Anabolic signaling and protein synthesis in human skeletal muscle after dynamic shortening or lengthening exercise

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Anabolic signaling and protein synthesis in human skeletal muscle after dynamic shortening or lengthening exercise. Am J Physiol Endocrinol Metab 290:E731–E738, 2006. First published November 1, 2005; doi:10.1152/ajpendo.00415.2005.—We hypothesized a differential activation of the anabolic signaling proteins protein kinase B (PKB) and p70 S6 kinase (p70s6K) and subsequent differential stimulation of human muscle protein synthesis (MPS) after dynamic shortening or lengthening exercise. Eight healthy men [25 ± 5 yr, BMI 26 ± 3 kg/m² (means ± SD)] were studied before and after 12 min of repeated stepping up to knee height, and down again, while carrying 25% of their body weight, i.e., shortening exercise with the “up” leg and lengthening exercise with contralateral “down” leg. Quadriceps biopsies were taken before and 3, 6, and 24 h after exercise. After exercise, over 2 h before the biopsies, the subjects ingested 500 ml of 3.0- and 2.4-fold, respectively) and 24 h increased significantly at 6 (over the period including exercise and 3 h recovery but had remained elevated at 6 and 24 h. After exercise, rates of myofibrillar protein synthesis rose faster after exercise (3.2- and 2.0-fold, respectively), independently of the mode of exercise. Eighty healthy men [25 ± 5 yr, BMI 26 ± 3 kg/m² (means ± SD)] were studied before and after 12 min of repeated stepping up to knee height, and down again, while carrying 25% of their body weight, i.e., shortening exercise with the “up” leg and lengthening exercise with contralateral “down” leg. Quadriceps biopsies were taken before and 3, 6, and 24 h after exercise. After exercise, over 2 h before the biopsies, the subjects ingested 500 ml of water containing 45 g of essential amino acids and 135 g of sucrose. Rates of muscle protein synthesis were determined via incorporation over time of [1-13C]leucine (≥6 h after exercise) or [1-13C]valine (21–24 h after exercise) and phosphorylation of signaling proteins by Western analysis. PKB and p70s6K phosphorylation increased ~3-fold after 3 h and remained elevated at 6 and 24 h. After exercise, rates of myofibrillar and sarcoplasmic protein synthesis were unchanged over the period including exercise and 3 h of recovery but had increased significantly at 6 (~3.0- and 2.4-fold, respectively) and 24 h (~3.2- and 2.0-fold, respectively), independently of the mode of exercise. Short-term dynamic exercise in either shortening or lengthening mode increases MPS at least as much as resistance exercise and is associated with long-term activation of PKB and p70s6K.

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which the muscles of one leg performed shortening exercise while those of the contralateral leg performed lengthening exercise. We chose this model to determine whether there were possible differences between the responses to dynamic lengthening and shortening exercise. Such differences might be detectable with a more dynamic form of exercise carried out continuously, rather than intermittently as during conventional resistance exercise. We chose to measure the synthesis rates of the myofibrillar proteins, the sarcoplasmic proteins, and muscle extracellular matrix collagen to provide more specific information on the responses than what was obtainable by analysis of the overall synthesis rate of mixed muscle.

The hypotheses we tested here were that, in the quadriceps of young, healthy, but untrained men, the mode of exercise, (either muscle shortening or lengthening) would differentially influence 1) activation of translational signaling proteins, 2) stimulation of muscle myofibrillar protein synthesis, and 3) muscle collagen synthesis, with the effects being more pronounced after muscle lengthening vs. shortening. To do this, we measured the phosphorylation states of two components of the mTOR signaling pathway (PKB and p70S6K) and rates of myofibrillar, sarcoplasmic, and collagen synthesis. We studied the subjects in the fed, rather than fasted, state to elicit a maximal anabolic response.

