Resistance training reduces the acute exercise-induced increase in muscle protein turnover

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Phillips, S. M., K. D. Tipton, A. A. Ferrando, and R. R. Wolfe. Resistance training reduces the acute exercise-induced increase in muscle protein turnover. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E118–E124, 1999.—We examined the effect of resistance training on the response of mixed muscle protein fractional synthesis (FSR) and breakdown rates (FBR) by use of primed constant infusions of \([^{2}H_5]\)phenylalanine and \([15N]\)phenylalanine, respectively, to an isolated bout of pleiometric resistance exercise. Trained subjects, who were performing regular resistance exercise (trained, \(T; n = 6\)), were compared with sedentary, untrained controls (untrained, \(UT; n = 6\)). The exercise test consisted of 10 sets (8 repetitions per set) of single-leg knee flexion (i.e., pleiometric muscle contraction during lowering) at 120% of the subjects’ predetermined single-leg 1 repetition maximum. Subjects exercised one leg while their contralateral leg acted as a nonexercised (resting) control. Exercise resulted in an increase, above resting, in mixed muscle FSR in both groups (UT: rest, 0.036 ± 0.002; exercise, 0.0802 ± 0.01; \(T\): rest, 0.045 ± 0.004; exercise, 0.067 ± 0.01; all values in %/h; \(P < 0.01\)). In addition, exercise resulted in an increase in mixed muscle FBR of 37 ± 5% (rest, 0.076 ± 0.005; exercise, 0.105 ± 0.01; all values in %/h; \(P < 0.01\)) in the UT group but did not significantly affect FBR in the T group. The resulting muscle net balance (FSR – FBR) was negative throughout the protocol (\(P < 0.05\)) but was increased in the exercised leg in both groups (\(P < 0.05\)). We conclude that pleiometric muscle contractions induce an increase in mixed muscle protein synthetic rate within 4 h of completion of an exercise bout but that resistance training attenuates this increase. A single bout of pleiometric muscle contractions also increased the FBR of mixed muscle protein in UT but not in T subjects.

hypertrophy; muscle damage; muscle protein synthesis; muscle protein breakdown

 Skeletal muscle is often referred to as a “plastic” tissue, having the ability to adapt to chronic changes in workload. Regular resistance training has been shown to result in muscle fiber hypertrophy (11, 14, 19, 22). Muscle fiber hypertrophy is thought to be due to the addition of new sarcomeres in a parallel force-producing arrangement (14, 19, 22). The training-induced addition of new muscle sarcomeres requires the synthesis of new myofibrillar and nonmyofibrillar proteins. In accordance with this suggestion, a number of investigators have demonstrated that, in human subjects, the rate of mixed muscle protein synthesis is increased within 3–24 h after an isolated bout of resistance exercise (3, 5, 17, 25, 26). In fact, the rate of mixed muscle protein synthesis remains elevated for up to 24 h in trained persons but returns to resting levels by 36 h postexercise (5, 13). However, data from this lab showed that mixed muscle protein synthesis remained elevated for ≈48 h postexercise in relatively untrained subjects (17). In the same investigation, we also reported that the rates of muscle protein synthesis and breakdown responded similarly after pleiometric or concentric exercise (17). On the basis of previous studies (10), this may have been because the exercise stimulus was insufficient to induce the different degrees of muscle damage that might have been expected.

Literature regarding the effect of resistance exercise training on muscle protein synthesis is difficult to interpret (25, 26). One report indicated that the mixed muscle protein fractional synthetic rate (FSR) increased 37% after 12 wk of resistance training (25). In a later publication, the same authors report that 2 wk of resistance training resulted in an increase in mixed muscle protein FSR of 55% in a group of young subjects and of 155% in a group of older subjects. In both of these studies, however, the initial pretraining measures of muscle protein synthesis were made before exercise, in a resting state, whereas the posttraining measures were made 3–24 h after the final training session (25, 26). Because a single exercise bout causes an elevation of mixed muscle FSR for up to 48 h after exercise (5, 17), the previous reports of elevations in mixed muscle FSR (25, 26) likely reflect, at least in part, the response to an acute bout of exercise.

