TRANSLATIONAL PHYSIOLOGY

Branched-chain amino acids increase p70^{S6k} phosphorylation in human skeletal muscle after resistance exercise

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[1]*Department of Health and Sport Sciences, University College of Physical Education and Sports, Stockholm S-114 86;*
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Karlsson, Håkan K. R., Per-Anders Nilsson, Johnny Nilsson, Alexander V. Chibalin, Juleen R. Zierath, and Eva Blomstrand. Branched-chain amino acids increase p70^{S6k} phosphorylation in human skeletal muscle after resistance exercise. *Am J Physiol Endocrinol Metab* 287: E1–E7, 2004. First published March 2, 2004; 10.1152/ajpendo.00430.2003.—The aim of the study was to investigate the effect of resistance exercise alone or in combination with oral intake of branched-chain amino acids (BCAA) on phosphorylation of the 70-kDa S6 protein kinase (p70^{S6k}) and mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK1/2), and p38 MAPK in skeletal muscle. Seven male subjects performed one session of quadriceps muscle resistance training (4×10 repetitions at 80% of one repetition maximum) on two occasions. In a randomized order, double-blind, crossover test, subjects ingested a solution of BCAA or placebo during and after exercise. Ingestion of BCAA increased plasma concentrations of isoleucine, leucine, and valine during exercise and throughout recovery after exercise (2 h postexercise), whereas no change was noted after the placebo trial. Resistance exercise led to a robust increase in p70^{S6k} phosphorylation at Ser^{424} and/or Thr^{421}, which persisted 1 and 2 h after exercise. BCAA ingestion further enhanced p70^{S6k} phosphorylation 3.5-fold during recovery. p70^{S6k} phosphorylation at Thr^{389} was unaltered directly after resistance exercise. However, during recovery, Thr^{389} phosphorylation was profoundly increased, but only during the BCAA trial. Furthermore, phosphorylation of the ribosomal protein S6 was also increased in the recovery period only during the BCAA trial. Exercise led to a marked increase in ERK1/2 and p38 MAPK phosphorylation, which was completely suppressed upon recovery and unaltered by BCAA. In conclusion, BCAA, ingested during and after resistance exercise, mediate signal transduction through p70^{S6k} in skeletal muscle.

exercise training; mitogen-activated protein kinase; protein synthesis; signal transduction

RESISTANCE EXERCISE leads to changes in protein turnover in skeletal muscle that occur up to 48 h into the recovery period (22). Regular resistance exercise training will induce an increase in protein synthesis and muscle mass. The anabolic benefits of resistance exercise training are likely to be mediated through changes in signal transduction. The signaling networks controlling protein synthesis through translational initiation involve phosphorylation of the mammalian target of rapamycin and sequential activation of the 70-kDa S6 protein kinase (p70^{S6k}) and the eukaryotic initiation factor 4E-binding protein 1 (13, 14, 20). However, the specific cascades linking growth stimuli to the activation of protein synthesis in human skeletal muscle are not fully resolved.

Increased amino acid availability and exercise both directly increase protein synthesis in skeletal muscle. Branched-chain amino acids (BCAA; leucine, isoleucine, and valine), and in particular leucine, have anabolic effects on protein metabolism by increasing the rate of protein synthesis and decreasing the rate of protein degradation in resting human skeletal muscle (17, 19). During recovery from exercise, BCAA have an anabolic effect in human skeletal muscle (4). Furthermore, administration of BCAA increases the phosphorylation of proteins involved in the regulation of protein synthesis, including p70^{S6k}, in human skeletal muscle (16). Exercise-induced p70^{S6k} activity correlates with increased skeletal muscle mass after 6 wk of resistance training (1a) and with elevated rates of protein synthesis in rat skeletal muscle at 12 and 24 h of recovery (12). Thus changes in p70^{S6k} phosphorylation in skeletal muscle after exercise can reflect activation of putative signaling pathways that may account for increases in protein synthesis during the early recovery phase after exercise. However, the effects of exercise and amino acid supplementation on p70^{S6k} phosphorylation directly after exercise and in the early recovery phase are unknown.

Muscle hypertrophy through increased protein synthesis may require activation of the mitogen-activated protein kinase (MAPK)-signaling cascades. Increased MAPK signaling in response to endurance exercise regulates the activity of various transcription factors in skeletal muscle (26, 29). Resistance exercise has recently been reported to increase phosphorylation of extracellular signal-regulated kinase (ERK)1/2 MAPK, but not p38 MAPK, in skeletal muscle from young male subjects (27). However, information regarding the effects of BCAA on MAPK signaling during and after exercise is lacking.

