Artificial gravity maintains skeletal muscle protein synthesis during 21 days of simulated microgravity

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Symons TB, Sheffield-Moore M, Chinkes DL, Ferrando AA, Paddon-Jones D. Artificial gravity maintains skeletal muscle protein synthesis during 21 days of simulated microgravity. J Appl Physiol 107: 34–38, 2009. First published April 23, 2009; doi:10.1152/japplphysiol.91137.2008.—We sought to determine the effects of longitudinal loading (artificial gravity) on skeletal muscle protein kinetics in 15 healthy young males after 21 days of 6° head-down tilt bed rest [experimental treatment (Exp) group: n = 8, 31 ± 1 yr; control (Con) group: n = 7, 28 ± 1 yr, means ± SE]. On days 1 and 21 of bed rest, postabsorptive venous blood samples and muscle biopsies (vastus lateralis and soleus) were obtained during a 1-h pulse bolus infusion protocol (0 min, t-[ring-13C6]phenylalanine, 35 μmol/kg; 30 min, t-[ring-15N]phenylalanine, 35 μmol/kg). Outcome measures included mixed muscle fractional synthesis (FSR) and breakdown rates (FBR). The Exp group experienced 1 h of longitudinal loading (2.5G at the feet) via a short-radius centrifuge during each day of bed rest. Mixed muscle FSR in the Con group was reduced by 48.5% (day 1, 0.081 ± 0.0006%/h vs. day 21, 0.042 ± 0.0006%/h; P = 0.001) in vastus lateralis after 21 days of bed rest, whereas the Exp group maintained their rate of protein synthesis. A similar but nonsignificant change in FSR was noted for the soleus muscle (Exp, −7%; Con, −22%). No changes in muscle protein breakdown were observed. In conclusion, 1 h of daily exposure to artificial gravity maintained the rate of protein synthesis of the vastus lateralis and may represent an effective adjunct countermeasure to combat the loss of muscle mass and functional during extended spaceflight.

MATERIALS AND METHODS

Participants. All subjects were recruited and screened through the Johnson Space Center Human Test Subject Facility. The protocol was approved by the Johnson Space Center Committee for the Protection of Human Subjects, the University of Texas Medical Branch (UTMB) Institutional Review Board, and the General Clinical Research Center (GCRC) Advisory Committee. Written informed consent was obtained from all participants. Subject eligibility was assessed by a series of medical screening tests including a National Aeronautics and Space Administration-modified Air Force class III physical examination; psychological evaluation; human immunodeficiency virus, hepatitis, and tuberculosis screening; illegal drug and alcohol screening; and a criminal background test. Subjects were free of cardiovascular, musculoskeletal, or sensory-motor dysfunction that would prevent the required centrifugal loading. Exclusion criteria included medication that could interfere with the interpretation of the results, nutritional deficiencies, history of thyroid dysfunction, renal stones, mental illness, smoking, family or personal history of thrombosis, failure to clear criminal background check, and history of gastroesophageal reflux disease.

A total of 168 individuals met basic inclusion criteria and underwent medical screening. Of the 43 that completed all screening tasks, 7 failed to meet minimum bone mineral density requirements, 6 did not meet the aerobic capacity requirement, and 4 failed the centrifuge tolerance test. Of the 20 subjects that started the study proper, 3 withdrew due to medical reasons and 2 withdrew due to personal reasons. A total of 15 healthy male volunteers completed the study. Subjects were randomly assigned to either an experimental treatment group (Exp; n = 8, 31 ± 1 yr) or a control group (Con; n = 7, 28 ± 1 yr). The Univ. of Texas Medical Branch, 301 Univ. Blvd., Galveston, TX 77555-1144 (e-mail: dpadd@utmb.edu).

WHEREAS TECHNOLOGICAL INNOVATION permits exploration of increasingly greater cosmic distances, the human body and its physiological systems remain firmly grounded on Earth. Prolonged exposure to microgravity perturbs the homeostatic balance of many physiological systems (7, 10, 27), including the musculoskeletal system. The most readily consequence of microgravity or inactivity is the loss of lean body mass and associated functional impairment (14, 18).

Mechanistically, a loss of lean body mass reflects a disturbance in protein turnover (6, 13, 15, 26). Specifically, it appears that a decrease in protein synthesis is the primary driving force behind muscle loss during exposure to microgravity, immobilization, or inactivity (14).