METHODS

Power Analysis

The coefficient of variation (CV) for repeated measures of muscle protein labeling in the same sample is ~3.8%, using [1-13C]leucine or [1-13C]valine to determine the rate of muscle protein synthesis. The population CVs are ~10% for young men. For Western analysis we commonly find CVs of ~20% in healthy young men. In fact, we have had no difficulty in detecting significant differences in protein amount or phosphorylation of 25% between groups of eight young men after physiological interventions such as feeding. A power calculation, assuming a population variance of 15%, studied using laboratory techniques with CVs of 15%, suggested that we need eight subjects to detect (with 80% confidence at the 5% significance level) differences of 20% between treatments (on a paired basis).

Subject Characteristics

Eight healthy men, 25 ± 5 yr, 1.78 ± 0.09 m, 81 ± 10 kg, and body mass index (BMI) 26 ± 3 kg/m², participated in the study. The subjects were habitually active at a recreational level. They were instructed to adhere to their usual diet and to refrain from strenuous physical activity for 2 days before the study. The subjects were informed of the experimental protocol both verbally and in writing before giving their informed consent. The study protocol was approved by the Tayside Ethics Committee and was carried out according to the Helsinki Declaration.

Experimental Protocol

The subjects attended the laboratory having fasted beginning at 2000 on the previous evening. A forearm vein of each arm was cannulated at the antecubital fossa for infusion of stable isotope tracer amino acids and for blood sampling. Four hours before exercise, an infusion of [1-13C]leucine (prime 1.0 mg/kg, 0.8 mg·kg⁻¹·h⁻¹) was started and continued until 6 h postexercise (Fig. 1). Between 21 and 24 h after exercise, a separate infusion of [1-13C]valine (prime 1.2 mg/kg, 1.0 mg·kg⁻¹·h⁻¹) was given. Two separate amino acid tracers were used to exclude the need for an additional muscle biopsy at 21 h and to determine baseline tissue enrichment, the latter being determined from the zero time biopsy for valine. Quadriceps muscle biopsies were taken, using 1% lignocaine anesthetic and the conchotome technique (15) at the start and end of the 4-h period preceding exercise from either leg and 3, 6, and 24 h after exercise from both legs. All biopsies were taken using separate incisions and made from distal to proximal areas of the quadriceps.

After 4 h at rest, the subjects stepped up with one leg onto a knee-high box (of 0.55 m) and stepped down with the other leg at ~0.5 Hz for 6 min, carrying 25% body weight. The “up” and “down” legs were randomly assigned by the investigators. A metronome and
verbal prompting guided the subjects to perform the exercise at the correct cadence. After 2 min of rest, they performed two additional 3-min bouts. Thus the protocol lasted for a total of 16 min, 12 min of which were during exercise. Work by the muscles raising the body was calculated as the product of the total weight (body wt plus 25% in backpack), the gravitational constant, the height of the step, and the number of steps climbed within the 12 min. The total work done was $197 \pm 23$ kJ with a power output of 274 $\pm 32$ W. Although a similar amount of energy must have been absorbed by the leg supporting the body during lowering, it is not possible to calculate the internal work done by the muscles of this leg, although it is likely to have been substantially less. On completion of the exercise bout, all subjects reported being fatigued, were unable to continue further, and had to be helped to a seat.

After exercise, in the 2 h preceding each biopsy, subjects were given a 500-ml drink containing 45 g of essential amino acids (EAA) and 135 g of sucrose. The amino acid-sucrose solution was given as an initial aliquot of $\sim 140$ ml followed by six subsequent aliquots of 70 ml every 20 min. The amino acid composition reflected the amino acid composition of muscle protein (42). Five percent of the total leucine or valine dissolved in the drink was given as $\left[^{1-13}\text{C}\right]$leucine or $\left[^{1-13}\text{C}\right]$valine, as appropriate, to maintain the isotopic labeling of the plasma and valine. This pattern of feeding was chosen to maintain a sufficient supply of amino acids to the muscle. The composition of the drinks, given over the three time periods, was designed to meet the subjects’ 24-h energy ($\sim 9$ MJ) and protein requirements. No other feeding was allowed until the end of the study.

