle obtained before and 120 min after exercise with sections stained for phospho-S6K1 are presented. Phosphorylation of S6K1 was increased after resistance exercise in both the type I and type II fibers (Fig. 7; $P < 0.05$). However, phosphorylation of S6K1 increased to a greater extent in the type II fibers than in the type I fibers (Fig. 7; $P < 0.05$). At 30 min after the resistance exercise session, nuclear phospho-S6K1 content was increased in the type II fibers only ($P < 0.05$).

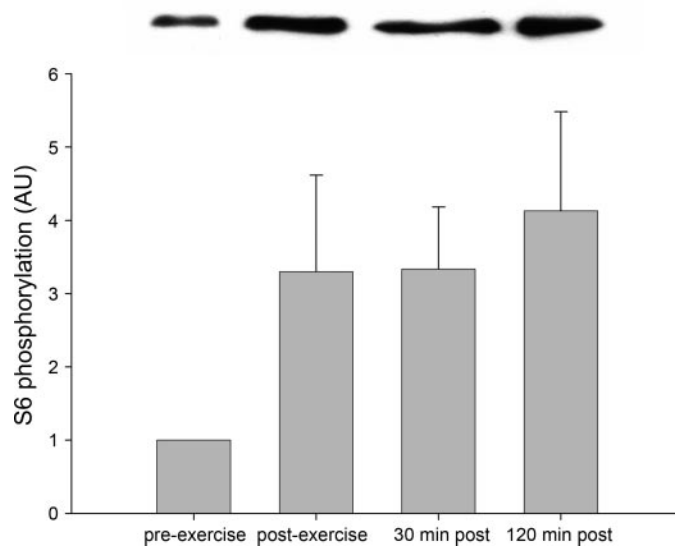


Fig. 4. Ribosomal S6 protein (S6) phosphorylation at Ser^{235/236} in vastus lateralis muscle at rest, immediately postexercise, and after 30 and 120 min of postexercise recovery. *Top*: representative immunoblots. Values are means + SE. No differences were observed over time.

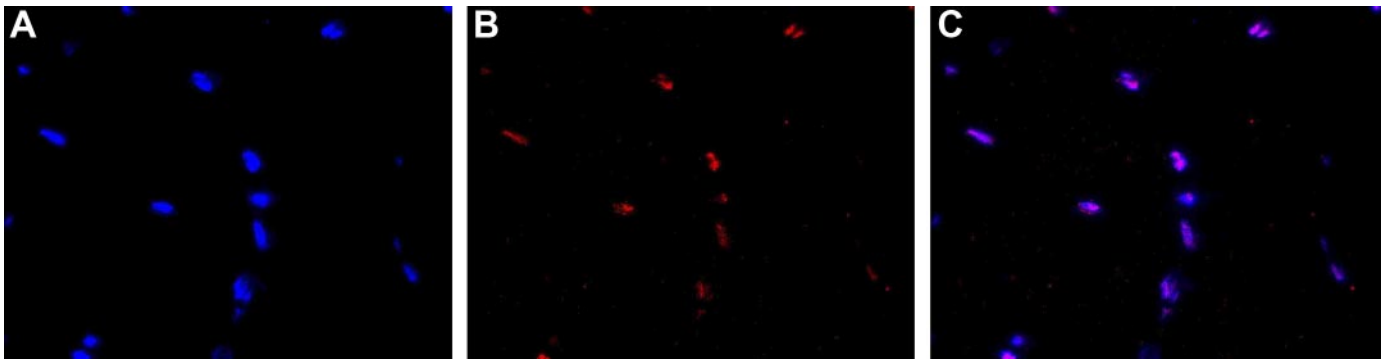


Fig. 5. Immunofluorescent images ($\times 360$ magnification) of muscle nuclei (A) and S6K1 (Thr⁴²¹/Ser⁴²⁴) (B) in the preexercise muscle biopsies showing nuclear localization of phospho-S6K1 (C). The nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI).