The present study was designed to examine whether regular resistance training minimizes the extent of muscle protein breakdown caused by pleiometric muscle contractions. It is known that previous exposure to a bout of pleiometric muscle contraction causes subsequent muscle damage (8, 15). We hypothesized that trained subjects would have a reduced degree of muscle damage and, subsequently, a reduced rate of muscle protein breakdown vs. untrained subjects after an intense bout of pleiometric muscle contractions. In addition, we compared the acute changes in muscle protein turnover of trained and untrained subjects to examine the effect of regular training on muscle protein turnover.

**METHODS**

Subjects. The project was approved by the Institutional Review Board and the Clinical Research Center (CRC) of The University of Texas Medical Branch. Subjects (n = 12; 6 males and 6 females) were volunteers who were advised of the purposes of the study and associated risks. All subjects gave written informed consent before participating in the study. The subjects’ descriptive characteristics are shown in Table 1.
All subjects were healthy, nondiabetic, and normotensive, and they had a normal cardiac rhythm with no abnormalities, as judged by medical history, physical examination, resting electrocardiogram, and laboratory blood and urine tests. Subjects were recruited with the intention of yielding two groups who were either untrained (UT, n = 6 males and 3 females) or trained (T, n = 6 males and 3 females); BMI, body mass index; 1RM, single repetition maximum (nondominant leg concentric knee extension repetition maximum; see methods for explanation). *Significantly different from UT.

Table 1. Subjects' descriptive characteristics

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<thead>
<tr>
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<th>UT (n = 6)</th>
<th>T (n = 6)</th>
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<tr>
<td>Age, yr</td>
<td>24.5 ± 2.6</td>
<td>26.3 ± 2.6</td>
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<tr>
<td>Wt, kg</td>
<td>70.4 ± 8.4</td>
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<td>Ht, cm</td>
<td>170 ± 4.0</td>
<td>178 ± 4.0</td>
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<td>BMI, kg/m²</td>
<td>24.2 ± 2.0</td>
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<td>1RM, kg</td>
<td>54.5 ± 9.2</td>
<td>73.3 ± 7.9</td>
</tr>
<tr>
<td>1RM/body wt</td>
<td>0.75 ± 0.04</td>
<td>0.97 ± 0.1*</td>
</tr>
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Values are means ± SE. UT, untrained (n = 6, 3 males and 3 females); T, trained (n = 6, 3 males and 3 females); BMI, body mass index; 1RM, single repetition maximum (nondominant leg concentric knee extension repetition maximum; see methods for explanation). *Significantly different from UT.

We have used female subjects in this study, as others have previously done (17, 26), because there do not appear to be any sex-based differences in postexercise muscle protein turnover (18). All of our female subjects were tested in the early to mid-follicular phase of their menstrual cycle.

Experimental protocol. The protocol was designed to examine the effect of a single bout of pleiometric resistance exercise on the acute response of mixed muscle protein FSR and fractional breakdown rate (FBR) in T and UT subjects. Subjects exercised only one leg, and their resting leg served as an internal control. All subjects in the T group were asked to refrain from performance of any weight-lifting exercise activities, not including weight lifting, for no more than 1.5 h/wk. T subjects engaged in a regular program of resistance training and had been doing so for at least 3 min, with at least 3 weight-lifting training sessions per week. All subjects reported to the Metabolism Unit for a 2-wk period before participating in the study, the following day. All subjects main-
The infusion rate for both [2H5]- and [15N]phenylalanine was 0.05 µmol·kg⁻¹·min⁻¹. The infusion was monitored in a calibrated Harvard syringe pump (Natick, MA). Isotopes were purchased from Cambridge Isotopes and dissolved in 0.9% saline before infusion. Isotopes were purchased from Cambridge Isotopes (Andover, MA). Infusion of the isotopes was performed using a calibrated Harvard syringe pump (Natick, MA). The infusion rate for both [2H5]- and [15N]phenylalanine was 0.05 µmol·kg⁻¹·min⁻¹ (priming dose 2.0 µmol/kg). All isotopes were filtered through a 0.2-µg filter before infusion. The infusion protocols were designed to achieve isotopic steadystate in both the muscle and plasma pools. That isotopic plateau would occur in these body pools has been shown in a number of previous investigations from this laboratory (3, 4, 17). Blood and muscle enrichments are shown in Fig. 2, A and B.

