The effect of rowing ergometry and resistive exercise on skeletal muscle structure and function during bed rest

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Prolonged exposure to microgravity induces structural and functional impairment of skeletal muscle. Key antigavity muscles undergo marked atrophy with decreased muscle mass and fiber size (1, 15, 33, 62). Muscle fiber phenotypes shift from slow-twitch fiber type I to fast-twitch fiber type IIa and hybrid myosin heavy chain expression, and there is a decrease in myofibril content (15, 19, 20, 61). Associated functional alterations include substantial reductions in absolute strength and torque-velocity relationship (1, 15, 61). As a result, astronaut performance and health are impacted in a number of ways: 1) the operation of structurally rigid and voluminous space suits is strenuous and requires high torque production; 2) long-term missions in partial gravity environments and return to Earth require preservation of muscle function and protection from injury; 3) impaired skeletal muscle negatively impacts cardiovascular (18, 38, 42) and bone homeostasis (16, 27, 29, 50). Current countermeasure (a strategy designed to prevent a loss of function after microgravity exposure) regimens applied both in spaceflight and bed rest studies have not been fully successful at maintaining skeletal muscle function and structure. Previous countermeasure research often focused on isolated exercise modalities and is limited to specific contraction modes and single muscle groups of the lower limb. Importantly, there is a lack of mission-specific targets for preservation of physical functional capacity; no quantitative strength and endurance goals are defined for individual astronauts or the mission requirements they are facing. This deficit in clear exercise objectives may contribute to suboptimal countermeasure outcomes both in ongoing missions and while new countermeasure concepts are being developed. An exercise countermeasure supporting space mission durations of 2–3 yr for Mars exploration or for extended stays on the International Space Station should guarantee astronaut function, minimize the risk for injury, and maintain acceptable levels of health, while requiring minimal crew time.

To achieve these goals, an integrative approach to countermeasure development that has the potential to condition endurance, strength, and bone properties would be beneficial. While there is evidence that the combination of resistive and endurance loading elements to an exercise modality has synergistic effects for skeletal muscle function and structure (33, 45), it also makes intuitive sense to make use of low-volume but high-intensity exercise concepts proven effective in professional athletics (5, 34), as athletes are models of human performance physiology. Rowing unifies these aspects as it provides an intense volume and pressure load stimulus on the heart, as well as marked activation of lower, upper body, and core musculature. Rowing ergometry is also a low-impact activity and easy to learn (47, 53, 55).

The purpose of our study was to explore the integrative effects of a novel countermeasure regimen consisting of rowing ergometry and resistive strength exercise. A separate publication from our laboratory showed that this countermeasure was able to preserve cardiovascular structure and function, including orthostatic tolerance (21). This paper investigates our hypothesis that this type of countermeasure will attenuate structural and functional alterations in skeletal muscle induced by prolonged bed rest, thereby preserving strength and endurance.

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Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>ExBR</th>
<th>BR</th>
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<tr>
<td>n</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Sex, M/F</td>
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<td>8/1</td>
</tr>
<tr>
<td>Race, C/AA/A/H</td>
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<td>4/2/1/1</td>
</tr>
<tr>
<td>Age, yr</td>
<td>34.5 ± 10.3</td>
<td>33.2 ± 13.4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>175.7 ± 9.1</td>
<td>174.1 ± 11.4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>77.8 ± 13.5</td>
<td>70.8 ± 10.3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.1 ± 2.7</td>
<td>23.3 ± 2.1</td>
</tr>
</tbody>
</table>

Values are absolute numbers for sex and race and means ± SD for all other parameters; n, no. of subjects. ExBR, bed rest with exercise countermeasure; BR, bed rest; M, male; F, female; C, Caucasian; AA, African-American; A, Asian; H, Hispanic; BMI, body mass index.

METHODS

Subjects

Twenty-seven healthy volunteers between the ages of 20 and 54 yr participated in the study and completed 5 wk of −6° head-down-tilt bed rest (HDBR). During bed rest, subjects were admitted to the General Clinical Research Center at the University of Texas Southwestern Medical Center using standard protocols for nutrition and preservation of circadian rhythms, as previously described (21). Subjects were confined to bed rest in the head-down-tilt position for the entire 5-wk period (except during exercise as noted below), including personal hygiene, although they were allowed to raise up on one elbow for meals only. Compliance with this position was confirmed by the use of bed alarms designed to detect a change in position and frequent nursing checks.

To obtain homogenous groups, subjects were stratified by baseline characteristics (Table 1) and then randomly assigned (balanced randomization) to two study groups in a 2:1 ratio: 1) ExBR, bed rest with exercise countermeasure (n = 18, 2 women); 2) BR, sedentary bed rest (n = 9, 1 woman). All subjects signed a consent form approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas. One subject in ExBR became ill just before post-HDBR muscle data collection and could not be included in the analysis.

Exercise Countermeasure

The countermeasure consisted of short-term but high-intensity endurance training combined with resistive strength exercise. Rowing ergometry was performed on a Concept2 Model D device 6 days/wk following a weekly periodized (varying in intensity and duration) exercise protocol (Figure 1). Before bed rest, subjects underwent maximal exercise testing to define five training zones based on maximal heart rate (HR) and the HR at ventilatory threshold which we also called “maximal steady state” (MSS). These five zones were labeled as follows: 1) recovery, 2) base training, 3) threshold or MSS, 4) critical power (also called “race pace”), and 5) intervals, as we (23, 34) and others (63) have described previously for the training of competitive athletes.