On average, muscle samples taken from the vastus lateralis muscle consisted of $53 \pm 2\%$ of type I and $47 \pm 3\%$ of type II muscle fibers. Type II muscle fibers had a significantly greater cross-sectional area than the type I fibers ($5,243 \pm 331$ vs. $4,668 \pm 240 \mu\text{m}^2$; $P < 0.05$). No differences between fiber types were observed in average number of myonuclei per muscle fiber cross section (2.1 ± 0.3 vs. 2.3 ± 0.2 nuclei per fiber for type I and II muscle fibers, respectively; not significant). In addition, nuclei area did not differ between type I and II muscle fibers (31.8 ± 0.9 vs. $31.7 \pm 1.0 \mu\text{m}^2$, respectively).

DISCUSSION

In the present study, we show that a single bout of resistance exercise leads to the phosphorylation of S6K1 in human skeletal muscle in a fiber type-dependent manner, with the most pronounced phosphorylation being observed in the type II

muscle fibers. The phosphorylation of S6K1 observed after exercise was not accompanied by a substantial activation of S6K1. The latter could, in part, be attributed to the observed immediate exercise-induced increase in AMPK phosphorylation, which has been shown to induce reductions in 4E-BP1 phosphorylation and S6K1 activation (5).

Although the metabolic response to resistance exercise has not been studied extensively, it has been shown that muscle lactate concentrations quickly increase up to ~ 17 mmol/kg wet muscle wt, during a single session of resistance exercise training in vivo in humans (13, 36). Furthermore, we observed that skeletal muscle fiber glycogen content declines substantially in both type I and II muscle fibers following resistance-type exercise tasks (20). In line with these findings, we observed a marked increase in the phosphorylation of AMPK in the present study (Fig. 1). This indicates that resistance exer-

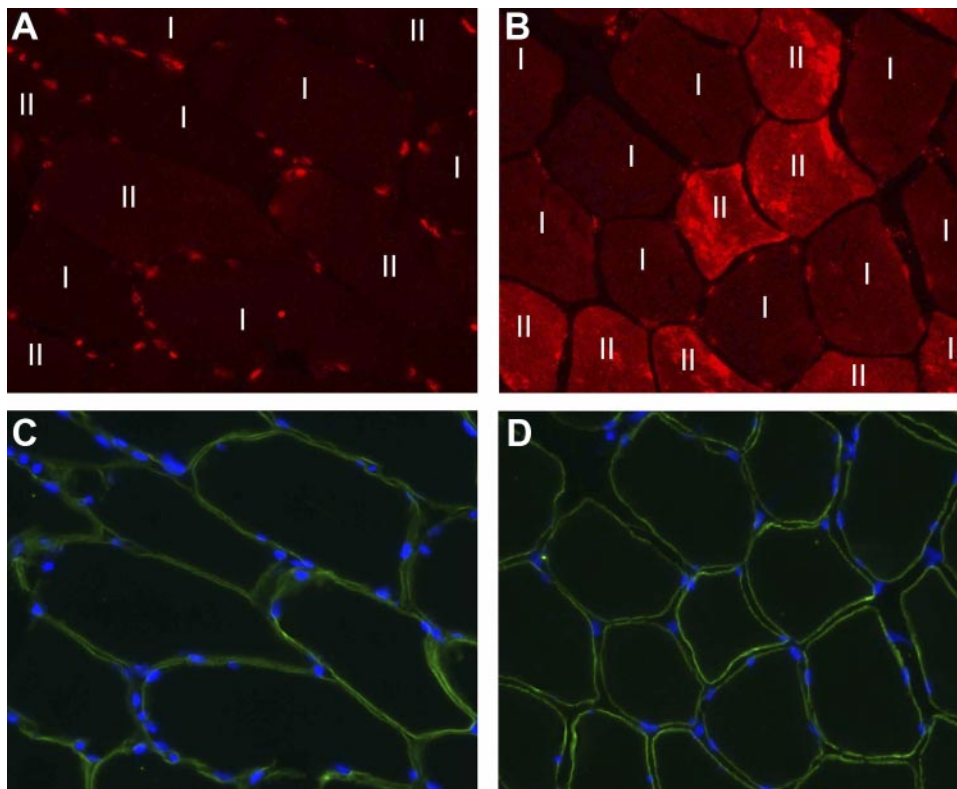


Fig. 6. Images ($\times 240$ magnification) of representative cross sections of vastus lateralis muscle showing primarily nuclear localization of phospho-S6K1 in muscle samples obtain before exercise (A). Phospho-S6K1 is primarily located in the cytosol of type II muscle fibers after 2 h of postexercise recovery (B). Staining for caveolin-3 and nuclei are shown in C and D (showing the cell membranes in green and nuclei in blue). The muscle fibers are labeled as type I and type II.