The purpose of the present study was to investigate the effect of resistance exercise alone or in combination with ingestion of BCAA on phosphorylation of p70^{S6k}, the ribosomal protein S6, ERK1/2 MAPK, and p38 MAPK. We hypothesized that BCAA

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Fig. 1. Recording of electromyographic (EMG) activity (arbitrary units) in vastus lateralis muscle (VL dxt and VL sin, right and left legs) and hamstring muscle (Ham sin, left leg), and displacement during concentric and eccentric action from 1 subject during repeated contractions during the prestudy trial. Right vertical bar denotes EMG amplitude at isometric maximal voluntary contraction (EMG IMVC).
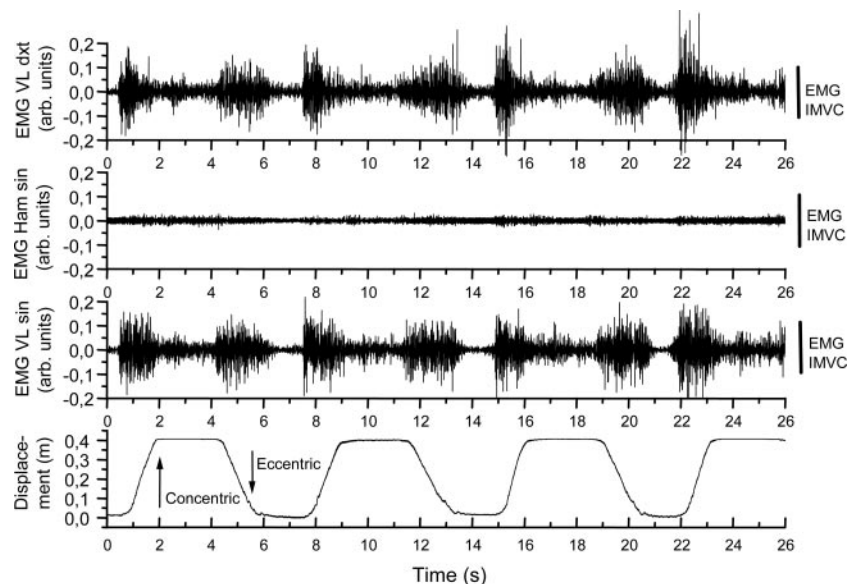
would potentiate the effects of resistance exercise on signal transduction. To test this hypothesis, healthy subjects ingested a solution of BCAA or placebo during and after resistance exercise. Skeletal muscle biopsies were obtained, and phosphorylation of p70^{S6k}, S6, ERK1/2 MAPK, and p38 MAPK was determined by immunoblot analysis by use of phosphospecific antibodies.

MATERIALS AND METHODS

Subjects. Seven healthy men of age 25 ± 1 yr, height 178 ± 2 cm, weight 74.4 ± 3.5 kg, and maximal oxygen uptake 3.78 ± 0.20 l/min participated in the study. The subjects reported performing endurance or resistance exercise training 1–2 times weekly. The Ethics Committee at the Karolinska Institute approved the study protocol. Subjects were fully informed of the nature and the possible risks associated with the study before they volunteered to participate. The investigation was performed according to the principles outlined in the Declaration of Helsinki.

Prestudy. Before the experiment, subjects participated in two preparatory tests. The first test was designed to determine one repetition maximum (1 RM). During the 1 RM test, a leg press was performed at a 90–180° knee angle. The load was progressively increased until the subject could not perform more than one single repetition. The subjects reached 1 RM within 5–6 trials. Subjects were allowed unlimited periods of rest between trials to avoid muscle fatigue. Electromyography (EMG) electrodes (Hellige Ag/AgCl surface electrodes with diameter of recording surface 9.5 mm) were placed over the right and left vastus lateralis muscles and the hamstring muscles on the left leg. A telemetry EMG system (model IC-600-G, Medinik, Uppsala, Sweden) was used to record muscle activity. A transducer (position transducer model 1850, Houston Scientific, Houston, TX) was connected between the chair and the stack wagon on the leg press machine to record the displacement amplitude and frequency of the exercise movement. A second preparatory exercise test was performed to familiarize the subjects with the intensity and the repetition frequency of the test exercise. During the second preparatory exercise test, subjects performed the actual standardized exercise routine scheduled to be performed during the study. The second preparatory test was performed ≥2 wk before the actual exercise study.

Maximal oxygen uptake was determined on a mechanically braked cycle ergometer (Monark 816E, Varberg, Sweden). The work rate was

gradually increased until exhaustion, as previously described (1). Oxygen uptake was measured using an on-line system (Amis 2001 Automated Metabolic Cart, Innovision, Odense, Denmark).

Experimental protocol. Subjects were asked to refrain from vigorous physical activity for the 2 days before the experiment. Subjects reported to the laboratory in the morning after an overnight fast. The subject was placed in a supine position, and a catheter was inserted into the antecubital vein for resting blood samples. In one subject, in the placebo trial, the catheter was not placed in the vein because of technical difficulties, and thus venous blood samples were taken before and 2 h after the exercise by venipuncture. Therefore, comparisons of plasma concentration of amino acids and insulin between the two conditions were made for six subjects. Muscle biopsy specimens were obtained from the lateral part of the right and left quadriceps (vastus lateralis) muscle with a Weil-Blakesley conchotome (AB Wisex, Mölndal, Sweden), as described (11).