Accordingly, during each week of bed rest, subjects completed one recovery (low intensity, typically <70% maximal HR), two base (moderate intensity, typically between 70 and 80% maximal HR), one MSS (vigorous intensity, typically 80–90% maximal HR), and two interval sessions (high intensity, typically 90–95% maximal HR or above), each lasting a total of 30–46 min and separate warm-up/cool-down phases lasting 5 min each. Intervals consisted of six cycles of 3 min at 90–95% of maximal HR, followed by 3 min at recovery pace. Heart rates were recorded using commercially available monitors (Polar), and subjects were guided and vigorously motivated by an experienced exercise physiologist or physician during every exercise session to ensure compliance with training goals.

ExBR subjects moved from the bed to the rowing ergometer and back, maintaining a semirecumbent sitting position at all times with the feet never touching the floor. BR subjects sat up on a stretcher in a position similar to that on a rowing ergometer for the same amount of time ExBR subjects spent with exercise.

On 2 days during each week of bed rest, biphasic resistive strength exercise was added to complement and maximize the presumed strength-preserving effects of rowing ergometry. Two sets of 8–12 repetitions of lower body exercises and one set of 8–12 repetitions of upper body exercises were performed in the supine position on a modified commercial Schwinn RippPro device (Nautilus), and loads were adjusted weekly to reach muscle fatigue during each set of exercise. Lower body exercises included leg press, planar flexion, knee flexion, hip flexion, and hip abduction. Upper body exercises included shoulder press, elbow flexion and extension, chest press, pullovers, and abdominal crunches. The time required to complete all sets amounted to 25–30 min. Because of calf muscle strength deficits in a pilot subject after 5 wk of bed rest, planar flexion exercises against an elastic band (TheraBand) were added for all remaining subjects in the ExBR arm. These consisted of 2 sets of 20 repetitions of planar flexion on each leg twice daily, requiring a total of 6–8 min.

Fig. 1. Study design and training schedule. Subjects underwent a pre-bed rest testing phase (pre) followed by 5 wk of −6° head-down-tilt bed rest (HDBR), as indicated by the revolving arrow. Subjects were assigned to two groups: 1) bed rest with exercise countermeasure (ExBR) and sedentary bed rest (BR). HDBR was followed by a post-bed rest (post) and reambulatory testing phase (reamb). Subjects trained every day of the week (days of the week abbreviated in capital letters) at different intensity levels [base, intervals, recovery, maximal steady state (MSS)] on a rowing ergometer and/or performed resistive strength exercise following a periodized exercise protocol. [Fig. in part adapted from Ref. 21].

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Testing

Skeletal muscle strength and endurance. Peak torque and fatigue index (FI) were assessed before (pre), immediately following 5 wk of HDBR (post), and following an unsupervised reambulatory phase of 4–6 wk (reamb) during which subjects returned to their regular daily activities and could follow individual exercise recommendations on a voluntary basis. Peak isokinetic torque (Nm) was assessed at 60°/s during ankle plantar/dorsiflexion and knee and hip extension/flexion. Two sets of five repetitions were performed, and the highest torque measurements from each set were averaged. Measurements were performed on a commercial torque-velocity dynamometer (Biodex Multi Joint System Pro 3). Identical device settings and angles for each subject were used during pre, post, and reamb measurements.

Strength endurance, expressed as FI, was determined at 240°/s for 30 repeated knee and elbow extension/flexion movements, accounting for initial repetitions of sporadically observed submaximal effort. FI was calculated as a ratio of the peak torque average from repetitions 26–30 and repetitions 1–5.

Fig. 2. Muscle strength. Skeletal muscle strength as measured by peak torque (Nm) at pre, post, and 4–6 wk of reamb for knee extensors (A), knee flexors (B), plantar flexors (C), and dorsiflexors (D). Pre and post HDBR comparisons, including group interactions, are from the primary prespecified statistical analysis, while comparisons with reamb data were analyzed separately.
Skeletal muscle volume. MRI volumes of upper and lower leg extensor and flexor muscle groups in the right leg were measured pre, post, and reamb using a 1.5 T clinical scanner (Philips Intera). Subjects rested for 30 min on the MRI gurney before measurements were started, and no strenuous physical exercise preceded data acquisition to avoid plasma volume and hyperemic changes affecting tomographic imaging (10). Scout scans were used for identification of landmarks and further planning of data acquisition. For both the upper and lower leg, continuous cross-sectional areas were acquired, of which 15 serial slices were analyzed, 6 mm thick each and spaced out to cover a length of 10.5 cm. Maximal cross-sectional area was always included in the slice series used for analysis. A custom-built software program (Wafter) was used to analyze flexor and extensor muscle groups using a filter excluding adipose tissue from muscle signal intensity distribution set at ±2.5 SD of a Gaussian fit to standardize for changes in signal intensity in between measurements (44). Analyzed muscle groups included the thigh muscle (M. quadriceps femoris), hamstring (Mm. semitendinosus, semimembranosus and biceps femoris), dorsiflexors (Mm. tibialis anterior, extensor digitorum longus, extensor hallucis longus, and, if present, peroneus tertius), and plantar flexors (Mm. triceps surae, flexor hallucis longus, flexor digitorum longus, tibialis posterior). Muscle groups were identified manually.

Fig. 3. Muscle strength. Skeletal muscle strength as measured by peak torque (Nm) at pre, post, and 4–6 wk of reamb for hip flexors (A), hip extensors (B), elbow flexors (C), and elbow extensors (D) is shown. Pre and post HDBR comparisons, including group interactions, are from the primary prespecified statistical analysis, although comparisons with reamb data were analyzed separately.
for each slice blinded to subject identification and time point of measurement. Data are expressed in milliliters of volume and respective percent changes between study phases. In two ExBR subjects, pre- and post data acquisition protocols for the lower leg were inconsistent due to human error and had to be excluded from the analysis.