Blood. Blood samples taken from the arterialized hand vein for determination of amino acid enrichment and concentration were immediately precipitated in preweighed tubes containing 15% sulfosalicylic acid, which contained a weighed amount of internal standards in whole blood, the tertiary butyl dimethylsilyl (t-BDMS) derivative of phenylalanine was made according to previously described procedures (17). Analysis of t-BDMS phenylalanine by gas chromatography-mass spectrometry (Hewlett-Packard 5890, series II) was performed using electron impact ionization and selected ion monitoring of mass-to-charge ratios (m/z) 234, 235, 239, and 240 for the m+0, m+1, m+5, and m+6 ions, respectively. Appropriate corrections were made for any spectra that overlapped, contributing to the tracer (t)-to-tracee (T) ratio (24).

Muscle. Muscle biopsy tissue samples were analyzed for protein-bound and free intracellular enrichment, as well as intracellular concentration, as previously described (3, 17). Muscle free intracellular enrichment was determined by making the heptafluorobutyric (HFB) derivative of the pooled intracellular fractions, as previously described (17). To calculate the muscle FSR, protein-bound amino acid enrichment was determined by making the HFB derivative of phenylalanine from the hydrolyzed muscle-protein pellet. The HFB derivative of phenylalanine was made to facilitate the determination of phenylalanine enrichment using chemical ionization. Enrichment for the protein-bound HFB-phenylalanine samples was determined using previously described procedures (17). Precursor enrichment of the free amino acid pool for calculation of mixed muscle FSR, was determined from intracellular enrichment of the infused [3H3]phenylalanine by using chemical ionization and monitoring the ratio of ions m/z 409 to 404 (m+5/m+0) of the HFB-phenylalanine derivative. For calculation of the mixed muscle protein FBR, the decay of intramuscular free [15N]phenylalanine was also measured on the HFB by monitoring the ratio of m/z 405 to 404 (m+1/m+0). Muscle free intramuscular phenylalanine concentration was measured, with corrections for overlapping spectra as described previously (17, 24).

For calculation of muscle protein FBR (36), the decay in plasma enrichment was determined by analyzing the decay in the [15N]phenylalanine enrichment of the t-BDMS-phenylalanine derivative and the intracellular decay of the HFB-phenylalanine derivative. Two different derivatives were used (i.e., t-BDMS-phenylalanine for plasma enrichments and HFB-phenylalanine for intramuscular enrichments) in the calculation of FBR. We have found, however, that using the decay in plasma HFB-phenylalanine, or conversely using the decay of intracellular t-BDMS-phenylalanine (n = 2, rest and exercised), had no significant effect on the calculated FBR (data not shown). Previously, we had calculated FBR by assuming that the enrichment of the muscle intracellular [15N]phenylalanine enrichment at plateau, before terminating the infusion (t = 300; see Fig. 1), could be calculated by using the ratio of the mean intracellular [3H3]phenylalanine (determined in 3 biopsies) to arterial [3H3]phenylalanine enrichment during infusion protocol. Group mean enrichments are presented for clarity. T, trained; UT, untrained.

Fig. 1. Schematic representation of infusion protocol. Note that time for the exercise bout was somewhat variable but averaged 32 ± 1 min. Time values are in min beginning at 0600 on the testing day (see METHODS for details).

Fig. 2. [15N]- and [3H3]phenylalanine enrichment (tracer-to-tracee ratio) during infusion protocol. A: blood [15N]- and [3H3]phenylalanine enrichment during infusion protocol. Group mean enrichments are presented for clarity. T, trained; UT, untrained. B: muscle [15N]- and [3H3]phenylalanine enrichment during infusion protocol, resting values. Values are means ± SE (n = 6/group).
enrichment and multiplying by the arterial \(^{15}\text{N}\)phenylalanine enrichment (17). As we had stated previously, this approach has been validated in rabbits (X.-J. Zhang, unpublished observations). Nonetheless, because in this protocol we took a muscle biopsy at plateau, we were able to measure intracellular \(^{15}\text{N}\)phenylalanine enrichment directly and compare it with the predicted value, obtained from the calculation outlined above. The ratio of the calculated to measured intracellular \(^{15}\text{N}\)phenylalanine enrichment from the biopsy taken at \(t = 300\) min was \(1.05 \pm 0.04, r = 0.97 (P < 0.001; \text{data not shown})\).

