WISE-2005: Supine treadmill exercise within lower body negative pressure and flywheel resistive exercise as a countermeasure to bed rest-induced bone loss in women during 60-day simulated microgravity

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Abstract

Bone loss associated with disuse during bed rest (BR), an analog of space flight, can be attenuated by exercise. In previous studies, the efficacy of either aerobic or resistive exercise countermeasures has been examined separately. We hypothesized that a regimen of combined resistive and aerobic exercise during BR would prevent bone resorption and promote bone formation. After a 20-day ambulatory adaptation to controlled confinement and diet, 16 women participated in a 60-day, 6° head-down-tilt BR and were assigned randomly to one of the two groups. Control subjects (CON, n = 8) performed no countermeasure. Exercise subjects (EX, n = 8) participated in an exercise program during BR, alternating between supine treadmill exercise within lower body negative pressure (3–4 d wk−1) and flywheel resistive exercise (2–3 d wk−1). By the last week of BR, excretion of helical peptide (CON, 79%±44 increase; EX, 64%±50, mean±SD) and N-terminal cross-linking telopeptide (CON, 51%±34; EX, 43%±56), markers of bone resorption, were greater than they were before BR in both groups (P<0.05). However, serum concentrations of the bone formation marker procollagen type I N propeptide were greater in EX than CON throughout and after bed rest (P<0.05), while concentrations of the bone formation marker bone alkaline phosphatase tended to be greater in EX than CON. Dual-energy X-ray absorptiometry results indicated that the exercise treatment significantly (P<0.05) attenuated loss of hip and leg bone mineral density in EX compared to CON. The combination of resistive and aerobic exercise did not prevent bone resorption but did promote bone formation, and helped mitigate the net bone loss associated with simulated microgravity.

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Keywords: Bone turnover markers; Exercise; Space flight; Bed rest; Simulated weightlessness

Abbreviations: ALP, alkaline phosphatase; ANOVA, analysis of variance; BMD, bone mineral density; BR, bed rest; BW, body weight; CNES, Centre National d’Études Spatiales; CON, control; CSA, Canadian Space Agency; CTX, C-terminal cross-linking telopeptide of type I collagen; DEXA, dual-energy X-ray absorptiometry; ELISA, enzyme-linked immunosorbent assay; ESA, European Space Agency; EX, exercise; LBNP, lower body negative pressure; MEDES, French Institute for Space Medicine and Physiology; NASA, National Aeronautics and Space Administration; NTX, N-terminal cross-linking telopeptide of type I collagen; PINP, procollagen type I N propeptide; PTH, parathyroid hormone; RIA, radioimmunoassay; RMR, resting metabolic rate; UCSD, University of California, San Diego.

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Introduction

Complete protection against bone loss associated with space flight has yet to be achieved. One promising countermeasure is exercise. Physical activity and loading enhance the mechanical competence of bone in ambulatory subjects [1–4], and ground-based evaluations of exercise have documented the ability to mitigate the negative effects of bed rest on bone metabolism [5–8]. However, the optimal exercise prescription (the modality, frequency, intensity, and duration) has not been identified for space travelers.

The efficacy of exercise countermeasures during space flight and simulated weightlessness (bed rest) may be compromised by an inability to achieve sufficient loading and hydrostatic pressure gradients within blood vessels and other fluid columns in the body [9,10]. Typically, crew members can tolerate only loads equivalent to 60–70% of body weight during bungee-cord treadmill exercise, one of the standard countermeasures during space flight [11,12], because of discomfort associated with the hip and shoulder harness used to hold the crew member on the treadmill. Lower body negative pressure (LBNP) is a proven method to provide greater, more comfortable loads during exercise, as well as transmural blood pressure gradients in the lower body. Supine treadmill exercise within LBNP mitigates the usual bed rest-induced increase in bone resorption in men [6] and tends to mitigate it in women [13].

Resistive exercise also has a protective effect against net bone loss during bed rest [5], apparently by increasing bone formation while having little effect on bone resorption. Although resistive exercise itself may have an independent effect on bone loading and preserve muscle strength during unloading, this exercise modality may preserve the capacity to produce muscle-generated force to protect bone. The protective effects of these two types of exercise countermeasures during bed rest seem to be different: the LBNP/treadmill exercise protocol reduces the resorptive response, and resistive exercise protocols increase bone formation.

To date, studies have focused on either aerobic or resistive exercise, but not both countermeasures utilized together in the same bed rest investigation. Because we previously documented that these separate countermeasures were effective through different mechanisms, we hypothesized that a combination of treadmill exercise within LBNP, plus resistive exercise countermeasures, would protect against bed rest-induced bone loss, as assessed by bone biochemical markers and dual-energy X-ray absorptiometry (DEXA).