**Muscle biopsy.** A needle muscle biopsy of 300 mg on average was obtained from the midbelly of the right (pre) and left (post) vastus lateralis muscle (VL) before and after 5 wk of bed rest. The pre-bed rest muscle biopsy was thus obtained before exposure to the exercise regimen. The leg was marked and then cleaned and draped in a sterile fashion. After local anesthesia was applied, a 1-cm incision was made with a scalpel. A Bergstrom needle was used to obtain the desired amount of muscle tissue using one to four needle passes through the same incision (22). Each muscle biopsy sample was inspected microscopically by experienced staff immediately on collection at the procedure table and then separated and flash frozen in liquid nitrogen for biochemical analyses and in liquid nitrogen-cooled isopentane for histochemical stains (ATPase pH 4.3, 4.6, and 9.4) and histoimmunostains (CD31) (see Fig. 6).

**31P-MRS.** 31P-magnetic resonance spectroscopy (MRS) was performed to study in vivo skeletal muscle metabolic kinetics during isometric knee extension exercise pre and post HDBR, as well as following reamb. Subjects were placed on a custom-built wooden exercise bench supporting the leg in a knee-bent position. The ankle was attached to a restraint device, allowing isometric knee extension against maximal resistance while ensuring minimal thigh and surface coil movement during data acquisition. Force output was displayed visually and recorded via data logging software (Windaq). 31P spectra were obtained in a clinical 1.5 T MRI scanner (Philips Intera) using a 6 cm 31P surface coil placed on the midbelly of the right VL in proximity to, but not covering, the muscle biopsy site. Following imaging sequences for coil localization, a voxel size appropriate for our surface coil’s penetration depth was selected for shimming. Due to incomplete relaxation of signal with high-resolution data acquisitions during isometric exercise (repetition time = 2 s), an average of 16 fully relaxed spectra (repetition time = 30 s) was collected on each test subject. These data were used for partial spin saturation correction, and resting [ATP] (where brackets denote concentration) was set at 8.2 mM to observe absolute metabolite concentrations (58). Following acquisition of resting spectra, the subjects performed steady knee extension at 80% maximal voluntary contraction (based on the average of three consecutive trials) with visual and auditory feedback on force output by digital display and an operator present in the scanner room. Spectra were continuously acquired, and live data were displayed to the console operator. Subjects were instructed to release force once phosphocreatine (PCr) and inorganic phosphate levels (Pi) had reached equal amplitudes with the intention of eliciting similar exercise challenges during each measurement. Continuous recovery spectra were then acquired during 20 min more. Spectra were analyzed (AMARES/3MRUI 4.0) for [PCr], [Pi], and time constants τ after using a monoexponential curve fit to the recovery data (SigmaPlot 12.0) (see Fig. 7). Free adenosine diphosphate (ADP) concentration, a key regulator of mitochondrial ATP synthesis, was calculated from [PCr] and pH using the creatine kinase equilibrium constant (K_\text{eq}) of 1.66 × 10^9 M^{-1} (32) and based on the assumption that 15% of total creatine is unphosphorylated at rest (26):

$$\text{[ADP]} = \left( \frac{[\text{ATP}] \times [\text{Cr}]}{([\text{PCr}] \times [\text{H}^+])} \times K_{\text{eq}} \right)$$

Intracellular pH (pHi) was calculated from the chemical shift (ΔpH) relative to the PCr peak (3, 57):

$$\text{pHi} = 6.75 + \log \left( \frac{\Delta \text{ppm} - 3.27}{5.69 - \Delta \text{ppm}} \right)$$

Recovery kinetics were calculated as initial [PCr] recovery rate

$$V_{\text{PCr}} = \left( \frac{1}{\tau_{\text{PCr}}} \right) \times \Delta [\text{PCr}]$$

and maximal ATP synthesis rate as a measure of maximum aerobic capacity

$$Q_{\text{max}} = V_{\text{PCr}} \times \left( 1 + \left( \frac{K_{\text{eq}}}{[\text{ADP}]_{\text{end}}} \right) \right)$$

where [ADP]_{\text{end}} is the ADP concentration at the end of exercise.

Data from six ExBR and one BR subject could not be included in the analysis owing to technological challenges inherent to 31P-MRS. Specifically, a low signal-to-noise ratio in some subjects, insufficient penetration depth in the legs in some subjects with thick subcutaneous tissue, and scanner maintenance issues at crucial data acquisition time points reduced the number of viable data sets.

**Foot forces.** In a pilot trial, foot forces were measured during rowing ergometry to obtain quantitative insight into its impact on the lower extremity. Two male ambulatory subjects (age: 27 and 25 yr, weight: 87 kg and 71 kg) rowed on an ergometer identical to the device used by our BR subjects at varying levels of intensity (base, MSS, and interval pace). In-shoe forces were measured with capacitance-based sensors (Novel) under the feet at a sampling rate of 50 Hz. Minimum and maximum values were extracted from each stroke, and their difference was the peak force value (G_F) (6, 8, 64). Data were integrated and normalized for multiples of body weight (BW) using Matlab (Mathworks).

