Resistance training alters the response of fed state mixed muscle protein synthesis in young men


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Tang JE, Perco JG, Moore DR, Wilkinson SB, Phillips SM. Resistance training alters the response of fed state mixed muscle protein synthesis in young men. Am J Physiol Regul Integr Comp Physiol 294: R172–R178, 2008. First published November 21, 2007; doi:10.1152/ajpregu.00636.2007.—Ten healthy young men (21.0 ± 1.5 yr, 1.79 ± 0.1 m, 82.7 ± 14.7 kg, means ± SD) participated in 8 wk of intense unilateral resistance training (knee extension exercise) such that one leg was trained (T) and the other acted as an untrained (UT) control. After the 8 wk of unilateral training, infusions of L-[ring-d5]phenylalanine, L-[ring-13C6]phenylalanine, and d3-o-ketoisocaproic acid were used to measure mixed muscle protein synthesis in the T and UT legs by the direct incorporation method [fractional synthetic rate (FSR)]. Protein synthesis was determined at rest as well as 4 h and 28 h after an acute bout of resistance exercise performed at the same intensity relative to the gain in single repetition maximum before and after training. Training increased mean muscle fiber cross-sectional area only in the T leg (type I: 16 ± 10%; type II: 20 ± 19%, P < 0.05). Acute resistance exercise increased mixed muscle protein FSR in both legs at 4 h (T: 162 ± 76%; UT: 108 ± 62%, P < 0.01 vs. rest) with the increase in the T leg being significantly higher than in the UT leg at this time (P < 0.01). At 28 h postexercise, FSR in the T leg had returned to resting levels; however, the rate of protein synthesis in the UT leg remained elevated above resting (70 ± 49%, P < 0.01). We conclude that resistance training attenuates the protein synthetic response to acute resistance exercise, despite higher initial increases in FSR, by shortening the duration for which protein synthesis is elevated.

IN HEALTHY INDIVIDUALS MUSCLE protein mass is regulated by changes in muscle protein synthesis (MPS), and to a lesser degree, muscle protein breakdown (MPB). In the fasted state, MPB exceeds MPS such that muscle protein balance is negative and there is a net loss of muscle protein (18). In the fasted state, resistance exercise increases both MPS and MPB, im-

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of each subject; the biopsy was performed under local anesthesia (2% xylocaine) using our custom suction-modified 5-mm Bergstrom needle. The leg to be trained was selected randomly, counterbalanced for dominance based on strength, such that equal numbers of subjects trained their dominant and nondominant legs. Training was performed 3 d/wk (Monday to Friday) initially (weeks 1–4) and then only 2 d/wk at the latter stages of the training (weeks 5–8); at least 1 day of rest was provided between each training session. For weeks 1–2, training began with three sets of knee extension exercise performed at a workload equivalent to each subject’s 10–12 RM. For weeks 3–4, participants performed four sets at their 8–10 RM. The number of sets was increased to five for weeks 5–6. Finally, for weeks 7–8, participants performed six sets at a workload equivalent to their 6–8 RM. Each subject performed all 20 training sessions, which were supervised by a study investigator.

Stable isotope infusion protocol. L-[ring-d5]phenylalanine (98% enriched), L-[ring-13C6]phenylalanine (99% enriched), and d3-3-ketoisocaproic acid (d3-3-KIC; sodium salt, 98% enriched) were purchased from Cambridge Isotope Laboratories (Andover, MA). All isotopes were dissolved in 0.9% saline, passed through a 0.2-μm filter, and stored in evacuated bottles prior to infusion. Isotopes were passed through an in-line 0.2-μm filter during the infusion by using a calibrated syringe pump (74900 Series; Cole Palmer Instrument, Niles, IL).

Stable isotope infusions were used to measure MPS in the T and UT legs on three occasions: at rest, 4 h following an acute bout of resistance exercise, and 28 h thereafter. The experimental infusion protocols are shown schematically in Fig. 1. Four days following their last training session, subjects participated in the infusion trial to measure MPS at rest. Following the resting infusion trial, subjects then performed two additional training sessions according to their last recorded training volume and workload. A second infusion trial was performed 1 wk following the resting infusion trial to examine the response of MPS 4 h after an acute bout of resistance exercise (6 sets of 8–10 repetitions at ~80% 1-RM knee extension at the same relative intensity for the T and UT leg) in the fed state. Subjects then returned to the lab 24 h following the bout of resistance exercise for the third and final infusion trial. Dietary intake was controlled 48 h prior to the infusions as well as between the infusion sessions by providing subjects with prepackaged diets. The diets contained standard food items as well as at least 30% of the subjects’ dietary energy in the form of a liquid meal replacement drink (Boost; Novartis, Mississauga, ON, Canada) to provide vitamins and minerals at the recommended levels. Diets were formulated based on the subjects’ estimated energy requirements according to the Harris-Benedict equation using a moderate activity level. Protein was held constant in each diet package at 1.2 g·kg⁻¹·d⁻¹. Compliance with the diets was checked by having subjects report to the lab on each day they were consuming the diet and providing dietary checklists as well as close monitoring of the subjects’ weight during this time.