Materials and methods

This study was a multinational effort among several space agencies to conduct an extensive study of countermeasures against bed rest-induced losses of bone and muscle mass, aerobic capacity, orthostatic tolerance, and muscle performance. We report here the impact of the combined exercise protocols on bone metabolism and bone mineral density.

Subjects

A total of 16 healthy, non-smoking women (mean ±SD, 32 years ± 4, 166 cm ± 7, 59 kg ± 5) volunteered to participate in the study. An intensive screening and evaluation protocol was used to ensure that subjects were healthy and fit for participation in the study. Among the inclusion criteria were a body mass index of 20–25 kg m⁻², regular menstrual cycles, no family history of chronic disease or psychiatric disease, active, and free from orthopedic, musculoskeletal, and cardiovascular disorders. Among the exclusion criteria were use of oral contraceptives in the 2 months before the study; completely sedentary or extremely fit subjects, bone mineral density (left hip and lumbar spine) more than 1.5 standard deviation above or below the age/sex-matched mean, fractures or tendon laceration within 1 year, history of genetic muscle or bone diseases of any kind, chronic back pain, history of thrombophlebitis, and presence of metallic implants. The protocol for this study was approved by the local ethics committee (CCPRPB of Toulouse, France); by the University of California, San Diego Institutional Review Board; and by the Johnson Space Center Committee for the Protection of Human Subjects.

Study design

The study was conducted at the Institute for Space Medicine and Physiology (MEDES) in Toulouse, France. It consisted of a 20-day ambulatory control period followed by 60 days of 6° head-down-tilt bed rest (BR). A 20-day ambulatory recovery period followed the BR period.

Subjects were recruited in two different bed rest campaigns (Feb 2005–May 2005, Sept 2005–Dec 2005). Four control subjects (CON) and four exercise subjects (EX) were recruited for each campaign, for a total of eight subjects in each group. Subjects were matched according to age and body mass index (BMI), and then assigned randomly to either CON or EX. CON subjects performed no countermeasures during bed rest. EX subjects performed an exercise countermeasure that consisted of both aerobic and resistive elements: supine aerobic treadmill exercise within LBNP, and flywheel resistive exercise.

Blood samples and two consecutive 24-hour urine pools were collected twice before bed rest (between days BR–10 and BR–1), two (blood) or three (urine) times during bed rest (only urine was collected on day BR 14/BR 15, and both were collected between BR 28 and BR 32, and again between BR 56 and BR 60), and once after bed rest ended (between days R+5 and R+7) for the measurement of markers of bone and calcium metabolism. Blood samples were collected fasting (>10 h), immediately after subjects were awakened, at the same time of day (0630–0800), to minimize the effect of diurnal changes in endocrine and biochemical markers [14]. Blood and urine were processed and frozen at −80 °C until analysis. In general, samples from each campaign were assayed at the same time to minimize inter-assay variations in the results.

Dietary control

During the 20 days before bed rest, resting metabolic rate (RMR) was determined by indirect calorimetry. For CON, the initial calorie intake was set at 110–125% of the RMR. For EX, calorie intake was set at 110–125% of the RMR plus an estimated energy expenditure due to physical activity. Every 15 days, RMR and fat mass were determined by indirect calorimetry and DEXA. Body weight was monitored daily. During the recovery period after bed rest, caloric intake was prescribed to be 130% of RMR for both groups. The goal for the ambulatory phases of the study was to maintain body mass. During the bed rest phase of the study the goal was to maintain fat mass (that is, to allow total body mass to change if necessary to prevent fat mass from increasing).

Subjects were restricted from ingesting caffeine, alcohol, and chocolate during the study. They were provided three meals and up to two snacks per day. Subjects had a choice of two menus from which to select meals for each day. For each subject, dietary guidelines aimed to have sodium intake at 1.2–1.6 mmol kg⁻¹ d⁻¹, potassium intake at 0.9–1.1 mmol kg⁻¹ d⁻¹, calcium intake at 1 g d⁻¹, and phosphorus intake at 1.2–1.6 mmol kg⁻¹ d⁻¹.

Exercise countermeasure protocols

The exercise countermeasure consisted of resistive exercise training and supine LBNP treadmill exercise, performed on separate days (each performed 2–3 d wk⁻¹). For the resistive exercise training, the thigh and calf muscle groups were trained using supine leg press and calf press exercises on an inertial ergometer [15–17]. A total of 19 sessions were scheduled for each subject about every third
A significant main effect of time was found for the data presented in Table 1. Different letters in each column represent significant \( (P < 0.05) \) differences between time points for that nutrient.

Subjects were women randomly assigned to the control (n=8) or exercise (n=8) group. Exercise was resistive exercise combined with treadmill exercise within lower body negative pressure. All data are presented as means±SD.