**Histological and Biochemical Methods.**

**Fiber types.** For ATPase stains, 6-μm serial cryo-sections were obtained on 30 x 30 coverslips kept frozen at −80°C and stained for ATPase at pH 4.3, 4.6, and 9.4, showing a checker board pattern of type 1 and type 2a, 2b, and 2c fibers (7). Serial photomicrographs of five different fields at ×20 magnification were collected (Nikon Eclipse 80i microscope, Nikon DXM1200F digital camera, Nikon NIS-Element-BR 3.10). Each fiber type was

Table 2. **Muscle fatigue**

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>Change Pre to Post, %</th>
<th>Change Post to Reamb, %</th>
</tr>
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<tbody>
<tr>
<td><strong>ExBR (n = 17)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee extension</td>
<td>70.9±9.6</td>
<td>72.5±12.2</td>
<td>66.4±11.4</td>
<td>3.2±17.1†</td>
</tr>
<tr>
<td>Knee flexion</td>
<td>63.0±16.1</td>
<td>64.5±9.5</td>
<td>63.1±12.7</td>
<td>8.8±32.4</td>
</tr>
<tr>
<td>Elbow extension</td>
<td>67.8±9.7</td>
<td>68.9±9.7</td>
<td>66.9±10.6</td>
<td>5.7±24.8</td>
</tr>
<tr>
<td>Elbow flexion</td>
<td>62.7±13.6</td>
<td>64.0±15.8</td>
<td>62.3±11.2</td>
<td>1.7±27.1</td>
</tr>
<tr>
<td><strong>BR (n = 9)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Knee extension</td>
<td>74.9±5.8</td>
<td>66.1±12.5</td>
<td>71.3±14.3</td>
<td>−12.1±12.7*†</td>
</tr>
<tr>
<td>Knee flexion</td>
<td>68.1±9.0</td>
<td>61.7±15.3</td>
<td>63.5±12.6</td>
<td>−7.9±26.2</td>
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<tr>
<td>Elbow extension</td>
<td>65.0±13.1</td>
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<td>6.8±33.3</td>
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<td>Elbow flexion</td>
<td>57.7±14.9</td>
<td>59.6±15.5</td>
<td>54.7±18.3</td>
<td>4.1±13.6</td>
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</table>

Values are means ± SD; n, no. of subjects. Fatigue indexes are in % = (average peak torque repetitions 26–30/average peak torque repetitions 1–5) × 100.

*P = 0.032 for change pre to post within BR. †P = 0.038 for pre-post group interaction.
identified by a blinded operator for an average of 300 fibers per data set (see Fig. 5).

**Fiber size.** The least diameters (in μm) of an average of 300 fibers per data set were measured manually on calibrated digital photomicrographs (ATPase stain at pH 4.6) using NIS-Element-BR 3.10 (Nokia).

**Capillarization.** Immunostaining of serial cryo-sections was performed using the monoclonal antibody for CD31 (Millipore). CD31 antibody is highly specific for the staining of vascular endothelial cells in normal and abnormal tissue and most widely used for studying angiogenesis and neo-vascularization, as it is strongly expressed by all endothelial cells (41). The streptavidin/horseradish peroxidase method was used, coupled with 3,3′-diaminobenzidine, chromogen (Vectastain), and a 0.1% Light Green counterstain (Sigma-Aldrich). Microscopic photography was performed. For each subject and condition, three optical fields were analyzed at ×10 averaging 300 cross-sectioned muscle fibers using a threshold mask to determine the average ratio of capillary area/cell and tissue surface (see Fig. 6). Differences in ratio pre and post HDBR were assessed and used to calculate percent change.

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**Fig. 4. Muscle volume.** Skeletal muscle volume (ml) by magnetic resonance imaging at pre, post, and following 4–6 wk of reamb for quadriceps (A), hamstring (B), plantar flexors (C), and dorsiflexors (D) is shown. Pre and post HDBR comparisons, including group interactions, are from the primary prespecified statistical analysis, while comparisons with reamb data were analyzed separately.
Oxidative chain enzymes. Muscle oxidative phosphorylation enzyme assays were assessed from frozen muscle biopsy tissue. Frozen muscle was washed, blotted, weighed, and minced in 10 vol/wt homogenization buffer (120 mM KCl, 5 mM MgCl₂, 1 mM EGTA, 20 mM HEPES, 0.5% fatty acid-free bovine serum albumin, 50 U/ml heparin, pH 7.2). The homogenate was centrifuged at 1,000 g, and aliquots of the supernatant were snap-frozen and stored at −80°C for complexes I or III activity assays. The remainder of the supernatant was freeze-thawed three times for complexes II or IV or citrate synthase (CS) activity assays. Complexes I–IV mean activities were normalized to mean CS activity within each group (40).

NADH/ubiquinone oxidoreductase (complex I) activity was measured as oxidation of NADH before and after addition of 4 g/ml rotenone [absorbance at 340 nm (ε₃₄₀ nm) = 6.22 mM⁻¹·cm⁻¹] (59). Succinate dehydrogenase (complex II) activity was measured as oxidation of dichlorophenolindophenol coupled to succinate oxidation to fumarate (ε₆₅₀ nm = 19.1 mM⁻¹·cm⁻¹) (13). Ubiquinol/cytochrome c reductase (complex III) activity was assayed as cytochrome c reduction coupled to ubiquinol-2 oxidation (ε₅₅₀ nm = 20 mM⁻¹·cm⁻¹) (59). Cytochrome c oxidase (complex IV) activity was determined as oxidation of reduced cytochrome c (65). CS activity was assayed by 5-thio-2-nitro-benzoic acid generation in the reaction of CoASH with 5,5'-dithiobis-2-nitro-benzoic acid to yield acetyl-CoA (ε₄₁₂ nm = 13.6 mM⁻¹·cm⁻¹) (52, 59).

Statistics

Data are expressed as means ± SD. Statistical analyses were performed using commercial software (SigmaStat 3.5). Two-way repeated-measures ANOVA was used to test for significant differences (P < 0.05) between pre, post HDBR, and reamb data, where applicable. The primary analysis was prespecified and powered to include only the pre and post HDBR comparisons, as the reamb period was not strictly controlled. Analysis of the reamb period was considered a separate, “safety” analysis and was, therefore, conducted after the primary analysis was completed. Interactions were detected using Tukey’s post hoc test when P < 0.05. Where indicated, a paired t-test was used to detect differences.