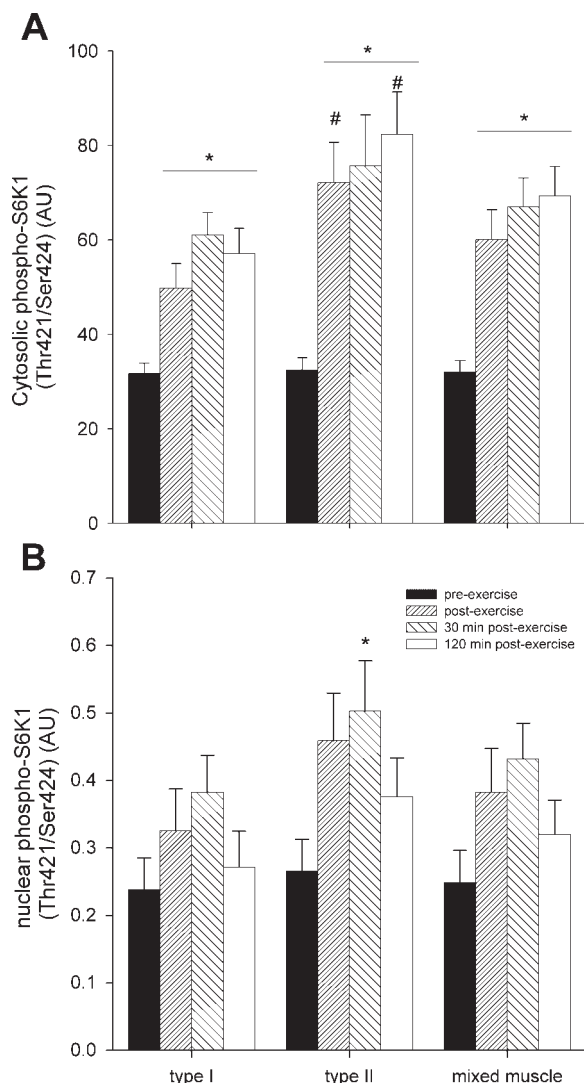


Fig. 7. Effect of resistance exercise on the phosphorylation status of S6K1 in the cytosol (A) and nuclei (B). Mean fiber type-specific intramyocellular phospho-S6K1 (expressed as staining intensity) and mean fiber type-specific nuclear phospho-p70/p85-S6K (expressed as % area stained) before and immediately after exercise and after 30 and 120 min of postexercise recovery as determined by fluorescence microscopy on stained muscle cross sections. Data provided are means + SE. *Significantly different from preexercise values ($P < 0.05$); #significantly different from values observed in type I fibers ($P < 0.05$).

cise leads to a reduction in the energy status of the muscle fiber, thereby stimulating the phosphorylation of skeletal muscle AMPK. As far as we know, this is the first study to report an increase in AMPK phosphorylation following resistance-type exercise activities. In accordance, earlier studies have reported that high-intensity endurance exercise (7, 30, 42) but not low- or moderate-intensity exercise (7, 39) augments AMPK activation. The latter requires AMPK phosphorylation on Thr¹⁷² by an AMPK kinase. The major AMPK kinase in skeletal muscle is LKB1 (32). In addition, AMPK phosphorylation has been associated with the concomitant decline in muscle glycogen content, as AMPK has been suggested to have a glycogen-binding domain (32), which would explain the observation that AMPK is activated to a greater extent when muscle glycogen concentrations are low (42). Although AMPK

is generally regarded as a main energy sensor in the cell, being responsible for the regulation of skeletal muscle glucose and fatty acid uptake and oxidation (18, 27), it was recently proposed that the AMPK might also play an important role in the regulation of muscle protein synthesis and/or degradation (5, 8).