The LBNP exercise device used for this study was similar to that used in previous 5-day [21], 15-day [22], and 30-day bed rest studies [11,13]. The negative pressure (−48 to −55 mmHg) required to produce 1.0 times body weight (BW) calibrated for each subject has been shown to produce cardiovascular, biomechanical, and metabolic responses similar to those produced by upright exercise in 1 G [23].

EX subjects performed 40 min of exercise on 2 to 4 d wk \(^{-1} \), followed by 10 min of resting LBNP. Target exercise intensities for this protocol consisted of supine running/walking for 7 min at 40% of pre-BR VO\(_2\)pk, 3 min at 60%, 2 min at 40%, 3 min at 60%, 2 min at 40%, 2 min at 50%, 2 min at 60%, 3 min at 50%, 2 min at 60%, 3 min at 60%, 2 min at 40%, 3 min at 60%, 3 min at 60%, and 5 min at 40%. Prescribed target speeds to achieve these exercise intensities were based on data from the pre-BR VO\(_2\)pk treadmill test performed in the upright posture. This protocol was similar to one that successfully preserved upright exercise capacity during 15 and 30 days of bed rest [6,22,24].

During the course of the 60-day BR, 29 LBNP exercise sessions were prescribed for each EX subject. Not all LBNP/exercise sessions were completed by all subjects. One subject was unable to complete her first two exercise sessions because of calf pain as a result of the pre-BR muscle biopsy for another part of this project. However, she subsequently completed all other sessions. One subject was unable to complete three exercise sessions because of recurrent back or hip pain, and two other subjects were unable to complete one exercise session because of a short-term illness (fever, upper respiratory symptoms). Post-exercise resting LBNP was terminated early (before 10 min) in three subjects due to pre-syncopal symptoms. Resting LBNP was terminated early in two of these subjects during two sessions each. In the third subject LBNP was terminated early in 7 of the 29 sessions.

Subjects were women randomly assigned to the control (n=8) or exercise (n=8) group. Exercise was resistive exercise combined with treadmill exercise within lower body negative pressure. All data are presented as means±SD. BR, bed rest day; Post-BR, post-bed rest.

![Fig. 1. Percent change in serum parathyroid hormone during and after 60 days of head-down-tilt bed rest for women in the sedentary control group (●) (n=5) and the exercise group (○) (n=5), resistive exercise combined with treadmill exercise within LBNP. Due to analytical issues (see Materials and methods section), these data represent data from subjects with all values obtained from the same assay technique. Values are means±SD. BR, bed rest day; Post, post-bed rest; Pre, before bed rest. Statistical analyses were performed on raw data, although data are expressed here as percent change from pre-bed rest. *Significantly (P<0.05) different from pre-bed rest (Pre).](image-url)
Table 3

Bone formation and turnover markers before, during, and after 60 days of bed rest

<table>
<thead>
<tr>
<th></th>
<th>Pre-BR</th>
<th>BR 30</th>
<th>BR 58</th>
<th>Post-BR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total alkaline phosphatase&lt;sup&gt;a,b&lt;/sup&gt; (serum, U l&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>Control 47±8</td>
<td>53±11&lt;sup&gt;*&lt;/sup&gt;</td>
<td>51±9</td>
<td>48±8</td>
</tr>
<tr>
<td></td>
<td>Exercise 50±10</td>
<td>62±16&lt;sup&gt;*&lt;/sup&gt;</td>
<td>62±13&lt;sup&gt;*&lt;/sup&gt;</td>
<td>63±14&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bone alkaline phosphatase (serum, U l&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>Control 22±8</td>
<td>23±7</td>
<td>22±7</td>
<td>20±9</td>
</tr>
<tr>
<td></td>
<td>Exercise 22±7</td>
<td>30±13</td>
<td>28±7</td>
<td>30±14</td>
</tr>
<tr>
<td>Osteocalcin&lt;sup&gt;c&lt;/sup&gt; (serum, ng ml&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>Control 12±3</td>
<td>13±3&lt;sup&gt;*&lt;/sup&gt;</td>
<td>13±2</td>
<td>12±3</td>
</tr>
<tr>
<td></td>
<td>Exercise 11±2</td>
<td>12±4&lt;sup&gt;*&lt;/sup&gt;</td>
<td>11±3</td>
<td>12±3</td>
</tr>
<tr>
<td>PINP&lt;sup&gt;c,d&lt;/sup&gt; (serum, ng ml&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>Control 72.1±25.1</td>
<td>64.6±19.7</td>
<td>68.1±18.6</td>
<td>78.6±18.9</td>
</tr>
<tr>
<td></td>
<td>Exercise 66.7±28.4</td>
<td>90.9±42.8&lt;sup&gt;*&lt;/sup&gt;</td>
<td>88.4±30.4&lt;sup&gt;*&lt;/sup&gt;</td>
<td>95.5±33.5&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Subjects were women randomly assigned to the control (n=8) or exercise (n=8) group. Exercise was resistive exercise combined with treadmill exercise within lower body negative pressure.