Subjects exercised at 100 W for 10 min on a cycle ergometer for a warm-up bout. Thereafter, subjects were seated in the leg press machine and performed resistance exercise at a workload corresponding to 80% of 1 RM. A metronome with a frequency of 50 beats/min, along with verbal assistance, guided the subjects to perform each repetition at a set pace so that the concentric and eccentric phases were performed for 2.4 s each. A short pause (~1.2 s) in the
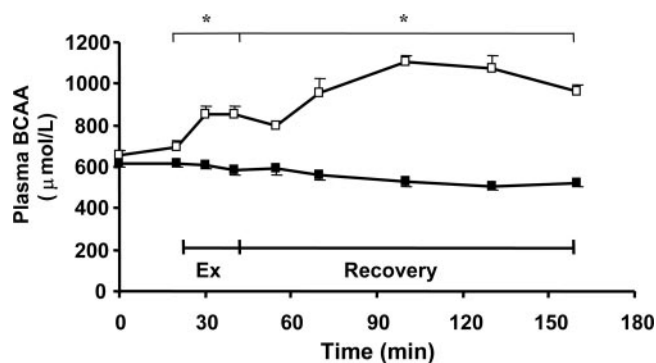

Fig. 2. Plasma concentration of branched-chain amino acids (BCAA; sum of leucine, isoleucine, and valine) before, during, and after 1 bout of resistance exercise (Ex) during placebo or BCAA trial. Open symbols, BCAA trial; filled symbols, placebo trial. Values are means $\pm$ SE for 6 subjects. *$P < 0.05$ for BCAA vs. placebo.

Table 1. *Plasma concentration of individual branched-chain amino acids*

Amino Acid	Condition	Rest	Exercise			Recovery, min				
			Before	During	After	15	30	60	90	120
Isoleucine	Placebo	100±3	98±3	98±3	92±5	90±5	85±4	79±4	75±3	80±4
	BCAA	110±2	121±5	155±8	154±10	141±9	176±19	209±9	184±10	169±8
Leucine	Placebo	187±5	187±6	185±7	176±7	175±10	164±6	153±6	148±5	153±6
	BCAA	200±9	220±14	278±15	277±13	256±9	322±28	374±10	340±16	320±12
Valine	Placebo	326±6	329±9	328±11	313±10	328±19	308±10	294±11	283±8	289±8
	BCAA	342±21	352±18	416±21	422±17	400±16	456±27	522±17	552±48	473±19

Values are means ± SE for 6 subjects in μmol/l. Plasma concentration of individual branched-chain amino acids (BCAA: isoleucine, leucine, and valine), as evaluated by comparison of corresponding areas under time-concentration curves, is significantly increased in the BCAA trial during and after exercise ($P < 0.05$ vs. placebo).

movement at both the upper (knee angle 180°) and lower (knee angle 90°) angles of the exercise increased the subjects' ability to control the force and speed of the exercise. The protocol consisted of four sets of 10 repetitions, with a 5-min rest between sets. Thus the resistance exercise protocol lasted for a total of 20 min.

Blood samples were taken at rest (before the warm-up and before any drink was supplied), immediately before resistance exercise, during resistance exercise at 10 min, immediately after resistance exercise, and during recovery at 15, 30, 60, 90 and 120 min after resistance exercise. Muscle biopsy specimens were taken at rest (before the warm-up period), immediately after resistance exercise, and during recovery at 1 and 2 h after resistance exercise. The first and third biopsies were taken from the right vastus lateralis muscle, and the second and fourth biopsies were taken from the left vastus lateralis muscle. The second biopsy in each leg was taken ~5 cm proximal to the first biopsy.

Subjects ingested a 150-ml solution of either a mixture of the three BCAA (45% leucine, 30% valine, and 25% isoleucine; Ajinomoto, Kanagawa, Japan) or flavored water before the warm-up exercise, immediately before the resistance exercise, during resistance exercise at 15 min, and during recovery at 15, 30, 60, and 90 min after resistance exercise. Both drinks contained lemon flavor, salts, citric acid, and an artificial sweetener. The two drinks were indistinguishable in taste. The subjects were provided a total of 100 mg of BCAA/kg body wt in 1,050 ml of flavored water. The amount of BCAA and the composition of the BCAA mixture were the same as in a previous study (4). The experiment was designed as a double-blind crossover test. The two experiments were performed with a 1-mo interval between them. Four subjects were supplied BCAA solution in the first experiment and placebo in the second; consequently, three subjects were supplied with placebo in the first and BCAA in the second experiment.

Blood chemistry. Blood samples were taken from the venous catheter and divided into two different glass tubes; one was heparinized for amino acid measurements, and one contained EDTA for measurements of insulin. The tubes were centrifuged (6,000 rpm for 2 min), and plasma was stored at −80°C. For amino acid determinations, plasma samples were deproteinized with 5% trichloroacetic acid (1:5) and centrifuged at 9,000 g for 2 min. The supernatant was stored at −80°C until analyzed. The concentration of amino acids was measured by reversed-phase HPLC, as described (21), with orthophthaldialdehyde as the derivatizing agent. Plasma insulin concentration was measured with a radioimmunoassay (Linco Research, St. Charles, MO).

