Muscle Atrophy, Pain, and Damage in Bed Rest Reduced by Resistive (Vibration) Exercise

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ABSTRACT

MIOKOVIC, T., G. ARMBRECHT, U. GAST, R. RAWER, H. J. ROTH, M. RUNGE, D. FELSENBERG, and D. L. BELAVÝ. Muscle Atrophy, Pain, and Damage in Bed Rest Reduced by Resistive (Vibration) Exercise. *Med. Sci. Sports Exerc.*, Vol. 46, No. 8, pp. 1506–1516, 2014. The purpose of this study was to investigate the effectiveness of a short-duration (5–6 min, 3 $d \cdot wk^{-1}$) resistive exercise program with (RVE) or without (RE) whole-body vibration in reducing muscle atrophy in the lower limb during prolonged inactivity when compared with that in an inactive control group. **Methods**: As part of the second Berlin BedRest Study, 24 male subjects underwent 60 d of head-down tilt bed rest. Using magnetic resonance imaging, muscle volumes of the individual muscles of the lower limb were calculated before and at various intervals during and after bed rest. Pain levels and markers of muscle damage were also evaluated during and after bed rest. Adjustment of *P* values to guard against false positives was performed via the false discovery rate method. **Results**: On the "intent-to-treat" analysis, RE reduced atrophy of the medial and lateral gastrocnemius, soleus, vasti, tibialis posterior, flexor hallucis longus, and flexor digitorum longus ($P \leq 0.045$ vs control group) and RVE reduced atrophy of the medial and lateral gastrocnemius and tibialis posterior ($P \leq 0.044$). Pain intensity reports after bed rest were lower in RE at the foot ($P \leq 0.033$) and whole lower limb ($P = 0.01$) and in RVE at the thigh ($P \leq 0.041$), lower leg ($P \leq 0.01$), and whole lower limb ($P \leq 0.036$). Increases in sarcomere-specific creatine kinase after bed rest were less in RE ($P = 0.020$) and RVE ($P = 0.020$). No differences between RE and RVE were observed. **Conclusions**: In conclusion, a short-duration RVE or RE can be effective in reducing the effect of prolonged bed rest on lower extremity muscle volume loss during bed rest and muscle damage and pain after bed rest. **Key Words:** TRAINING, HYPERTROPHY, IMMOBILIZATION, SPACE FLIGHT, CROSS-SECTIONAL AREA

BASIC SCIENCES

On the basis of current knowledge (6), high-load resistive exercise (RE) compared with low-load or aerobic-based exercise (8,34,39) is considered to be the most effective exercise countermeasure for reducing muscle atrophy in space flight and bed rest, a model used to simulate the effects of microgravity on the human body (25). The exercise programs performed to date typically involved multiple exercise sessions per day, multiple-set and/or several different exercises, thus requiring considerable time for implementation. For optimization of countermeasure exercises for astronauts, it is useful to understand whether a short-duration exercise program could be effective. Because a single-set RE can be effective in improving muscle strength in ambulant individuals (37), it seems reasonable that a single-set RE may also be effective in bed rest. Our primary hypothesis was that a short-duration (5–6 min of active exercise per exercise session), high-load RE program performed three times a week could reduce muscle atrophy during prolonged bed rest.

RE performed with additional whole-body vibration (RVE) during bed rest has previously shown to be effective in reducing muscle atrophy in the muscles of lower limbs during bed rest (6). Whole-body vibration increases muscle activation through high-frequency and high-velocity stimulation of spinal neuronal networks via a stretch reflex (12). Increased lower leg blood flow (20) and muscle tissue oxygenation (28) during vibration exercise may be indicative of increased neuronal and metabolic demands placed on the musculature with whole-body vibration. Thus, whole-body vibration *may* present a greater stimulus for the retention of muscle mass during bed rest. It is not known, however, if the addition of whole-body vibration to RE is more effective than RE alone as a countermeasure against muscle atrophy for the lower limbs. Thus, our secondary hypothesis was that RVE would result in greater prevention of lower limb muscle atrophy than that in RE alone during prolonged bed rest.

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Examination of atrophy in a large number of muscles is best performed via magnetic resonance (MR) imaging to examine both whole-muscle volume and intramuscular differences in the extent of atrophy (21). Investigation of muscle function is also of great importance particularly because declines in performance after bed rest cannot be wholly explained by muscle size changes (22). Peak neuromuscular performance is known to decline after bed rest (11) and space flight (2). Furthermore, as a consequence of disuse, the musculature seems to be more vulnerable to damage (26). This vulnerability can be expressed by reports of pain after bed rest (27), which often, but not always, resembles the clinical manifestation of ''delayed-onset muscle soreness'' (31). The vulnerability of muscle to damage after bed rest is also expressed by a spike in creatine kinase levels in the first few days after subjects end bed rest and undertake post–bed rest testing (7).

The main goal of the current study was to assess the effect of short-duration, high-load RE with and without whole-body vibration on muscle size loss, reports of pain, and serum markers related to muscle damage after prolonged bed rest. A secondary goal was to examine the relations between muscle size loss, functional loss, pain reports after bed rest, and alterations in serum markers of muscle damage.

MATERIALS AND METHODS

Subject and Bed Rest Characteristics

Twenty-four males participated in the second Berlin BedRest Study. The screening process ensured that subjects were medically and psychologically healthy. The recruitment process and the bed rest study protocol are discussed in detail elsewhere (4). Subjects attended the facility for the baseline data collection (BDC) 9 d before a 60-d, 6° head-down tilt (HDT) bed rest period and remained in the facility for a 7-d post–bed rest recovery period (R+1 to R+7). During the HDT phase, subjects performed all hygiene activities in the HDT position. Nutrition was strictly controlled, and subjects in each group were spread evenly into four campaigns of six subjects over a 1-yr period to avoid seasonal influences (4). Subjects then returned to the facility for follow-up appointments 14, 30, 90, and 180 d after bed rest (R+14, R+30, R+90, and R+180). For logistical reasons, the R+14 appointment was performed with two of the six subjects from each campaign 13, 14, or 15 days after bed rest. Exclusion criteria specifically relevant to the current study were balance disorders, any kind of cartilage or joint disease, previous knee surgery, and performance of RE in the last 6 months. To reduce interindividual variability, subjects who participated in competitive sports in the last 5 yr were excluded from the study. Three subjects had performed regular physical activity as part of their daily life (two jogging, one cycling) in the last 5 yr. The remaining subjects were sedentary. The study was approved by the ethics committee of the Charité Universitätsmedizin Berlin. All subjects gave their informed written consent before participation in the study. After bed rest, subjects returned to their normal daily lives. A short questionnaire for the measurement of habitual physical activity in epidemiological studies showed no differences in physical activity 180 d after bed rest compared with that before bed rest (3).