RESULTS

Muscle Strength and Fatigue

After 5 wk of HDBR, muscle strength (Fig. 2), as measured in peak torque (Nm) during isokinetic exercise, decreased significantly in BR subjects for plantar flexors (100 ± 26 vs. 78 ± 15 Nm, P = 0.001), and dorsiflexors (31 ± 7 vs. 26 ± 6 Nm, P = 0.016), while strength was increased for plantar flexors in the ExBR group and decreased for dorsiflexors (82 ± 30 vs. 93 ± 25 Nm, P = 0.011 and 30 ± 6 vs. 27 ± 5 Nm, P = 0.037). The pre-post interaction between BR and ExBR was significant for plantar flexors (P < 0.001), but not for dorsiflexors (P = 0.428). Substantial preservation of strength in ExBR was also observed in the knee extensors (BR: 146 ± 28 vs. 126 ± 26 Nm, −14%, P = 0.014; ExBR: 163 ± 36 vs. 156 ± 36 Nm, −4%, P = 0.238; interaction P = 0.167). No significant changes in strength of knee flexors, hip extensors and flexors, and elbow flexors and extensors were found for BR and ExBR post HDBR (Fig. 3).

Fatigue index for movement at the knee and elbow joint demonstrated decreased endurance of knee extensors in BR (P = 0.032) and preserved strength in ExBR (P = 0.558; interaction P = 0.038). No significant changes in fatigue indexes were found for knee flexion and elbow extension/flexion (Table 2).

Following bed rest, subjects assumed their previous daily routines or were able to follow individualized reambulation training recommendations before returning for testing after only 4–6 wk of unsupervised reambulation. In virtually all muscle groups where significant changes in strength had occurred after HDBR, peak torque measurements returned close to baseline in sedentary subjects (%change from baseline for plantar flexion: −5%, P = 0.793; dorsiflexion: −13%, P = 0.064; knee extension: +5%, P = 0.587). Notably, plantar flexion strength was even significantly elevated in the ExBR group comparing reambulation with baseline data (+29%, P < 0.001) (Fig. 2).

Skeletal Muscle Volume

Following 5 wk of HDBR, skeletal muscle volume loss was attenuated by the countermeasure (Fig. 4). Atrophy of the m. quadriceps femoris was twice as high in BR compared with ExBR (BR: 617 ± 75 vs. 362 ± 87 ml, −9%, P < 0.001; ExBR: 638 ± 110 vs. 606 ± 106 ml, −5%, P < 0.001), and the protective effect of exercise was significant (P = 0.018) (Fig. 4A). Hamstring volume significantly decreased in both groups by 7–8%, and there was no difference between ExBR and BR (P = 0.922) (Fig. 4B). Plantar flexor atrophy was more pronounced overall in both groups (BR: 362 ± 40 vs. 292 ± 32 ml, −19%, P < 0.001; ExBR: 363 ± 63 vs. 310 ± 46 ml, −14%, P < 0.001), but exercise tended to partially attenuate that loss (P = 0.076) (Fig. 4C). Similarly, dorsiflexor volume significantly decreased 5% in ExBR and 9% in BR, but exercise tended to be partially protective (P = 0.089) (Fig. 4D).

After 4–6 wk of unsupervised reambulation, atrophy in most muscle groups was reversed to baseline in ExBR and BR.

Muscle Fiber Types and Fiber Size

Changes in muscle fiber-type distribution within the VL showed high variability, but results from ExBR and BR show a diverging trend. There was a shift toward fatigue-sensitive fast-twitch 2b fibers in BR. Conversely, ExBR demonstrated a shift toward more fatigue-resistant 2a fibers (Table 3).