Tissue processing. Muscle biopsies (30–100 mg) were cleaned of visible surface blood and placed in liquid nitrogen. Samples were freeze-dried and homogenized in 90 μl/mg dry wt ice-cold buffer (137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 0.5 mM Na₃VO₄, 1% Triton X-100, 7.5% glycerol, 20 mM Tris pH 8.0, 5 μg/μl leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 10 mM NaF). Homogenates were rotated for 30 min and centrifuged at 12,000 g for 10 min at 4°C. Protein was determined in aliquots of the supernatant using a Bradford protein assay (Bio-Rad Laboratories, Richmond, CA).

Immunoblot analysis. Aliquots of muscle lysate were solubilized in Laemmli sample buffer (45 μg protein phosphorylation or 300 μg for gel mobility shift assay), and proteins were separated by SDS-PAGE. After gel electrophoresis, proteins were transferred to polyvinylidene fluoride or nitrocellulose membranes for S6 phosphorylation (Bio-Rad Laboratories). Membranes were blocked in Tris-buffered saline (TBS; 10 mM Tris, pH 7.6, and 100 mM NaCl) containing 5% nonfat milk for 1 h at room temperature and then incubated for 2 h at room temperature with primary antibodies. For phosphorylation determinations, membranes were incubated with commercially available phosphospecific antibodies that recognize p70^{S6k} phosphorylated at Ser^{424}/Thr^{421}, ERK1/2 (p44/p42) MAPK phosphorylated at Thr^{202}/Tyr^{204}, p38 MAPK phosphorylated at Thr^{180}/Tyr^{182}, S6 phosphorylated at Ser^{235}/Ser^{236} (Cell Signaling Technology, Beverly, MA), and p70^{S6k} phosphorylated at Thr^{389} (Santa Cruz Biotechnology, Santa Cruz, CA). For the p70^{S6k} gel mobility shift assay, membranes were incubated with a polyclonal antibody that recognizes p70^{S6k} protein (New England Biolabs, Beverly, MA). After incubation with primary antibodies, membranes were washed with TBS, containing 0.1% Tween 20 (TBST) and with 2.5% nonfat dry milk added, and incubated with appropriate secondary antibody for 1 h at room temperature, followed by washing in TBST. Proteins were visualized by enhanced chemiluminescence reagents (Amersham Pharmacia Biotech, Uppsala, Sweden) and quantified by densitometric scanning with a Gel Doc 1000, in combination with Molecular Analyst software (version 1.5; Bio-Rad Laboratories).

The membranes described above were incubated in buffer (Tris·HCl pH 6.7, 2% SDS, and 100 μM β-mercaptoethanol) for 30 min at 60°C and reprobed to confirm equal sample loading. Protein expression of p70^{S6k}, ERK1/2 MAPK, p38 MAPK, and S6 was determined by immunoblot analysis, as described above, with the respective polyclonal antibodies (Cell Signaling Technology).

Statistics. All data are presented as means ± SE. Differences between BCAA and placebo conditions for plasma concentrations of
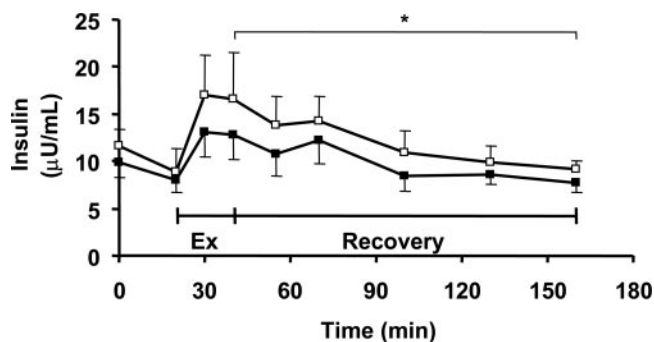

Fig. 3. Plasma concentration of insulin before, during, and after 1 bout of resistance exercise (Ex) during placebo or BCAA trial. Open symbols, BCAA trial; filled symbols, placebo trial. Values are means ± SE for 6 subjects. *$P <$ 0.05 for BCAA vs. placebo.