Subjects were randomized into three different groups: one that performed RE with whole-body vibration during bed rest (RVE: n = 8; mean (SD) age, 32.0 (10.7) yr; height, 179.5 (5.9) cm; weight, 80.5 (6.4) kg), one that performed RE only (RE: n = 8, 31.1 (5.1) yr, 179.3 (7.7) cm, 75.0 (12.8) kg), and, finally, one that performed no exercise and served as a control group (CTR: n = 8, 34.7 (6.8) yr, 182.4 (5.4) cm, 81.2 (5.3) kg).

Exercise Countermeasures

The countermeasure exercise protocol is discussed in detail elsewhere (4). In brief, exercise maneuvers were chosen to target those load-bearing regions of the body that are most affected by bed rest (i.e., lower quadrant and lumbar region). The training program was designed as high-load RE training with a goal to achieve muscle hypertrophy (37). A single-set regimen was chosen to minimize exercise time. Training was performed 3 $d \cdot wk^{-1}$ during the HDT phase. Subjects performed all exercises in the HDT posture. Greater detail about the exercise device including a figure of the device can be found elsewhere (4); however, in brief, the subject lay in a supine position on a sliding back rest with padded shoulder restraints and hand grips. The feet were positioned on the foot plate, which was set to vibrate during high-load RE in the RVE group. A pneumatic system generated the required force and was applied through the sliding back rest, against which the subject needed to resist and move (via the shoulder pads and hand grips). The force levels were monitored via sensors in the foot plate. In the RVE group, the vibration stimulus was generated via the side-alternating movement of the foot plate. After a short warm-up, the following exercises were performed on the Galileo Space exercise device (Novotec Medical GmbH, Pforzheim, Germany): bilateral leg press (approximately 75%–80% of pre–bed rest maximum voluntary contraction; in RVE group: vibration frequency, 24 Hz; amplitude, 3.5–4 mm; peak acceleration, approximately 8.7g where g = 9.81 $m \cdot s^{-2}$), single leg heel raises (approximately 1.3 times the body weight; in RVE group: vibration frequency, 26 Hz; amplitude, 3.5–4 mm; peak acceleration, approximately 10.2g), double leg heel raises (approximately 1.8 times the body weight; in RVE group: vibration frequency, 26 Hz; amplitude, 3.5–4 mm; peak acceleration, approximately 10.2g), and back and forefoot raise (performing hip and lumbar spine extension against gravity with ankle dorsiflexion but with approximately 1.5 times the body weight applied at the shoulders; in RVE group: vibration frequency, 16 Hz; amplitude, 3.5–4 mm; acceleration, approximately 3.9g). The RVE group performed the

same exercises as the RE group, except that whole-body vibration was applied (4). Note that the acceleration parameters stated refer to the acceleration of the platform itself. Effective accelerations on the subject are much lower and differ according to subject position, body region, joint alignment, and the neuronal regulation of stiffness of the muscle–tendon–bone system. Loading levels were increased by 5% per exercise when the subject could perform 12 exercise repetitions in two consecutive sessions (1). Exercise time comprised 5–6 min per session, and total time consumption was 22 min including rest periods.

MR Imaging Protocol

Baseline MR scanning was conducted on a 1.5-T Siemens Avanto scanner (Siemens, Erlangen, Germany) 9 or 8 d before the beginning of bed rest (BDC-9/-8) and subsequently on the 27th or 28th day (HDT27/28) and the 55th or 56th day (HDT55/56) of HDT bed rest. Scanning was also conducted 14 (R+14), 90 (R+90), and 180 d (R+180) after reambulation. Cushions provided by the MR manufacturer were positioned behind the knee to support the joint in slight flexion. The MR coil placed over the legs and feet helped to standardize ankle and foot position and ensured that the subject did not need to actively contract their lower limb muscles to maintain position. To allow time for shift of body fluids from the extremities, subjects remained in horizontal lying for at least 2 h before each scanning session. During the HDT bed rest phase, beds were placed in the horizontal position 2 h before scanning to ensure comparability of pre- and post–bed rest data. MR data from the inactive CTR subjects have been analyzed in detail in an earlier publication (21) and are drawn on here solely to evaluate the exercise countermeasures.

Depending on subject height, up to 180 axial images (slice thickness, 6 mm; interslice distance, 0.6 mm; echo time, 36 ms) were collected from the iliac crest to the end of the lateral malleolus to encompass the left and right sides of the body. Scan regions were divided into three blocks at the hip (repetition time, 8000 ms; field of view, 420 × 294 mm interpolated to 320 × 224 pixels), thigh (repetition time, 5820 ms; field of view, 450 × 270 mm interpolated to 320 × 192 pixels), and calf (repetition time, 6190 ms; field of view, 400 × 200 mm interpolated to 320 × 160 pixels). The images were stored for offline analysis.

Image Analysis and Further Data Processing

Each data set was coded with a random number (www.random.org) to blind the operator to study time point. The same experienced operator (T. M.) used ImageJ (http://rsb.info.nih.gov/ij/) to measure the area of muscles of the lower limb in every image on the left and right sides of the body (Fig. 1). The first and last few slices for each muscle typically contained a mixture of muscle (gray) and tendon (black), although the two were usually straightforward to distinguish. Intramuscular fat and nerves were carefully traced around during measurements. After measurement of a subject's entire data, the data were plotted and checked visually by the operator and another author while maintaining blinding, to screen for any errors. The volume of each muscle was calculated via linear interpolation, given the slice thickness of 6 mm and interslice gap of 0.6 mm.

Data from the left and right sides of the body were then averaged before statistical analysis. The differences in muscle size changes between the left and right legs were 3.1% of baseline volume in the adductor brevis, 2.6% in the adductor longus, and 2.1% in the flexor hallucis longus, with all other muscles showing side-to-side differences in atrophy of less than 1.8%.

Neuromuscular Performance: Countermovement Jump, 15-m Sprints, and Leg Press One-Repetition Maximum

For the comparison of changes in neuromuscular performance to muscle atrophy and pain, we used data from recently published work (15) where detailed methodology of these tests has been reported. For the correlation analyses, only data on percentage change between the baseline and first testing session after bed rest were used. Countermovement jump (n = 22) and 15-m sprint (n = 16) testing was performed within 2 h after end of bed rest (R+1). Leg press testing (n = 22) was performed the day after end of bed rest (R+2). Percentage change in maximal jump height from countermovement jump, percentage change in sprint time, and percentage change in maximal force during leg press were used in subsequent correlation analyses. Jump height was taken as the main parameter from countermovement jump testing because subjects were instructed to jump as high as possible.

