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## Blood flow restriction during low-intensity resistance exercise increases S6K1 phosphorylation and muscle protein synthesis

Satoshi Fujita,<sup>1,4</sup> Takashi Abe,<sup>4</sup> Micah J. Drummond,<sup>2,3</sup> Jerson G. Cadenas,<sup>1</sup> Hans C. Dreyer,<sup>2,3</sup> Yoshiaki Sato,<sup>4</sup> Elena Volpi,<sup>1</sup> and Blake B. Rasmussen<sup>2,3</sup>

Departments of <sup>1</sup>Internal Medicine and <sup>2</sup>Physical Therapy; and <sup>3</sup>Division of Rehabilitation Sciences, University of Texas Medical Branch, Galveston, Texas; and <sup>4</sup>Department of Human and Engineered Environmental Studies, Graduate School of Frontier Sciences, University of Tokyo, Chiba, Japan

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**Fujita S, Abe T, Drummond MJ, Cadenas JG, Dreyer HC, Sato Y, Volpi E, Rasmussen BB.** Blood flow restriction during low-intensity resistance exercise increases S6K1 phosphorylation and muscle protein synthesis. *J Appl Physiol* 103: 903–910, 2007. First published June 14, 2007; doi:10.1152/jappphysiol.00195.2007.—Low-intensity resistance exercise training combined with blood flow restriction (REFR) increases muscle size and strength as much as conventional resistance exercise with high loads. However, the cellular mechanism(s) underlying the hypertrophy and strength gains induced by REFR are unknown. We have recently shown that both the mammalian target of rapamycin (mTOR) signaling pathway and muscle protein synthesis (MPS) were stimulated after an acute bout of high-intensity resistance exercise in humans. Therefore, we hypothesized that an acute bout of REFR would enhance mTOR signaling and stimulate MPS. We measured MPS and phosphorylation status of mTOR-associated signaling proteins in six young male subjects. Subjects were studied once during blood flow restriction (REFR, bilateral leg extension exercise at 20% of 1 repetition maximum while a pressure cuff was placed on the proximal end of both thighs and inflated at 200 mmHg) and a second time using the same exercise protocol but without the pressure cuff [control (Ctrl)]. MPS in the vastus lateralis muscle was measured by using stable isotope techniques, and the phosphorylation status of signaling proteins was determined by immunoblotting. Blood lactate, cortisol, and growth hormone were higher following REFR compared with Ctrl ( $P < 0.05$ ). Ribosomal S6 kinase 1 (S6K1) phosphorylation, a downstream target of mTOR, increased concurrently with a decreased eukaryotic translation elongation factor 2 (eEF2) phosphorylation and a 46% increase in MPS following REFR ( $P < 0.05$ ). MPS and S6K1 phosphorylation were unchanged in the Ctrl group postexercise. We conclude that the activation of the mTOR signaling pathway appears to be an important cellular mechanism that may help explain the enhanced muscle protein synthesis during REFR.

mammalian target of rapamycin; ischemia; hypertrophy; protein metabolism; postexercise recovery

RESISTANCE EXERCISE is a potent stimulus for an increase in muscle protein synthesis and subsequent muscular hypertrophy. After an acute bout of high-intensity resistance exercise, muscle protein synthesis increases significantly within 1–2 h and remains elevated for up to 48 h (34, 36, 37). The mammalian target of rapamycin (mTOR) signaling pathway plays a significant role in stimulating translation initiation and muscle protein synthesis (50). Recent studies have shown that the activation of mTOR signaling pathway is gradually activated during the recovery phase of resistance exercise (5, 9, 20). In

fact, mTOR signaling to its downstream effector, ribosomal S6 kinase 1 (S6K1), is involved in the regulation of mRNA translation initiation and appears to be a critical regulator of exercise-induced muscle protein synthesis and training-induced hypertrophy (3, 4, 39). More recently, we have shown that following an acute bout of resistance exercise, the mTOR signaling pathway is activated in association with an increase in protein synthesis in human skeletal muscle (9). Furthermore, the phosphorylation of Akt/protein kinase B (PKB) increased and the phosphorylation of eukaryotic translation elongation factor 2 (eEF2) decreased during postexercise recovery (9). Akt activation promotes mTOR phosphorylation and signaling (50). Additionally, mTOR signaling to S6K1 inhibits eEF2 kinase, which reduces eEF2 phosphorylation and thus promotes translation elongation (50).