Fig. 4. 70-kDa S6 protein kinase (p70^{S6k}) phosphorylation at Ser^{424}/Thr^{421} in skeletal muscle at rest and after exercise during placebo and BCAA trials. Representative immunoblots are shown (*top*). Values in graph are arbitrary units (means $\pm$ SE for 7 subjects). #$P < 0.05$ vs. rest; *$P < 0.05$ for BCAA vs. placebo.
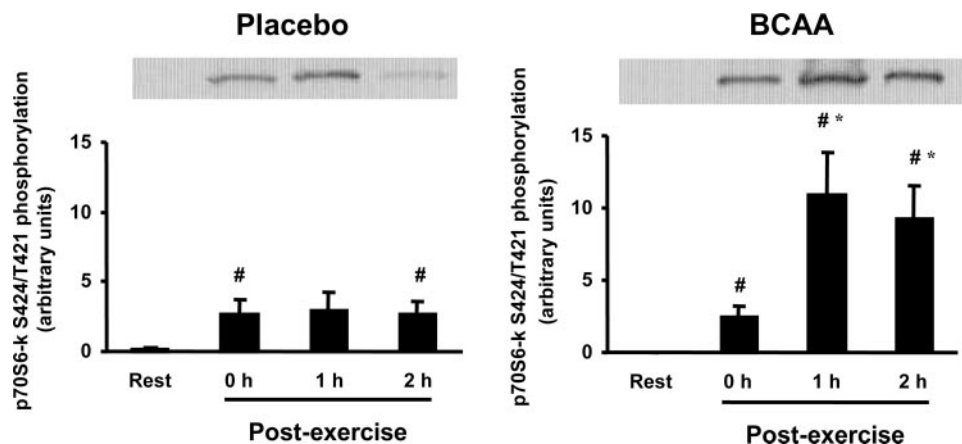
insulin and amino acids during exercise and recovery were evaluated by comparing the corresponding areas under the time vs. concentration curves. The concentrations at two consecutive time points were averaged, multiplied by the time span, and summed for the whole exercise or recovery period. The comparison between the areas was then made by Student's *t*-test for paired observations. A two-factorial (time, supplement) repeated-measures ANOVA was employed to compare changes in phosphorylation in skeletal muscle samples between the BCAA and placebo treatments. When a significant overall effect was indicated, a Pair-Wise Contrasts post hoc test was performed. Significance was accepted at $P < 0.05$.

RESULTS

Four subjects performed the stipulated 4×10 repetitions in both trials. The remaining three subjects did not complete the entire protocol; two subjects performed 1–2 fewer repetitions on one occasion, and one subject performed 10–11 fewer repetitions on both trials. EMG activity recorded during the first pretest revealed increased activity of the vastus lateralis muscle of both legs during repeated contractions at 80% of 1 RM with relatively little activation of the hamstring muscle (Fig. 1).

Ingestion of BCAA led to an increase in the plasma concentration of these amino acids, with levels still elevated 50–110% during the 2-h recovery period (Fig. 2). The individual BCAA, isoleucine, leucine, and valine (Table 1), were significantly elevated during and after exercise. Resistance exercise increased plasma insulin concentration in all subjects during the placebo and BCAA trials, respectively (Fig. 3). However, the change in plasma insulin concentration during exercise was not

significantly different between placebo and BCAA trials. Although plasma insulin concentration was significantly higher during the BCAA trial 2-h recovery period after exercise, this increase is unlikely to be physiologically relevant, because this particular parameter was within normal variability.

Ser^{424}/Thr^{421} phosphorylation of p70^{S6k} in skeletal muscle was increased after exercise (Fig. 4; $P < 0.05$). Ser^{424}/Thr^{421} phosphorylation of p70^{S6k}, assessed immediately after exercise, was similar between placebo and BCAA trials. The exercise effect on Ser^{424}/Thr^{421} phosphorylation of p70^{S6k} persisted into the recovery phase. This persistent effect of exercise on p70^{S6k} was further increased 3.5-fold ($P < 0.05$) during the BCAA trial. Thr^{389} phosphorylation of p70^{S6k} was unaltered immediately after resistance exercise in both the placebo and BCAA trials (Fig. 5). However, during recovery, Thr^{389} phosphorylation of p70^{S6k} was increased, but only during the BCAA trial (Fig. 5, $P < 0.05$). This was further confirmed by a decrease in the electrophoretic mobility of p70^{S6k} protein during recovery in the BCAA trial (Fig. 6). Importantly, phosphorylation of the ribosomal protein S6, a substrate of p70^{S6k}, was markedly increased in the recovery period only during the BCAA trial (Fig. 7, $P < 0.05$).

Resistance exercise led to a marked increase in phosphorylation of ERK1/2 MAPK (Fig. 8; $P < 0.05$) and p38 MAPK (Fig. 9; $P < 0.05$) immediately after exercise. One hour after exercise, ERK1/2 and p38 MAPK phosphorylation was similar to preexercise levels. In contrast to p70^{S6k}, phosphorylation of ERK1/2 and p38 MAPK was unaltered by BCAA.