All data are presented as means±SD. BR, bed rest day; Pre-BR, post-bed rest; PINP, procollagen type I N propeptide.

Biochemical analyses

Biochemical analyses were completed either in the NASA Nutritional Biochemistry Laboratory or the DLR Institute of Aerospace Medicine. Most biochemical analyses were performed by standard commercial techniques, and have been described in detail previously [13,25–30]. Briefly, circulating bone- and calcium-related factors were measured in blood serum. Serum 1,25-dihydroxyvitamin D was measured by radioimmunoassay (RIA) (DiaSorin, Stillwater, MN, USA) after extraction of samples with acetonitrile and purification on C18OH cartridges (16.2% CV). Serum 25-hydroxyvitamin D was determined by RIA (DiaSorin) after extraction with acetonitrile (9.1% CV).

Parathyroid hormone (PTH) was assayed for the intact peptide by RIA using two commercially available kits (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA, 5.9% CV, and Diagnostics Systems Laboratories, Inc., Webster, TX, USA, 4.2% CV). The Nichols Institute kit was unexpectedly discontinued in the period between the first and second phase of this study, and because of concerns about storage and other factors, samples were analyzed for all subjects after that phase of the study was completed. The number of subjects with a complete set of data from the same kit was smaller than the number with a complete data set. We performed the statistical analysis with each set of subjects and present the results of both analyses.

Bone alkaline phosphatase (bone ALP, 5.6% CV) was measured by ELISA (Quidel Corporation, Santa Clara, CA), and serum osteocalcin (6.0% CV) was measured by RIA (Biomedical Technologies, Stoughton, MA, USA). Another bone formation marker, procollagen type I N propeptide (PINP, 4.7% CV), was analyzed using a commercially available RIA kit (Orion Diagnostica, Espoo, Finland).

Bone resorption markers were also determined. Tartrate-resistant acid phosphatase (9.3% CV) was analyzed by commercially available ELISA (SBA SciencesBioCity, Turku, Finland) as were several of the urinary collagen crosslinks: C-terminal cross-linking telopeptide of type I collagen (CTX, Crosslaps, Osteometer BioTech, Herlev, Denmark, 7.5% CV), N-terminal cross-linking telopeptide of type I collagen (NTX, Osteomark, Ostex International Inc., Seattle, WA, USA, 5.4% CV), and pyridinoline and deoxypyridinoline (PYD and DPD, respectively, Pyrilinks, Quidel Corporation, 9.9 and 8.4% CV, respectively). Urinary and serum calcium (3% CV) concentrations were measured using atomic absorption spectrometry.

Table 4

Bone resorption markers before, during, and after 60 days of bed rest

<table>
<thead>
<tr>
<th></th>
<th>Pre-BR</th>
<th>BR 14/15</th>
<th>BR 30/31</th>
<th>BR 58/59</th>
<th>Post-BR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTX&lt;sup&gt;b&lt;/sup&gt; (urinary, nmol d&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>Control 327±126</td>
<td>399±135*</td>
<td>435±107*</td>
<td>475±150*</td>
<td>367±118</td>
</tr>
<tr>
<td></td>
<td>Exercise 434±206</td>
<td>544±214*</td>
<td>558±185*</td>
<td>563±223*</td>
<td>477±168</td>
</tr>
<tr>
<td>CTX&lt;sup&gt;c&lt;/sup&gt; (urinary, nmol d&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>Control 2045±631</td>
<td>2765±885*</td>
<td>3054±724*</td>
<td>3591±710*</td>
<td>2767±905*</td>
</tr>
<tr>
<td></td>
<td>Exercise 2628±1103</td>
<td>3517±1516*</td>
<td>3770±1396*</td>
<td>3695±1513*</td>
<td>3085±1259*</td>
</tr>
<tr>
<td>Helical peptide&lt;sup&gt;c&lt;/sup&gt; (urinary, μg d&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>Control 489±184</td>
<td>633±225*</td>
<td>729±255*</td>
<td>845±253*</td>
<td>613±217*</td>
</tr>
<tr>
<td></td>
<td>Exercise 579±250</td>
<td>771±297*</td>
<td>801±281*</td>
<td>876±326*</td>
<td>678±294*</td>
</tr>
<tr>
<td>Deoxypyridinoline&lt;sup&gt;c&lt;/sup&gt; (urinary, nmol d&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>Control 49±12</td>
<td>55±17</td>
<td>59±13</td>
<td>59±14*</td>
<td>49±8</td>
</tr>
<tr>
<td></td>
<td>Exercise 50±17</td>
<td>59±17</td>
<td>62±18</td>
<td>63±30*</td>
<td>57±20</td>
</tr>
<tr>
<td>Tartrate-resistant acid phosphatase&lt;sup&gt;b&lt;/sup&gt; (urinary, mg d&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>Control 2.78±0.59</td>
<td>3.69±0.72*</td>
<td>3.75±0.81*</td>
<td>3.23±0.73*</td>
<td>3.50±1.20*</td>
</tr>
<tr>
<td></td>
<td>Exercise 2.74±0.69</td>
<td>3.85±0.91*</td>
<td>3.56±1.14*</td>
<td>3.50±1.20*</td>
<td>3.50±1.20*</td>
</tr>
</tbody>
</table>