Pain Questionnaires

At baseline MR scanning (BDC-8/-9), 2 d before bed rest (BDC-2), every day during the first 2 wk of bed rest (HDT1 to HDT14), thence at weekly intervals (HDT18, HDT25, HDT32, HDT39, HDT46, HDT53, and HDT57), and at every post-bed rest recovery time point (R+1 to R+7, R+14, R+30, R+90, and R+180), subjects were asked to fill out a pain questionnaire. Subjects were asked to report whether they experienced pain in any part of their body and to mark its location on a body chart and its intensity (1–100) on a 100-mm visual analog scale (VAS). Reports of pain from the lower quadrant were then coded into the hip or buttock, thigh, knee or patellofemoral joints, lower leg, ankle joint, or foot regions, and the VAS score was recorded. Late-in-recovery (R+30 and beyond) subjects were also asked if they had experienced pain in a particular body region since the last appointment. In this case, no VAS scores were available.

The raw VAS score and the numbers of subjects reporting pain during bed rest or the first 2 wk after bed rest were used in further analyses. Average VAS score from "all lower limb regions" was also calculated.

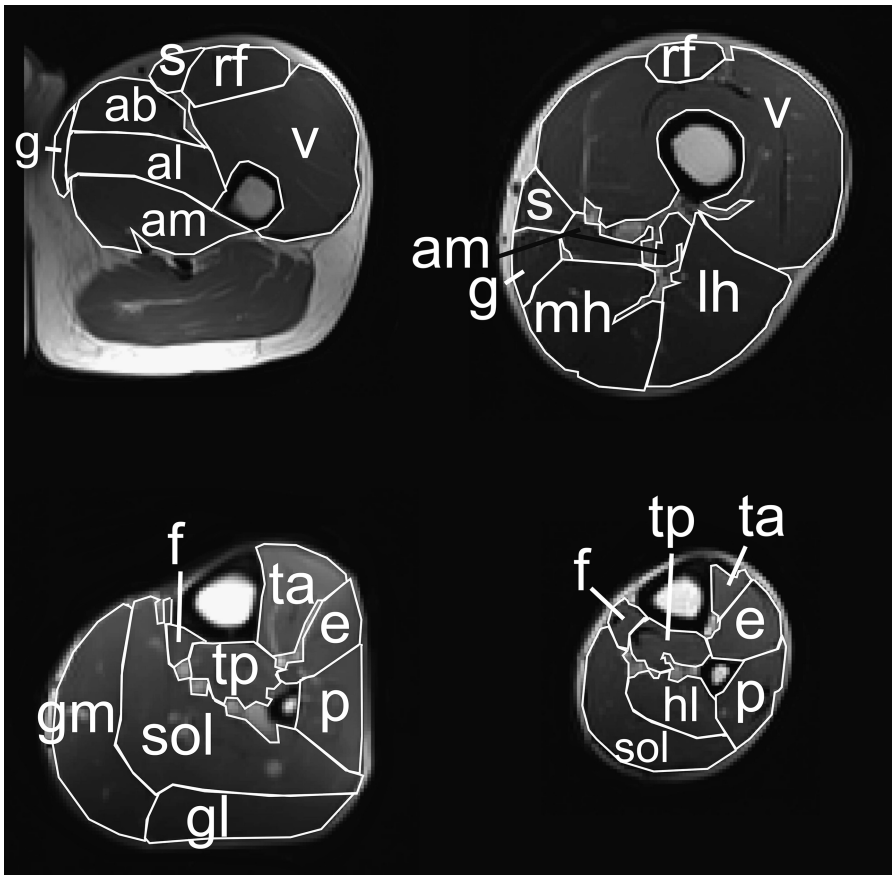

FIGURE 1—Muscle measurements on MR images. Images are from the proximal thigh (top left), distal thigh (top right), proximal lower leg (bottom left), and distal lower leg (lower right). Thigh: ab, adductor brevis; al, adductor longus; am, adductor magnus; g, gracilis; lh, lateral hamstrings (biceps femoris long and short heads); mh, medial hamstrings (semimembranosus and semitendinosus); rf, rectus femoris; s, sartorius; v, vasti. The pectineus is not shown. Lower leg: e, extensor digitorum longus; f, flexor digitorum longus; gl, lateral gastrocnemius; gm, medial gastrocnemius; hl, flexor hallucis longus; p, peroneals; sol, soleus; ta, tibialis anterior; tp, tibialis posterior.

Blood Drawings

Venous blood samples were taken between 07:00 h and 08:00 h after 12 h in fasting state during baseline collection 2 d before the start of bed rest (BDC-2), on HDT bed rest days HDT5, HDT12, HDT19, HDT26, HDT33, HDT40, HDT47, HDT54, and HDT60, and on post-bed rest recovery (R+) days R+3, R+7, R+14, R+30, R+90, and R+180. Samples were centrifuged at 3500 rpm for 10 min, 30 min after the blood was drawn. Serum was then obtained and subsequently stored at −80°C until analysis. Samples were shipped on dry ice to the laboratory (Labor Limbach, Heidelberg, Germany) and stored again at −80°C until analysis.

Serum Analyses

Sarcomeric (skeletal and cardiac) muscle-specific creatine kinase (CK-MM) is a marker of muscle damage and was measured using HYDRAGEL ISO-CK kit (Sebia, Fulda, Germany) designed for the identification and quantification of creatine kinase isoenzymes by agarose gel electrophoresis on the semiautomated HYDRASYS LC system (Sebia, Fulda, Germany). The coefficient of variation was 2.31% for CK-MM at an absolute concentration of 67 $U \cdot L^{-1}$. Tests and instruments were run strictly in accordance with the guidelines given by the manufacturer and were subject to continuous maintenance and service according to the laboratories' standard operating procedures for good laboratory practice. Serum samples were thawed at the day of analysis at ambient temperature, mixed on a head-over-head mixer, and centrifuged before measurement. Blinded samples were measured randomly. To further reduce imprecision of measurement, all samples were analyzed using one reagent lot.

Further Data Processing and Statistical Analysis

Muscle size. In the primary analysis, muscle volume was evaluated. Linear mixed-effects models were used to examine whether the countermeasures affected muscle volume at baseline, HDT27/28, and HDT55/56. Main effects of "group" and "study time" as well as their interaction were examined. Allowances for heterogeneity of variance (e.g., due to "group" and/or "study time") were made when necessary. Subsequently, in the secondary analyses, two-group (i.e., CTR vs RE, CTR vs RVE, and RE vs RVE) linear mixed-effects models were built using the same approach to examine which countermeasures had an effect and whether the response of the RVE and RE groups differed. Muscle

volume differences in recovery compared with those at baseline were also evaluated.

