WISE-2005: Countermeasures to prevent muscle deconditioning during bed rest in women

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Abstract

The objectives of this study were to evaluate the efficacy of two separate countermeasures, exercise and protein supplementation, to prevent muscle strength and lean tissue mass losses during 60 days of bed rest (BR) in women and whether countermeasure efficacy was influenced by pre-BR muscular strength, endurance, and leg lean mass in women during BR, while a nutritional countermeasure included resistive exercise training and a standardized diet. Twenty-four women were assigned to an exercise (EX, n = 8), a no-exercise control (CON, n = 8), or a no-exercise protein supplementation group (PROT, n = 8). EX performed supine treadmill exercise within lower body negative pressure 3–4 days/wk and maximal concentric and eccentric supine leg- and calf-press exercises 2–4 days/wk. PROT consumed a diet with elevated protein content compared with CON and EX (1.6 vs. 1.0 g·kg

1·day

1). Knee and calf isokinetic strength and endurance, isotonic leg-press strength, and leg lean mass were measured before and after BR. Post-BR knee extensor strength and endurance, ankle strength, and leg lean mass were significantly greater and leg-press strength tended to be higher in EX than in CON and PROT. Post-BR measures in PROT were not different than those in CON. Exercise countermeasure efficacy was less, and strength, endurance, and leg lean mass losses in CON and PROT were greater, in subjects who were more fit pre-BR. An exercise protocol combining resistive and aerobic exercise training protects against losses in strength, endurance, and leg lean mass in women during BR, while a nutritional countermeasure without exercise was not effective. Exercise countermeasures may require individualization to protect higher levels of strength and endurance.

Although the physical fitness requirements for critical tasks during spaceflight and exploration missions are not yet defined, it is assumed that relatively high levels of muscle strength and endurance will be required during prolonged orbital and planetary exploration missions. Astronauts must be capable of safely performing microgravity and partial gravity extravehicular activities and, if required, an emergency egress from the space habitat or spacecraft. Our laboratory (16, 27) and others (24, 44) have reported that exposure to long-duration spaceflight and bed rest, a spaceflight analog, significantly decreases muscle volume or mass, strength, and endurance. In spaceflight, these decrements occur despite performance of exercise countermeasures. Although in-flight countermeasures historically have focused on endurance-type or aerobic exercise (33), even recent reports from International Space Station missions that included resistive exercise training suggest that the current countermeasures are not completely effective in preserving muscle size and function (16, 45, 53). Thus the National Aeronautics and Space Administration (NASA) and its international partners continue to conduct research to identify and refine countermeasures to spaceflight-induced deconditioning.

Decreased muscle mass induced by unloading results primarily from a decrease in protein synthesis, rather than increased protein degradation (37), and thus countermeasures historically have been directed toward maintaining or increasing hypertrophic signals to skeletal muscle. Exercise, known for its hypertrophic and anatrophic properties, has been routinely performed by astronauts and cosmonauts during spaceflight for many years. The current International Space Station exercise protocol includes both resistive and aerobic exercise training performed daily to counteract musculoskeletal and cardiovascular deconditioning (33). An exercise countermeasure protocol that provides protection with a reduced time commitment (e.g., only one exercise modality per day) and total caloric consumption (decreased frequency and duration of exercise) would reduce crew time and preserve important resources. Thus the first objective of this bed rest study was to determine the efficacy of a combined exercise countermeasure protocol, which included resistive exercise, using an gravity-independent flywheel ergometer and aerobic exercise, supine treadmill exercise against lower body negative pressure. Resistive and aerobic exercise sessions were performed on alternating days approximately three times per week, a program that represents a substantial reduction compared with the current International Space Station exercise countermeasure programs.

Dietary intake also can have a significant effect on protein balance, and reduced protein synthesis may be exacerbated in spaceflight where inadequate dietary consumption historically has been a common finding (13, 46, 49). The postprandial anabolic effect associated with protein and/or amino acid consumption is well known, and dietary practices such as protein and/or amino acid supplementation have been advocated as potential countermeasures to unloading-induced muscle atrophy and decreased muscle performance (13, 36, 50, 51). Thus a second objective of this study was to determine whether protein supplementation to a standardized diet would be protective of lean tissue mass and muscle strength during bed rest. While some previous studies have provided promising results,

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the findings across studies have not been consistent (48), and data are limited in women.

This study was part of a larger group of investigations known as the Women’s International Space Simulation for Exploration (WISE-2005), sponsored by NASA, the Canadian Space Agency, the French National Space Agency, and the European Space Agency. WISE-2005 was undertaken to evaluate the effectiveness of two separate countermeasure protocols, a combined resistive and aerobic exercise protocol and a protein supplementation protocol, to the deconditioning effects of 60 days of bed rest on multiple physiological systems in women. In this arm of the study, we hypothesized that subjects who performed the combined resistive and aerobic exercise countermeasures would be protected from the loss of lean tissue mass and muscle strength and endurance compared with control subjects who did not participate in the countermeasures. Additionally, we hypothesized that subjects who participated in a specific protein supplementation protocol would benefit from an attenuation of the loss in lean tissue mass and muscle strength and endurance during bed rest. Furthermore, we hypothesized that subjects who did not participate in any countermeasures would experience losses in muscle strength, endurance, and lean tissue mass in a manner that was inversely proportional to their pre-bed rest muscular fitness, a response similar to other physiological systems (10, 30), but that subjects who participated in countermeasures would be equally protected independent of their pre-bed rest condition. Countermeasures to spaceflight-induced deconditioning should be effective for all crewmembers, regardless of their preflight muscular fitness (26). Results from the WISE-2005 study are important to the spaceflight and bed rest deconditioning literature because few long-duration studies have been conducted with women volunteers (19), particularly with regard to muscle function. A unique aspect of this study is that it uses the same testing modalities chosen to evaluate astronauts before and after International Space Station missions.

**METHODS**

**Overall study protocol.** Twenty-four healthy, nonsmoking women volunteered to participate in the WISE-2005 bed rest study. The study was completed in two campaigns (Feb 2005–May 2005, Sept 2005–Dec 2005), with 12 women participating in each campaign. Within each campaign, subjects were matched according to pre-bed rest aerobic fitness levels, one of the primary variables of interest in the investigations central to the overall project (43), and were assigned in a balanced manner to one of three groups of four subjects each: control (Control), exercise countermeasures (Exercise), or a nutritional countermeasure (Protein). Subjects participated in pre- and post-bed rest measures of strength and endurance in select muscle groups and leg lean tissue mass to determine the effects of countermeasures relative to the control condition and to determine whether pre-bed rest muscular fitness parameters influence post-bed rest outcomes. Control subjects did not participate in any countermeasures during the bed rest period. Exercise subjects performed an exercise countermeasure protocol that consisted of both flywheel exercise and treadmill exercise against lower body negative pressure sessions throughout the bed rest (described in detail below). Protein subjects did not participate in exercise countermeasures, but consumed additional dietary protein daily, ~160% of the protein consumed by the other two groups. The three groups were similar before bed rest in height (Exercise: 164.9 ± 2.5 cm, Control: 162.8 ± 2.2 cm, Protein: 170.3 ± 1.9 cm; mean ± SE), weight (Exercise: 58.1 ± 2.2 kg, Control: 55.8 ± 1.4 kg, Protein: 61.1 ± 1.6 kg), pre-bed rest aerobic fitness (Exercise: 39.0 ± 0.7 ml·kg⁻¹·min⁻¹, Control: 38.0 ± 1.7 ml·kg⁻¹·min⁻¹, Protein: 38.9 ± 1.7 ml·kg⁻¹·min⁻¹), but Protein subjects (31 ± 1 yr) were younger (Exercise: 33 ± 1 yr, Control: 34 ± 1 yr) (43).

