Resistance exercise maintains skeletal muscle protein synthesis during bed rest

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Resistance exercise maintains skeletal muscle protein synthesis during bed rest. J. Appl. Physiol. 82(3): 807–810, 1997.—Spaceflight results in a loss of lean body mass and muscular strength. A ground-based model for microgravity, bed rest, results in a loss of lean body mass due to a decrease in muscle protein synthesis (MPS). Resistance training is suggested as a proposed countermeasure for spaceflight-induced atrophy because it is known to increase both MPS and skeletal muscle strength. Therefore, we hypothesized that scheduled resistance training throughout bed rest would ameliorate the decrease in MPS. Two groups of healthy volunteers were studied during 14 days of simulated microgravity. One group adhered to strict bed rest (BR; n = 5), whereas a second group engaged in leg resistance exercise every other day throughout bed rest (BREx; n = 6). MPS was determined directly by the incorporation of infused L-[ring-13C6]phenylalanine into vastus lateralis protein. After 14 days of bed rest, MPS in the BREx group did not change and was significantly greater than in the BR group. Thus moderate-resistance exercise can counteract the decrease in MPS during bed rest.

spaceflight; microgravity; biopsy

Prolonged inactivity or bed rest has been shown to result in loss of body nitrogen (10, 12, 23, 25) and lean body mass (9, 13, 23, 26). This loss of lean body mass is most prevalent in the ambulatory muscle groups (13, 19). Because the bed rest-induced loss mimics that resulting from spaceflight (20), it is often used as a ground-based model to study the effects of microgravity (16). Although the basis for a loss of lean body mass in microgravity remains unclear, bed rest has been shown to decrease skeletal muscle protein synthesis (MPS) (12). Thus interventions known to increase MPS, such as resistance exercise (6, 7, 21), might be of potential benefit. Previous studies (7, 21) noted a doubling of MPS by acute bout of resistance exercise. Thus it was hypothesized that resistance training throughout bed rest could ameliorate the effects of bed rest on MPS.

Materials and Methods

Clinical protocol. Six healthy men [28 ± 7 (SD) yr; 81.8 ± 16.6 kg; 179.0 ± 4.1 cm] were recruited for a study investigating the effects of simulated microgravity and resistance exercise on MPS. An additional six healthy men [30 ± 6 yr; 64.8 ± 6.2 kg; 171.8 ± 4.1 cm] were recruited for a study investigating the effects of simulated microgravity on protein metabolism. These data have been presented previously (12); however, only five of the six subjects from this group were infused with stable isotopes, as described in the METHODS section of that study, and served as the control group for this intervention. These data (12) have been included in the present study because the conditions of the bed rest and dietary protocols were identical, except that one group performed scheduled resistance exercise (BREx), whereas the other adhered to strict bed rest (BR). The BR group was not strength tested or exercised before the isotope-infusion studies. After written consent was obtained, each subject was admitted to the General Clinical Research Center (GCRC) at the University of Texas Medical Branch at Galveston. This study protocol was reviewed and approved by the institutional review boards at the University of Texas Medical Branch and National Aeronautics and Space Administration, Johnson Space Center.

This study involved a 15-day stay at the GCRC. The first day served as the pre-bed rest (BR pre) measure of MPS. This was followed by 14 days of strict 6° head-down bed rest. A second determination of MPS was made on bed rest day 14 (BR 14). The head-down tilt was utilized to simulate the effects of microgravity on initial fluid volume shifts (15) for unrelated studies. Subjects were not permitted to deviate from this position and were monitored continuously by the GCRC staff.

Subjects were given a mixed diet on the basis of the Harris-Benedict equation designed to maintain body weight throughout the study. The caloric distribution was 14% protein, 27% fat, and 59% carbohydrate. All subjects consumed ~1.1 g protein·kg body wt−1·day−1.

Resistance training. The knee extensors were exercised isotonically every other day during bed rest from the supine position by utilizing a horizontal leg-training device (Cybex Strength Systems, Ronkonkoma, NY). Resistance exercise subjects were first familiarized with the training protocol 1 day before bed rest. After learning the proper exercise form, subjects practiced by completing 3 sets of 10–12 repetitions. To preclude the likelihood of studying the effects of acute resistance exercise on MPS (7), the final training session (BR 13) again consisted of 3 sets of 10–12 repetitions.

During bed rest, muscle soreness was minimized in these untrained subjects by progressively increasing volume and intensity during the first three sessions. By session 3, five sets to volitional muscle failure were completed. The number of repetitions for all sets was set at 8 (range 7–9) throughout training. If a set was performed outside this range, the load of subsequent sets was adjusted accordingly. Progressive resistance was therefore incorporated into the program. Exercise was performed at a volume and intensity known to effectively induce gains in strength and muscle mass (1, 18). Rest between sets was set at 90 s to permit restoration of creatine phosphate (27). Loads for warm-ups were set at two-thirds of the previous training session’s eight repetition maximum (RM). All training was supervised by one of the investigators.