Table 3. Microscopic structure of skeletal muscle

<table>
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<th>Pre</th>
<th>Post</th>
<th>Change, %</th>
</tr>
</thead>
<tbody>
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<td>ExBR (n = 17)</td>
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<td></td>
<td></td>
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<tr>
<td>Fiber type, %</td>
<td></td>
<td></td>
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<tr>
<td>Type 1</td>
<td>43.1 ± 13.6</td>
<td>44.1 ± 12.4</td>
<td>+7.1 ± 7.0</td>
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<tr>
<td>Type 2a</td>
<td>33.0 ± 8.9</td>
<td>36.1 ± 9.7</td>
<td>+11.7 ± 6.1</td>
</tr>
<tr>
<td>Type 2b</td>
<td>23.2 ± 10.5</td>
<td>19.2 ± 11.0</td>
<td>−17.8 ± 11.1†</td>
</tr>
<tr>
<td>Fiber diameter, μm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>60.9 ± 8.0</td>
<td>57.2 ± 7.2</td>
<td>−5.6 ± 2.0</td>
</tr>
<tr>
<td>Type 2a</td>
<td>64.6 ± 6.6</td>
<td>65.9 ± 8.8</td>
<td>+0.1 ± 2.6</td>
</tr>
<tr>
<td>Type 2b</td>
<td>53.9 ± 8.4</td>
<td>53.6 ± 9.2</td>
<td>−0.3 ± 4.2</td>
</tr>
<tr>
<td>Capillarization, %</td>
<td>1.28 ± 0.18</td>
<td>1.54 ± 0.29*</td>
<td>+21.6 ± 24.4</td>
</tr>
<tr>
<td>BR (n = 9)</td>
<td></td>
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</tr>
<tr>
<td>Fiber type, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>38.9 ± 9.1</td>
<td>37.5 ± 10.5</td>
<td>−0.1 ± 9.3</td>
</tr>
<tr>
<td>Type 2a</td>
<td>40.4 ± 7.4</td>
<td>37.5 ± 10.1</td>
<td>−6.5 ± 7.4</td>
</tr>
<tr>
<td>Type 2b</td>
<td>20.0 ± 8.3</td>
<td>24.3 ± 15.2</td>
<td>+22.2 ± 19.3*</td>
</tr>
<tr>
<td>Fiber diameter, μm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>60.0 ± 5.5</td>
<td>59.0 ± 9.3</td>
<td>−1.8 ± 3.6</td>
</tr>
<tr>
<td>Type 2a</td>
<td>63.2 ± 4.1</td>
<td>65.3 ± 8.3</td>
<td>+3.4 ± 4.0</td>
</tr>
<tr>
<td>Type 2b</td>
<td>52.8 ± 4.0</td>
<td>54.3 ± 4.4</td>
<td>+2.8 ± 4.9</td>
</tr>
<tr>
<td>Capillarization, %</td>
<td>1.21 ± 0.39</td>
<td>1.34 ± 0.25*</td>
<td>+20.1 ± 23.4</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects. †P ≤ 0.014. *P = 0.043 for interaction.
shift from fatigue-sensitive type 2b to more fatigue-resistant type 2a fibers and mainly oxidative metabolism-dependent type 1 fibers (mean %change in fiber type: type 1: BR −0.1 ± 9.3, P = 0.729; ExBR +7.1 ± 7.0, P = 0.708; type 2a: BR −6.5 ± 7.4, P = 0.399; ExBR +11.7 ± 6.1, P = 0.114; type 2b: BR +22.2 ± 19.3, P = 0.272; ExBR −17.8 ± 11.1, P = 0.072). Even though within-group changes failed to reach significance, interaction between study phase and intervention for type 2b fiber gain in BR and loss in ExBR was significant (P = 0.043) (Table 3, Fig. 5).

Despite thigh muscle volume loss in both groups, VL fiber size was largely unaffected except for a significant 6% reduction in type I fiber diameter in ExBR (P = 0.01) (Table 3).

Capillarization

Capillarization expressed as the ratio of capillary to fiber area did not change significantly in BR (1.21 ± 0.39 vs. 1.34 ± 0.25%, P = 0.189), but increased by 22% in ExBR from 1.28 ± 0.18 to 1.54 ± 0.29%. Although absolute changes are small, the increase in ExBR was significant (P < 0.001) (Table 3, Fig. 6).

Oxidative Chain Enzyme Activity

Changes in mitochondrial enzyme activity clearly mirrored shifts seen in muscle fiber type and support the tentative observation of a shift toward fatigue resistance by demonstrating significant increases in oxidative chain enzyme activity levels in the ExBR group. The opposite was shown in the sedentary group. Complexes I–IV and CS activity levels fell by 1–12% in BR. Conversely, there was a 20–44% increase in enzyme activity in ExBR (interaction P ≤ 0.003) (Table 4).

31P-MRS Metabolic Kinetics

31P-MRS was performed on the VL to obtain in vivo data of metabolic properties (Fig. 7). While ExBR did not cause any changes in energy kinetics, we found evidence of impaired metabolism in the sedentary group with a significant 20% prolongation of initial PCr recovery as measured by PCr time constant (BR: 49 ± 7 vs. 59 ± 12, P = 0.037; ExBR: 48.3 ± 10.1 vs. 48.0 ± 11.0, P = 0.90) (Fig. 7A, Table 5) and a 17% decrease in Qmax (BR: pre 0.38 ± 0.10 vs. 0.30 ± 0.07, P =
Foot Forces

Mean peak forces (Gz) for base, MSS, and interval pace were $0.68 \pm 0.05$, $0.89 \pm 0.06$, and $0.84 \pm 0.04$ BW, respectively, for subject 1, and $0.58 \pm 0.02$, $0.67 \pm 0.03$, and $0.74 \pm 0.03$ BW, respectively, for subject 2.

DISCUSSION

The principal findings from the present study are that a novel short-duration but high-intensity exercise program consisting of rowing ergometry and resistive strength exercise: 1) preserved muscle functional properties, including skeletal muscle strength and energy metabolism kinetics in key antigravity muscles; 2) attenuated losses in skeletal muscle volume; 3) largely preserved or improved skeletal muscle structural properties, including increased capillarization and higher levels of oxidative chain enzyme activity, while fiber type data tended to show an increased proportion of fatigue-resistant fibers in ExBR subjects. Furthermore, 4–6 wk of unsupervised reambulation was sufficient to eliminate strength deficits in the sedentary group, while muscle volume approached baseline levels in virtually all measured areas in both exercise and sedentary subjects.

Discordance Between Skeletal Muscle Volume Loss and Preserved Functional Properties

A key aim of this integrated countermeasure study was to assess the effectiveness of highly dynamic/static rowing ergometry and resistance exercise in attenuating the loss of skeletal muscle structure and functional capacity. Disuse muscle atrophy has been studied in microgravity and bed rest as its ground-based analog in both humans and more abundantly so in rodents. The consequences of atrophy affect every aspect of muscle structural and functional properties, including loss of muscle volume and strength (15, 46), changes in fiber type (15, 19, 20, 62), decreases in myofibril content (60), reduced capillarization, and impaired metabolic capacity (2, 4, 14, 35). Novel resistance exercise regimens using flywheel technology...
with high concentric and eccentric force loads have been encouraging by preserving quadriiceps muscle volume and strength, while calf muscle volume was not sufficiently protected (19,61).

Of note, our study did not use a resistance exercise regimen as the primary exercise modality. In fact, resistance exercise was used to compliment a training regimen primarily accomplished by rowing ergometry with its high-intensity combination of both dynamic and static properties.