The study was conducted at the Institute for Space Medicine and Physiology (MEDES) in Toulouse, France and consisted of a 20-day ambulatory control period followed by 60 days of strict 6° head-down-tilt bed rest. Showering, transport to all testing and countermeasure sessions, defecation, and urination were conducted in the head-down posture. A 20-day ambulatory recovery period followed the bed rest period. Standardized conditions were maintained throughout the study, including diet, fluid consumption (discussed below), sleep-wake cycle, and consistency of testing schedules. Subjects maintained a standard sleep/wake schedule in which subjects arose at 0700 and retired at 2300. Testing was performed before and after bed rest at the same time of day to avoid any potential circadian effects. The protocol for this study was reviewed and approved by the NASA Johnson Space Center Committee for the Protection of Human Subjects, the University of California-San Diego Institutional Review Board, and the local ethics committee (CCPBR of Toulouse, France). Before participation, subjects received verbal or written explanation of all study procedures and provided written, informed consent.

Inclusion criteria for the subjects included body mass index between 20 and 25 kg/m², regular menstrual cycles, no family history of chronic disease or psychiatric disease, physically active with at least average aerobic capacity for age and sex (39), and freedom from orthopedic, musculoskeletal, blood clotting, and cardiovascular disorders. Subjects self-reported that they were eumenorrheic and were excluded if they had used oral contraceptives in the 2 mo before the study, their pre-bed rest bone mineral density measured by dual-energy X-ray absorptiometry (DEXA) scans of the hip or lumbar vertebrae was 1.5 SDs above or below the age/sex-matched mean, or they were orthostatically intolerant before bed rest, defined as an inability to complete a 10-min 80° head-up tilt test. Preparticipation medical screening included a medical history, clinical and psychological examinations, chest X-ray, ECG, echocardiogram, Doppler examination of the lower limb veins, a head-up tilt test, cycle exercise test, DEXA scans, and standard laboratory tests (hematology, blood chemistry, urine analysis) (12, 43, 49).

**Exercise countermeasures.** The exercise countermeasure protocol consisted of flywheel exercise and treadmill exercise against lower body negative pressure training (Fig. 1) performed during bed rest in separate sessions on separate days, except on 1 day when all Exercise subjects performed both flywheel and treadmill exercise on the same day because of scheduling constraints of the in-bed rest MRI (Table 1). Flywheel exercise was performed 2–3 days/wk, and exercise against lower body negative pressure was performed 3–4 days/wk, based on scheduling with other test sessions.

During flywheel exercise, the thigh and calf muscle groups were trained using supine leg-press and calf-press exercises on an inertial ergometer using a protocol similar to those used in previous bed rest investigations (2, 3). The inertial ergometer was positioned so that all flywheel exercise sessions were performed in the 6° head-down-tilt position. Ten minutes of light supine cycling and submaximal leg-press and calf-press repititions were completed as warm-up. The leg-press exercise consisted of 4 sets of 7 maximal concentric and eccentric repetitions, and the calf-press exercise consisted of 4 sets of 14 maximal concentric and eccentric repetitions. Subjects were encouraged verbally to perform maximal efforts with each repetition and received immediate digital feedback as to their performance on a repetition-by-repetition basis. Subjects rested for 2 min between sets. Force and flywheel rotational velocity were measured, and work and power were calculated throughout each repetition. Results from these training sessions are reported elsewhere (54).

The treadmill exercise against lower body negative pressure countermeasure protocol that was used for this study was similar to that used in previous 15- and 30-day bed rest studies (29, 30, 55) with two
exceptions. First, because the flywheel exercise was documented as an effective countermeasure (2, 3) and because treadmill exercise against lower body negative pressure countermeasure attenuated the loss of muscle strength and endurance when performed 6 days/wk (42, 55), in this study the number of treadmill exercise training sessions was reduced to 3 days/wk. Second, although the exercise profile of the countermeasure was not different from previous studies, the postexercise orthostatic stress period was longer. Specifically, EX subjects performed 40 min of interval exercise at 40–80% of their pre-bed rest peak oxygen consumption, followed immediately by 10 min of resting lower body negative pressure (Fig. 2). During exercise, subjects were exposed to a level of lower body negative pressure that produced a footward force of ~1.0 body wt at the start of bed rest. The magnitude of lower body negative pressure during exercise was increased over the course of the bed rest period, similar to previous investigations, in some subjects to produce as much as 1.15 body wt based on the subject’s tolerance of the exercise countermeasure. The postexercise lower body negative pressure was held constant at 1.0 body wt throughout the study. The amount of lower body negative pressure required to produce the desired level of body weight was calibrated for each subject before the study started. The level of lower body negative pressure required to produce 1.0 body wt of loading ranged from ~47 to ~56 mmHg. Heart rate and rating of perceived exertion data were collected as indicators of exercise intensity, and during each exercise session the treadmill speed was adjusted if needed to obtain the target training heart rate. Heart rate data from these sessions are reported elsewhere (43).

As a consequence of various medical situations that arose during the bed rest period, including the impact of the combined treadmill exercise and flywheel exercise countermeasure program (soreness and injury), discomfort from muscle biopsies, and mild illness, not all exercise sessions were completed as prescribed (43, 54). Exercise subjects completed an average of 96% of the prescribed treadmill exercise sessions. After ~30 days of bed rest, all Exercise subjects completed their exercise at an lower body negative pressure level that produced at least 1.0 body wt. Thereafter, as their tolerance to exercise increased, all but one of the eight subjects completed at least some exercise sessions with a footward force greater than 1.05 body wt. Of the exercise sessions performed, the mean treadmill exercise time (including postexercise rest with lower body negative pressure) was

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Fig. 1. Line drawings of the exercise countermeasure devices used during 60 days of bed rest (BR). Top: example subject using the flywheel exercise device to perform supine leg-press exercises while tilted 6° head down. Bottom: example subject performing supine treadmill exercise while being pulled to the treadmill surface using lower body negative pressure (LBNP).
and Protein groups. For the Exercise group, caloric intake during bed adjusted downward to 110% of resting metabolic rate for the Control pre-bed rest and recovery periods. During bed rest, caloric intake was determined by indirect calorimetry. Caloric intake for all three groups was prescribed to be 140% of their resting metabolic rate during the 20 days before bed rest, resting metabolic rate was 52 ± 3 mmHg, which corresponded to a mean loading of 1.0 ± 0.1 body wt. During the flywheel exercise sessions, subjects completed 82% of the leg-press exercise as planned, 13% were conducted at a reduced level of effort, and 5% were not performed at all. Similarly, 74% of the calf-press exercises were performed as prescribed, 20% were performed at a reduced level of effort, and 6% were not accomplished. The number of reduced-effort and missed flywheel exercise sessions varied among the volunteers and were scattered throughout the 60-day bed rest period (43, 54).