Subjects were women randomly assigned to the control (n=8) or exercise (n=8) group. Exercise was resistive exercise combined with treadmill exercise within lower body negative pressure.

All data are presented as means±SD. For urinary analytes, 24-hour urine pools were collected on 2 consecutive days. The means of individual values from the 2 days at each time point were used to determine the group mean and SD. BR, bed rest day; Post-BR, post-bed rest; CTX, C-terminal cross-linking telopeptide of type I collagen; NTX, N-terminal cross-linking telopeptide of type I collagen.

<sup>*</sup>Different (P<0.05) from pre-bed rest (Pre-BR), as determined by a Bonferroni post hoc t-test analysis.
same operator to ensure consistency of positioning and measures, and daily calibrations were performed with a manufacturer-supplied phantom. All DEXA scan images were analyzed by one individual in the Johnson Space Center Bone and Mineral Laboratory, using Hologic software (Version 12.3.7), in accordance with procedures established and used for analyzing and reporting earlier space flight and bed rest data [5,31,32]. Hip scans were manually analyzed, with the global region of interest comprising the area from the most superior margin of the femoral head proximally to a horizontal line placed 15 spaces inferior to the base of the lesser trochanter and from the most lateral margin of the greater trochanter to the most medial margin of the femoral head. The inferior margin of the trochanter subregion touched the base of the greater trochanter laterally. The femoral neck subregion was defined using the default mode (“Whole Mode”), with the box positioned manually along the midline of the femoral neck and the lateral inferior corner of the box in the soft tissue immediately adjacent to the medial margin of the greater trochanter, with the other three corners in soft tissue. The intertrochanter margins were defined by the femoral neck box inferior margin, the inferior margin of the greater trochanter, and the inferior margin of the global region of interest. After the first scan was analyzed manually, subsequent scans were analyzed using the “Compare to Previously Analyzed Scan” selection.

Leg bone mineral density was determined from the whole-body scans, and cut lines were automatically placed and manually adjusted by the same technician per standard JSC Bone and Mineral Lab procedures. Precision of the manual procedure is equivalent to that of the automated procedures available on current DEXA analysis software. Data from left and right legs were averaged prior to statistical analysis.

The root mean square standard deviation (RMS SD) for trochanter was 0.007, for total hip was 0.007, and for legs was 0.014. The RMS SD was determined on 48 sets of 3 samples, as defined by the International Society of Clinical Densitometry.

### Statistical analysis

Data were analyzed using repeated-measures analysis of variance (ANOVA) techniques. A Bonferroni post hoc t-test was used to identify differences between time points (an a priori decision was made to only compare all time points to pre-bed rest). Statistical analyses were performed using Sigma Stat 3.11 (Systat Software, Inc., San Jose, CA). Statistical significance was defined as $P<0.05$.

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Fig. 2. Percent change in urinary and serum markers of bone and calcium metabolism during and after 60 days of head-down-tilt bed rest for women in the sedentary control group (●) ($n=8$) and the exercise group (□) ($n=8$, resistive exercise combined with treadmill exercise within LBNP). Values are means ± SD. BR, bed rest day; Post, post-bed rest. Statistical analyses were performed on raw data, although data are expressed here as percent change from pre-bed rest. ALP, bone alkaline phosphatase; NTX, N-terminal cross-linking telopeptide of type I collagen; PINP, procollagen type I N propeptide; TRAP, tartrate-resistant acid phosphatase.