CK-MM. Residuals from parametric analyses of these variables were not normally distributed. Thus, a nonparametric analog of repeated-measures ANOVA was implemented (10). To assess the effect of the countermeasures on impeding muscle damage after bed rest, the primary analysis of the serum parameters evaluated the changes on R+3 versus those at end of bed rest(HDT60). Previous data (7) have shown a peak of CK at this time after bed rest. Secondary analyses evaluated the changes during bed rest compared with those during baseline as per other parameters. The Mann–Whitney test was performed, comparing each day during and after bed rest with baseline.

Pain questionnaires. The raw VAS data were analyzed with a nonparametric analog of repeated-measures ANOVA performed to assess the changes over time and the effect of subject group (10). The bed rest and recovery phases were analyzed separately. The ANOVA-type test statistic is reported for these data (10). Chi square analyses were performed on the number of subjects reporting pain either in bed rest or in the first 2 wk after bed rest.

Pearson correlation coefficient was calculated for the following variables:

- *Function versus muscle volume*: between the percentage change in muscle volume at end of bed rest and percentage change in each functional parameter at first testing session after bed rest.
- *Pain versus muscle volume change and functional deterioration after bed rest*: presence and intensity of pain on R+2 were correlated with the percentage change in muscle volume at end of bed rest.
- *Serum parameters*: The percentage change in CK-MM between end of bed rest (HDT59) and day 3 after bed rest was correlated to intensity of pain on R+2, extent of muscle atrophy at end of bed rest, and functional deterioration after bed rest on R+2.

The ''R'' statistical environment (version 2.10.1, www.r-project.org) was used for all analyses. Unless otherwise stated, the P values given in the results section refer to the study time–group interaction on ANOVA. An α level of 0.05 was taken for statistical significance on ANOVA. Unless otherwise specified, results are presented as mean (SD).

TABLE 1. Volume of the posteromedial and anterolateral leg muscles during and after bed rest: ITT analysis.

		Study Time				
Group	**BDC (cm^3)**	**HDT27/28 (%)**	**HDT55/56 (%)**	**R+14 (%)**	**R+90 (%)**	**R+180 (%)**
Flexor digitorum longus (study time × group, P = 0.004; A)						
CTR	31.3 (3.4)	−11.3 (8.6)**	−15.2 (10.1)***	−8.4 (7.5)**	−0.2 (6.5)	1.5 (7.4)
RE	29.3 (8.3)	1.7 (5.0)	1.3 (8.8)	3.0 (9.1)	0.7 (4.0)	2.6 (6.7)
RVE	25.9 (7.5)	0.4 (9.9)	−5.5 (11.0)	−2.8 (10.5)	0.2 (7.4)	−0.6 (8.6)
Flexor hallucis longus (study time × group: P < 0.001; A)						
CTR	87.8 (7.2)	−10.5 (4.0)***	−16.7 (8.7)***	−10.2 (7.7)**	−5.9 (5.8)*	0.9 (6.9)
RE	82.1 (26.3)	2.7 (4.6)	−3.0 (5.6)	−7.1 (5.4)**	−0.5 (2.3)	1.6 (4.6)
RVE	75.4 (11.2)	−1.3 (12.6)	−8.3 (14.0)	−11.3 (9.6)**	−6.2 (9.5)	−7.4 (13.5)
Tibialis posterior (study time × group: P < 0.001; A, b)						
CTR	119.6 (18.4)	−13.0 (4.2)***	−15.9 (6.0)***	−3.4 (7.2)	−0.9 (5.2)	0.4 (3.5)
RE	100.9 (20.2)	0.5 (3.7)	−2.2 (5.1)	1.3 (4.1)	1.8 (2.9)	0.3 (2.8)
RVE	109.0 (21.2)	−4.0 (8.6)	−5.7 (10.1)	−1.2 (9.1)	−2.5 (8.2)	−0.7 (10.0)
Lateral gastrocnemius (study time × group, P = 0.046; a, b)						
CTR	188.0 (32.8)	−12.3 (11.7)**	−19.5 (12.5)***	−13.4 (10.5)**	−2.5 (8.9)	−2.2 (8.5)
RE	195.8 (34.1)	−2.7 (8.4)	−2.9 (7.5)	1.5 (9.6)	0.4 (7.9)	−3.7 (7.3)
RVE	167.9 (30.2)	0.4 (10.0)	−3.1 (11.5)	−2.3 (11.5)	1.0 (11.4)	0.4 (13.2)
Medial gastrocnemius (study−time × group, P = 0.022; A, b)						
CTR	297.6 (50.0)	−17.0 (9.0)***	−24.8 (10.5)***	−8.6 (6.4)**	1.5 (4.2)	0.3 (5.0)
RE	301.8 (35.6)	−4.2 (7.5)	−7.9 (5.9)**	−2.9 (4.7)	1.0 (4.7)	−2.5 (4.6)
RVE	272.1 (40.3)	−5.3 (6.2)	−10.8 (8.6)**	−1.8 (8.3)	0.6 (9.4)	0.4 (12.3)
Soleus (study time × group, P = 0.005; A)						
CTR	574.9 (93.8)	−16.8 (6.7)***	−22.7 (9.5)***	−7.2 (6.7)*	−0.3 (2.8)	−1.0 (3.2)
RE	527.0 (96.2)	−7.3 (3.8)***	−12.7 (4.7)***	−4.8 (2.9)***	1.2 (2.1)	1.0 (4.3)
RVE	564.4 (59.0)	−9.8 (7.3)**	−16.4 (8.4)***	−7.4 (7.4)*	−1.6 (7.1)	−3.3 (7.6)
Extensor digitorum longus						
CTR	122.3 (20.8)	−3.0 (4.0)	−5.2 (4.2)**	−2.3 (5.2)	−1.9 (4.5)	−2.5 (4.3)
RE	113.8 (14.1)	−2.2 (3.9)	−4.2 (3.2)**	−1.5 (5.6)	0.5 (4.5)	1.0 (2.8)
RVE	110.6 (12.7)	−0.8 (5.0)	−4.7 (5.5)	−2.2 (6.3)	−0.9 (6.4)	−1.2 (7.5)
Tibialis anterior						
CTR	169.3 (21.2)	−7.4 (3.8)***	−12.0 (4.2)***	−4.9 (4.7)*	−0.4 (2.7)	−1.9 (2.1)
RE	159.6 (32.6)	−6.8 (4.1)***	−10.9 (3.2)***	−1.4 (5.9)	−0.1 (3.8)	0.4 (4.1)
RVE	142.4 (11.7)	−6.2 (4.6)**	−8.9 (6.7)**	−2.6 (5.0)	1.5 (4.3)	0.6 (4.6)
Peroneals						
CTR	158.5 (29.5)	−11.6 (6.0)***	−16.4 (9.6)***	−9.4 (5.6)***	−1.2 (3.1)	−0.5 (5.4)
RE	143.8 (9.5)	−4.3 (3.0)**	−8.2 (3.6)***	−0.7 (3.5)	0.3 (3.6)	1.6 (2.7)
RVE	137.1 (17.0)	−6.0 (3.8)***	−10.2 (6.2)***	−4.5 (4.8)*	−1.1 (6.1)	−0.2 (4.0)