Diet. During the 20 days before bed rest, resting metabolic rate was determined by indirect calorimetry. Caloric intake for all three groups was prescribed to be 140% of their resting metabolic rate during the pre-bed rest and recovery periods. During bed rest, caloric intake was adjusted downward to 110% of resting metabolic rate for the Control and Protein groups. For the Exercise group, caloric intake during bed rest was set to 110% of resting metabolic rate plus estimated energy expenditure related to physical activity (43). Subjects were restricted from ingesting caffeine, alcohol, and chocolate during the study. They were provided three meals/day, and up to two snacks. Subjects had a choice of two menus from which to select meals for each day. For each subject, dietary guidelines aimed to have the sodium intake at 1.2–1.6 mmol·kg⁻¹·day⁻¹, potassium intake at 0.9–1.1 mmol·kg⁻¹·day⁻¹, calcium intake at 1 g/day, and phosphorus intake at 1.2–1.6 mmol·kg⁻¹·day⁻¹. Subjects were encouraged to consume fluids throughout the study, but consumption of coffee, tea, and alcohol was prohibited. The maximum liquid intake was the same for all three groups (60 ml·kg⁻¹·day⁻¹), except on the days when the Exercise subjects performed physical countermeasures (75 ml·kg⁻¹·day⁻¹) (43). The protein intake in the Control and Exercise groups was controlled at 1.0 g·kg⁻¹·day⁻¹. The protein intake in the Protein group was increased to 1.45 g·kg⁻¹·day⁻¹ of dietary protein plus an additional 3.6 g/day of free leucine, 1.8 g/day of free isoleucine, and 1.8 g/day of free valine). The additional protein was divided and supplied equally across the three meals. The diet has been reported in detail elsewhere (54).

Table 1. Bed rest schedule showing the primary measures acquired in the integrated study of women in bed rest (Women’s International Space Simulation for Exploration, WISE)

<table>
<thead>
<tr>
<th>Week</th>
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<td>During Bed Rest</td>
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<td>OTT, TM sub</td>
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Details of other test protocols are reported elsewhere (17, 39, 52). *In the in- and post-bed rest sections of this table, asterisks designate the overlap between the two sections. MRI, MRI for muscle volume measurements (52); LBNPex, treadmill exercise session within lower body negative pressure (Exercise group only), familiarization during pre-bed rest period; FWex, resistive exercise session using flywheel ergometer (Exercise group only); OTT, orthostatic tolerance test using head-up tilt and lower body negative pressure (17); Cycle: cycle ergometer test to peak exertion; data not reported elsewhere; FW MS, muscle strength measures using flywheel ergometer (52); Biopsy, muscle biopsies obtained from the vastus lateralis and soleus (50, 51); Isok, isokinetic and isoinertial testing performed for the current report; TM Max, treadmill test to maximal exertion (39); TM sub, treadmill exercise test to submaximal levels (39); Combo, day on which both exercise countermeasures were performed (Exercise group only).

For each subject, dietary guidelines aimed to have the sodium intake at 1.2–1.6 mmol·kg⁻¹·day⁻¹, potassium intake at 0.9–1.1 mmol·kg⁻¹·day⁻¹, calcium intake at 1 g/day, and phosphorus intake at 1.2–1.6 mmol·kg⁻¹·day⁻¹. Subjects were encouraged to consume fluids throughout the study, but consumption of coffee, tea, and alcohol was prohibited. The maximum liquid intake was the same for all three groups (60 ml·kg⁻¹·day⁻¹), except on the days when the Exercise subjects performed physical countermeasures (75 ml·kg⁻¹·day⁻¹) (43). The protein intake in the Control and Exercise groups was controlled at 1.0 g·kg⁻¹·day⁻¹. The protein intake in the Protein group was increased to 1.45 g·kg⁻¹·day⁻¹ of dietary protein plus an additional 3.6 g/day of free leucine, 1.8 g/day of free isoleucine, and 1.8 g/day of free valine). The additional protein was divided and supplied equally across the three meals. The diet has been reported in detail elsewhere (54).

Isokinetic strength and endurance. During the pre-bed rest ambulatory control period, subjects completed two isokinetic testing sessions. Testing was conducted 18 and 9 days before beginning head-down tilt bed rest using a standard dynamometer (HUMAC NORM, CSMI, Stoughton, MA). The pre-bed rest testing was scheduled early in the pre-bed rest (in-hospital) stabilization period so as not to interfere with the primary variables of interest in the other studies (43, 54). Post-bed rest testing was conducted on the 6th day of reambulation. Post-bed rest strength tests were not conducted until the primary measures of other studies were collected, but also were scheduled to coincide with the approximate timing of testing performed for International Space Station astronauts after long-duration missions. Testing during the first 5 days of reambulation that preceded isokinetic testing included submaximal and maximal treadmill tests (43), tests of...
orthostatic tolerance (18), and strength testing using the flywheel exercise device (54).

The first testing session was considered a familiarization session, although the subjects were instructed to perform maximal efforts. Subjects were positioned and restrained in a manner similar to the manufacturer’s recommendations, but some additional straps were added to stabilize the ankle and transport during ankle plantar- and dorsiflexion testing. Alignment of the subject to the dynamometer was recorded so that it could be reproduced during subsequent sessions. The dynamometer was calibrated each day before testing using the manufacturer-provided procedures. Gravity correction was used to remove the effect of limb and limb adapter weight from torque measurements. Using the procedures described here, these isokinetic tests have a high degree of reliability (intraclass correlation coefficient > 0.90) in our laboratory, except for ankle dorsiflexion (intraclass correlation coefficient = 0.67).

Before isokinetic testing was conducted, subjects pedaled a cycle ergometer at a workload of 25–50 W at a cadence of 60–80 rpm for 5 min. Standard joint-specific warm-up procedures were followed and consisted of five submaximal repetitions and two to three maximal repetitions utilizing the actual testing movements and speeds. After the warm-up, subjects rested at least 2 min before data collection began. Strength tests were performed such that subjects exerted a maximal effort in only one direction for each set of repetitions. At the end of the range of motion, subjects were instructed to relax, and the test operator returned the limb to the starting position.

Knee extension and flexion tests were conducted in the seated position. Knee range of motion was set from 0.35 rad (20°) to 1.66 rad (95°) from full extension (0 rad; 0°) for both the knee strength and endurance measures. Strength testing data collection began with five maximal concentric knee extension repetitions at 1.05 rad/s (60°/s). Five maximal concentric knee flexion repetitions were performed separately in a similar manner. The isokinetic speed was changed to 3.14 rad/s (180°/s) for endurance testing, and subjects performed two to three submaximal repetitions to familiarize themselves with the new test speed. After 2 min of rest, subjects performed 21 uninterrupted repetitions of extension and flexion at 3.14 rad/s (180°/s). Subjects were instructed not to pace themselves and to give a maximal effort with every repetition.

Ankle plantar- and dorsiflexion strength tests were conducted in the prone position. Ankle range of motion was determined by the subject’s maximum plantar- and dorsiflexion with a minimum of 0.52 rad (30°) and 0.26 rad (15°), respectively. The anatomical neutral position was defined as 0 rad (0°). Data collection began with five maximal concentric plantar flexion repetitions at 0.52 rad/s (30°/s). Subjects then performed five maximal concentric dorsiflexion repetitions at the same speed.