*Significantly ($P<0.05$) different from pre-bed rest (Pre). #Significant difference between groups ($P<0.05$).
Results

Diet and body weight

Actual dietary intakes for the control and exercise groups before, during, and after bed rest are listed in Table 1. Energy intake was lower during bed rest in the CON but not the EX subjects, by design (because CON subjects had lower energy expenditure). This was reflected in lower carbohydrate and fat intakes in CON subjects, while protein intake was held relatively constant. Mean body weights for CON and EX were not significantly different from each other before the study (56.2 kg ± 3.6 and 59.1 kg ± 6.5, respectively, \( P = 0.12 \)) or after bed rest (CON, 52.2 kg ± 3.7; EX, 54.8 kg ± 6.2; \( P = 0.06 \)). The average rate of body mass loss was 0.06 kg d\(^{-1}\) ± 0.01 for CON and 0.04 kg d\(^{-1}\) ± 0.01 for EX (no significant difference, \( P > 0.05 \)). In the CON group, the weight loss was mostly lean tissue (7.4% average change in lean tissue mass, 2.6% average change in fat tissue mass, data not presented). In the EX group, the opposite was observed, as expected (3.2% average change in lean tissue mass, 14.3% average change in fat tissue mass). Muscle volume changes have been published elsewhere [20].

Exercise countermeasures

Data compiled at the end of both bed rest campaigns provided a profile of the exercise sessions. As a consequence of various medical situations that arose during the bed rest period, including the impact of the combined aerobic and resistive exercise countermeasure program (soreness and injury) and mild illness, not all exercise sessions were completed as planned. EX subjects completed an average of 96% of the prescribed LBNP treadmill exercise sessions. No subject completed less than 90% of her sessions. Subjects completed an average of 95% of the post-exercise resting LBNP exposures; all but one subject completed more than 90%. A subject who had recurrent post-exercise pre-syncopal symptoms was able to complete only 75% of the post-exercise resting LBNP exposures (note: the biochemical data from this subject were not remarkably different from other subjects’ data).

Regarding the leg press sessions, 82% were conducted as planned, 13% were reduced effort, and 5% were missed. Regarding the calf press sessions, 74% were conducted as planned, 20% were reduced effort, and 6% were missed. The submaximal and missed resistive exercise sessions varied among the volunteers and were scattered throughout the 60-day bed rest period.

After about 30 days of bed rest, all EX subjects completed their exercise at an LBNP level that produced at least 1 BW of loading. Thereafter, as their tolerance to exercise increased, all but one of the eight subjects completed at least some exercise sessions at greater than 1.05 times BW. For the subject who had pre-syncopal symptoms, the average exercise and post-exercise LBNP sessions were reduced to about 0.9 times BW for part of the countermeasure period. During the exercise sessions, the mean LBNP exercise time (including rest time) was 50 min ± 2.

Biochemical markers

Bed rest had a significant effect on 25-hydroxyvitamin D and serum calcium (Table 2). In both groups, urinary calcium excretion was greater during BR than before BR. Although there was a significant main effect of time on PTH (serum concentrations tended to be lower during bed rest at BR30 in both groups, but more pronounced in CON), when a post hoc test was performed for pre-bed rest and each later time point, PTH was not significantly different from pre-bed rest at any time (Table 2). When only subjects whose data were analyzed using a single assay were included (see Materials and methods for details), there was a significant group (\( P < 0.05 \)) and time (\( P < 0.03 \)) effect (Fig. 1).

Bed rest had a significant effect on total ALP, osteocalcin, and PINP (Table 3). The increase in total ALP and PINP in EX was evident at BR 30 and BR 58, and after BR. Bone ALP tended to be greater in EX than in CON (\( P = 0.108 \)). No significant differences between groups were found for any urinary markers of bone metabolism (Table 4). In both groups, excretion of NTX, CTX, and helical peptide was greater during BR than before BR. These markers were variable between subjects, but they were still greater during BR in both groups when the data were expressed as percent change from pre-bed rest values (Fig. 2), with no treatment effect evident. Serum tartrate-resistant acid phosphatase was elevated in both groups (Table 4, Fig. 2), indicating increased bone resorption during bed rest, with no exercise effect.

Table 5

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<thead>
<tr>
<th>Location</th>
<th>Pre-BR</th>
<th>Mid-BR</th>
<th>Post-BR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral neck</td>
<td>1.006 ± 0.065</td>
<td>1.009 ± 0.078</td>
<td>1.006 ± 0.083</td>
</tr>
<tr>
<td>Spine</td>
<td>1.056 ± 0.081</td>
<td>1.068 ± 0.083</td>
<td></td>
</tr>
<tr>
<td>Total hip</td>
<td>0.858 ± 0.057</td>
<td>0.870 ± 0.055</td>
<td>0.854 ± 0.066</td>
</tr>
<tr>
<td>Leg (L+R averaged)</td>
<td>1.012 ± 0.06</td>
<td>1.011 ± 0.057</td>
<td>1.004 ± 0.06</td>
</tr>
<tr>
<td>Control</td>
<td>0.935 ± 0.069</td>
<td>0.935 ± 0.069</td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>1.213 ± 0.079</td>
<td>1.204 ± 0.086</td>
<td>1.213 ± 0.079</td>
</tr>
</tbody>
</table>

Subjects were women randomly assigned to the control (\( n = 8 \)) or exercise (\( n = 8 \)) group. Exercise was resistive exercise combined with treadmill exercise within lower body negative pressure. All data are presented as means ± SD, g cm\(^{-2}\). BR, bed rest day; Mid-BR, after about 30 days of bed rest; Post-BR, post-bed rest (3 days after reambulation).