According to the size principle (9, 14, 15), motor units with a smaller cell size, i.e., slow-twitch type I muscle fibers, are recruited first at lower exercise intensities, whereas at higher exercise intensities, larger motor units and their associated type II muscle fibers are recruited. These fast-twitch type II fibers respond to high-intensity resistance exercise with a greater amount of hypertrophy than type I fibers (28, 31). Therefore, the American College of Sports Medicine (22) recommends that resistance exercise be performed at an intensity of at least 70% of an individual's one repetition maximum (1-RM) to achieve the maximal hypertrophy. Although these guidelines are optimal for healthy people, there are numerous circumstances in which it would be extremely difficult to achieve such a high exercise intensity level in populations such as the frail elderly, in patients with osteoarthritis, or in patients undergoing the immediate rehabilitation phase following surgery. Therefore, interventions designed to prevent muscle atrophy and/or enhance muscle hypertrophy using exercise protocols consisting of lower intensities may be useful for counteracting a variety of muscle wasting conditions, such as sarcopenia, cancer cachexia, AIDS, chronic obstructive pulmonary disease, stroke, trauma/surgery, etc. Low-intensity resistance exercise during blood flow restriction may be a useful intervention to enhance muscle growth in these clinical conditions.

A series of recent studies have shown that a low-intensity (20–50% of 1-RM) resistance exercise training combined with a moderate reduction of blood flow to the working muscle (REFR) produces similar increases in muscle size and strength compared with traditional high-intensity resistance training (1,

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42, 46, 47). However, the metabolic and molecular mechanisms responsible for inducing the increase in muscle hypertrophy during REFR are currently unknown.

Therefore, the purpose of the present study was to determine the effect of an acute bout of low-intensity resistance exercise (with or without blood flow restriction) on the muscle mTOR signaling pathway (an important regulator of translation initiation, elongation, muscle protein synthesis, and muscle cell growth) and muscle protein synthesis. We hypothesized that an acute bout of REFR would enhance mTOR signaling and stimulate muscle protein synthesis.

## EXPERIMENTAL PROCEDURES

### Subjects

We studied six young male subjects on two separate occasions. All subjects were healthy and physically active but were not currently engaged in an exercise training program. All subjects gave informed written consent before participating in the study, which was approved by the Institutional Review Board of the University of Texas Medical Branch. Screening of subjects was performed with clinical history, physical examination, and laboratory tests, including complete blood count with differential, liver and kidney function tests, coagulation profile, fasting blood glucose, and oral glucose tolerance test (OGTT), hepatitis B and C screening, HIV test, thyroid-stimulating hormone, lipid profile, urinalysis, drug screening, and ECG. The subjects' characteristics are summarized in Table 1. The subjects were initially randomized to an infusion study in which they performed resistance exercise during blood flow restriction (REFR) or a control group in which the subjects performed resistance exercise with no restriction of blood flow (Ctrl).

### Study design

Each subject was admitted to the General Clinical Research Center of the University of Texas Medical Branch the day before the exercise study, and a dual-energy X-ray absorptiometry (DEXA) scan (Hologic QDR 4500W, Bedford, MA) was performed to measure body composition and lean mass. The subjects were then fed a standard dinner, and a snack was given at 2200. The subjects were studied following an overnight fast under basal conditions and refrained from exercise for 24 h before study participation. The morning of the infusion study, at 0600, an 18-gauge polyethylene catheter was inserted into an antecubital vein for tracer infusion. Another 18-gauge polyethylene catheter was inserted retrogradely in a hand vein of the opposite arm, which was kept in a heated pad for arterialized blood sampling. After drawing a background blood sample, a primed continuous infusion of L-[ring- $^{13}\text{C}_6$ ]phenylalanine (Cambridge Isotope Laboratories, Andover, MA) was begun (time = 0 h at 0800) and maintained at a constant rate until the end of the experiment (Fig. 1). The priming

Table 1. Subject characteristics

Characteristic	Value
Age, yr	32 ± 2
Weight, kg	84 ± 4
Height, m	1.70 ± 0.02
BMI, kg/m <sup>2</sup>	29 ± 1
Leg lean mass, g	
Left leg	9,840 ± 447
Right leg	9,974 ± 541

Values are means ± SE; n = 6 subjects. Each subject was tested once during blood flow restriction (REFR) and a second time without the pressure cuff (Ctrl). REFR, resistance exercise with blood flow restriction; Ctrl, resistance exercise without blood flow restriction. BMI; body mass index.

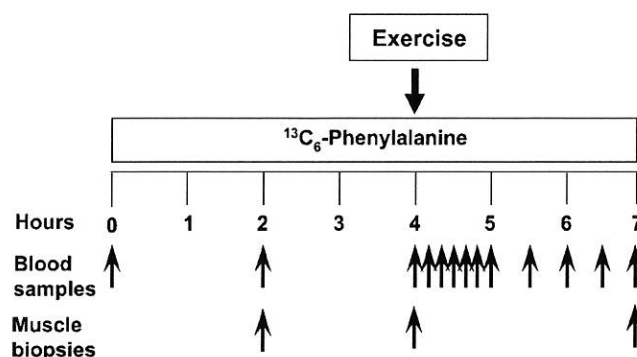


Fig. 1. Study design. Blood and muscle samples were taken at the times indicated by the arrows. Exercise was performed immediately after the second biopsy.

dose for the labeled phenylalanine was 2  $\mu\text{mol}/\text{kg}$ , and the infusion rate was 0.05  $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ .

Two hours following the initiation of the tracer infusion, the first muscle biopsy was obtained from the lateral portion of the vastus lateralis of the leg with the biopsy site between 15 and 25 cm from the midpatella. The biopsy was performed using a 5-mm Bergström biopsy needle under sterile procedure and local anesthesia (1% lidocaine). Muscle tissue was immediately blotted and frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  until analysis.

**REFR group.** After the second biopsy was obtained, a lower-extremity pressure cuff (Kaatsu-Master Mini, Sato Sports Plaza, Tokyo, Japan) was placed around the most proximal portion of each leg. While the subject was seated on a chair, the pressure cuff was increased to 120 mmHg for 30 s, and the air pressure was released. The pressure cuff was then inflated four more times with each period being increased by 20 mmHg. Each period lasted 30 s, and then the cuff was released for 10 s between periods until a final pressure of 200 mmHg was reached. With the pressure maintained at 200 mmHg, the subjects then performed a set of 30 repetitions of bilateral leg extension exercise at 20% of 1-RM, followed by a 30-s rest period. Subsequently subjects then performed three more sets of 15 repetitions with 30-s rest intervals for a total of four sets and 75 repetitions. Immediately after the fourth set, the pressure was released from the cuff. Total time for the exercise period was approximately 4–5 min.

**Resistance exercise without blood flow restriction: Ctrl group.** The subjects in the Ctrl group performed the identical exercise protocol as the REFR group except that the cuff was not inflated and no pressure was applied to the legs.

**Infusion study 2.** Three weeks after the first visit, subjects assigned to the REFR group during infusion study 1 repeated the protocol without blood flow restriction (Ctrl). Subjects assigned to the Ctrl group during infusion study 1 then completed the blood flow-restricted exercise protocol (REFR). Subjects were initially randomized to either the Ctrl or the REFR group, and therefore six subjects were studied for both groups.

### Hormones and Glucose/Lactate

Serum concentrations of growth hormone (GH), IGF-1, total testosterone, and cortisol were determined by chemiluminescent enzyme immunoassay using the Immunolite Automated Analyzer (Diagnostic Products, Los Angeles, CA). Plasma glucose and lactate concentration was measured using an automated glucose and lactate analyzer (YSI, Yellow Springs, OH).

### SDS PAGE and Western Blot Analysis

Details of the immunoblotting procedures have been previously published (9). Briefly, ~30–50 mg of frozen tissue was homogenized

(1:9, wt/vol), centrifuged for 10 min at 4°C, followed by the removal of the supernatant. Total protein concentrations were determined by using the Bradford assay (Bio-Rad, SmartSpec Plus spectrophotometer). The supernatant was diluted (1:1) in a sample buffer mixture containing 125 mM Tris (pH 6.8), 25% glycerol, 2.5% SDS, 2.5% B-mercaptoethanol, and 0.002% bromophenol blue and then boiled for 3 min at 100°C. Fifty micrograms of total protein was loaded into each lane, and the samples were separated by electrophoresis (100 V for 60 min) on a 7.5% polyacrylamide gel (Bio-Rad, Criterion). A molecular weight ladder (Bio-Rad, Precision Plus protein standard) was also included on each gel. Following electrophoresis, protein was transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA) at 50 V for 60 min. Blots were incubated in a single primary antibody overnight at 4°C (antibody concentrations are described below). The next morning, blots were incubated in secondary antibody for 1 h at room temperature. Chemiluminescent solution (ECL plus, Amersham BioSciences, Piscataway, NJ) was applied to each blot. After a 5-min incubation, optical density measurements were obtained with a phosphorimager (Bio-Rad) and densitometric analysis was performed using Quantity One software (version 4.5.2) (Bio-Rad). Preliminary experiments were performed to assess if protein abundance changed over the 7 h of the experiment. We found that protein abundance did not change over the short time frame of the study; therefore, the data were expressed as the change in phosphorylation (in arbitrary units) normalized to a rodent standard.

#### Antibodies

The primary antibodies used were all purchased from Cell Signaling (Beverly, MA): phospho-mTOR (Ser2448; 1:1,000), phospho-p70 S6K1 (Thr389; 1:500), phospho-Akt (Ser473; 1:500), and phospho-eEF2 (Thr56; 1:1,000). Anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody was purchased from Amersham Bioscience (1:2,000).