Peak torque values were used as measures of knee and ankle extensor and flexor strengths in pre- and post-bed rest sessions. For each joint movement, peak torque was defined as the highest torque value achieved across each of the five repetitions. For knee extensor and flexor tests, isokinetic endurance was defined as the total work performed during repetitions 2–21. The first pre-bed rest test was considered a familiarization session and was not used for subsequent analyses.

**Leg-press strength.** The leg-press one-repetition maximum (1 RM) was performed using the CYBEX 5320 Plate-Loaded Leg Press Machine (CYBEX International, Medway, MA). The subject sat inside the device and placed her feet on the sled approximately shoulder-width apart. The test started with the legs extended. The subject lowered the weight until her knees were flexed 1.57 rad (90°) before pushing the weight back to the starting position. Subjects performed a warm-up set of 5 reps with only the weight of the sled. Additional warm-up sets were performed consisting of 8 reps at 30–40% of 1 RM, 5 reps at ~50%, and 3 reps at ~60% of 1 RM. These loads were based upon a pretest estimate of the 1-RM score for the familiarization session and the actual familiarization session 1 RM for the second pre-bed rest test. The subject then performed single repetitions of increasing intensity until she could no longer lift a given weight. When single rep trials were performed, the weight was increased by 5–10% for each trial, and the subject was allowed to rest 2–3 min between attempts. The leg-press strength score was the maximum load lifted through the full range of motion using the correct form and technique.

**Leg lean tissue mass.** Measures of leg lean tissue mass, excluding bone mineral content, were acquired from whole body scans using DEXA (Hologic Discovery, Hologic, Bedford, MA). All DEXA scans were acquired by the same operator to ensure consistency of positioning and measures. Measurements were made in triplicate and averaged at each time point: before bed rest, 30 days into bed rest (mid-bed rest), and 3 days post-bed rest. Regions of interest were defined using standard protocols, with the same operator from the Johnson Space Center Bone and Mineral Laboratory analyzing the scans for the same subject. Using these procedures, precision for lean mass measures is ~1.3% for the legs (44). Whole body lean tissue mass, fat mass, and body mass results from these subjects have been reported previously (43).

**Statistical analysis.** To determine the effectiveness of each countermeasure to prevent or attenuate losses relative to performing no countermeasures, we used analysis of covariance (ANCOVA) with the pre-bed rest measurement as a covariate. Used in this way, the ANCOVA model helps control for any baseline differences still present after the subjects were assigned to the groups. Other statistical models of change, such as assuming mean change within groups is a constant (ANOVA or analysis of differences) or assuming mean relative change is a constant (analysis of relative or percent change), also are possible to use, but departures from these assumptions can lead to incorrect inference or loss of statistical power. For lean leg mass, which also was measured during bed rest, a mixed-model regression analysis was used to compare treatments in and after the bed rest. Post hoc comparisons for the strength and endurance outcomes were based on t-tests with finite degrees of freedom, whereas comparisons for lean leg mass were based on asymptotic Wald tests (z-values). Residuals for all analyses were checked for the underlying assumptions of normality and homogeneity of variance. To account for multiple testing, we used the Hochberg procedure (22) to adjust P value thresholds for significance to control the familywise error rate to ≤0.05 for 16 comparisons (Exercise vs. Control groups and Protein vs. Control groups for each of 8 parameters). All tests were two-sided.

For two of the outcomes, single outliers were identified during statistical analysis. One subject in the Exercise group demonstrated a much larger ankle plantar flexor strength loss (~54%) than expected, likely attributable to calf muscle pain secondary to a muscle biopsy obtained for a companion study (54). One subject in the Protein group demonstrated a much larger loss in knee extensor strength (~73%) than expected, given pre-bed rest values of knee extensor strength and lean leg mass. We know of no circumstances that explain this outlier, but reanalysis without this data point did not change the basic inference about the effectiveness of the Protein treatment. Statistical analyses of these two parameters, knee extensor strength and ankle plantar flexor strength, are reported in the RESULTS section, with and without outliers.

An important reason for using pre-bed rest performance as a covariate in an ANCOVA was to enable the comparison of treatments after adjusting for unforeseen baseline differences. However, the regression coefficient of this covariate also provides additional information that furthers the interpretation of these results. Specifically, we sought to determine whether subjects with relatively high pre-bed rest muscular fitness may lose more strength or endurance during bed rest and may require more aggressive countermeasures than those with lower levels of fitness. To examine whether pre-bed rest muscular fitness affected our results, consider the following simplification of the ANCOVA model for one treatment group:
Table 2. Modeled post-bed rest outcomes in the Control, Exercise, and Protein groups as a function of pre-bed rest scores for a subject with average pre-bed rest values

<table>
<thead>
<tr>
<th>Measure</th>
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<th>Pre-Bed Rest</th>
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<th>Exercise</th>
<th>Protein</th>
</tr>
</thead>
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<td>Knee extension</td>
<td>Peak torque</td>
<td>121.9</td>
<td>23</td>
<td>83.1 ± 4.8</td>
<td>112.5 ± 4.7*</td>
<td>83.7 ± 5.0</td>
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<td></td>
<td>(N·m)</td>
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<td></td>
<td>0.0003 (0.0033)</td>
<td>0.93 (0.025)</td>
<td></td>
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<tr>
<td>Knee flexion</td>
<td>Peak torque</td>
<td>65.9</td>
<td>24</td>
<td>52.7 ± 3.2</td>
<td>53.8 ± 3.2</td>
<td>55.8 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>(N·m)</td>
<td></td>
<td></td>
<td>0.83 (0.13)</td>
<td>0.49 (0.007)</td>
<td></td>
</tr>
<tr>
<td>Knee extension</td>
<td>Total work</td>
<td>1046</td>
<td>24</td>
<td>873 ± 32</td>
<td>1050 ± 32*</td>
<td>851 ± 32</td>
</tr>
<tr>
<td></td>
<td>(N·m)</td>
<td></td>
<td></td>
<td>0.0009 (0.0038)</td>
<td>0.63 (0.008)</td>
<td></td>
</tr>
<tr>
<td>Knee flexion</td>
<td>Total work</td>
<td>618</td>
<td>24</td>
<td>555 ± 36</td>
<td>563 ± 35</td>
<td>555 ± 35</td>
</tr>
<tr>
<td></td>
<td>(N·m)</td>
<td></td>
<td></td>
<td>0.88 (0.017)</td>
<td>0.99 (0.05)</td>
<td></td>
</tr>
<tr>
<td>Ankle plantar flexion</td>
<td>Peak torque</td>
<td>103.0</td>
<td>23</td>
<td>72.1 ± 4.3</td>
<td>98.6 ± 4.6*</td>
<td>84.6 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>(N·m)</td>
<td></td>
<td></td>
<td>0.0005 (0.0036)</td>
<td>0.055 (0.0045)</td>
<td></td>
</tr>
<tr>
<td>Ankle dorsiflexion</td>
<td>Peak torque</td>
<td>27.1</td>
<td>24</td>
<td>22.5 ± 0.9</td>
<td>25.0 ± 0.8</td>
<td>24.3 ± 0.9</td>
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<tr>
<td></td>
<td>(N·m)</td>
<td></td>
<td></td>
<td>0.045 (0.0042)</td>
<td>0.19 (0.006)</td>
<td></td>
</tr>
<tr>
<td>Leg press</td>
<td>Load lifted</td>
<td>66.5</td>
<td>23</td>
<td>51.5 ± 3.1</td>
<td>59.4 ± 2.8</td>
<td>46.4 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>(kg)</td>
<td></td>
<td></td>
<td>0.081 (0.005)</td>
<td>0.024 (0.006)</td>
<td></td>
</tr>
<tr>
<td>Leg lean tissue mass</td>
<td>Lean mass</td>
<td>13.1</td>
<td>24</td>
<td>11.6 ± 0.1</td>
<td>12.5 ± 0.1*</td>
<td>11.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>(kg)</td>
<td></td>
<td></td>
<td>&lt;0.0001 (0.0031)</td>
<td>0.66 (0.01)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. Outliers were removed in some analyses as described in the Statistical analysis section. Exact P values are reported below each outcome in the Exercise and Protein groups, with the threshold values required to reject the null hypothesis in parentheses. Threshold values were obtained using the Hochberg procedure for controlling the overall Type I error rate = 0.05 (19). *Significantly greater than Control. There were no significant differences between Control and Protein groups.