Fig. 5. p70^{S6k} phosphorylation at Thr^{389} in skeletal muscle at rest and after exercise during placebo and BCAA trials. Representative immunoblots are shown (*top*). Values in graph are arbitrary units (means $\pm$ SE for 7 subjects). #$P < 0.05$ vs. rest; *$P < 0.05$ for BCAA vs. placebo.
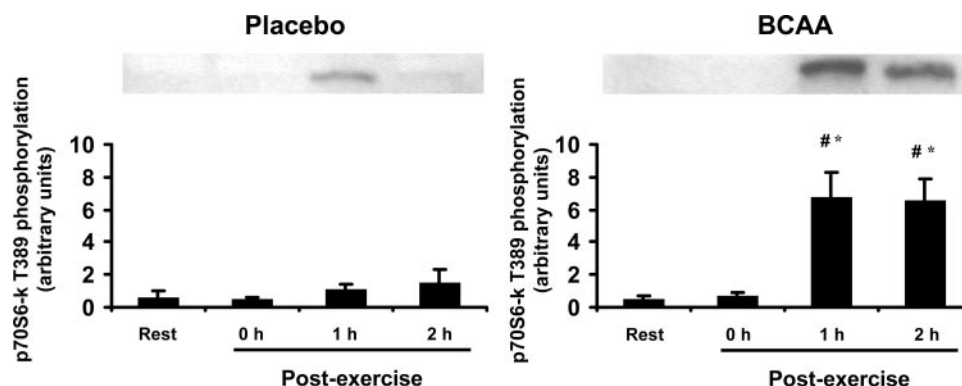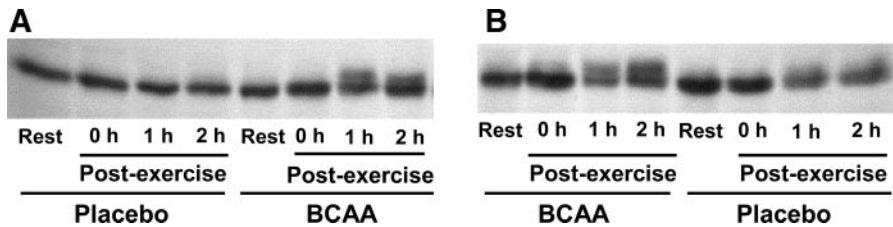
Fig. 6. p70^{S6k} phosphorylation as determined by 6% SDS-PAGE mobility shift assay. Muscle biopsies were obtained before and after exercise and during recovery in placebo and BCAA trials. Representative immunoblots (*A* and *B*) are shown for 2 subjects.

DISCUSSION

The major finding of the present study is that branched-chain amino acid ingestion during and after acute resistance exercise increases site-specific phosphorylation and activation of p70^{S6k} in skeletal muscle. This is evident by our findings of increased site-specific phosphorylation at Ser^{424}/Thr^{421} and Thr^{389}, decreased electrophoretic mobility of p70^{S6k} protein, and increased phosphorylation of the ribosomal protein S6, a substrate of p70^{S6k} in skeletal muscle, during recovery from exercise in the BCAA trial. Resistance exercise was also associated with increased phosphorylation of ERK1/2 and p38 MAPK. However, in contrast to p70^{S6k}, exercise effects on MAPK were transient, and levels returned to basal within 1 h after exercise. Furthermore, BCAA were without effect on MAPK, providing evidence that changes in p70^{S6k} phosphorylation are a specific, rather than a generalized, effect of BCAA.

p70^{S6k} is activated by phosphorylation at several Ser/Thr residues. Phosphorylation of four residues (Ser^{411}, Ser^{418}, Thr^{421}, and Ser^{424}) in the COOH-terminal autoinhibitory domain is required for altering the conformation of p70^{S6k} and making Thr^{389} and Ser^{404} in the linker domain and Thr^{229} in the catalytic domain available for phosphorylation, thereby activating p70^{S6k} (6, 23). One session of resistance exercise was not sufficient to fully phosphorylate and activate p70^{S6k}. However, exercise in combination with BCAA ingestion led to a striking effect on p70^{S6k}, as evident by site-specific phosphorylation at Thr^{389}. Phosphorylation of this residue is key for the activity of p70^{S6k} (6, 23). Moreover, phosphorylation of the ribosomal protein S6 was increased in skeletal muscle, indicating that p70^{S6k} was activated.

Repeated isometric contractions for 30 min (induced by stimulation of the sciatic nerve in rats) increase phosphorylation of p70^{S6k} at Thr^{389} in gastrocnemius muscle, with persistent effects noted 2 and 6 h after contraction (10). Furthermore, a skeletal muscle fiber type-specific effect of sciatic nerve

stimulation (60 contractions of 3 s over a 20-min period) has been reported on p70^{S6k} activity, as noted by a decrease in the electrophoretic mobility of p70^{S6k} protein, with largest effects in fast-twitch (extensor digitorum longus and tibialis anterior) muscles and no effect noted in slow-twitch (soleus) muscle (1a, 18). However, this apparent tissue-specific effect is likely to be related to the mode of contraction (concentric vs. eccentric) and intensity of the exercise, rather than to a fiber type difference, because a prolonged increase in p70^{S6k} activity was observed only after eccentric exercise (18). Our results provide evidence that acute resistance exercise increases p70^{S6k} phosphorylation in human skeletal muscle composed of a mixture of fibers with different contractile and metabolic characteristics (7, 8). However, the exercise effect on p70^{S6k} was limited to Ser^{424}/Thr^{421}, because phosphorylation of Thr^{389} and S6 was unaltered, indicating that the kinase was incompletely activated. This finding is in contrast to studies in rodents (1a, 10, 18), whereby p70^{S6k} activity is increased in skeletal muscle in response to either isometric or eccentric contractions achieved by electrical nerve stimulation. Thus a contraction-specific effect, rather than a fiber type-specific effect, may be responsible for the exercise-induced increase in p70^{S6k} activity. However, because the exercise regimen employed in the present study involved eccentric and concentric movements, other factors including exercise intensity or nutritional status may account for differences between responses in humans (present study) and rodents (1a, 10, 18). Future studies to explore whether exercise effects on p70^{S6k} activity in human skeletal muscle are dependent on mode of contraction (eccentric or concentric), exercise intensity, nutritional status, or skeletal muscle fiber type composition per se are warranted.