#### Muscle Fractional Synthetic Rate

Muscle intracellular free amino acids and muscle proteins were extracted as previously described (51, 52). Muscle intracellular free concentration and enrichment of phenylalanine was determined by gas chromatography-mass spectrometry (GCMS, 6890 Plus GC, 5973N MSD, 7683 autosampler, Agilent Technologies, Palo Alto, CA) using appropriate internal standard ( $L$ -[ $^{15}\text{N}$ ]phenylalanine) (51, 52). Mixed muscle protein-bound phenylalanine enrichment was analyzed by GCMS after protein hydrolysis and amino acid extraction (51, 52), using the external standard curve approach (6). We calculated the fractional synthetic rate of mixed muscle proteins (FSR) by measuring the incorporation rate of the phenylalanine tracer into the proteins ( $\Delta E_p/t$ ) and using the precursor-product model to calculate the synthesis rate:

$$\text{FSR} = (\Delta E_p/t) / [(E_{M1} + E_{M2})/2] \cdot 60 \cdot 100$$

where  $\Delta E_p$  is the increment in protein-bound phenylalanine enrichment between two sequential biopsies,  $t$  is the time between the two sequential biopsies, and  $E_{M1}$  and  $E_{M2}$  are the phenylalanine enrichments in the free intracellular pool in the two sequential biopsies. Data are expressed as percent per hour.

#### Statistical Analysis

All values are expressed as means  $\pm$  SE. Comparisons were performed using ANOVA with repeated measures, the effects being group (REFR, Ctrl) and time (baseline, postexercise). Post hoc testing was performed using Tukey-Kramer when appropriate. Significance was set at  $P < 0.05$ .

## RESULTS

### Blood pH and Lactate

There were no significant differences in blood pH and plasma lactate between groups before performing the bout of resistance exercise. However, peripheral blood pH decreased immediately after exercise in both groups ( $P < 0.05$ , Fig. 2A) and gradually returned to baseline levels. There were no differences between groups in the blood pH response to exercise; however, blood pH tended to return to the baseline value more slowly in the REFR group ( $P = 0.10$ ).

Plasma lactate concentration increased immediately after exercise and stayed high for 40 min after exercise in the REFR group ( $P < 0.05$ , Fig. 2B). Similarly, plasma lactate increased significantly after exercise in the Ctrl group, but the values were significantly lower than those of REFR group ( $P < 0.05$ , Fig. 2B).

### Hormonal Response

There were no significant differences in serum GH, cortisol, IGF-1, or total testosterone between groups at baseline ( $P > 0.05$ , Fig. 3).

Serum GH concentration significantly increased at 10 min postexercise and remained elevated for 40 min postexercise in

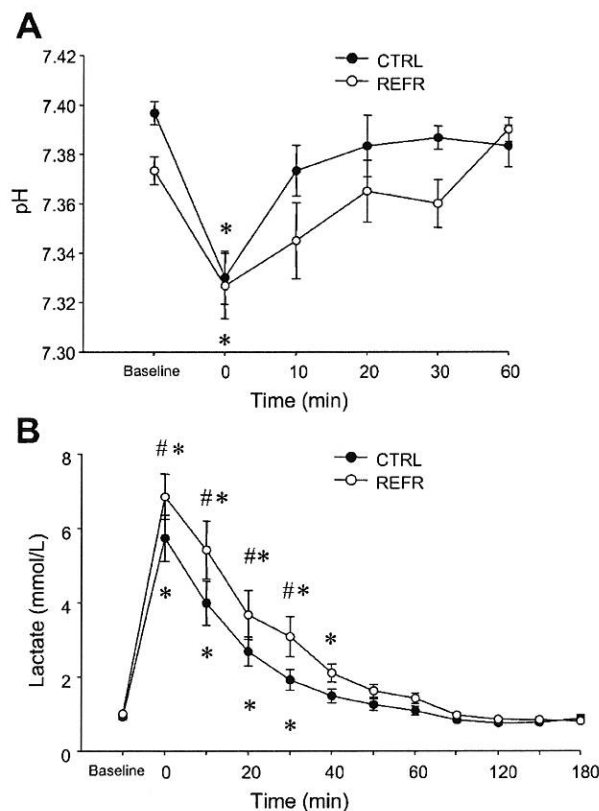
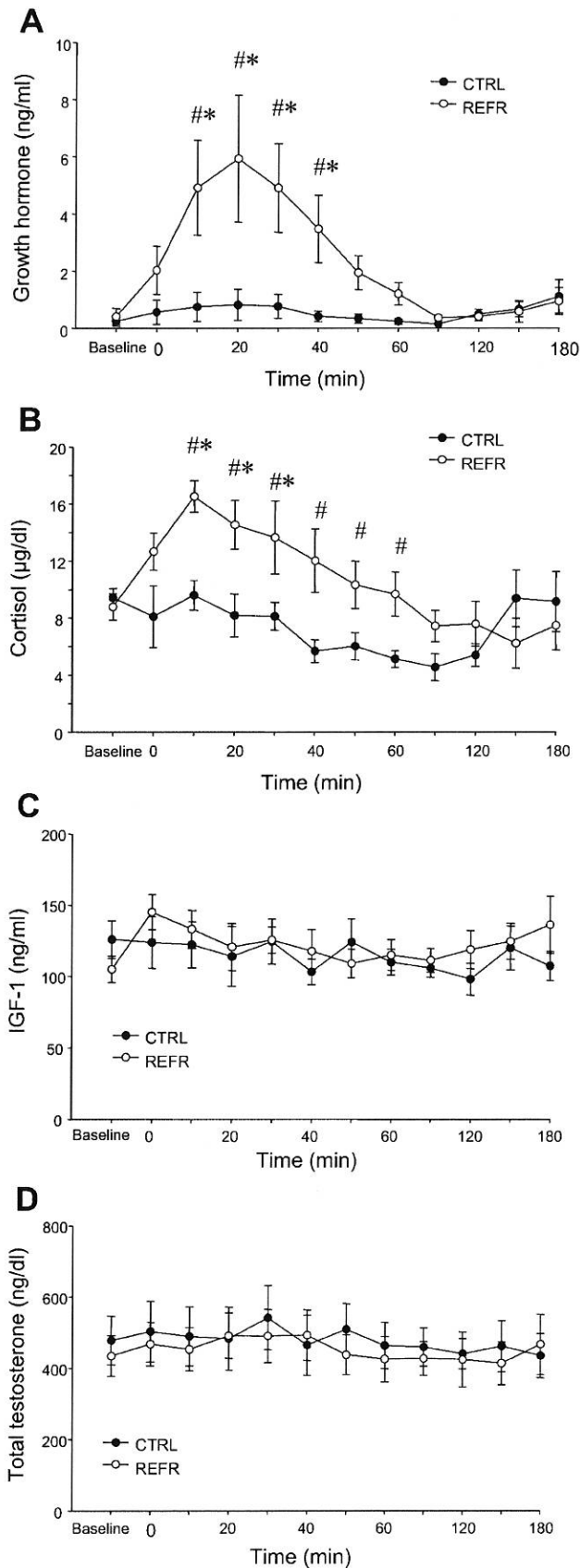