\[
\text{post} = C + \beta \cdot \text{pre} + e 
\]  

(1)

where \( C \) is a constant that in general depends on the treatment the subject receives, pre and post are observed muscle strength or endurance for pre- and post-bed rest sessions respectively, and \( e \) is a random error term. \( \text{Equation 1} \) also can be written in the form

\[
\text{post-pre} = C + (\beta - 1) \cdot \text{pre} + e 
\]  

(2)

If in Eq. 2, \( \beta < 1 \), this is interpreted to mean that, for the same treatment, subjects who have higher levels of muscular fitness would have greater loss of muscular fitness during bed rest than those who had lower initial levels of muscular fitness. In \( \text{Eq. 1} \), \( \beta \) is thought of as a gain parameter that measures how much “output” (post-bed rest performance) changes per unit change in “input” (pre-bed rest performance). For the strength and endurance outcomes, instead of taking the estimates of \( \beta \) directly from the fitted ANCOVA models, we used leg lean mass as an instrumental variable (IV) in our regression model to account for bias due to uncertainty in the pre-bed rest variable. \( \text{IV regression} \) has been used previously in a similar manner in clinical studies (21, 35). Comparisons between \( \beta \) estimated from the original ANCOVA models and with the IV models show that, in every case, \( \beta \) was higher (more gain) when estimated by the IV model (see APPENDIX). For analysis of change in leg lean mass relative to pre-bed rest leg lean mass, no IV was utilized; the ANCOVA estimate of \( \beta \) is unbiased because pre-bed rest leg lean mass is an objective measure, and its measurement error is relatively small.

All statistical computations were made with Stata 12 statistical software (StataCorp LP, College Station, TX).

RESULTS

Effectiveness of countermeasures. Model-predicted post-bed rest means of each outcome by treatment group (Control, Exercise, and Protein) are shown in Table 2 for hypothetical “typical” subjects, i.e., those with mean pre-bed rest outcomes equal to the actual pre-bed rest average for all subjects in this study. These results indicate that the countermeasure protocol was effective in reducing or preventing loss in four of eight measures in the Exercise subjects. In particular, post-bed rest knee extensor strength and endurance, ankle plantar flexor strength, and leg lean mass were significantly higher in the Exercise subjects than in the Control subjects (Table 2). However, the exercise countermeasures provided no protection against losses in knee flexor strength, knee flexor endurance, and ankle dorsiflexor strength. As noted in the Statistical analysis section above, these results reflect the exclusion of one outlier from the Exercise group in the analysis of plantar flexor strength. By including this subject who demonstrated very low post-bed rest plantar flexor strength in the analysis, the test for comparing the Exercise and Control groups for this outcome would result in a \( P \) value of 0.027, which would lead us to report a finding of no statistically significant difference between the Exercise and Control groups for this outcome after downward adjustment of the critical \( P \) value (0.0033) to control overall type I error. On the other hand, the post-bed rest measures in the Protein group were not significantly different from those of the Control group for any of the outcomes studied. As described in the Statistical analysis section, one subject was excluded from the analyses of knee extensor strength. The inference about the effectiveness of the protein countermeasure to protect knee extensor strength was essentially the same whether all of this subject’s data were included (\( P = 0.60 \)) or whether the outlier was excluded (\( P = 0.93 \)).

Effect of pre-bed rest condition. Of the eight muscular fitness parameters evaluated in this study, the changes in four post-bed rest outcomes were significantly related to pre-bed rest condition (\( \beta < 1 \)). Specifically, the bed rest-induced loss of knee extensor endurance, ankle flexor strength, leg-press strength, and lean leg mass was greater in subjects with higher pre-bed rest levels of knee extensor endurance, ankle flexor strength, leg-press strength, and lean leg mass, respectively. Table 3 gives estimates of the gain (\( \beta \)), along with 95% confidence limits for each outcome. Evidence also was fairly strong that \( \beta = 1 \) for knee extensor strength. Figures 3–5, showing the change in muscle strength or total work in indi-
individual subjects from pre- to post-bed rest for knee extensor strength and total work, ankle plantar flexor and dorsiflexor strength, and leg-press strength measures, appear to corroborate these findings. There was little evidence that the protein countermeasure was effective, and thus the Control and Protein groups were combined in these figures but not in the analyses. Figure 6, depicting the change in leg lean tissue mass during and after bed rest at three levels of modeled pre-bed rest leg lean mass, also demonstrates these results.

DISCUSSION

Three primary findings resulted from this study. First, 3 days/wk of resistive exercise performed with the flywheel exercise device combined with 3 days/wk of treadmill exercise within lower body negative pressure using an aerobic, interval-style protocol mitigated the lower body muscle deconditioning normally associated with long-duration bed rest. Specifically, the reductions in muscle strength and endurance of the knee extensors and in muscle strength of the ankle extensor muscles were prevented in women participating in 60 days of bed rest when subjects performed an exercise countermeasure program that included aerobic and resistive exercise components. Importantly, both muscle performance and aerobic capacity (43) were protected using this combined countermeasure protocol. Second, increasing protein intake during bed rest, from 1.0 g·kg⁻¹·day⁻¹ in the Control group to 1.6 g·kg⁻¹·day⁻¹ in the Protein group, did not protect either muscle performance or leg lean tissue mass. Table 3. The effect of pre-bed rest muscular fitness on post-bed rest performance based on the estimates of gain (β) from pre-bed rest to post-bed rest