Bolster and coworkers (5) were the first to show that AMPK activation in skeletal muscle tissue, by 5-aminoimidazole-4-carboxamide 1- β -D-ribofuranoside (AICAR) administration in rats, reduces protein synthesis rates. The latter was associated with a reduced phosphorylation state of mTOR at Ser²⁴⁴⁸, S6K1 at Thr³⁸⁹, and 4E-BP1 at Thr³⁷ (5). More recently, it was proposed that activation of AMPK results in mTOR phosphorylation at Thr²⁴⁴⁶ (8), which decreases mTOR phosphorylation at Ser²⁴⁴⁸, thereby diminishing the ability of insulin (and other growth factors) to phosphorylate S6K1 (8). These observations are indicative of the presence of another metabolic master switch mechanism in which AMPK plays a key role by integrating signals associated with the energy state of the cell and growth factors associated with protein translation.

The present study shows that a single session of high-intensity resistance exercise in vivo in humans significantly increases AMPK phosphorylation and reduces the phosphorylation status of 4E-BP1 in skeletal muscle tissue immediately after cessation of exercise (Fig. 2). The exercise bout did not significantly increase S6 phosphorylation (Fig. 4), indicating that S6K1 activity was not substantially increased. The latter is in close agreement with observations in vitro in H2K myotubes (8) and in vivo in rats (5) showing AMPK phosphorylation to suppress S6K1 activity. The kinase activity of S6K1 is controlled by a series of phosphorylation steps at several Ser/Thr residues (12, 40). Phosphorylation of S6K1 in the carboxy-terminal auto-inhibitory domain (at Ser⁴¹¹, Ser⁴¹⁸, Thr⁴²¹, and Ser⁴²⁴) results in a conformational change in the protein, allowing the phosphorylation of Ser⁴⁰⁴, Thr³⁸⁹ in the linker domain and Thr²²⁹ in the catalytic domains, which activates the kinase (29, 40). Consequently, the ribosomal protein S6 on the 40S subunit is phosphorylated, which is thought to alter the interaction of the protein with the eIFs and the mRNA (7). Because S6 is one of the main substrates of S6K1, the phosphorylation status of S6 can be used as a measure of the activity of S6K1 (34). However, S6 might not be as important as previously thought because translational activation of specific mRNAs containing terminal oligopyrimidine tracts adjacent to the 5' cap has been shown to occur in the absence of phosphorylation of S6 (34, 35).

In the present study, we show that a single session of high-intensity resistance exercise was not sufficient to fully activate S6K1. The exercise effect of S6K1 was likely to be limited to Thr⁴²¹/Ser⁴²⁴, as we did not observe a significant increase in the phosphorylation of S6. The extracellular signal-regulated kinase-1/2 (ERK1/2) has been suggested to play an important role in phosphorylation of S6K1 on Thr⁴²¹/Ser⁴²⁴. In accordance, ERK1/2 phosphorylation has been shown to be substantially increased immediately after exercise (17, 26). Our observations are in agreement with data reported by Karlsson et al. (17) showing that a single session of resistance exercise (4 sets, 10 repetitions of leg extension) does not fully activate S6K1. However, they showed that postexercise ingestion of branched-chain amino acids has a striking effect on S6K1, which was evident by site-specific phosphorylation at Thr³⁸⁹

(17). These data indicate that intake of an ample amount of protein/amino acids is needed to fully activate skeletal muscle translation initiation. In contrast to these and our observations, there are some studies in rodents that have reported an increase in S6K1 activity in response to either isometric or eccentric contractions (2, 3, 16, 25). The apparent contradictory findings could possibly be attributed to differences in the exercise model that was applied, i.e., electrical stimulation vs. voluntary contraction (14).