Our data demonstrated preserved or even increased strength in key antigravity muscles of the knee extensors and plantar flexors in ExBR, while BR showed significant decreases in strength in these muscle groups. However, significant muscle volume losses still occurred in both groups, although exercise reduced quadriceps atrophy by a factor of two and attenuated volume losses still occurred in both groups, although exercise strength reached borderline statistical significance for the interaction between BR and ExBR. Data from a recently published short-term (14 days) horizontal bed rest study integrating high-load resistive (3 days/wk) and high-intensity interval-type aerobic (6 days/wk) exercise demonstrated preservation of lower extremity muscle strength and volume, even in the calf muscle (43). Further studies would be needed to establish this effect over a longer period of bed rest, such as was used in the present study, as well as to establish the ideal combination of endurance, high intensity, and strength training for long-duration (>6 mo) spaceflight.

### Alterations in Skeletal Muscle Microstructural Properties

The effect of spaceflight and unloading on metabolic indexes in rodents has produced conflicting results. Possibly as a result of methodological differences, there is no clear adaptive response in oxidative metabolism capacity (2,4,14,35). However, while Baldwin et al. (4) found no impairment of pyruvate oxidative capacity in skeletal muscle after exposing rodents to microgravity, there appeared to be a shift in substrate preference to carbohydrate use over fatty acid use. In humans, regular endurance exercise has been shown to largely ameliorate age-related reductions of oxidative capacity in antigravity muscles (30) and enhances oxidative phosphorylation in the volume shifts and losses occurring during bed rest almost certainly impacted the assessment of skeletal muscle volume by MRI with a net-negative effect and may thus account for some of the volume losses (10). Changes in innervation ratio and motor unit recruitment could explain preservation of strength during an isokinetic assessment mostly dependent on glycolytic contraction. This may be especially true for the plantar flexors, as strength tended to increase by 13% while volume still decreased significantly by 14% in ExBR. Contractile protein turnover was not assessed in this study, but it is conceivable that a difference in myofibril homeostasis in exercising vs. sedentary subjects may contribute to the observed discordance. Finally, some of the observed pre-post changes in muscle strength reached borderline statistical significance for the interaction between BR and ExBR.

#### Table 4. Oxidative chain enzyme activity

<table>
<thead>
<tr>
<th>Complex</th>
<th>Pre</th>
<th>Post</th>
<th>Change, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate synthase</td>
<td>12.9 ± 2.1</td>
<td>16.6 ± 2.9*</td>
<td>+31.2 ± 6.8‡</td>
</tr>
<tr>
<td>Complex I (UR)</td>
<td>5.2 ± 1.4</td>
<td>6.3 ± 1.7*</td>
<td>+24.3 ± 6.1‡</td>
</tr>
<tr>
<td>Complex II (SD)</td>
<td>2.3 ± 0.5</td>
<td>2.8 ± 0.7*</td>
<td>+20.8 ± 5.1‡</td>
</tr>
<tr>
<td>Complex III (CCR)</td>
<td>11.4 ± 3.6</td>
<td>15.8 ± 4.8*</td>
<td>+42.5 ± 9.0‡</td>
</tr>
<tr>
<td>Complex IV (CCO)</td>
<td>4.5 ± 1.4</td>
<td>6.2 ± 1.5*</td>
<td>+43.5 ± 10.4‡</td>
</tr>
</tbody>
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Values are means ± SD in U·min⁻¹·mg⁻¹; n, no. of subjects. UR, NADH ubiquinone reductase; SD, succinate dehydrogenase; CCR, ubiquinone cytochrome c reductase; CCO, cytochrome c oxidase. *P < 0.001 and ‡P ≤ 0.05 by paired t-test. †P = 0.003 for interaction.

![Image](https://via.placeholder.com/150)

**Fig. 7.** 31P-magnetic resonance spectroscopy (MRS). A: recovery of phosphocreatine concentration ([PCr]) in right thigh pre/post HDBR for ExBR and BR. PCr time constant τ is a measurement of velocity of [PCr] recovery obtained by a monoexponential fit to [PCr] recovery. [PCr] recovery was prolonged in BR and unchanged in ExBR after 5 wk of HDBR. B: 31P-MRS spectra acquired every 8 s during knee extension exercise test at 80% peak force. Shown are rest (time t − 80 s), exercise (descending PCr peaks), end of exercise (PCr trough at t + 0 s), and early recovery (ascending PCr peaks). ppm, Parts per million.

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setting of mitochondrial proliferation in patients with mitochondrial myopathies (56). In contrast, nonpostural muscle does not typically show major morphological or biochemical changes after bed rest (11).

In the present study, there were marked increases in enzyme activity levels in CS and complexes I–IV by up to 44% in ExBR, indicating increased oxidative metabolism capacity, typical of the response to endurance training. Levels in BR were unchanged or showed a moderate 10% reduction similar to those previously observed (12). While the capillary-to-fiber area ratio in sedentary subjects did not change after bed rest, similar to what has been shown in the past (12), capillarization in ExBR significantly increased by 22%, although this relative change represented small change in absolute terms. This increase in capillarization may be a reflection of the high-intensity endurance training stimulus achieved with this exercise regimen in our subjects who were unfamiliar with high-intensity training (24). As previously published by our laboratory, metabolic capacity as a function of maximal \( \dot{V}O_2 \) uptake was preserved by the countermeasure (21), and \(^{31}\)P-MRS data showing preserved in vivo energy kinetics for ExBR further support the concept that the apparent improvements in microstructural properties in ExBR may stabilize muscle microstructural properties. Conversely, significant reductions in PCr recovery and ATP resynthesis rates in BR reflect impaired functional properties.