Pre- and post-bed rest strength were measured (BREx group only) by 1 RM on the same training device. Pre- and post-bed rest strength were also measured in a separate control group of a similarly designed but unrelated study by one of the investigators (3). Identical equipment and strength-testing procedures were utilized, and the comparison of
Skeletal MPS. We examined the effects of bed rest and bed rest plus resistance exercise on MPS. On days BR − 1 and BR 14 after a 12-h fast, a catheter was inserted into an antecubital vein for stable isotope infusion. Subjects received a 5-h primed (2 µmol/kg) continuous (0.05 µmol·kg\(^{-1} \cdot \text{min}^{-1}\)) infusion of \(^{13}\)C\(_6\)-phenylalanine (Phe; 99% enriched; Cambridge Isotope Laboratories, Woburn, MA). Subjects were recumbent and horizontal throughout the 5-h infusion period. At 2 h, a muscle needle biopsy was taken from the lateral portion of the vastus lateralis to measure isotopic carbon enrichment of bound and free Phe in the muscle. A second biopsy was performed at the end of the study at 5 h. Biopsies were performed according to the Bergström technique as previously described (5).

MPS was calculated from the determination of the rate of tracer incorporation into the protein and the enrichment of the intracellular pool as the precursor

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\text{MPS} = \left[\frac{E_{p2} - E_{p1}}{1.5(E_u + t)}\right] \times 60 \times 100
\]

where \(E_{p1}\) and \(E_{p2}\) are the enrichments of the protein-bound Phe tracer at the start (2 h) and end (5 h) of the sampling period, respectively, \(E_u\) represents the average intracellular Phe enrichment over the time of incorporation, and \(t\) is the time in minutes. The numerator is multiplied by 1.5 to normalize the combustion measurement of the protein-bound Phe by isotope ratio mass spectrometry with the measurement of the enrichment of the precursor by gas chromatography-mass spectrometry. Only six out of the nine carbons in the Phe tracer are enriched, so a tracer/tracee ratio of 0.01 determined by gas chromatography-mass spectrometry would yield a ratio of 0.0067 if measured by isotope ratio mass spectrometry. The factors 60 and 100 are required to express MPS in percentage per hour.

Analysis of samples. Tissue biopsies of the vastus lateralis were immediately blotted and frozen in liquid nitrogen. Samples were then stored at −70°C until processed. The tissue was weighed and protein was precipitated with 0.5 ml of 10% perchloric acid. The tissue was then homogenized and centrifuged, and the supernatant was collected. This procedure was repeated two more times, and the pooled supernatant (∼1.3 ml) was stored at −70°C until analysis.

The remaining pellet of muscle tissue was further washed three times with absolute ethanol and twice in 0.9% saline. It was then placed overnight in an oven and dried at 50°C. The dried pellet was then hydrolyzed at 110°C for 36 h with 6 N HCl. The protein hydrolysate was then passed through acid-washed Celite to remove carbon particles. Phe was isolated and purified from the filtered solution by high-pressure liquid chromatography (LKB Bromma). Samples containing pure Phe were dried under nitrogen, placed in tin containers, and combusted by using a carbon-nitrogen analyzer (Nitrogen Analyzer 1500, Carlo Erba, Serono, Italy). After combustion, the CO\(_2\) was automatically injected into an isotope ratio mass spectrometer (VG Isogas, Middlesbrough, UK) for determination of the \(^{13}\)C/\(^{12}\)C ratio in protein-bound Phe. The precision of this procedure has been previously reported (5).

On thawing, the pooled supernatant of cellular water was passed over a cation-exchange column (Dowex AG 50W-8X, 100- to 200- mesh H\(^+\) form; Bio-Rad, Richmond, CA) and dried under vacuum by using a Speed Vac (Savant Instruments, Farmingdale, NY). One hundred microliters of N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (Pierce, Rockford, IL) and 100 µl of acetonitrile were added to the dry residue. The mixture was then heated at 95°C for 45 min. The isotopic enrichment of the in vivo labeled Phe was determined by using an HP 5890/5989B GC/MS engine (Hewlett-Packard, Palo Alto, CA). The ions 234 and 240 (mass-to-charge ratio) were selectively monitored to determine precursor Phe enrichment.

Data presentation and statistical analysis. Data are presented as means ± SE. BR − 1 and BR 14 MPS and 1-RM values in each group were compared by paired t-test. The change in MPS from BR − 1 to BR 14 for each group was compared between groups by t-test. A P value ≤ 0.05 was considered statistically significant.