In the present study, we also aimed to study muscle fiber type-specific effects of resistance exercise on S6K1 phosphorylation. Using immunohistochemical techniques, we were able to quantify changes in phosphorylation of S6K1 at Thr⁴²¹/Ser⁴²⁴ in a fiber type-specific manner. Phosphorylation of S6K1 was increased after resistance exercise in both the type I and type II fibers (Fig. 6). However, phosphorylation of S6K1 increased to a greater extent in the type II fibers compared with the type I fibers ($P < 0.05$). In accordance, the phosphorylation of PKB (or Akt), mTOR, and S6K1 following resistance exercise in rats has been reported to be more pronounced in muscle groups containing a greater proportion type II vs. type I muscle fibers (e.g., tibialis anterior vs. soleus) (3, 27, 31). Moreover, by the application of immunohistochemistry in rat tibialis anterior muscle of rats, it has recently been shown that mTOR phosphorylation is selectively increased in type IIa fibers several hours after a bout of resistance exercise (27). These observations are most likely due to specificity in muscle fiber type recruitment. Interestingly, we have recently shown that a single session of resistance exercise results in a substantial reduction in muscle fiber glycogen content. Moreover, these changes are fiber type specific and are closely related to fiber type oxidative capacity. As such, net changes in muscle fiber glycogen content were more pronounced in type II fibers than in type I muscle fibers, which can be attributed to the greater recruitment of these fibers during high-intensity resistance exercise (14). One could, therefore, speculate that AMPK phosphorylation will increase to a greater extent in these fibers, phosphorylating mTOR on Thr²⁴⁴⁶, thereby decreasing the capacity of the kinase to phosphorylate S6K1 at Thr³⁸⁹. This could explain why we did not observe a substantial increase in S6 phosphorylation during the 2 h of postexercise recovery, while phosphorylation of S6K1 on Thr⁴²¹/Ser⁴²⁴ was increased.

The localization of proteins involved in the regulation of translation initiation is thought to play an important role in the modulation of protein synthesis (18). Proteins like mTOR and S6K1 have been reported to be localized both in the cytosol and in the nuclei of HEK-293 cells (18). Interestingly, it has been demonstrated that in vitro nuclear import of mTOR enhances 4E-BP1 phosphorylation and S6K1 activation, whereas nuclear export of mTOR attenuated the phosphorylation/activation, suggesting that both activation of S6K1 and phosphorylation of 4E-BP1 require nuclear mTOR (18). Similar nuclear transport has also been reported for S6K1 (18). In light of these observations, the present study also examined the subcellular location of S6K1, using immunofluorescence staining of the muscle membrane, nuclei and S6K1 (Thr⁴²¹/Ser⁴²⁴). In the preexercise muscle biopsies, we show a predominant nuclear localization of phospho-S6K1 (Figs. 5 and 6, A and C), whereas after the resistance exercise session, phosphorylated S6K1 is primarily located in the cytosol (Fig. 6, B and D),

especially in type II fibers. Interestingly, we show that 30 min after cessation of exercise nuclear phospho-S6K1 content was increased in the type II fibers. The latter could imply that the nucleus plays a key role in the activation of S6K1 and/or that S6K1 functions as a transcription factor. However, because the antibody that was used recognizes both p70^{S6K} and p85^{S6K}, we cannot differentiate between nuclear import of S6K1 and phosphorylation of p85^{S6K}, the latter of which is known for its unique nuclear localization (11, 22). More research is warranted to determine the role of subcellular location of S6K1 and its potential regulatory role.

We conclude that a single bout of resistance exercise in vivo in humans activates skeletal muscle AMPK, resulting in a decrease in the phosphorylation state of 4E-BP1. During recovery, S6K1 phosphorylation is increased and shown to be fiber type specific, indicating that the signaling response to S6K1 is muscle fiber type specific. However, in the absence of food intake, the phosphorylation status of S6K1 on Thr⁴²¹/Ser⁴²⁴ is not accompanied by a substantial increase in the phosphorylation of S6 under fasting conditions. As such, in the absence of food intake, exercise does not fully activate the translation initiation process in skeletal muscle tissue.

ACKNOWLEDGMENTS

We gratefully acknowledge the enthusiastic support of the subjects who volunteered to participate in these trials.

The monoclonal antibody A4.840 developed by Dr. Blau was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health and Human Development and maintained by the University of Iowa (Department of Biological Science, Iowa City, IA 52242).

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