Significant difference between treatment groups, \( P < 0.05 \). Significant interaction between treatment group and time, \( P < 0.01 \). Different (\( P < 0.05 \)) from pre-bed rest (Pre-BR), as determined by a Bonferroni post hoc t-test analysis. Significant difference between groups, as determined by a Bonferroni post hoc t-test analysis (\( P < 0.05 \)).
In the trochanter, the BMD of CON was significantly lower than pre-bed rest during and after bed rest and the BMD of EX was lower only after bed rest (Table 5). In the total hip, the BMD of CON and EX was significantly lower during and after bed rest. BMD losses were significantly less \( (P<0.05) \) for EX than for CON (Fig. 3). A significant interaction was found in leg BMD, where more BMD loss occurred in CON than EX post BR (Table 5). No bed rest or group effect was observed for the femoral neck or the spine.

**Discussion**

No consensus exists on the type, frequency, and duration of exercise that is optimal for preventing bone loss associated with space flight. In this study, we assessed the effectiveness of a combination of resistive and aerobic exercise in bed rest as a model for space flight. Contrary to our hypothesis, concentrations of bone resorption markers were greater during and after bed rest than before bed rest in both groups, and the response of the resorption markers was not attenuated by exercise countermeasures. However, the concentrations of bone formation markers were increased and the loss of bone mineral density in the total hip and trochanter was attenuated in exercise subjects.

Exercise tended to increase serum concentrations of bone formation markers in our female subjects during bed rest, but the concentrations were not as great as the ones we previously observed in a 17-week bed rest study (men and women) in which resistive exercise alone was used as a countermeasure for bone loss [5]. In that study, bone ALP did not change in controls but significantly increased (+64%) in the resistive exercise group [5]. In the current study, bone ALP increased 37%, total ALP increased 25%, and PINP increased 45% in EX (Fig. 2). It is intriguing that the bone formation response was approximately half of that seen in the previous study, while the resistive exercise volume also was, by rough estimate, somewhat less than half (completed every other day as opposed to every day, with fewer sets and reps).

Vitamin D stores, as reflected by circulating 25-hydroxyvitamin D concentrations, were reduced during (and after) bed rest in both groups. This phenomenon has been observed in astronauts during and after flight [27,28], and likely reflects the need for greater intakes of vitamin D given the lack of ultraviolet light exposure and general inadequacy of vitamin D intake. However, the lack of change in 1,25-dihydroxyvitamin D, the active form of the vitamin/hormone, observed here suggests that the decline in vitamin D stores likely did not exacerbate bone loss. Although maintaining optimal vitamin D status is important for bone and other physiological systems, vitamin D alone is not likely to mitigate weightlessness-induced loss of bone.

Several differences between the present study and the 17-week study [5] with regard to the resistive exercise countermeasure prescription might help explain the disparity in the magnitude of change in the bone formation markers. Shackelford et al. [5] utilized a whole-body resistive exercise protocol that stimulated a greater total bone mass, whereas in the current study subjects trained with only the lower body exercises. Exercise countermeasure subjects in the 17-week study performed resistive exercise 6 d wk\(^{-1}\), training the upper body 3 d wk\(^{-1}\) and the lower body 3 d wk\(^{-1}\), but the EX subjects in the current study performed only the lower body countermeasure 3 d wk\(^{-1}\). Additionally, subjects in the 17-week study performed some single-leg stance exercises to maximize loads on the lower extremities by putting the entire load on the leg and hip on each side independently. Bone responses observed in subjects who performed double-leg exercises in a pilot study for the 17-week bed rest study by Shackelford et al. [5] were similar to those observed in the current study, suggesting that the biomechanical aspects of the load application may be a contributing factor to region-specific protection of BMD, and may be more important than machine characteristics (beyond the basic force requirements). Furthermore, in the 17-week study [5], subjects performed a progressive resistive exercise protocol that increased the number of exercise sets and the intensity of the loads applied, but in the present study subjects consistently performed 4 sets of each exercise at maximal effort. Shackelford et al. [5] varied the volume and intensity of the stimulus, but the stimulus was relatively constant in the current protocol. Finally, the loading stimuli...
were potentially different between the inertial flywheel in the current study and the weight stack device in the 17-week study [5].