<table>
<thead>
<tr>
<th>Measure</th>
<th>Gain (β)</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee extension Peak torque</td>
<td>0.70</td>
<td>0.28</td>
<td>1.13</td>
</tr>
<tr>
<td>Knee flexion Peak torque</td>
<td>0.93</td>
<td>0.41</td>
<td>1.46</td>
</tr>
<tr>
<td>Knee extension Total work</td>
<td>0.59*</td>
<td>0.29</td>
<td>0.90</td>
</tr>
<tr>
<td>Knee flexion Total work</td>
<td>0.81</td>
<td>0.19</td>
<td>1.42</td>
</tr>
<tr>
<td>Ankle plantar flexion Peak torque</td>
<td>1.14</td>
<td>0.26</td>
<td>2.02</td>
</tr>
<tr>
<td>Ankle dorsiflexion Peak torque</td>
<td>0.57*</td>
<td>0.36</td>
<td>0.79</td>
</tr>
<tr>
<td>Leg press Load lifted</td>
<td>0.76*</td>
<td>0.54</td>
<td>0.98</td>
</tr>
<tr>
<td>Lean leg tissue mass Lean mass</td>
<td>0.86*</td>
<td>0.79</td>
<td>0.92</td>
</tr>
</tbody>
</table>

CI, confidence interval. If β < 1, then, for the same treatment, stronger subjects would be expected to have more loss of strength or lean leg tissue mass post-bed rest than those who had lower initial levels of strength or lean leg tissue mass, respectively. Estimates of β were corrected to allow for within-subject variation in the pre-bed rest measurement using instrumental variable regression. Estimates of β were calculated without outliers. *β significantly < 1, P < 0.05.
lean tissue mass. Similarly, our laboratory previously reported that the Protein countermeasure did not prevent the loss of whole body lean tissue mass during bed rest (43). The level of protection observed in previous shorter bed rest studies utilizing a nutritional countermeasure (36, 51) was not evident in this study. Third, we observed that the amount of muscle strength, endurance, and lean tissue mass loss resulting from bed rest was inversely proportional to the pre-bed rest muscular

Fig. 4. Change in ankle extensor (top) and flexor strength (bottom) in individual subjects in the exercise (solid triangles; left), control (open circles; right), and protein-supplementation groups (shaded squares; right). Subjects who were less fit pre-BR (lower strength scores) appear on the left side of each plot, while subjects with higher levels of pre-BR muscle strength are depicted on the right side of each plot. Dashed horizontal line depicts no change in the measured parameter from pre- to post-BR. When a subject who reported calf pain during post-BR testing was not included in the analyses, participation in the exercise countermeasure protocol was shown to protect against strength losses in the ankle extensors. The exercise countermeasure protocol also protected against strength losses in the ankle flexors. Post-BR ankle strength in the protein-supplementation countermeasure subjects was not different from that of controls.

Fig. 5. Change in leg-press strength in the individual subjects as a function of pre-BR strength scores in exercise (solid triangles; left), control (solid circles; right), and protein-supplementation subjects (shaded squares; right). Subjects who were less fit pre-BR (lower strength scores) appear on the left side of each plot, while subjects with higher levels of pre-BR muscle strength are depicted on the right side of each plot. Dashed horizontal line depicts no change in the measured parameter from pre- to post-BR. Participation in the exercise countermeasure protocol tended to protect against losses in leg-press strength. Post-BR leg-press strength in the protein-supplementation countermeasure subjects was not discernable from that of controls.
fitness of the subjects. Importantly, performing exercise countermeasures during bed rest maintained or improved muscle performance measures in subjects with lower levels of muscular fitness before bed rest, but may not provide complete protection in subjects who were more fit.

These overall findings of this study corroborate those reported previously in the same subjects but using different testing modalities. Trappe et al. (54) measured changes in muscle volume using magnetic resonance imaging and tested muscle performance using isometric and dynamic explosive leg-press and calf-press exercises using the flywheel exercise device. We utilized testing modalities that have been used in numerous other studies, although perhaps less specific to the countermeasure training performed during bed rest in these subjects. The consistency of muscle strength and endurance results across different testing modalities and protocols confirms the utility of the combined resistive and aerobic exercise countermeasures and the inability of this nutritional supplementation to provide protection. However, two additional points from our measurements likely are important in the development of countermeasures for spaceflight and bed rest-induced deconditioning.

First, Trappe et al. (54) focused on the effects of these countermeasures on only the extensor muscles, those most likely to be affected by unloading-induced deconditioning during spaceflight and spaceflight analogs (1). Using a testing protocol that surveyed muscle strength and endurance in additional leg muscles, we observed that this combined exercise countermeasure failed to protect the knee flexor muscles, which also become deconditioned during long-duration spaceflight (16, 27) and bed rest (25, 44). Countermeasures historically have been directed toward protecting the extensor muscle because of their apparent faster rate of deconditioning (1, 25), but results from bed rest and spaceflight indicate that this approach to exercise countermeasures has the potential to propagate muscle strength imbalances (7). Protection against loss of knee flexor strength was not achieved in this study, likely because neither of the exercise countermeasures, flywheel and treadmill exercise, specifically targeted this muscle group. Not protecting a specific muscle group such as the knee flexors increases the risk for injury; atrophic muscles may be more susceptible to muscle damage during reambulation after bed rest and spaceflight (41).

Second, the commonality between testing protocols in this study and other work allows us to compare changes in muscle strength and lean tissue mass in women, a generally understudied population in bed rest and spaceflight literature (19). A unique aspect of this report is that these measures are the same ones used routinely to assess lean tissue mass and muscle performance as a medical requirement in astronauts participating in flights to the International Space Station and in other NASA-sponsored bed rest investigations. NASA had chosen this muscle testing battery to characterize performance in isolated muscle groups (isokinetics) known to be affected by spaceflight and bed rest as well as in a multijoint protocol (leg press) to assess integrated function while minimizing strong eccentric contractions that are related to post-spaceflight muscle damage (1). As expected but not previously demonstrated in women using the same testing protocols, the bed rest-induced loss of muscle mass and endurance is progressive as bed rest duration increases. For example, losses in knee extensor peak torque (−25 vs. −5%), knee flexor peak torque (−18 vs. −3%), and leg lean mass (−14 vs. −4%) are greater after 60 days than after 30 days of bed rest in control subjects (42). Interestingly, preliminary results from women astronauts (n = 8) reveals that the mean loss of knee extensor (−15%) and flexor muscle strength (−18%) after ~6 mo of spaceflight (measured with the same testing protocol used in this bed rest) is similar to the losses observed in our control subjects after 60 days of bed rest (Kirk English, personal communication). Thus, assuming that muscle deconditioning would have progressed to a greater extent with longer duration of spaceflight without countermeasures (1, 24), it would appear that the in-flight countermeasures used on the International Space Station are at least partially effective in women. The adoption of an exercise countermeasure program by International Space Station astronauts that is more similar to the one tested in this bed rest study may provide better protection against muscle deconditioning.

Exercise countermeasures. Spaceflight- and bed rest-induced deconditioning are not limited to reductions in skeletal muscle size and function, but also includes reductions in aerobic and anaerobic exercise performance (28, 33). Thus both high-intensity resistive and aerobic exercise are likely necessary to preserve functional capacity of astronauts during long-duration spaceflight (6). Countermeasures against skeletal
An additional benefit of fewer aerobic and resistive exercise sessions as tested in this bed rest study compared with the current International Space Station protocol may be a reduced risk of overuse injuries (54). However, the exercise countermeasure protocols tested in this bed rest study likely are not optimized, but represent an amalgamation of two previously successful protocols that previously had not been tested concurrently (43). While the combination of these two protocols into a single countermeasure program does not appear to interfere with their effectiveness demonstrated in previous studies when tested independently (3, 29, 30), the systematic manipulation of each of the components of these countermeasures, and others, may further enhance their efficacy.