Calculations. Mixed muscle protein FSR (\%/h), FBR (\%/h), and net balance (\%/h) were calculated as outlined previously (17). Briefly, mixed muscle protein FSR was determined using the free intracellular phenylalanine enrichment as the precursor pool, which appears to be a superior surrogate for the true precursor, phenylalanine mRNA enrichment, over blood phenylalanine enrichment (1, 12). The actual mixed muscle protein FSR was calculated as the mean of the incorporation of \(^{15}\text{N}\)phenylalanine into mixed muscle proteins over the time period from \(t = 120\) min to \(t = 320, 340,\) and 360 min divided by the intracellular precursor enrichment (3, 5, 17).

Statistics. Data were analyzed using a two-way repeated-measures analysis of variance (ANOVA), with condition (rest or exercise) as the within factor and group (training status) as the between factor. Wherever ANOVA revealed significant \((P < 0.05)\) differences, a Tukey post hoc procedure was used to locate the pairwise difference. Significant differences of independent means from zero were performed by t-test. Correlations were performed using a Pearson-product correlation and analyzed according to the appropriate degrees of freedom. A value for \(P \) of <0.05 was considered significant. All data are expressed as means ± SE.

RESULTS

Subjects’ characteristics. Table 1 shows the subjects’ descriptive characteristics. Subjects that were recruited to the T group had been performing regular weight-lifting training bouts for 10.5 ± 1.3 yr and trained on average 4.3 ± 0.6 times per week. All T subjects’ weight-training routines were split-body rotations that incorporated at least one leg workout per week and were, for the most part, free-weight training sessions. There was no difference between the T and UT groups with respect to age, weight, height, body mass index, or 1RM. 1RM was not significant because of the high degree of variation in this estimate, as much as both males and females are included in the mean. When 1RM was expressed relative to body weight, the T group was significantly stronger than the UT group (Table 1).

Muscle and blood phenylalanine enrichments and concentrations. Figure 2A shows the mean blood \(^{15}\text{N}\)phenylalanine and \(^{15}\text{H}\)phenylalanine enrichments throughout the protocol. Figure 2B shows the mean muscle free \(^{15}\text{N}\)phenylalanine and \(^{15}\text{H}\)phenylalanine enrichments in the nonexercised leg throughout the protocol. The mean enrichments are presented for clarity, although no individual subject’s blood or muscle free amino acid enrichments had any significant degree of fluctuation, which would indicate that plateau was not achieved in either the blood or muscle pools. Blood concentration of phenylalanine was essentially constant throughout the protocol at 55 ± 5 nmol/ml whole blood. Muscle intracellular phenylalanine concentration was 86 ± 9, 69 ± 14, 72 ± 15, and 79 ± 13 nmol/ml intracellular water at \(t = 120, 300, 340,\) and 360 min, respectively, in the T group and 81 ± 7, 70 ± 11, 75 ± 10, and 82 ± 15 nmol/ml intracellular water at the same time points in the UT group (mean effects, \(P = 0.17; \text{group effect } P = 0.84)\).

Muscle. Mixed muscle protein FSR was calculated according to the steady-state precursor-product equation (5, 6, 23). There was no significant difference in mixed muscle protein FSR between the T and UT groups. Muscle FSR was increased from nonexercised (resting) levels, however, by 118 ± 18% (\(P < 0.01; \text{Fig. 3A})\) in the UT group and by 48 ± 6% in the T group (\(P < 0.01; \text{Fig. 3A})\) as a result of exercise. The response in the UT group was greater than the response in the T group (\(P < 0.05)\). Tukey’s multiple comparison procedure did not result in significant differences in the T or UT groups between rest and after exercise.

Resistance training had no apparent effect on muscle protein FBR at rest (Fig. 3B). Mixed muscle protein FBR increased by 37 ± 5% in the UT group after exercise (\(P < 0.01; \text{Fig. 3B})\). FBR after exercise was higher by 15 ± 3% than at rest in the T group, but this
difference was not statistically significant (P > 0.05; Fig. 3B).

In accordance with our previous observations (17), we found that there was a significant correlation between mixed muscle protein FSR and FBR (r = 0.84, P < 0.001; Fig. 4). In addition, there was a significant correlation between the change, from rest to exercise, in muscle protein FSR and that in muscle protein FBR (r = 0.91, P < 0.001; data not shown).