Fig. 2. REFR group, low-intensity resistance exercise training combined with blood flow restriction; Ctrl group, low-intensity resistance exercise training without blood flow restriction. A: Peripheral vein blood pH response in REFR and Ctrl subjects before, immediately after, and 1–3 h postexercise. B: plasma lactate response in REFR and Ctrl subjects before, immediately after, and 1–3 h postexercise. \*Significantly different from baseline ( $P < 0.05$ ). #Significantly different from Ctrl ( $P < 0.05$ ).



the REFR group compared with both baseline and Ctrl concentrations ( $P < 0.05$ , Fig. 3A). GH concentration did not change following exercise in the Ctrl group ( $P > 0.05$ ).

Serum cortisol concentration increased at 10 min postexercise and remained elevated (compared with baseline and Ctrl) for 30 min postexercise in the REFR group ( $P < 0.05$ , Fig. 3B). Cortisol concentrations remained significantly higher than Ctrl at 40, 50, and 60 min postexercise ( $P < 0.05$ ). Serum cortisol concentration did not change during or following exercise in the Ctrl group.

No significant changes in IGF-1 were observed in either the REFR or the Ctrl group ( $P > 0.05$ ). Similarly, serum total testosterone concentration did not change in either group following the bout of resistance exercise ( $P > 0.05$ , Fig. 3D).

#### Phosphorylation of Translation Initiation and Elongation Regulatory Proteins

Phosphorylation status of Akt, mTOR, S6K1, or eEF2 were not different between groups before performing the bout of resistance exercise ( $P > 0.05$ ). Therefore, the data in Fig. 4 are expressed as the percent change in phosphorylation status 3 h postexercise compared with baseline values for both groups.

Phosphorylation of Akt at Ser473 tended to increase in both the REFR and Ctrl group (time effect = 0.054, Fig. 4A); however, no group differences were observed ( $P > 0.05$ ).

The phosphorylation status of mTOR at Ser2448 did not change significantly 3 h postexercise in either group (Fig. 4B).

Phosphorylation of S6K1 at Thr389 was increased by three-fold postexercise in the REFR group ( $P < 0.05$ , Fig. 4C). S6K1 phosphorylation at 3 h postexercise was also significantly higher in the REFR group compared with the Ctrl group ( $P < 0.05$ ). S6K1 phosphorylation status in the Ctrl group was not different from baseline 3 h postexercise ( $P > 0.05$ ).

Interestingly, phosphorylation of eEF2 at Thr56 decreased to a similar extent in both the REFR and Ctrl group ( $P < 0.05$ , Fig. 4D) in response to exercise.

#### Phenylalanine Concentration and Enrichment

Phenylalanine concentration within the muscle free pool was similar between groups at baseline without a significant group difference ( $P > 0.05$ , Table 2). In addition, muscle phenylalanine concentration at 3 h postexercise did not change in either group ( $P > 0.05$ ). Muscle intracellular enrichment of phenylalanine was at a steady state throughout the infusion study with no significant differences between groups ( $P > 0.05$ , Table 2).