Analytical Methods

Plasma. Plasma was separated from whole blood by centrifugation (300 g) immediately after collection. Labeling of leucine and valine were measured in their tert-butyldimethylsilyl (t-BDMS) derivatives (36) and that of $\alpha$-ketoisocaproic acid (KIC) enrichment in its quinoxalinol-t-BDMS derivative (35). The plasma labeling of the ketoacids of leucine and valine, KIC, and ketoisovaleric acid (KIV), respectively, were taken as representing the value of the true precursors (aminoacyl tRNA) for calculation of the fractional synthesis rates.

Muscle

Myofibrillar and sarcoplasmic protein extraction. All procedures were performed as previously described (6). Frozen muscle biopsy samples (60–80 mg) were ground in liquid nitrogen, and the frozen powder was transferred to 1.6 ml of homogenization buffer containing protease inhibitors and phosphatase inhibitors [0.15 M NaCl, 0.1% Triton, 0.02 M Tris, 50 $\mu$M DTT, 0.1 M EDTA, and 1 mM phenylmethylsulfonyl fluoride (PMSF)] to prevent dephosphorylation of the signaling proteins (6). All procedures were performed on ice.

The homogenate was centrifuged at 1,600 g for 20 min to produce a myofibrillar pellet and a supernatant containing the sarcoplasmic fraction, which was removed. The myofibrillar pellet was washed and centrifuged twice in a low-salt buffer and then washed twice with 70% ethanol. The myofibrillar pellet was then solubilized in 0.3 N NaOH, and an aliquot was removed for the determination of protein content using the Bradford assay (Bio-Rad Laboratories, Hercules, CA). The supernatant was subjected to high-speed centrifugation (7,000 g, 15 min), and the sarcoplasmic proteins were precipitated with ethanol and then washed twice with buffer. The sarcoplasmic pellet was then solubilized in 0.3 N NaOH, and an aliquot was removed for the determination of protein content using the Bradford assay.

HCl (6 N) was added to the myofibrillar and sarcoplasmic pellets, and the protein-bound amino acids were released by heating at 110°C overnight. The HCl was evaporated under nitrogen, the amino acids were purified by ion exchange chromatography on Dowex H+ resin, and an aliquot was used to determine the rate of protein synthesis as described below.

Collagen extraction. Frozen muscle biopsy samples, weighing between 20 and 30 mg, were powdered under liquid nitrogen and extracted with 0.15 M NaCl buffer and centrifuged, with the supernatant removed. Next, 0.7 M KCl was added to the pellet containing myofibrillar proteins and collagen, which was also centrifuged. The pellet containing collagen was then washed with acetic acid and acetic acid-pepsin (0.1% w/vol), dissolving immature collagen and procollagen and leaving an insoluble collagen pellet, which was almost pure type I collagen, according to PAGE analysis with immunoblotting. The myofibrillar, soluble, and insoluble collagen proteins were hydrolyzed in 0.1 M HCl-Dowex H+ slurry at 110°C overnight, the liberated amino and imino acids were separated using Dowex 50 W-X8 H+ ion exchange resin, and an aliquot was used to determine the rate of protein synthesis as described below.

Determination of the rate of muscle protein synthesis. The liberated amino acids were dried and derivatized as N-acetyl-n-propyl esters for measurement of tracer incorporation by gas chromatography-combustion-isotope ratio mass spectrometry (Finnigan DeltaPlus XL; Finnigan, Bremen, Germany). Fractional synthetic rates (%/h) were calculated by comparing the incorporation of $\left[^{13}\text{C}\right]$leucine or $\left[^{13}\text{C}\right]$valine over time into isolated protein fractions with the plasma KIC or KIV enrichment, as previously described (40).

Western Blotting

Muscle (20–40 mg) was homogenized using a polytron in a buffer containing 50 mM Tris-HCl, 100 mM sodium fluoride, 10 mM EDTA, 2 mM EDTA, 1 mM bezamidine, 0.2 mM PMSF, 10 mg/ml leupeptin hemisulfate, 10 mM Na3VO4, and 10 mM okadaic acid. After homogenization, the protein concentration was determined using the DC protein assay (Bio-Rad Laboratories). PKB phosphorylation was quantified by using an antibody for phospho-PKB (Thr308 and Ser327) and total PKB (Cell Signaling Technologies, Beverly, MA); p70 S6K phosphorylation was quantified by using an antibody for phospho-p70 S6K (Thr389) and total p70 S6K (Santa Cruz Biotechnol- ogy, Santa Cruz, CA).