P value from three-group ANOVA is presented. "A" or "B" ($P < 0.01$) and "a" or "b" ($P < 0.05$) refer to differences on two-group ANOVA for CTR versus RE or CTR versus RVE. All P values are adjusted for false positives via the "false discovery rate" method.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ and indicate significance of difference to baseline value.

An ''intent-to-treat'' (ITT) analysis approach was used. Results from the ''per-protocol'' (PP) analysis are reported only when the outcome differs from the ITT analysis. A large number of statistical tests were performed. We therefore implemented the ''false discovery rate'' method to adjust the *P* values and reduce the risk of false positives.

RESULTS

Study events and missing data. One RVE subject transferred to the CTR group at the beginning of the HDT phase of the study because of exercise-induced headaches, which prevented him from performing the exercises (4). This subject was classified as RVE group on the ITT analysis and as CTR group on the PP analysis. This subject also experienced ankle pain and swelling on reambulation and could not complete the post-bed rest performance-based tests. One other subject (RE) was withdrawn from the study on day 30 of bed rest because of a medical problem in the thoracic spine (5). Two subjects (one RE and one CTR) did not return for testing 90 or 180 d after bed rest. Sprint test data were not collected from the first bed rest campaign (two CTR, two RE, and two RVE) because the measuring system was not ready for use at this stage. No differences existed at baseline between groups, except for adductor longus muscle size ($P = 0.006$). Additional analyses with baseline data as a covariate did not change the study outcomes for this muscle. Insufficient serum material was available from day 33 of bed rest for five subjects (one RE, one RVE, and three CTR) for determination of CK-MM. From another six subjects (two from each group), serum samples were lost on day 180 after bed rest because of a laboratory error.

Muscle size. Atrophy of the medial and lateral gastrocnemius, soleus, vasti, tibialis posterior, flexor hallucis longus, and flexor digitorum longus was reduced in the RE group ($P \leq 0.045$) (Tables 1 and 2). On the ITT analysis, atrophy of the medial and lateral gastrocnemius and tibialis posterior was reduced in the RVE group compared with that in the CTR group ($P \leq 0.044$) (Table 1). On the PP analysis only, RVE also reduced atrophy of the soleus, vasti, flexor hallucis longus, and flexor digitorum longus ($P \leq 0.043$) (see tables, Supplementary Digital Content (SDC) 1: Volume of

TABLE 2. Volume of the knee extensors, knee flexors, and hip/thigh adductors during and after bed rest: ITT analysis.

	Study Time					
Group	BDC (cm^3)	HDT27/28 (%)	HDT55/56 (%)	R+14 (%)	R+90 (%)	R+180 (%)
Rectus femoris						
CTR	311.8 (41.8)	−1.9 (7.2)	−5.5 (7.8)	−4.4 (8.0)	1.1 (4.0)	1.6 (5.1)
RE	324.8 (47.9)	0.4 (4.4)	1.8 (3.9)	3.6 (3.7)	6.0 (4.7)*	4.6 (6.9)
RVE	300.7 (39.3)	−2.5 (5.8)	−0.6 (5.0)	1.6 (4.8)	1.8 (5.4)	2.6 (8.0)
Vasti (study time × group, P = 0.003; A)						
CTR	1948.6 (147.9)	−9.1 (6.9)**	−15.2 (8.5)***	−7.2 (6.2)**	−2.9 (6.9)	1.0 (7.9)
RE	2017.9 (366.3)	−1.7 (6.2)	−1.4 (6.1)	−0.7 (6.7)	3.5 (7.4)	1.8 (9.1)
RVE	1916.1 (270.5)	−4.4 (9.0)	−6.7 (10.6)	−1.8 (8.2)	0.1 (8.4)	−1.0 (8.1)
Lateral hamstrings						
CTR	379.4 (45.7)	−5.7 (7.7)	−13.4 (8.3)***	−5.1 (8.4)	−1.9 (5.9)	0.1 (6.4)
RE	380.4 (66.4)	−6.2 (5.4)**	−9.8 (6.0)***	−2.7 (5.7)	3.1 (5.1)	0.6 (5.3)
RVE	339.0 (38.9)	−6.9 (7.7)*	−10.8 (8.0)**	−2.0 (6.4)	3.0 (6.5)	1.4 (6.9)
Medial hamstrings						
CTR	521.7 (54.2)	−6.7 (5.8)**	−12.5 (5.6)***	−5.5 (5.8)*	−1.2 (6.3)	0.3 (6.4)
RE	528.2 (98.3)	−6.5 (5.0)**	−9.7 (5.0)***	−2.6 (5.3)	3.3 (4.3)	1.4 (5.1)
RVE	470.5 (56.6)	−7.4 (7.5)*	−10.9 (8.1)**	−0.8 (6.3)	3.6 (5.8)	2.1 (6.0)
Adductor brevis						
CTR	108.6 (15.0)	0.2 (12.5)	−1.4 (5.1)	0.9 (9.9)	2.6 (6.1)	2.8 (13.7)
RE	125.3 (28.8)	−1.0 (15.3)	−3.1 (21.2)	4.7 (14.5)	6.2 (10.6)	2.0 (15.3)
RVE	107.5 (11.7)	7.7 (8.2)	12.9 (12.9)	13.0 (10.2)*	13.9 (18.9)	9.4 (20.2)
Adductor longus						
CTR	185.1 (17.2)	−1.6 (5.6)	−5.1 (4.8)*	−2.6 (4.1)	2.0 (6.4)	−1.2 (5.8)
RE	212.4 (26.7)	−3.3 (5.2)	−4.6 (7.5)	−3.7 (4.9)	1.3 (6.0)	0.4 (5.8)
RVE	162.5 (16.1)	3.2 (7.3)	1.0 (8.6)	1.5 (8.1)	4.5 (6.7)	4.8 (8.8)
Adductor magnus						
CTR	600.6 (82.9)	−5.2 (8.2)	−9.2 (8.7)*	−2.5 (6.8)	−1.1 (4.5)	0.6 (5.7)
RE	633.9 (147.2)	−1.0 (4.6)	−1.3 (4.6)	2.1 (2.9)	4.5 (3.7)*	3.5 (4.0)
RVE	558.5 (52.0)	−2.6 (3.7)	−4.3 (4.8)*	−0.1 (5.0)	2.7 (4.9)	1.1 (6.0)
Pectineus						
CTR	72.8 (10.4)	−1.7 (8.5)	−0.6 (8.4)	−4.0 (8.0)	0.7 (8.1)	−6.4 (2.5)***
RE	79.7 (11.0)	−2.7 (4.6)	−1.8 (6.4)	−0.6 (7.6)	0.4 (6.2)	−2.0 (14.6)
RVE	65.0 (11.8)	0.4 (6.0)	−2.0 (9.8)	2.2 (6.7)	2.3 (8.4)	0.1 (9.8)
Gracilis						
CTR	109.9 (18.1)	4.3 (7.6)	−1.8 (8.1)	2.8 (9.0)	1.4 (8.2)	3.6 (7.1)
RE	122.5 (26.0)	0.5 (4.4)	−0.3 (5.1)	1.4 (5.7)	4.9 (4.4)	3.7 (5.3)
RVE	112.3 (19.4)	−1.7 (10.7)	−4.9 (11.3)	1.6 (10.3)	5.6 (10.6)	0.3 (11.7)
Sartorius						
CTR	184.3 (27.5)	−1.4 (4.8)	−6.7 (4.4)***	−1.4 (4.6)	−0.5 (3.9)	0.2 (4.7)
RE	173.5 (48.5)	−0.5 (4.6)	0.0 (5.1)	3.8 (5.5)	7.6 (4.4)**	4.0 (9.2)
RVE	175.6 (34.0)	−3.5 (13.2)	−6.0 (13.2)	2.4 (10.3)	1.0 (13.0)	3.2 (11.6)