#### Muscle Protein Synthesis

Whereas the phosphorylation statuses of signaling proteins are indicators of enhanced translation initiation and elongation, we also directly measured muscle protein synthesis, the end product of translation initiation and elongation. We found that the mixed muscle protein FSR, a direct measure of the incorporation of amino acids into protein, was significantly increased 3 h postexercise in the REFR group compared with

Fig. 3. Peripheral vein serum hormone concentrations in REFR and Ctrl subjects before, immediately after, and for 1–3 h postexercise. A: serum growth hormone concentration. B: serum cortisol concentration. C: serum IGF-1 concentration. D: serum total testosterone concentration. \*Significantly different from baseline ( $P < 0.05$ ). #Significantly different from Ctrl ( $P < 0.05$ ).

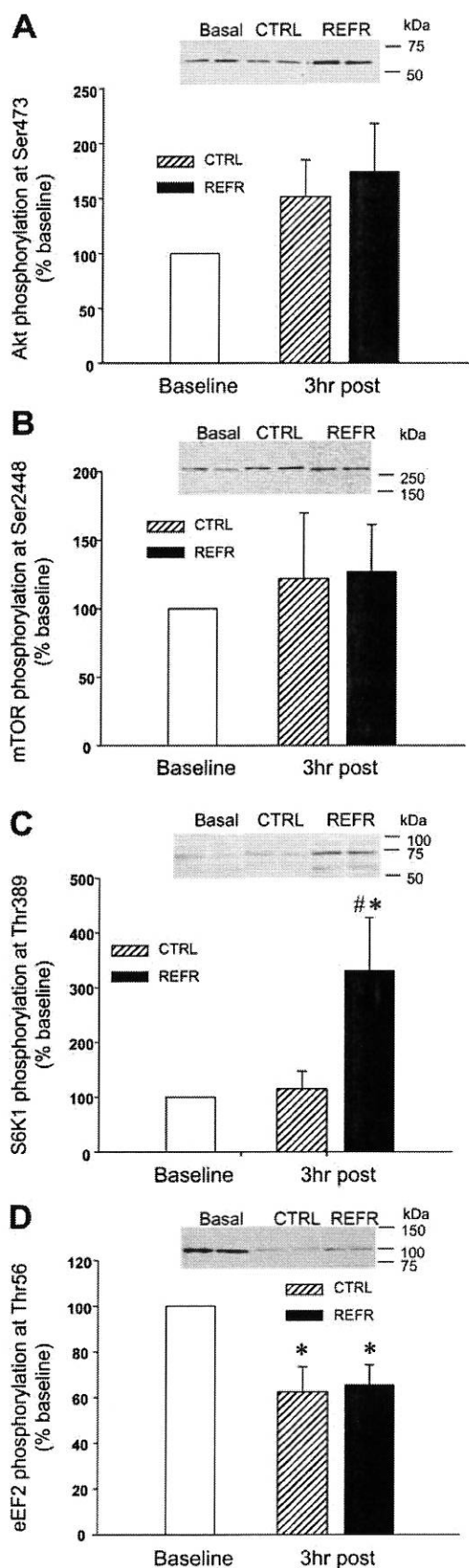


Table 2. Muscle free phenylalanine enrichment and concentration

	Ctrl	REFR
IC phenylalanine concentration, $\mu\text{mol/l}$		
Baseline	95.4 $\pm$ 4.7	95.5 $\pm$ 6.8
Exercise	99.8 $\pm$ 8.1	97.0 $\pm$ 7.4
IC phenylalanine enrichment, %		
120 min	5.6 $\pm$ 1.0	5.4 $\pm$ 1.2
240 min	6.7 $\pm$ 0.7	5.5 $\pm$ 0.3
420 min	5.8 $\pm$ 0.4	5.4 $\pm$ 0.5

Values are means  $\pm$  SE. IC, intracellular.

baseline and the Ctrl group ( $P < 0.05$ , Fig. 5). Muscle FSR did not change following exercise in the Ctrl group ( $P > 0.05$ ).

#### DISCUSSION

The major and novel finding from our study was that a key downstream effector of the mTOR signaling pathway, S6K1, became phosphorylated and muscle protein synthesis was stimulated following an acute bout of low-intensity (20% 1-RM) resistance exercise combined with blood flow restriction (REFR). Specifically, muscle FSR increased by 46% ( $P < 0.05$ ) 3 h after a bout of exercise in REFR, while muscle protein synthesis was not increased in the control-exercise (Ctrl) group without the blood flow restriction. Furthermore, the increased muscle protein synthesis in REFR group was associated with a significant increase in the phosphorylation of S6K1 and a significant decrease in eEF2 phosphorylation, suggesting enhanced translation initiation and elongation after REFR. Therefore, enhanced mTOR signaling may be an important cellular mechanism that may in part explain the hypertrophy induced by low-intensity resistance exercise during blood flow restriction.