Overall muscle protein balance (net balance) was calculated as the difference between FSR and FBR and is shown in Fig. 5. Net balance was significantly negative (i.e., significantly different from 0; P < 0.05) at all time points examined. Muscle protein net balance was significantly improved by exercise in both the UT and T groups by 37 ± 4 and 34 ± 11%, respectively. There was no effect of training status on muscle net balance at rest or after exercise, or on the magnitude of change in net balance (Fig. 5).

**DISCUSSION**

The acute response of human mixed muscle protein synthesis (FSR) was shown to be significantly increased, vs. a nonexercised (resting) leg, in the period of ∼4 h after an intense bout of plyometric contractions of the quadriceps. The finding of a rapid increase in muscle FSR after exercise in humans has been shown earlier (5, 26) and has been confirmed by experiments from our laboratory (3, 4, 17). The plyometric exercise bout also resulted in an increase in mixed muscle protein FBR in the UT group. In contrast, muscle protein FBR was unchanged after exercise in the T group. Collectively, the finding of a smaller increase, vs. a nonexercised control leg, in muscle protein FSR (Fig. 3A) and FBR (Fig. 3B) after plyometric exercise in the T group demonstrates that the repeated stimulus of resistance exercise appears to reduce muscle protein turnover after exercise. It may be that this training-induced suppression of muscle protein turnover is related, at least in part, to the training-induced reduction in contraction-induced muscle damage (8, 15).

An obvious constraint when the results of the present study are interpreted is the use of a cross-sectional design. We chose to use a cross-sectional design to test the hypothesis that trained subjects would have a reduced degree of muscle damage (8, 15) and, hence, a reduced muscle protein breakdown vs. the untrained subjects, because this design would optimize any potential differences between such groups. However, it is possible that the reduced postexercise muscle protein turnover seen in the T group may occur after only one previous bout of plyometric contractions (8) and may not necessarily require the numerous bouts of resistance exercise in which the T subjects had engaged. However, this hypothesis remains to be directly tested in humans.

We had hypothesized that muscle damage, characterized by disruption of the Z line and cytoskeletal structures (10), would be less in the T than in the UT subjects (8, 15). This hypothesis was based on data showing that training significantly attenuates, or even abolishes, increases in skeletal muscle enzyme efflux, which has been used as a marker of muscle damage (6, 8, 15). To our knowledge, only one report has actually documented that there is less ultrastructural damage at the myofibrillar level in trained vs. untrained humans (9). Accompanying the hypothesized reduction in muscle damage, we also anticipated that muscle protein breakdown, which we have previously reported is increased after resistance exercise (3, 17), would also be reduced. Our results supported this hypothesis, because the UT group was the only group to show a significant increase in FBR as a result of the plyometric exercise (Fig. 3B).

The mechanism responsible for the increase in muscle protein breakdown that we have reported (3, 17, present results) is not known. There are three pathways: calcium-sensitive calpain, lysosomal, and ATP-ubiquitin dependent, which are responsible for proteolysis in skeletal muscle, that can account for a varying proportion of total muscle proteolysis (for review, see Ref. 7).
Results from studies of rats have shown that exercise with a pleiometric contraction component can activate both the calpain (2) and lysosomal pathways (21). In addition, studies in which human subjects completed a bout of eccentric-isokinetic actions of the biceps muscle showed an increase in free and protein-conjugated ubiquitin in muscle biopsies taken 2 days postexercise (23).