From the sarcoplasmic fraction, 40 $\mu$g of total protein in Laemmli buffer were loaded per lane and subjected to SDS-PAGE (10%) gel. After electrophoretic separation, proteins were transferred to a polyvinylidene difluoride membrane, and the membranes were blocked in 5% powdered milk in Tris-buffered saline-1% Tween 20 for 1 h, followed by overnight incubation at 4°C with the following primary antibodies: phospho-PKB, phospho-p70 S6K, p70 S6K, and PKB. After overnight incubation, the blot was washed and then probed with anti-rabbit antibody (Vector Laboratories, Burlingame, CA) for 45 min at room temperature. The membranes were then washed, and antibody binding was detected autoradiographically, using the Amer sham Life Sciences enhanced chemiluminescence detection kit. Densitometric measurements were carried out using the FluorSmax Imager with QuantityOne Software (Bio-Rad Laboratories). Once the appropriate image was captured, membranes were stained with Ponceau S to verify equal loading in all lanes.

All results were determined using the ratio of the intensity of the phosphorylated form to the total intensity (both phosphorylated and unphosphorylated forms). Data was normalized to the control preexercise values and expressed in arbitrary units.

Data and Statistical Analysis

Data are expressed as means ± SD ($n=8$). The mean values of subject characteristics were compared with an unpaired $t$-test, but results from the plasma and muscle analyses were compared using one-way ANOVA with the Bonferroni correction. The null hypothesis was rejected at the 5% level ($P<0.05$).
RESULTS

Metabolites and Tracer Labeling

Amino acid, glucose, and insulin concentrations. During the periods of feeding, plasma glucose, insulin, and total and EAA concentrations increased; initially, plasma concentrations of non-EAA decreased slightly but remained stable thereafter (Fig. 2).

KIC and KIV labeling. Plasma labeling of [13C]KIC and [13C]KIV achieved plateaus (within 30 min of infusion) of 5.2 ± 0.2 and 6.3 ± 0.3% atom percent excess, respectively, and remained steady throughout, even during oral ingestion of amino acids.

Signaling Proteins

PKB phosphorylation. Compared with values before exercise in the postabsorptive state, phosphorylation of Ser473 and Thr308 increased markedly, peaking (3.2 ± 1.6-fold, P < 0.001) at 3 h and falling thereafter but remaining above baseline at 6 h (2.3 ± 1-fold, P < 0.05) and 24 h (2.5 ± 0.9-fold, P < 0.01; Fig. 3). There were no significant differences at any time points between the values of muscle PKB phosphorylation in the two legs, which, therefore, have been averaged.

p70S6 kinase phosphorylation. Phosphorylation of p70S6K accounted for 20% of the total amount of p70S6K (Fig. 3). At 3 h after exercise, p70S6K phosphorylation had increased above resting values by 3.5 ± 2.1-fold in both legs, and this was sustained up to 24 h (6 h, 3.4 ± 1.8; 24 h, 3.4 ± 1.5; all P < 0.01). As for PKB phosphorylation, there were no differences between the two legs, and so the values have been averaged.

Muscle Protein Synthesis

Myofibrillar protein synthesis. The fractional synthetic rate of myofibrillar protein before exercise was 0.042 ± 0.012%/h (Fig. 4). There was no significant increase in protein synthesis over the period between the preexercise biopsy and that at 3 h after exercise in the muscle of either leg (lengthening, 0.051 ± 0.015%/h vs. shortening, 0.048 ± 0.014%/h). However, the rates of protein synthesis were significantly increased above basal at 6 h (lengthening, 3.1 ± 1-fold to 0.133 ± 0.016%/h vs. shortening, 2.8 ± 0.7-fold to 0.118 ± 0.023%/h; P < 0.001) and 24 h (lengthening, 3.1 ± 0.8-fold to 0.132 ± 0.011%/h vs. 3.3 ± 0.9-fold to 0.139 ± 0.011%/h; P < 0.001). Because the difference in the rates of myofibrillar protein synthesis between the muscles of the two legs were not significant at any time point, the values were averaged.