Ingestion of BCAA markedly enhanced site-specific phosphorylation of p70^{S6k} at Ser^{421}/Thr^{424} and Thr^{389} in human skeletal muscle 1 and 2 h after resistance exercise. Infusion of leucine for 2 h is associated with increased p70^{S6k} phosphorylation in skeletal muscle from human subjects at rest (9).
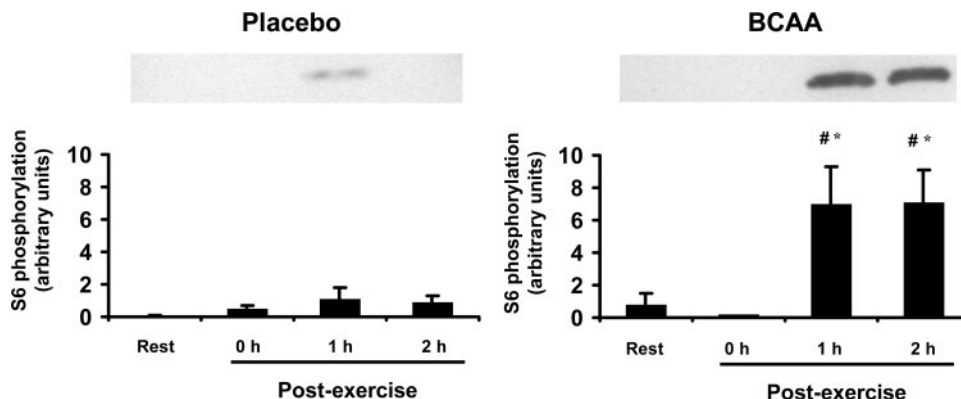

Fig. 7. Phosphorylation of the ribosomal protein S6 in skeletal muscle at rest and after exercise during placebo and BCAA trials. Representative immunoblots are shown (*top*). Values in graphs are arbitrary units (means ± SE for 7 subjects). #$P < 0.05$ vs. rest; *$P < 0.05$ for BCAA vs. placebo.

Fig. 8. ERK1/2 phosphorylation at Thr^{202}/Tyr^{204} in skeletal muscle at rest and after exercise during placebo and BCAA trials. Representative immunoblots are shown (*top*). Values in graphs are arbitrary units (means ± SE for 7 subjects). #$P < 0.05$ vs. rest.
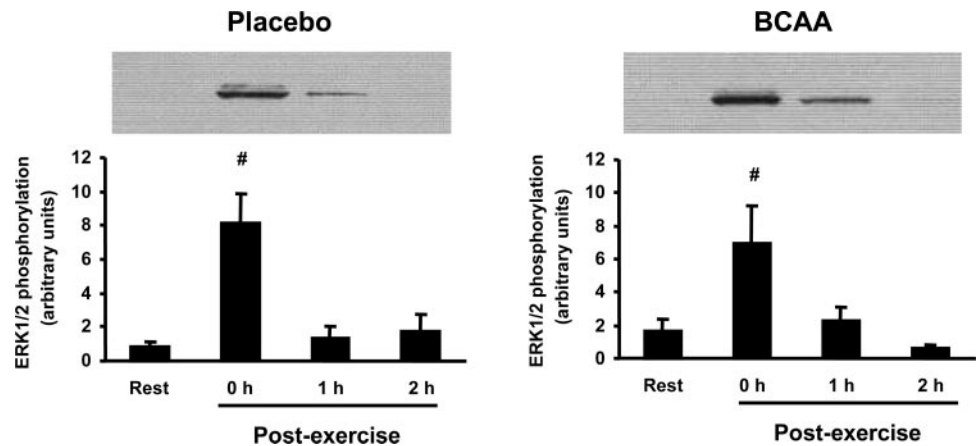
Coinfusion of leucine and insulin led to a further increase in p70^{S6k} phosphorylation, providing evidence that leucine and insulin may increase p70^{S6k} activity through different pathways (9). In a recent study (2), a combination of insulin and amino acids was required to stimulate protein synthesis and inhibit protein degradation. In the present study, plasma insulin concentration was increased during exercise, which is in agreement with a recent study (25). However, this is not a universal finding, since in some studies insulin is not altered by resistance exercise in the fasting state (3, 15). In any case, the increase in insulin levels observed in this study was mild, and at the time when p70^{S6k} phosphorylation was greatest, insulin levels were not higher than preexercise levels.