Each infusion trial began with subjects arriving in the lab at ~0700–0800 after an overnight fast (~10 h). Subjects had a 20-gauge catheter inserted in an antecubital vein from which blood samples were drawn throughout the infusion period (t = 0, 60, 90, 120, 180, 240, and 270 min; see Fig. 1). The catheter was kept patent by periodic flushing with 1–2 ml of 0.9% saline. Following the

Fig. 1. Schematic representation of the infusion sessions following training. ¹³C₆ Phe, L-[ring-¹³C₆]phenylalanine; d₅ Phe, L-[ring-d₅]phenylalanine; d₃-α-KIC, d₃-α-ketoisocaproic acid.
baseline blood sample, a second catheter was inserted in the contratellar arm in which participants received a primed constant infusion of L-[ring-d5]phenylalanine, L-[ring-13C6]phenylalanine, or d3-leucine in which participants received a primed constant infusion of baseline blood sample, a second catheter was inserted in the contralateral arm in which participants received a primed constant infusion of 

The dried pellet was placed in 2 ml of 6 N HCl and hydrolyzed for 24 h at 100°C. The acid hydrolysate was passed over acid-washed cation exchange column (Dowex Analytical Grade 50W-X8, 100–200 mesh hydrogen form; Bio-Rad Labs, Hercules, CA) for amino acid isolation/purification to determine the protein-bound enrichments. The samples were dried by using a rotary evaporator, and the dried pellet was then derivatized in the same manner as the blood samples. Intracellular enrichments were determined in the same manner as the blood, while bound muscle enrichments were determined using the standard curve approach (16).

Calculations. Fractional synthetic rate (FSR) was calculated from the determination of the rate of tracer incorporation into muscle protein and using the muscle intracellular free phenylalanine or leucine enrichment as a precursor, according to the following equation: FSR (%/h) = (Et - E0) / [Ep * (t1 - t0)] * 100%, where Et is the enrichment of the protein-bound isotope tracer from the first biopsy, E0 is the enrichment of the protein-bound isotope tracer from the second biopsy, Ep is the mean intracellular tracer enrichment during the time period for determination of protein incorporation, and (t1 - t0) is the incorporation time.

Statistics. Strength, amino acid, blood glucose, and insulin data were analyzed using a two-factor repeated-measures ANOVA. Fiber cross-sectional area data were analyzed using a paired t-test for two sample means (pre and post) for each fiber type. Rates of mixed MPS were analyzed using a two-factor ANOVA with training status (T and UT) and condition (rest, 4 h postexercise, and 28 h postexercise) as within-subject factors. Where ANOVA revealed significance, a Tukey’s post hoc procedure was used to identify pairwise differences. Residuals and comparisons of FSR determined by the phenylalanine and α-KIC tracers were made using GraphPad Prism (version 5.00, GraphPad Software). Significance was accepted at P < 0.05. All data are expressed as means ± SD.

RESULTS

Strength measurements. Prior to training there was no difference between legs in unilateral knee extension 1-RM strength (Fig. 2). Unilateral 1-RM strength increased in both legs with training (T = 62.3 ± 27%; UT = 19.7 ± 13%, P < 0.01; Fig. 2). However, by the completion of the training protocol, the increase in the T leg was threefold greater than that seen in the UT leg (P < 0.01; Fig. 2). There was no pretraining difference between legs in isometric strength (T = 284.5 ± 43.5; UT = 297.4 ± 46.8 N·m, P = 0.64). After training, isometric strength increased in the T leg (17.7 ± 12%, P < 0.01), with no change in the UT leg (P = 0.86).

Fig. 2. Knee extension 1 repetition maximum (RM) strength assessments.

*Significantly different from pretraining (PRE) in the same leg (P < 0.01). UT trained. +Significantly different from posttraining (POST) in the untrained (UT) leg (P < 0.001). Values are means ± SD (N = 10).
Muscle fiber cross-sectional area. The mean fiber cross-sectional area of both type I and type II fibers increased with training (type I: 16 ± 10%, P < 0.05; type II: 20 ± 19%, P < 0.05; Fig. 3) in the T leg. There was no change in type I or type II fiber cross-sectional area after the training protocol in the UT leg.