Sarcoplasmic protein synthesis. The fractional synthetic rate of sarcoplasmic protein at rest was 0.061 ± 0.007%/h (Fig. 4), which was, as reported earlier (6), higher than the rate of myofibrillar protein synthesis. As for myofibrillar protein synthesis, no significant increase in sarcoplasmic protein synthesis above basal was observed during exercise and at 3 h after exercise (lengthening, 0.060 ± 0.013%/h vs. shortening, 0.066 ± 0.018%/h). However, as observed for the myofibrillar fraction, sarcoplasmic protein synthesis was significantly stimulated at 6 and 24 h, with no discernible difference in the
responses between the two types of contractile activity. Mean rates of sarcoplasmic protein synthesis for lengthening and shortening contractions were significantly elevated from baseline at 6 h [2.4 ± 0.7-fold to 0.146 ± 0.028%/h vs. 2.3 ± 0.7-fold to 0.140 ± 0.027%/h (P < 0.001)] and at 24 h [2.1 ± 0.3-fold to 0.125 ± 0.019%/h vs. 1.9 ± 0.9-fold to 0.117 ± 0.014%/h (P < 0.001)], respectively (Fig. 3). The stimulation of rates of sarcoplasmic protein synthesis was ~20% less than that of myofibrillar protein synthesis.

Collagen protein synthesis. As expected from recent work (3), the fractional synthetic rate of collagen at rest was lower than that for myofibrillar or sarcoplasmic proteins (0.016 ± 0.002 vs. 0.042 ± 0.012 and 0.061 ± 0.007%/h, respectively; Fig. 5). At 3 h after exercise in both lengthening and shortening legs, the rate of collagen synthesis increased significantly above resting values (0.048 ± 0.006 and 0.032 ± 0.004%/h, respectively; lengthening vs. rest P < 0.001, shortening vs. rest P < 0.05, lengthening vs. shortening P < 0.05). At 6 h after exercise, no further increase in the rate of collagen synthesis from 3 h was observed in the muscle of the leg that had undergone lengthening contractions (0.051 ± 0.010 vs. 0.048 ± 0.006%/h), but there was a rise in collagen synthesis in the shortening muscle from 3 h (6 vs. 3 h, 0.058 ± 0.012 vs. 0.032 ± 0.004%/h; P < 0.001) so that any differences between exercise type had disappeared by 6 h.

DISCUSSION

The major findings of this study are that, after intense stepping exercise, 1) there are marked increases in the phosphorylation state of PKB and p70S6K within 3 h of the end of exercise, which are largely sustained for up to 24 h; 2) there are marked increases in the synthetic rates of myofibrillar and sarcoplasmic protein synthesis, which are only apparent after 6 h but are largely sustained for up to 24 h; 3) the magnitude of the increase in the rates for contractile and soluble protein synthesis are larger than previously reported (see, e.g., Refs. 4, 12, 25–27) for any type of exercise; 4) the rates of muscle collagen synthesis also rapidly increase, with increased rates being apparent within 3 h of exercise; and 5) there appears to be no effect of the mode of contraction on the magnitude or time course of the activation of the signaling proteins or on rates of myofibrillar and sarcoplasmic protein synthesis.