Intake of BCAA did not alter p70^{S6k} phosphorylation in skeletal muscle directly after exercise; rather, the effects were noted in the hours postexercise. This finding may possibly reflect the latent effect of amino acids on protein synthesis. In a recent study (5) in which a mixture of amino acids was infused to human subjects at rest, skeletal muscle protein synthesis was not altered during the first 30 min of amino acid infusion, but then rose rapidly between 30 and 60 min of infusion, and remained elevated for another hour of amino acid infusion.

A link between the MAPK-signaling pathway and translation initiation of protein synthesis has been suggested via MAPK-integrating kinase-1 (24). Resistance exercise was associated with increased ERK1/2 and p38 MAPK phosphorylation immediately after exercise. In a previous report, one session of knee extensor resistance exercise in young male

subjects was associated with an increase in ERK1/2 MAPK phosphorylation, with no effect on p38 MAPK phosphorylation (27). However, here we report that resistance training is associated with increased phosphorylation of ERK1/2 and p38 MAPK. The reason for the divergent results between these studies may be explained by the different exercise regimens. Eccentric contractions during the leg press exercise employed in the present study may have been more pronounced than those elicited during knee extensor exercise in the previous report (27). Consistent with this hypothesis, in isolated rat skeletal muscle, concentric contractions have been reported to increase ERK1/2 MAPK phosphorylation, with no effect on p38 MAPK, whereas eccentric contractions increase phosphorylation along both kinase cascades (28). Alternatively, the divergent effects on MAPK signaling may be related to the training status of the subjects, because exercise-induced signaling responses may be greater in trained subjects (30). The effect of resistance exercise on MAPK signaling is transient. Activation of ERK 1/2 and p38 MAPK returned to resting levels 1 h after resistance exercise, a finding consistent with previous work from our laboratory (26). However, the lack of an effect of BCAA on MAPK responses after exercise provides evidence that effects on p70^{S6k} are not generalized, but rather specific effects.

In summary, resistance exercise increased Ser^{424}/Thr^{421} phosphorylation of p70^{S6k} in skeletal muscle. This effect was sustained ≤2 h after exercise. Resistance exercise was without effect on phosphorylation of p70^{S6k} on Thr^{389} and S6 in skeletal muscle. ERK1/2 and p38 MAPK phosphorylation was

Fig. 9. p38 MAPK phosphorylation at Thr^{180}/Tyr^{182} in muscle biopsies in skeletal muscle at rest and after exercise during placebo and BCAA trials. Representative immunoblots are shown (*top*). Values in graph are arbitrary units (means ± SE for 7 subjects). #$P < 0.05$ vs. rest.
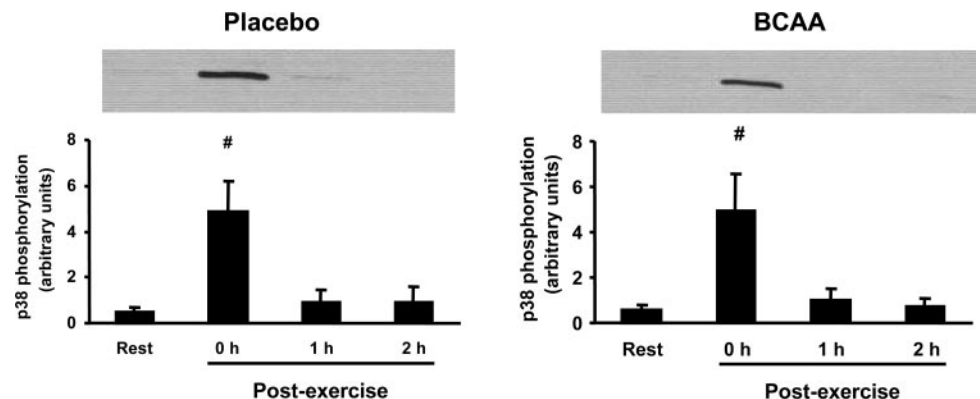
also increased after resistance exercise. However, this effect was transient, because ERK1/2 and p38 MAPK phosphorylation was indistinguishable from basal levels 1 h after exercise. Ingestion of BCAA during and after resistance exercise further increased phosphorylation of p70^{S6k} protein at Ser^{424}/Thr^{421}. Moreover, BCAA ingestion increased phosphorylation of p70^{S6k} protein at Thr^{389}, decreased electrophoretic mobility of p70^{S6k} protein, and increased S6 phosphorylation in skeletal muscle. In contrast to results for p70^{S6k}, BCAA ingestion was without effect on ERK 1/2 and p38 MAPK. Thus we speculate that BCAA may enhance protein synthesis in skeletal muscle during recovery from resistance exercise training through a p70^{S6k}-dependent (MAPK-independent) signaling cascade.

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