Nutritional countermeasure. The aggregate of the literature pertaining to the effectiveness of protein or amino acid supplementation as a countermeasure to muscle atrophy and decreased muscle strength during bed rest has been inconclusive (48). In the present study, the higher amount of protein supplied in the diet in nonexercising Protein subjects failed to have an impact on either muscle strength or lean tissue mass. In contrast, increasing protein consumption from 0.6 g·kg⁻¹·day⁻¹ to >1.0 g·kg⁻¹·day⁻¹ maintained nitrogen balance during 7 days of bed rest and prevented a decrease in protein synthesis (51). Additionally, Paddon-Jones et al. (36) used a nutritional countermeasure, consisting of essential amino acids plus carbohydrate (16.5 g essential amino acids and 30 g carbohydrate, 3 times per day), that maintained leg lean tissue mass (+0.2 kg) measured by DEXA and partially prevented the decrease in isotonic leg extension strength (−8.8 kg) compared that of control subjects (−17.8 kg) (36). Interestingly, supplementation with essential amino acids in that study resulted in a slightly lower protein intake compared with our present study (1.4 vs. 1.6 g·kg⁻¹·day⁻¹), but with more positive results. Perhaps the differences between these studies might be explained by the addition of carbohydrate to the nutritional supplement, resulting in an increased caloric intake and an elevated insulin response that might protect against protein degradation. Alternatively, differences between these studies might be explained by differences in protein intake in the control groups (0.8 vs. 1.0 g·kg⁻¹·day⁻¹). Stein and Blanc (48) have noted that in the three bed rest studies that have demonstrated beneficial effect of protein supplementation, control subjects receive protein intakes <1.0 g·kg⁻¹·day⁻¹, an amount likely below the normal protein intake of most subjects before entering the study. Thus they speculate that the “protein supplementation” in the nutritional countermeasure groups in those studies simply returned the subjects to their normal consumption levels.

As was demonstrated in the early International Space Station missions, exercise hardware is not free from failures, and adherence to exercise prescriptions is not guaranteed (23), particularly in the event of injury or trauma (37, 44). Therefore, it is important to understand the potential benefits of alternative or adjunct countermeasures (48). Current NASA flight rules call for the abandonment of the International Space Station if all exercise hardware fails for more than 30 days consecutively (NASA Flight Rules, NSTS-12820, Aeromedical, B13–113), and it is important to know whether this can be extended if other countermeasures are available. Furthermore, abandoning an exploration mission to Mars or an asteroid will not be possible. The success of some earlier bed rest studies led to the suggestion that protein and/or amino acid supplementation may...
be an effective countermeasure in these situations, but dietary countermeasures have not been previously tested during long-duration bed rest and spaceflight or in women. Unfortunately, the results of this study do not support the use of this nutritional countermeasure in this form.

Although protein supplementation alone during bed rest may not be an effective countermeasure, the combination of a nutrition countermeasure with exercise might be effective during spaceflight. A recent study by Brooks et al. (9) did not observe an additive effect of amino acid supplementation and resistive exercise during 28 days of bed rest when caloric restrictions were imposed during bed rest. Whether subjects performed resistive exercise only or performed resistive exercise and consumed amino acid supplementation, the losses in muscle strength and lean tissue mass were reduced relative to subjects whose only countermeasure was amino acid supplementation, but there were no differences between the countermeasure groups. Perhaps the effectiveness of the combination of nutritional supplementation and exercise would have been improved had sufficient calories been consumed, as has been documented during spaceflight (45). Future work in this area should consider the composition and timing of nutritional supplementation relative to exercise, as some studies have suggested that myofibrillar protein synthesis can be enhanced when these factors are optimized (4, 34, 52).

Effect of pre-bed rest muscular fitness. It is not surprising that the amount of bed rest-induced deconditioning in nonexercising subjects is related to the level of pre-bed rest muscle strength and lean tissue mass, as this has been reported for other physical fitness parameters, such as aerobic capacity (10, 30). To our knowledge, the examination of the pre-bed rest condition with respect to the loss of muscle strength and lean tissue mass has been limited (56), and we believe that this is the first report with regard to the effectiveness of countermeasures during bed rest. In support of our observations in bed rest, the effectiveness of exercise countermeasures to protect muscle volume in early International Space Station missions was affected by preflight condition; the change in calf muscle volume during spaceflight was inversely proportional to initial calf muscle volume measured by magnetic resonance imaging (53). Unfortunately, not all of the astronauts studied perform the same exercise countermeasures in a controlled manner during their missions, such as afforded by bed rest studies, and, therefore, interpretation of these results has some inherent limitations.

As a group, the exercise countermeasures in our study were protective of lean tissue mass and muscle strength, but examination of the individual responses to bed rest and exercise revealed an important observation. That is, we observed that the effectiveness of these combined exercise countermeasures may be attenuated in individuals with higher levels of pre-bed rest muscle fitness; the less fit subjects experienced an increase in muscle strength and lean tissue mass when performing the exercise countermeasures during bed rest, whereas the more fit subjects experienced a decrease. This occurred even though the intensity of the countermeasures was prescribed relative to the individual subjects (i.e., maximal effort or percentage of maximal capacity). Thus it appears that a “one-size-fits-all” approach to exercise countermeasures for spaceflight and bed rest may not be entirely appropriate. Countermeasure prescriptions to maintain muscle mass and strength for less fit individuals require high-intensity effort, but protection in more fit individuals likely will require optimization of other elements of the exercise prescription (i.e., frequency, total volume, periodization). Routine monitoring of countermeasure effectiveness using standardized testing during spaceflight missions might aid in the individualization and refinement of countermeasure protocols to maximize efficacy. The results of this bed rest study also highlight the importance of recruiting bed rest subjects whose fitness level is similar to that of astronauts to demonstrate the effectiveness of countermeasures in the target population. In general, the muscle strength and endurance measures in the women in our study were at the low end of the range of that observed in International Space Station astronauts, but similar to that observed in female astronauts (Kirk English, personal communication).

Spaceflight applications. It is generally assumed that the most physically taxing part of a spaceflight mission, particularly for the trunk and lower body musculature, is the return to normal gravity when the astronauts are deconditioned. In most cases, medical and support personnel are readily available to assist with egress from the vehicle on return to Earth, but this has not always been true. For example, a spacecraft malfunction during reentry of the Soyuz vehicle carrying the Expedition 6 crew caused the capsule to land 475 km from the intended landing zone, and the crew had to fend for themselves for 5 h until the support team’s arrival (40). Additionally, support personnel will not be available during exploration missions to extraterrestrial surfaces when astronauts will be required to function autonomously. Under nominal conditions, astronauts likely will be required to safe the vehicle, as well as perform construction and exploration activities soon after arrival on an extraterrestrial surface. The required amount of physical activity may be even greater than anticipated should an emergency arise (8) or should the exploration vehicle not perform as expected (33). Protection against losses in muscle strength and mass during weightlessness would be important in these mission scenarios, because full recovery of muscle strength has been reported to require 1 mo or more (15, 24).