In the present study, the increased phosphorylation of PKB and p70S6K helps explain some, but not all, of the increases in the rates of protein synthesis. After 3 h, phosphorylation of PKB and p70S6K was increased when the rate of protein synthesis was unchanged. However, at 6 and 24 h, the persisting activation of the signaling proteins was in line with the stimulation of muscle protein synthesis. The finding of a prolonged activation of the signaling proteins in response to exercise in the present study is in agreement with those of several studies in rats (2, 18, 30), although Bolster et al. (8) observed a much more transient response in the activation of the signaling proteins in jumping rats in vivo. Previously, studies examining activation of the signaling proteins after exercise in human skeletal muscle demonstrated increases of eIF4-BP1 and p70S6K phosphorylation for up to 4 h after an acute bout of resistance exercise (23, 34). Measurements of MPS were not made in the study by Karlsson et al. (23), but our earlier work (34) showed that isometric exercise of the quadriceps muscle enhanced the effects on both anabolic signaling and MPS.

In the present study, we observed a similar activation of the signaling proteins with muscle lengthening or shortening, whereas other workers studying rat muscle in vivo found a greater activation of p70S6K or PKB with muscle lengthening (2, 30). However, in those studies using rats, muscle contractions were induced by electrical stimulation (rather than voluntary exercise), which might have added an unknown factor.

The basal rates of myofibrillar and sarcoplasmic protein synthesis and of collagen synthesis are similar to those previously reported (3, 13). The response of sarcoplasmic protein synthesis to exercise in the fed state is the same as previously reported after an acute bout of resistance exercise (26), but the response of myofibrillar protein is substantially greater, possibly because of the exhausting nature of the exercise, stepping while carrying 25% of body weight. Whatever the reason for
the increased response, the results support previous findings (11, 25, 38, 39) that dynamic exercise is capable of inducing significant increases in MPS.

The lack of an early rise (3 h after exercise) in the rates of MPS requires explanation. In the rested state, a single oral bolus of 10 g of EAA significantly stimulates myofibrillar and sarcoplasmic protein synthesis after 3 h (13). Yet in the present study, no stimulation of myofibrillar or sarcoplasmic protein synthesis was observed during the 3-h period after the start of exercise despite subjects being fed 45 g of EAA for 2 out of the ∼3 h of the measurement. One possibility is that the normally observed stimulation of MPS at 3 h after feeding was inhibited after exercise due to a fall in muscle energy status (e.g., ATP/ADP ratio), as suggested previously (10).

Consistent with this, we recently showed that a pattern of muscle stimulation likely to mimic dynamic long-term exercise was associated with an inhibition of the PKB phosphorylation of TSC2, an upstream regulator of mTOR, probably as a result of activation of AMPK (1). However, if this were the case, the effect is surprisingly long lasting, being maintained beyond the 16 min of the exercise protocol into a further 3 h of postexercise recovery. Furthermore, at 3 h we observed no inhibitory effect on anabolic signaling as reported to occur in rats after exercise (7), but rather an activation of PKB and p70S6K.

Whatever the explanation, any inhibition on MPS during and immediately after exercise did not affect synthesis of collagen, possibly because this is not synthesized in myofibers, but in the fibroblasts within the extracellular matrix of muscle.

The persistence of stimulation of MPS at 6 and 24 h with this protocol is also a novel finding. An elevated rate of MPS after resistance exercise normally persists for up to 48 h, but the response slowly diminishes over this time (12, 32). However, we observed a virtually undiminished elevation over 24 h with a greater magnitude of stimulation of MPS observed (a three- and twofold rise in myofibrillar and sarcoplasmic protein synthesis, respectively), an effect not commonly observed in previous studies. This may have been because of the intense nature of the exercise protocol we used or the high rates of amino acid ingestion. It has previously been shown that the stimulation of MPS by an amino acid infusion declines rapidly after 2.5 h despite continued amino acid availability (6). The strategy we adopted to avoid this, of only providing nutrients for 2 h in each measurement period, may have overcome any tachyphyaxis of MPS to amino acid stimulation.