Blood amino acid concentrations. Table 1 shows the concentration of plasma total amino acids, essential amino acids, and branched-chain amino acids during the three testing sessions. There was a main effect for time in total amino acids, essential amino acids, and branched-chain amino acid levels in the plasma (P < 0.01). Feeding stimulated an increase in plasma amino acid levels; however, the level remained constant at the times when biopsies were taken. There was also a main effect for condition where the amino acid concentrations seen at 24 h postexercise condition were lower than immediately postexercise (P < 0.05; Table 1).

Plasma glucose and insulin concentrations. Plasma glucose levels were not significantly affected by feeding or by condition. However, there was a main effect of time where insulin concentrations were significantly elevated over resting (t = 0 h) values at all time points (P < 0.01; Table 2).

Mixed muscle protein FSR. Plasma enrichments were stable over the incorporation period of each trial, with no differences between trials. There was no difference in resting mixed muscle FSR between the T and UT leg (P = 0.97; Fig. 4). The acute bout of resistance exercise elevated FSR above resting values (T: 162 ± 76%; UT: 108 ± 62%, P < 0.01, P < 0.01; Fig. 4); however, the increase in the T leg was significantly higher than that seen in the UT leg 4 h postexercise at the same relative intensity (P < 0.01; Fig. 4). At 28 h postexercise the increase in FSR in the T leg returned to resting levels; however, FSR remained elevated in the UT leg (70 ± 49%, P < 0.01; Fig. 4).

DISCUSSION

We report here that 8 wk of unilateral resistance training (20 total training sessions) results in hypertrophy and alters the response of fed state-mixed MPS to an acute bout of resistance exercise performed at the same relative intensity (i.e., greater work performed by the T leg). In the present study the subjects were fed a meal supplement to maximize anabolic potential by creating a hyperinsulinemic and hyperaminoacidemic environ-

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Values are means ± SD in micromolars. Zero hours had significantly lower total amino acids (TAA), essential amino acids (EAA), and branched-chain amino acids (BCAA) concentrations than all other time points (P < 0.01). Main effect for condition where there were lower TAA, EAA, BCAA concentrations 24 h postexercise than immediately postexercise (P < 0.05).

The limited number of studies that have aimed to define the time course of increased MPS following a bout of resistance exercise suggests that protein synthesis rises rapidly (within 3–4 h) postexercise and remains elevated for at least 24 h (7, 13, 18). Combined cross-sectional data from Chesley et al. (7) and MacDougall et al. (13) suggests that MPS rises at 4 h postexercise, peaks at 24 h, and subsequently returns to resting levels by 36 h postexercise in trained subjects. Conversely, Phillips et al. (18) demonstrated that protein synthesis peaks at 3 h postexercise, and remains elevated above resting levels, albeit declining progressively both at 24 h and 48 h postexercise after an acute bout of resistance exercise. It is difficult to reconcile these previous results, both to each other and to the

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Values are means ± SD. Zero hours had significantly lower insulin concentrations than all other time points (P < 0.01).

Fig. 3. Mean fiber cross-sectional area (CSA). *Significantly different from PRE (P < 0.05). Values are means ± SD (n = 7 men).
present study, as there are considerable differences in the training status of the participants and the study designs employed (i.e., cross-sectional vs. longitudinal). Indeed, Chesley et al. (7) and MacDougall et al. (13) utilized different groups of trained individuals for each time point studied, while Phillips et al. studied the same subjects at each time in a longitudinal design. Moreover, Chesley et al. (7) and MacDougall et al. (13) studied subjects in the fed state while the participants in the study by Phillips et al. (18) were fasted. To our knowledge, the present study is the first study to longitudinally examine the time course of changes in MPS in the fed state following an acute bout of resistance exercise after training. We found an increase in mixed MPS in the immediate period after exercise. However, while MPS remained elevated by 70% at 28 h postexercise in the UT leg, rates of MPS had returned to baseline by 10.2 ± 0.3 % by 28 h postexercise compared with the UT leg. These results are in agreement with the general concept of adaptation to stress, manifest here as a reduction in the duration of the postexercise stimulation of protein synthesis. While there has been some evidence to suggest that rate of protein synthesis is dependent on the tracer used (25, 27), a residual plot (Fig. 5) of our FSR shows that changes in MPS in the T state are initially greater in amplitude but are reduced in duration in the T state (Fig. 6). Indeed, as an estimate of the aggregate synthetic response following isolated resistance exercise, the area under the curve for the increase in MPS over 28 h is lower in the T vs. UT leg despite the greater initial rise in MPS in the T leg (Fig. 6). It is worth noting that our previous measures of MPS after training were made in the fasted state and likely result in an underestimation of the net MPS over 28 h after exercise. Nonetheless, the reciprocal pool model used with the α-KIC tracer did not unduly influence our data in magnitude or overall pattern of response.