Thus an important observation from this work is that the means by which countermeasures are currently evaluated may be flawed. Countermeasure effectiveness generally is judged by the ability to maintain fitness and function at pre-bed rest or pre-spaceflight levels. However, because the physical tasks that an astronaut might be required to perform under normal or emergency situations (5, 8) are not scaled to the pre-spaceflight fitness levels, the efficacy of countermeasures to maintain pre-bed rest or pre-flight performance levels may not be an appropriate test. Our results suggest that it is easier to maintain muscle strength and lean tissue mass in less fit subjects, but in a real space mission these strength and endurance levels may be insufficient to perform mission-critical tasks. For example, 2 of 13 Shuttle astronauts studied before spaceflight, even without deconditioning, had insufficient levels of physical fitness to successfully complete a simulated emergency egress (20). Thus future countermeasure studies might focus on the ability of a countermeasure prescription to maintain physical capabilities of mission-critical tasks expected to be performed by astronauts during exploration missions (5).

Limitations. Although bed rest is considered by many to be an adequate analog of spaceflight to assess changes in muscle performance (1, 13), limitations to the study design, particu-
larly when combining work from multiple investigator teams, should be considered. First, like all bed rest and spaceflight studies, the number of subjects participating in this investigation was small (1, 28, 38). While extrapolation of these results to a larger population of astronauts may be limited, we attempted to counter this limitation by assessing the effects of bed rest and the countermeasures during bed rest to the range of pre-bed rest muscular fitness levels in the individual subjects. Second, in an ideal situation, we would have included an additional study group, combining exercise and protein supplementation, to investigate the interactive effects of countermeasures, but logistical and financial considerations precluded this. Future studies should address exercise and nutrition countermeasure, including consideration of the timing of protein ingestion relative to countermeasure performance (4). Third, the authors recognize that using isokinetic testing and DEXA to assess muscle performance and lean tissue mass, respectively, after bed rest and spaceflight may have lower specificity than some other testing methodologies. However, these measures have been used in other studies and platforms (spaceflight and bed rest), and utilizing similar test protocols across studies allows for the comparison of the effectiveness of different countermeasures (32). Fourth, there was extensive physiological testing in the last week of bed rest and immediately upon reambulation, and it is possible that the volume and nature of the testing may have influenced performance- and motivation-related measurements, including the strength and endurance tests reported here. Although scheduling of pre- and post-bed rest testing was constrained by the limited amount of time available to collect data for all investigative teams, the MEDES-JSC Science Management Team attempted to mitigate confounding factors across measurements, including the delay of rehabilitation activities until after these isokinetic tests were conducted.

Conclusions. Results from this study and others in WISE-2005 suggest that resistive and aerobic exercise countermeasures can be reasonably performed during bed rest, and likely during spaceflight, to preserve lower body lean tissue mass and skeletal muscle function, as well as upright aerobic capacity, and to attenuate the loss of bone mineral density (43, 47, 54). Importantly, these benefits can be achieved by resistive and aerobic exercise countermeasures (2–4 days/wk for each modality) that can be reasonable performed in the course of a normal week. If translated to spaceflight, this represents a substantial savings of the astronauts’ time relative to current exercise prescriptions consisting of aerobic and resistive exercise 6 days/wk for each modality (33). Unfortunately, the nutritional countermeasure employed in this study was not effective on its own, but perhaps if it were combined with a properly prescribed exercise countermeasure program, additional benefits might be realized (45). As with all other reports from WISE-2005, these data are an important contribution to the understanding of the human response to spaceflight because so few women have been studied in the past, either in spaceflight or spaceflight analogs (19).

APPENDIX

IV Regression

The notion of gain described in the Statistical analysis section does not account for variability in the input as represented by only one pre-bed rest test. Strength and endurance performance, as measured in this study, depend to some extent on the subject’s motivation to exert maximum effort, which can vary from day to day, even if a subject’s actual physical condition is unchanged. As a result, the one pre-bed rest strength or endurance measurement is only an imperfect measure of a subject’s actual inherent condition in terms of strength or endurance. This inherent condition could be thought of as a hypothetical mean pre-bed rest performance that would be obtained with a large number of tests per subject. We, therefore, redefine gain to mean how much average post-bed rest performance changes relative to a unit change in inherent condition. The model now becomes

\[ \text{post} = C + \beta \mu + e \]  \hspace{1cm} (A1)

where \( \mu \) denotes the subject’s inherent condition.

Although the ANCOVA model using the one pre-bed rest result as a surrogate for \( \mu \) in Eq. A1 is adequate for making inferences on the differential effects of the treatments, the resulting estimate of \( \beta \) is attenuated toward zero and needs adjustment to obtain an unbiased estimate of the gain, as defined above. This situation is known as a “measurement-error model” because the one pre-bed rest measurement can be expressed as

\[ \text{pre} = \mu + d \]  \hspace{1cm} (A2)

where \( d \) is a random error term not related to \( e \) above. One way of correcting the estimate of \( \beta \) is to use IV regression. IV regression allows one to compensate for measurement error in a predictor variable by use of an “instrumental variable” that is expected to be correlated with the true predictor (in this case \( \mu \)) and is uncorrelated with the error term \( d \) in Eq. A2. In this application, lean leg mass serves as an excellent IV because it is an objective and accurate measure (being an average of triplicate DEXA measurements) and should be fairly well correlated with the subjects’ inherent strength or endurance measures studied here. Furthermore, measurement errors in leg lean tissue mass would not be expected to be correlated with the error in using one pre-bed rest measurement to estimate \( \mu \). Estimates of \( \beta \) using IV regression and corresponding test-based 95% confidence limits using the method of Finlay and Magnusson (14) are provided for each outcome. IV regression has been used in clinical studies (21, 35) and to correct for noncompliance in epidemiological studies (17).

Figure 7 shows the estimates of the gain with and without IV regression along with 95% confidence limits. Note that the point estimates are higher with IV for every outcome. This suggests using

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Fig7}
\caption{Comparison of point estimate of gain (\( \beta \)) with the 95\% confidence interval using ordinary least squares (OLS) and instrumental variable (IV) regression. KES, knee extensor strength; KFS, knee flexor strength; KEE, knee extensor endurance; KFE, knee flexor endurance; AES, ankle extensor strength; AFS, ankle flexor strength; LP: leg press.}
\end{figure}
IV regression is indeed removing downward bias induced by uncertainty in the baseline. However, this is at the expense of greater uncertainty (wider confidence limits).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S.M.C.L., S.M. Schneider, B.R.M., B.R.M., D.E.W., and A.R.H. performed experiments; S.M.C.L. and A.H.F. analyzed data; S.M.C.L., S.M. Schneider, A.H.F., B.R.M., B.R.M., S.M. Smith, D.E.W., and A.R.H. approved final version of manuscript; D.E.W., and A.R.H. edited and revised manuscript; S.M.C.L., S.M. Schneider, A.H.F., B.R.M., S.M. Smith, D.E.W., and A.R.H. performed at MEDES, Institute for Space Physiology and Medicine in Toulouse, France. This study was the “Promoteur” of the study according to French law. The study was supported by NASA Grant NNJ04HF71G to A. R. Hargens.

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