Integrated Countermeasure Approach and Role of Rowing Ergometry

Intense resistive strength exercise successfully averts or mitigates losses in muscle volume, while endurance training optimizes metabolic capacity during a detraining stimulus such as bed rest. An optimal training device and regimen will benefit the entire skeletal muscle system, and such a countermeasure is likely to have a wide impact on cardiovascular fitness, as well as bone homeostasis. At the same time, this countermeasure needs to be practical and easy to implement aboard the International Space Station or on a spaceship to Mars.

Rowing ergometry may be an optimal countermeasure to fit these basic needs. Competitive rowers are exposed to substantial loading of the axial skeleton and antigravity muscles, and rowing uses >75% of total muscle mass of the body (47, 54, 55). Rowers develop marked cardiac hypertrophy (9, 51) and increased bone mineral density at the lumbar spine and proximal femur (28, 31, 36, 37, 39). Despite the aforementioned low-impact properties of rowing based on foot forces in a ground reaction force model, force outputs created during rowing result in significant joint reaction forces of up to 4.6 BW at the spine, stimulating myotendinous and osseous structures (28, 37). As such, rowing has proven to be effective at maintaining fitness, strength, and bone mineral density in the aged (28). Modern training techniques applied by elite athletes use periodized training programs, including high-intensity interval exercise bursts and bouts of submaximal and base intensity, to increase training efficacy and were the key models for the program employed in this study.

Impact of the Present Study

To our knowledge, the countermeasure design of the present study represents the first to integrate endurance and strength training in a high-intensity short-duration paradigm during microgravity exposure. Although ExBR subjects followed a shorter duration exercise routine than applied in other studies (49), rowing for an average of 38 min/day with only 2 days/wk of classic resistive strength exercise preserved functional properties of key antigravity muscles. As recently published by our laboratory, this training program also maintained crucial cardiovascular parameters, including preservation of cardiac mass, left ventricular compliance, and orthostatic tolerance (21), demonstrating for the first time that a single countermeasure strategy could be effective for multiple systems. Thus this strategy may be particularly effective as a multisystem countermeasure during a prolonged space mission. Finally, our laboratory has shown that a countermeasure based on rowing and strength training restores exercise capacity and quality of life in young patients suffering from the Postural Orthostatic Tachycardia Syndrome (17, 48), a human disease model of cardiovascular and musculoskeletal deconditioning that becomes manifest in the upright posture (a gravitational stimulus) (25).

Conclusion

While partially attenuating muscle atrophy, rowing ergometry and supplemental resistive strength training preserved

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**Table 5. \(^{31}\)P-magnetic resonance spectroscopy**

<table>
<thead>
<tr>
<th>Pre</th>
<th>Post</th>
<th>Reamb</th>
<th>Change Pre to Post, %</th>
<th>Change Post to Reamb, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Delta)PCr, mM</td>
<td>10.4 ± 3.9</td>
<td>9.6 ± 2.4</td>
<td>10.6 ± 3.1</td>
<td>+19.3 ± 36.1</td>
</tr>
<tr>
<td>(\Delta)P, mM</td>
<td>12.0 ± 5.7</td>
<td>9.7 ± 2.6</td>
<td>9.7 ± 2.7</td>
<td>-7.8 ± 31.0</td>
</tr>
<tr>
<td>(\tau) PCR, s</td>
<td>48.3 ± 10.1</td>
<td>48.0 ± 11.0</td>
<td>46.3 ± 11.5</td>
<td>+0.4 ± 18.9</td>
</tr>
<tr>
<td>(\tau) Pi, s</td>
<td>35.8 ± 9.3</td>
<td>34.1 ± 5.5</td>
<td>34.2 ± 5.3</td>
<td>-1.1 ± 21.5</td>
</tr>
<tr>
<td>(V_{P_{Cr}})</td>
<td>0.21 ± 0.05</td>
<td>0.21 ± 0.06</td>
<td>0.23 ± 0.05</td>
<td>+2.4 ± 36.7</td>
</tr>
<tr>
<td>(Q_{max})</td>
<td>0.37 ± 0.07</td>
<td>0.34 ± 0.09</td>
<td>0.37 ± 0.12</td>
<td>-4.6 ± 30.0</td>
</tr>
</tbody>
</table>

Values are means ± SD; \(n\), no. of subjects. \(\Delta\), Change; \(\tau\) PCr, phosphocreatine; \(\tau\) Pi, inorganic phosphate; \(\tau\), time constant; \(V_{P_{Cr}}\), PCR recovery rate; \(Q_{max}\), maximal ATP synthesis rate. *\(P < 0.037\) by paired \(t\)-test, pre vs. post. †\(P < 0.01\) by paired \(t\)-test, post vs. reamb.
Rowing and Strength Training Prevent Bed Rest Deconditioning • Krainski F et al.

skeletal muscle structure and function in key antigravity muscles after 5 wk of bed rest. A preservation or even increase in muscle strength was accompanied by enhanced metabolic capacity, as evidenced by increased levels in oxidative enzyme activities and preserved in vivo energy kinetics, thus clearly demonstrating an endurance training response, despite 5 wk of bed rest. This high-intensity, short-duration exercise regimen averted cardiovascular deconditioning (21) and has been easily implemented in the training and treatment of young and aged patients (17, 28, 48). It may thus be a primary component of an integrated exercise prescription and countermeasure benefitting astronauts and patients affected by chronic deconditioning.

The author(s) declare no conflict of interest.

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DISCLOSURES

Concept2, Inc. provided the rowing ergometer. Nautilus, Inc. provided the resistive exercise device. No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