Several attempts have been made to combat the physiologic deconditioning associated with physical inactivity (1, 2, 16, 19). However, these potential in-flight countermeasures have been largely unsuccessful despite positive outcomes in the bed rest model (16, 17). In many instances, it would not be inconceivable to speculate that returning crew members could require several weeks of rehabilitation to return to preflight conditioning after lengthy missions (3, 12). Perhaps the most detrimental consequence of the loss of lean body mass is the associated loss of muscular strength and endurance. In addition, the loss of lean body mass appears to largely occur in the postural and ambulatory muscles, further compromising functional capacity during postflight reconditioning. As suggested by Convertino (11), a countermeasure that replicates the muscle actions and forces that occur in the normal 1-G environment would be particularly advantageous.

The aim of this study was to examine the effects of artificial gravity (AG) on skeletal muscle protein kinetics. We hypothesized that daily, 1-h bouts of longitudinal loading would preserve the capacity for muscle protein anabolism. To test this hypothesis, we measured skeletal muscle protein synthesis and breakdown after 21 days of bed rest with and without longitudinal loading.
or a control group (Con; n = 7, 27 ± 1 yr). The Exp group received 1 h of AG (centrifugation) daily during their 21 days of 6° head-down bed rest.

**Experimental protocols.** All subjects were studied in the UTMB GCRC. The general experimental time line and stable isotope protocol are depicted in Fig. 1. Subjects were admitted to the GCRC for 11 days of pre-bed rest baseline data collection. During this period, subjects remained ambulatory and diet stabilization occurred. The subjects diets were adjusted to ensure a constant body weight; starting from a baseline caloric intake of 35.7 kcal/kg body wt (2,500 kcal/day for 70 kg) and a fluid intake of 28.5 ml/kg body wt (2,000 ml of fluid for 70 kg). Food and fluid intake was increased from baseline to account for extra calories and sweat lost during countermeasure testing. The carbohydrate, fat, and protein ratio was 55:30:15. Intake of phosphorus (1,400 mg/day), sodium (2 mmol/kg/day), potassium (1.3 mmol/kg/day), and dietary calcium (1,000 mg/day) were standardized. Caffeine, cocoa, chocolate, tea, or herbal beverages were not allowed. All food and fluid intake was recorded.

Stable isotope infusion studies (days 1 and 21). At ~0630 on days 1 and 21 of bed rest, an 18-gauge polyethylene catheter (Insite-W; Becton Dickinson, Sandy, UT) was inserted into the forearm vein of each arm, one for blood sampling and one for bolus stable isotope infusion. A blood sample was drawn to establish background amino acid enrichment before the start of the stable isotope tracer study. We measured both muscle protein synthesis and breakdown to completely incorporate 13C into 15N-labeled phenylalanine (13C-Phe), after 21 days of bed rest. Subjects were permitted to use a bedside commode for bowel movements, but the time out of bed was limited to ~5 min.

**Artificial gravity.** Subjects in the Exp group received 1 h of AG (centrifugation) each day during bed rest. A description of the centrifuge and protocol is described in detail in companion articles (8a, 23a, 31). Briefly, subjects were spun on a 3-m-short-radius human-rated centrifuge. The padded subject station extended from above the subject’s head to the waist. It was designed to glide freely in the radial (body z-axis) direction and permit the AG load to be applied to the legs. A counterweight system minimized (<4 kg) the z-axis loading added to the subject by the moving components of the subject station. Loading was standardized for subjects of varying body heights by adjusting the radial distance of the foot support surface from the center of rotation (218–229 cm) and the angular velocity of the centrifuge arm (30.7–32.1 rpm) to achieve z-axis loading of 2.5 G at the feet. Video and auditory monitoring of subjects was maintained throughout each session. Con group subjects were transported to the centrifuge daily and placed in the setup position, but they were not spun.

**Recovery phase.** After the completion of the 21 days of bed rest, subjects remained in the GCRC for 9 days. With medical and nursing supervision, subjects slowly resumed weight-bearing activities and were discharged after medical evaluation on day 41.

**Analytical methods.** Cation exchange chromatography (Dowex AG 50W-8X, 100–220 mesh H+) and ter-butylidimethylsilyl derivative using GC-MS (HP model 5989; Hewlett-Packard, Palo Alto, CA) were used to extract blood amino acids from 750 l of 10% perchloroacetic acid. Approximately 1.5 ml of super-

**Table 1. Physical characteristics of participants**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Con</th>
<th>Exp</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>27.3±0.7</td>
<td>30.5±1.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>175.9±2.8</td>
<td>175.3±2.2</td>
</tr>
<tr>
<td>Mass, kg</td>
<td>81.8±3.1</td>
<td>81.2±3.3</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.5±0.8</td>
<td>26.4±0.9</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>61.8±2.3</td>
<td>61.9±1.5</td>
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Values are means ± SE; n = 7 for the control (Con) group and n = 8 for the experimental treatment (Exp) group.