P value from three-group ANOVA is presented. "A" or "B" ($P < 0.01$) and "a" or "b" ($P < 0.05$) refer to differences on two-group ANOVA for CTR versus RE or CTR versus RVE. All *P* values are adjusted for false positives via the "false discovery rate" method.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ and indicate significance of difference to baseline value.

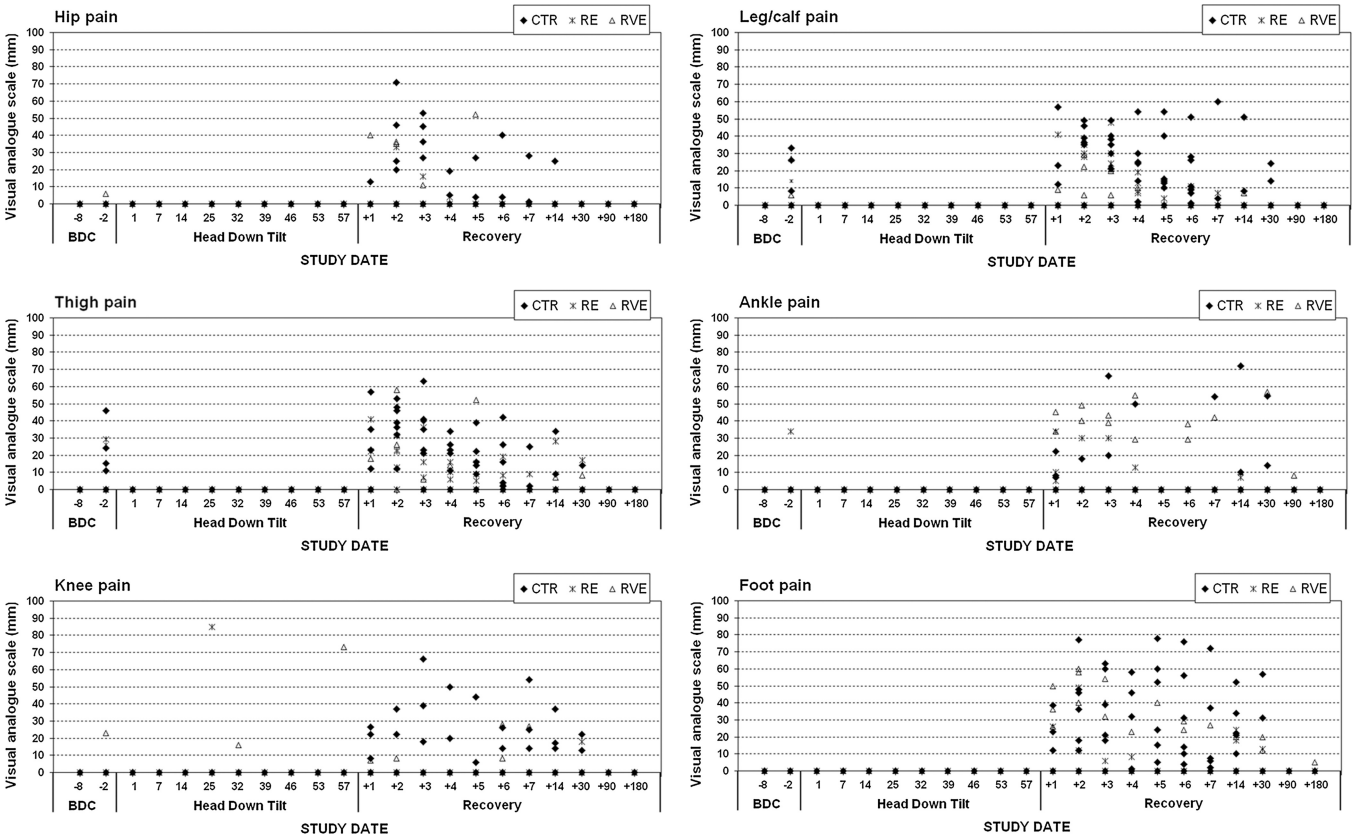

FIGURE 2—Pain reports at the hip, thigh, knee, calf, ankle, and foot. Data are raw VAS scores from all subjects in each group. On the questionnaire days not shown above (HDT2–6, HDT8–13, and HDT18), no pain was reported. BDC (-), day of BDC; HDT, day of HDT bed rest; recovery (+), day of post-bed rest recovery. Pain on BDC-2 is associated with maximal strength tests performed by other research groups.

the posteromedial and anterolateral leg muscles during and after bed rest: PP analysis, http://links.lww.com/MSS/A365; and SDC 2: Volume of the knee extensors, knee flexors, and hip/thigh adductors during and after bed rest: PP analysis, http://links.lww.com/MSS/A366). Furthermore, on the PP analysis, RE had a significant effect on reducing sartorius muscle atrophy ($P = 0.014$) (see table, SDC 2: Volume of the knee extensors, knee flexors, and hip/thigh adductors during and after bed rest: PP analysis). There were no significantly different responses in muscle volume between the RE and RVE groups during the bed rest phase.