Nevertheless, the results of this study, like those of Phillips et al. (32), showed there was no significant difference in stimulation of MPS between muscles undergoing different modes of contraction. However, in these studies comparisons were made by using a model of resistance exercise that consisted of eight sets of eight repetitions of raising or lowering weights at 80% of up to one repetition, so that the amount of external work done was substantially less in the muscles lowering the weight, i.e., with lengthening contractions. Furthermore, the studies were carried out in two groups of mixed male and female subjects, with only four per group randomized to one of the two modes of exercise, and all were studied in the postabsorptive state. The rate of synthesis of mixed muscle, rather than that of the specific muscle subfractions, was measured. It does seem that the lack of a greater effect is not due to a lack of amino acid availability; instead, it may be due to a difference in the intensity of work done by the muscles of the two legs. When work is matched between legs operating while shortening or lengthening, it is possible to see a rapid increase in myofibrillar MPS after 4.5 h, and a greater area under the myofibrillar time curve over 8.5 h after lengthening compared with shortening contractions (29). In that study by Moore et al. (29), the shortening and lengthening exercise were carried out sequentially on the same day, with comparisons of rest and exercise on different days. Also, all of the measurements of rates of myofibrillar and collagen (but not sarcoplasmic) protein synthesis were made in the fed state. An alternative interpretation of the Moore study is that it may have been the provision of food, rather than the increased work done per se, that stimulated the greater response, because both factors were changed simultaneously. Also, the lengthening contractions were carried out first, and although the timing of the biopsies between legs was adjusted to account for the exercise time, some unknown systemic factor may have influenced the result, e.g., cytokines released differentially according to differential stress in the two modes.

The greater overall stimulation of MPS observed in our study relative to that seen in the study by Moore et al. (29), irrespective of contraction type, requires explanation. The total exercise time in the present study was much greater than in many previous resistance exercise studies (12 min total exercise vs. repetitions; see, e.g., Refs. 4, 12, 27, 32), and thus there would have been a greater turnover of ATP. The hypothesis of ATP turnover being an important determinant of the stimulation of MPS is supported by the preliminary results of another study by our group (9) in which exercise consisted of different intensities and duration. Despite these differences, the total work done was constant, and the exercise protocol stimulated MPS markedly. However, if a disturbance of muscle energy status is a major factor in stimulating the adaptive postexercise response, it might be expected that lengthening contractions would be less efficient at stimulating synthesis because of a greater energetic efficiency and a supposedly smaller perturbation in the ATP/ADP ratio. Recent studies have implicated AMP protein kinase in regulating mTOR activity, linking mTOR regulation with the energy status of the cell (16, 37). The results of the present study provide evidence against this idea because the extent of stimulation of MPS was identical, even though the likely internal energy cost, borne presumably by ATP hydrolysis, was less in the muscle of the lengthening leg. In the present study, we may simply have achieved some threshold value for total ATP turnover that resulted in the same total stimulation of MPS, irrespective of mode.

Skeletal muscle collagen synthesis increased rapidly after lengthening and shortening exercise. This response was greater in the lengthened leg 3 h after exercise, which is in contrast to the findings observed by Moore et al. (29) 4.5 h after exercise. It has been demonstrated that as sarcomeres lengthen, the collagen fibers are stretched into a linear confirmation (17), and during repeated lengthening contraction some sarcomeres are extended beyond the myofilament overlap (43). These sarcomeres then remain extended, which leaves the collagen fibers under increased tension. Therefore, even though less work is done in lengthening contractions, there may be greater tension in the extracellular matrix.

We have previously shown (33) that amino acids and resistance exercise have a synergistic effect on the stimulation of skeletal muscle protein synthesis. The synergism between
amino acids and exercise on eIF4-BP1 and p70S6K activity that we (33, 34) and others (23) observe provides a plausible molecular mechanism for the enhanced effects of amino acids on MPS postexercise (4, 42). The results from the present study extend knowledge of the effects of feeding on postexercise increases in signaling protein phosphorylation and protein synthesis up to 24 h.

In addition, however, the results raise fascinating questions about the effects of different types of exercise, dose responses of anabolic signaling and MPS in relation to tension and duration of exercise, and the possible inhibitory effect of very intense exercise on MPS in the immediate postexercise period. Nevertheless, it seems clear that, even in the fed state, lengthening and shortening exercise carried out in conditions in which the amount of work done is not equalized, there is no difference in the responses of anabolic signaling activation and MPS.

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