**Fig. 1.** Experimental and stable isotope infusion protocols.

**Fig. 2.** Plasma L-[ring-13N]phenylalanine (15N-Phe) and L-[ring-13C]phenylalanine (13C-Phe) enrichment across the 60-min infusion protocol (combined day 1, day 21, treatment group, and control group data). Values are means ± SE.
protein-bound L-[ring-C13]phenylalanine enrichments were similar in the Exp and the Con groups on day 21 (P < 0.034, significant interaction effect). There were no changes in vastus or soleus mixed muscle FBR for either group during the study (Fig. 4, A and B).

**DISCUSSION**

The loss of lean body mass, specifically lower limb skeletal muscle protein (18, 24), can have a deleterious impact on a crew’s functional capacity during prolonged missions and may prolong recovery following gravity reloading. We examined the effectiveness of longitudinal loading (i.e., AG) on skeletal muscle protein kinetics. Our data demonstrates that daily 1-h bouts of longitudinal loading during prolonged inactivity preserve the capacity for postabsorptive muscle protein synthesis.

![Graph](image_url)

Fig. 3. Vastus lateralis (A) and soleus (B) mixed muscle fractional synthesis rate (FSR) before (d 1) and after 21 days (d 21) of bed rest in the experimental treatment group (Exp; n = 8) and the control group (Con; n = 7). Values are means ± SE. *P < 0.034, significant interaction effect. †P < 0.001, significant within-group difference: d 1 vs. d 21. Pairwise multiple comparison procedure (Tukey’s test) showed the following: #P < 0.05, significant difference from d 1; ##P < 0.05, significant difference between Exp and Con groups at d 21.
In a companion article, Caiozzo et al. (8) report on the effects of AG on muscle strength and fiber cross-sectional area. Their findings are largely consistent with our metabolic study results, suggesting that AG has the capacity to maintain functional and structural homeostasis during prolonged inactivity.

The mechanistic determinant of any countermeasure aimed at preserving muscle mass during period of inactivity is its ability to maintain or enhance skeletal muscle protein synthesis. In this study, longitudinal loading maintained mixed muscle FSR, whereas Con subjects experienced a 22–48% reduction. This reduction in protein synthesis in the absence of any countermeasure was similar to the reduction observed after 14 days of bed rest in a similar subject group (13). The maintenance of protein synthesis by daily 1-h bouts of longitudinal loading is also consistent with the findings of Ferrando et al. (16), who examined the effects of resistance exercise during 14 days of bed rest. In that study, three sets of 10–12 repetitions performed every other day during the 14 days of bed rest maintained muscle protein synthesis in the vastus lateralis, whereas the nonexercising Con group experienced a 46% reduction. The similarities between these studies supports the potential efficacy of longitudinal loading as a countermeasure.

However, despite the positive effect of AG on muscle protein synthesis in this instance, there are a number of limitations to the current study design that diminish our ability to comment on the practical significance of our data. Most importantly, the simultaneous assessment of muscle protein synthesis and breakdown via the pulse-bolus stable isotopic technique can only be performed during postabsorptive periods. We cannot be certain that AG would have a similar stimulatory effect on protein metabolism in the fed state; however, data from previous bed rest studies suggests that anabolic stimuli, such as amino acid supplementation, has a positive 24-h response (20, 21). Unlike previous countermeasures that have employed more traditional resistance or aerobic exercise interventions, the nature of stimulus provided by longitudinal loading is less obvious (i.e., resistance/anabolic or endurance/aerobic). However, on the basis of the duration of the AG sessions (1 h) and the gravitational load at the feet (2.5 G), it is likely that the stimulus approximated endurance/aerobic training rather than resistance training (28). Furthermore, because our measure of skeletal muscle protein synthesis is a gross measure of all the protein synthesized in skeletal muscle, it could be argued that the maintenance in mixed muscle FSR found in the current study may reflect an increase in mitochondrial proteins rather than myofibrillar proteins (28).

With the exception of situations where inactivity is accompanied by a stress response or hypercortisolemia (15), indexes of protein breakdown have not previously been found to be influenced by bed rest (13) or microgravity (25). In the current study, mixed muscle FBR of the vastus lateralis and the soleus was not affected by AG or bed rest.

In conclusion, artificial gravity preserved the rate of protein synthesis during 21 days of 6° head-down tilt bed rest and may represent an effective adjunct countermeasure to prevent or reduce the physiological deconditioning associated with prolonged microgravity or bed rest.

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REFERENCES

ARTIFICIAL GRAVITY AND MUSCLE PROTEIN SYNTHESIS