High-intensity resistance exercise is a potent stimulus for muscle protein synthesis and muscular hypertrophy (9, 21, 37, 54). Muscle fibers respond to overload stress with an increase in the cross-sectional area of muscle fibers and an increase in force-generating capacity. In general, a training intensity of over 70% of 1-RM is required to achieve a substantial muscle hypertrophy. On the contrary, exercise with an intensity of <65% of 1-RM generally induces an improvement of muscular endurance with no substantial increases in muscular size or the strength (24). The basis of this adaptive response is supported by a number of acute studies that reported a significant increase in muscle protein synthesis after a bout of resistance exercise (9, 34, 41). We have recently shown that a single bout of resistance exercise with 70% 1-RM significantly increased muscle protein synthesis within 1–2 h in combination with an increase in the phosphorylation of mTOR, Akt/PKB, and S6K1 and a reduced phosphorylation of eEF2 (9). A recent *in vivo* study has shown that during high-intensity eccentric contrac-

Fig. 4. Phosphorylation status of human muscle mammalian target of rapamycin (mTOR)-associated signaling proteins in REFR and Ctrl subjects before exercise and 3 h postexercise. A: Akt/protein kinase B (PKB) phosphorylation at Ser473. B: mTOR phosphorylation at Ser2448. C: S6 kinase 1 (S6K1) phosphorylation at Thr389. D: eukaryotic elongation factor 2 (eEF2) phosphorylation at Thr56. Data are expressed in arbitrary units and presented as percentage of baseline values. \*Significantly different from baseline ( $P < 0.05$ ). # Significantly different from Ctrl ( $P < 0.05$ ).

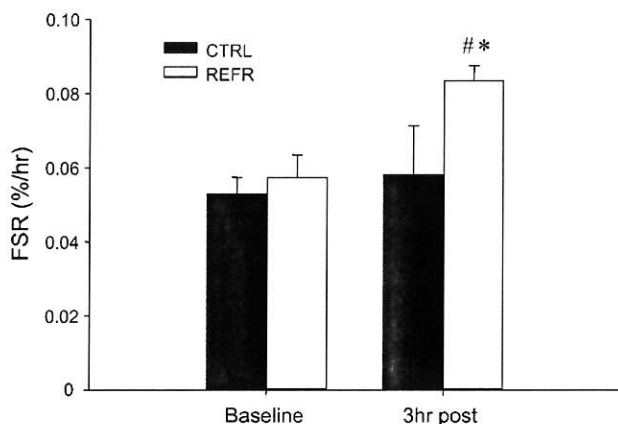


Fig. 5. Muscle protein synthesis as expressed by the mixed muscle fractional synthetic rate (FSR) in REFR and Ctrl subjects before exercise and 3 h postexercise. \*Significantly different from baseline ( $P < 0.05$ ). #Significantly different from Ctrl ( $P < 0.05$ ).

tion, there is a significant increase in the phosphorylation of S6K1, whereas no significant change was observed with sub-maximal contraction (10). It appears that during the recovery phase following high-intensity resistance exercise, an increase in translation initiation and elongation leads to the muscle anabolic response; however, there seems to be a threshold for the exercise intensity below which the anabolic response is not properly triggered.

Recent studies have shown that low-intensity (approximately 20–50% of 1-RM) resistance training combined with reduced muscular blood flow (REFR) leads to significant elevations in muscle strength and muscle fiber cross-sectional area (1, 42, 46, 47), with the increases comparable to those with high-intensity resistance training. Our present study indicates for the first time that low-intensity resistance exercise acutely stimulates muscle protein synthesis when combined with blood flow restriction. Furthermore, the increase in the phosphorylation of S6K1 associated with REFR suggests an upregulation of translational efficiency allowing for the synthesis of specific mRNAs essential for the muscle growth. There is a direct correlation between the increase in S6K1 measured at 6 h after an acute exercise bout and the percent change in muscle mass measured after 6 wk of training (3), suggesting the phosphorylation of S6K1 could be a marker for the long-term increase in muscle mass. Although the mechanism(s) of how mTOR signaling to S6K1 is enhanced during REFR was not identified in the present study, it is possible that either changes in the hormonal response, metabolic stress, and/or mechanotransduction signaling may be involved.

An interesting finding was that eEF2 phosphorylation decreased in the Ctrl group during low-intensity exercise despite the fact that the phosphorylation status of Akt, mTOR, and S6K1 was unchanged. Since eEF2 lies downstream of mTOR, we are not completely sure how to explain the disconnect between mTOR and eEF2 phosphorylation. However, it is possible that Akt/mTOR/S6K1 phosphorylation may have transiently increased in the Ctrl group before obtaining the 3-h postexercise biopsy. This may have occurred since it appears that muscle protein synthesis (although not significant) was slightly elevated in the Ctrl group. Another possibility may be

that other regulatory proteins influenced eEF2 kinase during the low-intensity control trial.