Pain questionnaires. The VAS scores reported for each study time are shown in Figure 2. Data from day of reambulation (R+1) were unavailable in six subjects. One subject (RE group) reported lower leg pain on R+2 but did not complete a VAS. The number of subjects reporting pain at the knee in HDT did not differ between groups, and the intensity of knee pain or "all lower limb pain" also did not differ between groups in the HDT phase. Further statistical testing of other regions of the lower limb could not be performed because of insufficient reports of pain.

After bed rest, in comparison with that in the CTR group, there was some evidence that pain intensity in the RE group was lower at the foot ($P \leq 0.033$) and "all lower limb regions" ($P = 0.01$) and in the RVE group at the thigh ($P \leq 0.041$), lower leg ($P \leq 0.01$), and "all lower limb regions" ($P \leq 0.036$). There were no differences between RE and RVE groups for pain intensity reports.

Sarcomeric muscle-specific creatine kinase. Compared with those on HDT60, the increases in CK-MM levels on R+3 differed between the three groups ($P = 0.019$) (Fig. 3). These increases were less in both the RE ($P = 0.020$ vs CTR) and RVE groups ($P = 0.020$ vs CTR). These effects were not statistically significant on the PP analysis. CK-MM level reductions during bed rest (Fig. 3) did not differ between groups.

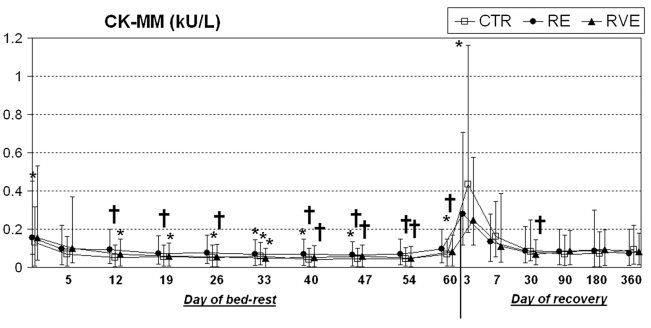

FIGURE 3—Sarcomere-specific creatine kinase during the study. Values are medians, and *error bars* indicate interquartile range. *$P <$ 0.05, †$P < 0.01$, and ‡$P < 0.001$ indicate significance of difference to baseline on the Mann–Whitney *U* test.

Correlation analyses. The correlations between muscle atrophy and decrements in neuromuscular tests are presented in Table 3. Atrophy of major prime movers, such as the vasti in leg press testing and the gastrocnemius in the sprinting tasks correlated significantly with decrements for those tasks. Atrophy of other muscles of the foot and ankle, such as the flexor hallucis longus and tibialis posterior muscles, correlated moderately with decrements in sprinting and countermovement jump tasks, respectively. Atrophy of several lower leg muscles also related to decrements in the leg press task. The presence of pain in the lower leg on R+2 was associated with greater atrophy in the peroneals ($P = 0.0006$), flexor hallucis longus ($P = 0.013$), extensor digitorum longus ($P = 0.035$), and medial gastrocnemius ($P = 0.011$). The extent of atrophy of the thigh muscles was not significantly associated with the presence of thigh pain on R+2. The change in CK-MM on R+3 versus that on HDT60 correlated with the intensity of pain on R+2 in the foot ($P = 0.017$).

DISCUSSION

The main finding of the current study is that a short-duration RE program can be effective in preventing or reducing muscle atrophy in the lower limbs during bed rest, pain during bed rest, and the increase in a marker of muscle damage (CK-MM) after bed rest. This reinforces the idea that high-load, muscle-specific RE is one of the most effective known current methods for prevention of muscle atrophy and muscle performance decline during space flight simulation. In comparison with our study, previous vibration bed rest studies, which used low-load, nonspecific muscle exercises, for example, vibration training in a standing or supine position, to target the lower limb (39) or spinal (16) musculature, were unsuccessful at reducing muscle atrophy in these regions. In addition, bed rest works, which incorporated aerobic-based countermeasure exercises such as cycling (34) and lower body negative pressure (8), have also been shown to be ineffective at reducing lower limb muscle atrophy. In contrast to a previous study where training was performed 11 times per week (27), the incidence or intensity of pain in the lower limbs in the current study was not higher than that in the CTR group during bed rest, although some overuse-type injuries did occur in the training groups. High-loading levels are less likely to cause overuse-type injuries when performed on a less frequent schedule during bed rest and/or allowed more time for healing of any acute injuries occurring during exercise (32).

The countermeasure program implemented in our study was, however, not 100% efficient. The calf musculature, for example, is particularly difficult to maintain during prolonged bed rest. Statistically significant muscle volume atrophy still occurred in previous studies where daily training of the calf muscles was performed (6). When less frequent training is performed, as in the current study, the musculature does not seem to be as well preserved. For example, the soleus (RVE group) showed a 15% loss in muscle volume after 56 d of bed rest in the current study compared with a 7% loss in muscle volume after 56 d of bed rest in a previous study where training was performed 11 times a week (6).

It might therefore be worth considering that high-load resistive plantarflexion exercise may not be the best approach for maintaining the calf musculature in space flight simulation. It has been shown that the forces generated at the ankle during jumping (36) seem to be higher than those at

TABLE 3. What is the relation between muscle atrophy and functional performance decrements?