Despite the many differences between the present study and the 17-week study in the particulars of the resistive exercise protocol and the magnitude of the bone formation marker changes, the pattern of response of the bone biochemical markers was remarkably similar in the two studies. Specifically, while exercise had essentially no impact on resorption, it was associated with a marked increase in bone formation markers, observed in the first blood sample collected (6 weeks into the 17-week protocol, 4 weeks into the bed rest reported here). These markers remained consistently elevated throughout bed rest.

The exercise group in this study did not display hypercalciuria despite the increased bone resorption. Evidence from our earlier resistive exercise bed rest study [5] suggests that this is related to increased flux of calcium out of the bone with reincorporation of the calcium into bone as a result of the increased bone formation. The lack of a change in the excretion pattern of calcium isotopes in exercising bed rested individuals, compared to sedentary controls, has been documented [33].

In a separate study in which flywheel resistive exercise (similar to the type reported here) was used as the sole exercise countermeasure by male subjects during 90 days of bed rest [7,8,15,34], bone resorption markers were unaffected by the exercise intervention, as seen here, while bone formation markers were increased by the exercise protocol [7]. In this previous study, the authors concluded that the flywheel exercise significantly mitigated bone loss in some, but not all, regions [7,8]. A significant reduction in proximal femur BMD loss occurred in subjects who performed flywheel exercise [8] compared with untreated controls. Trochanter and total hip BMD losses were mitigated in our study (Fig. 3), albeit not completely prevented compared with pre-bed rest values. Developing a suite of countermeasures, including exercise, nutrition, and potentially pharmacological agents, will likely allow a more complete inhibition of bone loss during bed rest and space flight. Pharmacological means may be able to accomplish this alone, but with far greater risk of side effects, and without the muscle, cardiovascular, and bone site-specific benefits of exercise.

In our previous bed rest studies with identical twins, treadmill exercise within LBNP had a protective effect on bone by mitigating the typical bed rest-induced increase in bone resorption [6,13]. Although resorption was unaffected by exercise treatment in the current study, key differences exist between this study and the earlier study [6,13]. First, the treadmill exercise within LBNP was the only exercise treatment in the previous investigation, but it was performed for 40 min d−1, 6 d wk−1. More frequent aerobic exercise bouts might be necessary to prevent the elevated bone resorption. Additionally, male subjects in that study were generally more fit and completed a larger percentage of their exercise countermeasure by running [24]. The female subjects in our current study had a lower fitness level, and the treadmill speeds prescribed to achieve the desired exercise intensities therefore incorporated more walking. For this reason, the women in this study, like those in our previous female identical twins study [13], likely received lower impact loading during the performance of this countermeasure. Nonetheless, it is clear that replacing half of the LBNP treadmill exercise sessions with this flywheel resistive exercise did not provide sufficient stimulus to mitigate bone resorption. Whether this relates to the reduced aerobic exercise, impact forces, or fluid dynamics associated with the LBNP/treadmill exercise remains unanswered.

Furthermore, gender effects may differentiate these results in women from our previous experiences with male subjects. For example, in our earlier studies, dietary acid load and bone resorption marker excretion were positively associated in male subjects [35] but not in females [13]. Gender differences may be related to menstrual cycle effects or other factors. In the present study, Dr. Charles Wade (personal communication) found a greater incidence of oligomenorrhea during bed rest than before bed rest, but the numbers of subjects were too small to define the effects of exercise on this phenomenon. Menstrual cycle dysfunction could have an impact on bone metabolism and other systems and may serve, at a minimum, to increase variability in observed responses.

Bed rest studies provide a valuable model for space flight-induced changes in many systems. Bed rest has qualitative effects on bone and calcium homeostasis similar to the effects of actual space flight, with generally reduced quantitative effects [5,29,36–41]. A primary limiting factor in the application of any ground or flight data on bone loss is that the rate of loss is nonlinear. Thus, while a short-duration study may be valuable in evaluating countermeasure effectiveness, the amount of bone loss in 1 month is not equivalent to one-sixth the amount of bone loss in 6 months (L. Shackelford et al., unpublished data).

The combination of resistive and aerobic exercise in this study represents an important step forward in understanding the relative importance of different exercise prescriptions. To date, most studies have used only one exercise modality, even though astronauts on the International Space Station perform both aerobic and resistive exercise countermeasures, and future exploration missions will likely have similar exercise protocols. It would have been ideal to include a balanced design here and test the combined as well as individual exercises, but the logistics and cost of two additional groups made this impossible.

Resistive exercise and aerobic exercise within LBNP present unique capabilities but seem to have different effects on bone. Although our results are informative, the current protocol for the combination of exercises that we used does not seem to provide an optimal bone countermeasure. Perhaps some combination of these two exercise modalities, manipulating frequency (>3 d wk−1 for each type), intensity, volume, and method of loading, could be developed to provide a more efficient stimulation of bone formation and prevention of degradation to protect bone during disuse.

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References