The greater initial increase in MPS observed in the T vs. UT leg seems counterintuitive to the general principle of adaptation, which would predict a lower protein synthetic response with training. Combining our present results with previous data from our lab (12), where we reported a reduced protein synthetic response 16 h postexercise, albeit in the fasted state, after training (at the same relative pretraining intensity), it provides a picture of the time course for changes in protein synthesis through the first 28 h following exercise (Fig. 6). This graph shows that changes in MPS in the T state are initially greater in amplitude but are reduced in duration in the T state (Fig. 6). Indeed, as an estimate of the aggregate synthetic response following isolated resistance exercise, the area under the curve for the increase in MPS over 28 h is lower in the T vs. UT leg despite the greater initial rise in MPS in the T leg (Fig. 6). It is worth noting that our previous measures of MPS after training were made in the fasted state and likely result in an underestimate of the net MPS over 28 h after exercise. Nonetheless,
we would propose that the change in MPS at 16 h, were the measures made in the fed state would be altered in magnitude but would still demonstrate that both UT and T legs, which showed a relative stimulation over basal of 172 and 28% respectively, respond similarly to nutrition. Thus, our data suggest that resistance training may increase the sensitivity of MPS, expressed as a more rapid rise in MPS, but on balance reduces the overall response of MPS to an acute bout of resistance exercise. This is consistent with the notion that the exercise stimulus becomes progressively less novel with training. Consequently, there is an attenuation of the perturbation from homeostasis induced by resistance exercise, minimizing the anabolic effect of training over time (1, 12). Therefore, the reduced protein synthetic response observed in the T leg over the 28-h period postexercise is consistent with a previously suggested reduction in protein turnover after exercise in the trained state (12, 17, 19). We are not suggesting, however, that the subjects in the present study had reached a peak in terms of the response of MPS with training. In practice, those attempting to gain lean muscle mass would manipulate a variety of variables including relative load, exercise mode, rest interval, and feeding patterns in an attempt to prevent the eventual plateau in gains of strength and mass that invariably accompanied long-term strength training (23).

The greater amplitude of the protein synthetic response we observed in the T leg may be relevant to the timing of nutrient provision when performing resistance exercise. It is known that amino acid availability is a key regulator of protein synthesis (4, 22). Moreover, the provision of amino acids in the immediate postexercise period is important for maximizing protein synthesis and, ultimately, muscle mass accrual (8, 11, 28). We recently reported that failure to consume protein 2 h after resistance exercise resulted in lower lean mass gains over 12 wk of training in young men (11). Furthermore, one study previously found that resistance training-induced hypertrophy was completely suppressed in older individuals when nutrient provision was delayed for 2 h after exercise over a 12-wk training study (9). Further, Andersen et al. (2) only reported hypertrophy in young men who resistance trained for 12 wk who consumed protein postexercise, whereas those who consumed only carbohydrate did not show any hypertrophy. These data (2, 8, 11, 28) suggest, therefore, that it is important for individuals to consume protein in the immediate postexercise period to maximize protein accretion during training.

We report here that 8 wk of progressive unilateral leg training reduces the mixed muscle protein synthetic response over 28 h to an acute bout of resistance exercise performed at the same relative intensity before and after training. Despite the reduced anabolic response, MPS was higher initially in the T leg (4 h postexercise) but returned to baseline by 28 h postexercise at which time rates of MPS in the UT leg remained elevated. We conclude, therefore, that resistance training alters the time course of the increase in mixed MPS in the fed state.

**Perspectives and Significance**

As the protein synthetic response becomes attenuated with training, our results suggest that it likely becomes increasingly important for individuals to consume a source of amino acids in the immediate postexercise period during training to maximize MPS and thus protein accretion. While these results may also suggest that the frequency or intensity of lifts being performed during training sessions needs to be increased as individuals progress through their training, consideration must be made for adequate recovery time between training sessions and the risk of overtraining. Finally, additional research is required to determine the mechanisms that underlie the training-induced changes in the protein synthetic response to exercise.

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