	Functional Test (Outcome Parameter)		
Muscle	**15-m Sprint (Time)**	**Leg Press One-Repetition Maximum (Force)**	**Countermovement Jump (Height)**
Medial thigh muscles			
Adductor brevis	−0.38	0.37	0.18
Adductor longus	−0.31	0.57*	0.36
Adductor magnus	−0.50	0.46	0.28
Pectineus	0.21	0.07	0.06
Gracilis	−0.53	0.25	−0.11
Sartorius	−0.31	0.50	0.03
Knee extensors			
Rectus femoris	−0.21	0.54*	0.02
Vasti	−0.57	0.80***	0.40
Hamstrings			
Medial hamstrings	−0.31	0.32	0.01
Lateral hamstrings	−0.33	0.34	0.10
Anterolateral leg muscles			
Extensor digitorum longus	−0.29	0.38	0.21
Tibialis anterior	−0.37	0.50	0.28
Peroneals	−0.56	0.52	0.38
Posteromedial calf muscles			
Flexor digitorum longus	−0.47	0.54*	0.45
Flexor hallucis longus	−0.70*	0.49	0.51
Tibialis posterior	−0.56	0.84***	0.64**
Lateral gastrocnemius	−0.70*	0.78***	0.49
Medial gastrocnemius	−0.63*	0.72**	0.43
Soleus	−0.43	0.74**	0.47

Values are Pearson correlation coefficient between percentage change in muscle volume at end of bed rest and percentage change in each functional test parameter at the first test after bed rest.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ and were adjusted for false positives via the "false discovery rate" method.

isokinetic plantar flexion maneuvers (14). Expanding countermeasure programs to include tasks such as "reactive jumps" (19) may provide an additional stimulus for the calf muscles. In daily life, however, these muscles are not frequently used in explosive tasks such as jumping but rather for many lower load activities such as walking (24). Endurance-type exercises may also be of benefit for the calf muscles.

There was no difference between the RE-only and RVE groups for the outcome parameters examined in this study. Hence, we were not able to confirm our hypothesis that less muscle atrophy occurs in the lower limb muscles in RVE versus that in RE.

It is worthwhile to discuss the changes in the serum parameters during bed rest. CK-MM level was reduced during bed rest in all groups. Immobilization in rats (40) and humans (17) leads to a reduction in creatine kinase activity in the immobilized muscle. Although muscle atrophy occurs during bed rest, muscle "damage" and subsequent release of CK-MM from the muscle fiber (9) due to mechanical overload does not occur. On the other hand, baseline phases of bed rest studies are quite busy with numerous tests, including maximal strength testing. There were also some reports of muscle pain 2 d before bed rest. This could mean that CK-MM levels are actually elevated above the subject's true normal level before bed rest. The fact that CK-MM levels were lower late in recovery in some groups fits this idea.

After bed rest, greater increases in CK-MM level were seen in the inactive CTR subjects. The CK-MM level increases after bed rest correlated with greater intensity of lower limb pain. Lower limb pain reports peaked on the second or third day after bed rest and largely dissipated in the countermeasure groups by 14 d after bed rest and by 30 d after bed rest in the CTR group. Pain reports in the hip, thigh, lower leg, and foot largely followed a profile observed in clinical practice of "delayed-onset muscle soreness" (31). Available data (26) indicate that muscle damage occurs in weakened muscles during initial reloading after unloading. At the ankle joint, our clinical examinations of the subjects indicated that because of prolonged changes in ankle posture during bed rest, the joint structures are initially shortened. After reambulation, relengthening of these tissues occurs in addition to the corresponding changed muscle recruitment and coordination demands. This relative mechanical overload undoubtedly leads to muscle damage and pain. Delayed-onset muscle soreness and increases in creatine kinase level have been associated in ambulant studies with further functional decrements (38). Recovery of muscle size and function typically occurred earlier and/or quicker in the countermeasure groups than in the CTR group, with most muscles regaining their pre–bed rest volumes by day 14 of recovery. This is understandable because of the effect of the countermeasures in the preceding bed rest phase.

The correlation analyses give an indication of what effect muscle-specific atrophy has on function. During interpretation of these results, it should be kept in mind that muscle volume is just one factor that can contribute to performance in functional tasks. It is not unreasonable to speculate that for tests requiring higher levels of neuromuscular coordination, the relative effect that changes in muscle volume will have on performance will be reduced. Hence, we would expect to find the strongest correlations between muscle atrophy and muscle performance on the functional tests where factors such as muscle strength play a more predominant role.

Examples of this can be seen in the role of the vasti as a prime mover in a leg press (13) and the role of the triceps surae in sprinting and jumping (23) tasks. In addition, the limited correlation between hamstring muscle atrophy and sprint time increase or jump height loss can be understood through biomechanical modeling findings (23), which show that these muscles are more important for transmission rather than generation of force in jumping and sprinting. On the other hand, some findings are not so readily understood such as the correlations between muscle atrophy in the long toe flexors, peroneals, and tibialis posterior and performance decrements in sprinting, hopping, and jumping. These muscles are all important for control of the ankle and foot (30). A biomechanical modeling study indicated that the metatarsophalangeal joint absorbs approximately 32% of all energy absorbed at the lower limb joints during sprinting (33). This energy dissipation must invariably depend largely upon the musculature, such as the flexor hallucis and digitorum longus. In addition, the tibialis posterior and peroneus longus are considered to act in a force couple to control the arch of the foot during ambulation (18). Furthermore, it seems from the correlation analyses that gastrocnemius atrophy is more important for sprint performance after bed rest than soleus. Neurophysiological studies (35) have indicated that soleus recruitment is saturated at lower loads, with the gastrocnemius being recruited more in higher-load tasks. In addition, biomechanical modeling studies (29) have indicated that the gastrocnemius is more important in forward propulsion during running whereas the soleus seems to have a greater role in vertical body support. Overall, these correlation analyses indicate that the muscle-specific atrophy seen in the lower extremity musculature is important for understanding the losses in functional performance and for the subsequent development of appropriate countermeasure exercises.

The current work has some important limitations. As is typical of bed rest studies, the number of subjects was limited because of logistical and financial restraints. Because of the limited number of subjects, some nonsignificant results for muscular atrophy may represent false negatives. For some subjects, their participation in the countermeasure exercise group represents their first exposure to a structured, frequent, and intense exercise protocol. Astronauts are considered to be highly physically trained individuals, and hence, the application of the current protocols to space flight could not occur on a 1:1 basis. Furthermore, although one of the strengths of the correlation analyses is that the data were collected prospectively, one of their limitations is that, like all correlations analyses, some of the findings may simply be

due to covariation rather than a causal link *per se*. The correlations of calf muscle atrophy to leg press performance loss could be considered in this light.

In conclusion, the current work showed that a short-duration RE (5–6 min time under tension, 3 $d \cdot wk^{-1}$) program with or without whole-body vibration prevented or reduced atrophy in several lower limb muscles during 60 d of bed rest, reduced the incidence of pain in early recovery after bed rest, and reduced the increase in a marker of muscle damage after bed rest. There were no differences detected in the effect on muscle size when whole-body vibration was added to RE.

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The results of the present study do not constitute endorsement by American College of Sports Medicine.

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