Previous studies that have examined the effects of an isolated bout of exercise on human muscle protein synthesis have reported that muscle protein FSR was increased in the postexercise period (3, 5, 17, 25, 26). The effect of regular resistance training on muscle protein synthesis has been addressed in two previous reports (25, 26), although it is difficult to distinguish a training effect from an acute effect of resistance exercise in these studies. Yarasheski et al. (25) reported that the muscle protein FSR in young males increased 37% after 12 wk of resistance training. The same authors (26) also reported that 2 wk of resistance training resulted in an increase in mixed muscle protein FSR of 55% in a group of young control subjects and of 155% in a group of older control subjects. We have recently confirmed the results of Chesley et al. (5), that an isolated bout of resistance exercise causes a persistent elevation of mixed muscle FSR for up to 48 h postexercise (17). The time course of the elevation in mixed muscle protein FSR after exercise may be shorter in trained subjects (5, 13). Because muscle protein FSR is elevated for \( \geq 24 \) h, and \( \leq 48 \) h, postexercise (5, 17), the previous reports of elevations in mixed muscle FSR after training (25, 26), in which measures of FSR were reported between 3 and 24 h after the last training bout, are likely due to an acute bout of exercise. The present finding, that training attenuates the increase in muscle protein FSR, may explain why Yarasheski and co-workers (25, 26) reported increases of between 37 and 55% in quadriceps (lateral vastus) muscle protein FSR in trained subjects, whereas we have reported large (112%) increases in relatively untrained subjects (17). In a recent report (20), a group of resistance-trained subjects completed a single bout of resistance exercise consisting of two modes (knee extension and leg press) of leg exercise to stimulate muscle protein FSR. The results from this study showed that muscle protein FSR was increased only 6% above baseline in a placebo condition, whereas consumption of a glucose supplement resulted in FSR being 35% higher in the exercised leg, which was also not a significant increase (20). The subjects in the previous report (20) were quite well trained; this, according to the present findings, may have resulted in a suppression of muscle protein synthesis postexercise. In the present study, we found a larger increase (49%) in muscle protein FSR in our trained subjects than reported by Roy et al. (20). This may have been due to the nature (completely pleiometric), intensity (120% of concentric 1RM), and volume (80 repetitions) of the exercise protocol.

In the present study, as in others (3, 17), we reported that muscle net balance, although improved by the exercise bout, was still negative in both groups of subjects (Fig. 5). This finding is not surprising, since the subjects in both of the previous studies were studied in a fasted state. Recently, Biolo et al. (4) demonstrated that, at rest, muscle balance became positive only when amino acids were supplied. Interestingly, the effect of exercise along with postexercise amino acid provision resulted in a synergistic effect, enhancing muscle protein synthesis above and beyond the amino acids alone in the resting condition (4). In fact, a recent study has demonstrated that early provision of amino acids and glucose may result in a greater postexercise stimulation of protein synthesis (16). The results of the present study and the previously published studies (3, 4) demonstrate, however, that exercise induces an increased intramuscular “recycling” of amino acids from protein breakdown. Exercise alone results in increased inward transport, from the blood to the intramuscular pool, of several amino acids, including alanine, leucine, and lysine, but not phenylalanine (3). Hence, muscle balance can be increased but does not become positive until exogenous amino acids are provided (4), and until that time the most readily available source of amino acids for utilization is from an increase in the rate of protein breakdown (3, 17). We have demonstrated a close correlation between FSR and FBR (17 and present results), which we believe is indicative of coupling between these two processes. Support for an interaction between the processes of synthesis and breakdown comes from the present findings, where a larger exercise-induced increase in muscle protein FSR in the UT group was associated with a larger increase in muscle protein FBR. The nature of the relationship between these two processes remains to be elucidated.

The increase in muscle protein synthesis in the current study occurred rapidly within the postexercise period. This rapid response of muscle FSR has been observed previously in response to exercise and in response to increased amino acid supply (3–5, 17, 25). Such a rapid increase in muscle protein synthesis implies that the mechanism(s) responsible for the increase in FSR is (are) almost certainly posttranscriptional in nature, at least for the majority of newly synthesized proteins. Hence, it appears that amino acid supply to the muscle intracellular compartment, either through amino acid transport (6, 21) into the muscle (4, 16) or through protein breakdown, may be an important regulatory factor in determining the rate of muscle protein synthesis.

We thank the staff and nurses of the General Clinical Research Centre (GCRC) at Univ. of Texas Medical Branch (UTMB), Galveston for their time and competent technical assistance. The medical support of Drs. J. Cortiella and S. Matin was also greatly appreciated. Thanks also to the volunteers who participated in this project.

This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-38010 and Shriners Hospital Grant 8490 to R. R. Wolfe. The GCRC at UTMB, Galveston is supported by National Institutes of Health Grant M01–0073. S. M. Phillips was supported by a postdoctoral fellowship from the National Sciences and Engineering Research Council of Canada.

Received 30 March 1998; accepted in final form 29 September 1998.
REFERENCES