It has been suggested the acute change in anabolic hormones such as testosterone and GH after resistance exercise are critical for skeletal muscle growth (25). In response to an acute bout of high-intensity resistance exercise, GH concentration increases significantly above resting values (17, 23, 49). Similarly, REFR has been shown to induce a significant GH response (35, 38, 44, 45). GH has been shown to be a positive regulator of cellular differentiation in a variety of cells, including muscle and liver cells (11, 33). In animal and in vitro studies, GH treatment is associated with increased muscle protein synthesis rates and decreased muscle protein breakdown (40). Although the signaling pathways involved in the GH stimulation of muscle protein synthesis are unclear, GH has been shown to activate phosphatidylinositol 3-kinase and S6K1 in 3T3 fibroblasts (29). More recently, Hayashi and Proud (13) have shown that GH stimulates protein synthesis in H4IIE hepatoma cells through mTOR signaling. Thus it is plausible that there may be a link between GH signaling and translational control in our study. However, in humans there is no evidence that GH enhances muscle protein synthesis when combined with traditional resistance exercise training (53). In our study, the peak GH response after REFR was 10-fold higher in the REFR group compared with the control-exercise group and was similar to values reported following high-intensity resistance exercise (23). Circulating IGF-1 concentrations were also elevated immediately postexercise, and serum cortisol concentrations were increased in the REFR group during postexercise recovery. Thus blood flow restriction during low-intensity exercise produced a hormonal response similar to high-intensity exercise without vascular occlusion (25). Although the acute increase in GH following high-intensity resistance exercise has been thought to be a key player in producing the muscle anabolic response, it is not clear if the acute increase in GH concentration after REFR in our study plays a role in the activation of mTOR signaling.

Another possibility relates to the increased muscle fiber recruitment associated with REFR. Two previous studies have shown that the integrated EMG values during REFR were significantly higher than a control group (32, 47), suggesting that a greater number of normally inactive muscle fibers are recruited to lift the a similar load when the blood flow is restricted. Although the mechanism of enhanced muscle fiber recruitment during REFR is not clear, it may involve a premature fatigue of active muscle fibers and resultant recruitment of normally inactive muscle fibers. Although in the present study the magnitude of decrease in blood pH after exercise was not different between groups, the increase in plasma lactate concentration was significantly higher in REFR compared with the Ctrl group, suggesting a pooling and local accumulation of lactate. A recent study has shown that the phosphorylation of S6K1 increased to a greater extent in the type II fibers compared with type I fibers after an acute bout of high-intensity resistance exercise (20). If the higher proportion of type II muscle fibers is activated during REFR, similar to the condition during high-intensity resistance exercise, it may explain the substantial increase in the phosphorylation of S6K1 in our present study. Thus metabolic stress may also be playing a role in stimulating type II fiber recruitment.

Blood flow restriction induced by the pressure cuff during exercise may have caused a mild ischemia, and with the release of the pressure cuff a subsequent hyperemia could have occurred (i.e., an ischemia-reperfusion condition). Although prolonged hypoxia has been shown to decrease mTOR signaling and muscle protein synthesis (2, 16, 27), ischemic preconditioning of the heart has been shown to protect against myocardial infarction (8, 30), and this ischemic preconditioning may involve the cell survival and cell growth control through the mTOR signaling pathway (7, 12, 18, 19). On the contrary, Reeves et al. (38) have shown that the low-intensity resistance exercise combined with blood flow restriction resulted in a significant growth hormone response, whereas blood flow restriction alone did not induce such an effect. Furthermore, Takarada et al. (48) demonstrated that 8 wk of low-intensity resistance training (twice a week) caused a significant increase in muscle cross-sectional area of knee extensors and muscular strength, whereas no such response was observed with blood flow restriction alone. Therefore, blood flow restriction alone apparently is not an adequate stimulus to induce an acute muscle anabolic response; however, future work is required to determine if hypoxia/occlusion (without exercise) can also alter mTOR signaling and protein synthesis in human muscle. Although we did not directly measure whether an ischemia-reperfusion condition was present in our subjects, it is interesting to speculate that muscular contraction and ischemia-reperfusion stress, when combined, additively stimulate the mTOR signaling pathway and muscle protein synthesis by promoting an upregulation of cell survival mechanisms.

In summary, we have shown that an acute bout of low-intensity resistance exercise combined with blood flow restriction stimulates S6K1 phosphorylation (a downstream component of the mTOR signaling pathway and a key regulator of translation initiation) and protein synthesis in human skeletal muscle. The mTOR signaling pathway and muscle protein synthesis did not change in the low-intensity resistance exercise group (without blood flow restriction). Therefore, we conclude that the activation of the mTOR signaling pathway appears to be an important cellular mechanism that may help to explain the enhanced muscle protein synthesis during low-intensity resistance exercise with blood flow restriction. Future studies are required to determine the cellular mechanism(s) responsible for activating S6K1 and whether the stimulation of the mTOR signaling pathway is the primary mechanism for inducing muscle hypertrophy during low-intensity resistance exercise with blood flow restriction.

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