RESULTS

Bed rest resulted in a 46% decrease in MPS in the BR group (0.074 ± 0.011 to 0.040 ± 0.007%/h; \(P < 0.04\)). However, resistance exercise maintained MPS in the BREx group (0.066 ± 0.011 to 0.094 ± 0.026%/h; \(P = 0.20\)) (Fig. 1). The demonstrated change in MPS from the beginning to the end of bed rest was significantly different between groups (\(P < 0.04\)). The BR group experienced a mean decrease of 0.034 ± 0.015%/h, whereas MPS in the BREx group demonstrated a nonsignificant increase of 0.028 ± 0.019%/h.

1-RM strength was maintained in the BREx group (208.3 ± 14.7 kg pre-bed rest vs. 214.2 ± 12.3 kg post-bed rest, respectively; \(P = 0.65\)).

DISCUSSION

Acute bouts of resistance training have been shown to double protein synthesis up to 24 h postexercise (7, 21). Because the subjects in this study engaged in resistance training every other day, the intent was to maintain a stimulation of MPS such that the noted decrease with inactivity could be prevented (12). The present study demonstrates the cumulative effects of...
repeated stimulation of MPS. In addition to the elimination of a decreased MPS, this exercise protocol maintained muscle strength.

Muscular unloading by inactivity or bed rest has been shown to decrease muscle strength (26). In particular, locomotor muscle strength is compromised with the continued unloading of bed rest (3, 26). In this respect, bed rest is a viable ground-based model for the perturbations of spaceflight (20). Weightlessness results in a more-pronounced loss of strength in the legs, particularly in the knee extensors (8). The ability to maintain strength and function in the locomotor muscle groups is of prime concern to the astronaut. Impaired muscle function is detrimental to the astronaut's ability to perform shuttle landing, egress, and extravehicular activity with prolonged exposure to microgravity. Thus a countermeasure that is effective in ameliorating these losses would enhance the astronaut's performance capability.

On the basis of the results of the present study and resistance training's established influence on muscle strengthening (1) and MPS (6, 7, 21), it is tempting to speculate that a relationship exists between the chronic stimulation of skeletal MPS and muscular strength. This notion is supported by the results of previous studies analyzing the effects of inactivity or immobilization on MPS and strength. An identical research protocol as the present study (with bed rest only) demonstrated a decrease in 1-RM leg extensor strength (229.1 ± 7.4 kg pre-bed rest vs. 208.0 ± 8.2 kg post-bed rest, respectively; P < 0.01) (3). The present study provides evidence that the decrease in strength corresponds to a decreased fractional synthetic rate of protein synthesis. With muscular stimulation, however, the relationship between MPS and strength is maintained. In addition to the present findings, Gibson et al (14) noted that the fractional synthetic rate of muscle protein and fiber cross-sectional area could be maintained in the casted (immobilized) leg by electrical stimulation. Electrical stimulation has also been shown to prevent reductions in leg volume, muscle compartment size, and muscle strength with bed rest (11).

These findings, however, do not establish a direct relationship between MPS and strength. Indeed, MPS is greatly increased immediately after resistance exercise (6), yet it cannot be suggested that strength would acutely increase. Rather, the relationship between MPS and muscular strength is most likely dependent on a net MPS balance over time. Circumstances resulting in hypermetabolism, such as burn injury, will produce elevated protein synthetic rates yet a net loss in skeletal muscle protein and strength (22). Resistance exercise alone provides a more favorable net muscle protein balance (6), which could be further enhanced by hyperinsulinemia and hyperaminoacidemia (4). The limited data involving spaceflight have shown an increase in whole body protein synthesis (24), yet spaceflight is known to result in a loss of lean body mass (20) and strength (8). Protein breakdown was not assessed in this study; however, previous work has demonstrated that the loss of lean body mass (12) and strength (3) during bed rest is a result of altered protein balance (12). Thus periodic stimulation of MPS with resistance exercise most likely results in a positive net protein balance over time that preserves muscle strength.

Most importantly, this study illustrates that scheduled periodic resistance exercise will prevent the decline in MPS and strength with inactivity. The results of the present study indicate that resistance training may be a viable countermeasure to the loss of muscle strength and mass with spaceflight (2). Endurance exercise is currently employed in space; however, it is unsuccessful in ameliorating reductions in muscle strength (17). Resistive devices are currently being developed but have yet to be flown as a countermeasure. This study suggests that repeated stimulation of MPS, or most likely the maintenance of net protein synthesis, may be integral to the maintenance of muscular strength.

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REFERENCES


