

Resistive vibration exercise reduces lower limb muscle atrophy during 56-day bed-rest

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Abstract

Objectives: The current study aimed to examine the effectiveness of a resistive vibration exercise countermeasure during prolonged bed-rest in preventing lower-limb muscle atrophy. **Methods:** 20 male subjects underwent 56-days of bed-rest and were assigned to either an inactive control, or a countermeasure group which performed high-load resistive exercises (including squats, heel raises and toe raises) with whole-body vibration. Magnetic resonance imaging of the lower-limbs was performed at two-weekly intervals. Volume of individual muscles was calculated. **Results:** Countermeasure exercise reduced atrophy in the triceps surae and the vastii muscles ($F>3.0$, $p<.025$). Atrophy of the peroneals, tibialis posterior and toe flexors was less in the countermeasure-subjects, though statistical evidence for this was weak ($F\leq 2.3$, $p\geq .071$). Atrophy in the hamstring muscles was similar in both groups ($F<1.1$, $p>.38$). The adductor longus, sartorius and rectus femoris muscles showed little loss of muscle volume during bed-rest ($F<1.7$, $p>.15$). **Conclusions:** The countermeasure exercise programme was effective in reducing atrophy in the extensors of the knee and ankle but not the hamstrings.

Keywords: Spaceflight, Berlin Bed Rest Study, Magnetic Resonance Imaging, Microgravity, Countermeasures

Introduction

With space agencies and governments striving towards manned missions to Mars, an important research question is the development of exercise countermeasures to maintain various body systems for optimal function upon landing. As part of this, it is important to assess the effectiveness of countermeasures in maintaining the musculature of the lower-limbs. The role of the lower-limb musculature, particularly with regard to the plantarflexors and knee and hip extensors¹⁻³, in upright posture and locomotion imply that the maintenance of these muscle systems during long-duration spaceflight, an environment where such functions are not required, would be critical. Prolonged bed-rest

is a frequently used ground-based methodology to simulate the effects of spaceflight on the human musculoskeletal system⁴. Basic principles of exercise physiology suggest that low load endurance exercise is inappropriate to maintain muscle mass during prolonged bed-rest and that higher load resistance exercise is required⁵. Indeed, bed-rest studies implementing aerobic countermeasures^{6,7} or low-load exercise⁸ have found that they were ineffective for the maintenance of the lower-limb musculature, whereas bed-rest studies implementing resistive exercise were much more successful, though the effect on particular muscle groups depended upon the types of exercise performed⁹⁻¹⁴.

More recently, whole body vibration during resistive exercise has received attention in sport science as a method to provide an additional stimulus during training¹⁵. It is thought that the vibratory inputs stimulate additional muscle activity during contraction¹⁶ via the muscle spindle system¹⁷⁻¹⁹, to produce a greater force of muscle contraction²⁰, and hence stimulus for muscle maintenance and/or hypertrophy. Vibratory stimuli, applied at the feet are transmitted up to the hip and lumbar spine²¹ and hence can potentially modulate muscle activity throughout the entire lower quadrant. We hypothesized that high-load resistive exercise with whole body vibration could be an effective countermeasure against muscle atrophy in the lower-limbs during prolonged bed-rest.

Dieter Felsenberg and Jörn Rittweger are acting as consultants to the European Space Agency and Novotec Medical for the exploitation of this study's results. All other authors have no conflicts of interest.

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Figure 1. Resistive vibration exercise during bed-rest. Subjects were required to perform lower-limb exercises against a resistive force transmitted via belts at the waist and shoulders and via hand-grips. The feet were placed on a suspended platform. Vibratory stimuli in the lower-limbs were generated by rotation of the platform around a vertically oriented axis.

Magnetic resonance imaging (MRI) is a commonly used methodology to assess muscle size. In studies where subject numbers are limited, such as bed-rest, measures of muscle atrophy during bed-rest and response to countermeasures are best performed using measures of muscle volume, thus avoiding greater imprecision associated with taking single cross-sectional area measures^{22,23}. In the current work, we wished to examine the effectiveness of resistive exercise with whole body vibration during prolonged bed-rest on changes in muscle volume in the lower-limbs as measured with MRI.

Materials and methods

Bed-rest protocol

The “Berlin Bed-Rest Study” was undertaken at the Charité Campus Benjamin Franklin Hospital in Berlin, Germany, from February 2003 to June 2005. Twenty medically and psychologically healthy male subjects underwent 8-weeks of strict bed-rest. The sample size of the Berlin Bed-Rest Study was based on power analyses of expected distal tibia bone density changes, rather than muscle measures. It is reasonable to expect, however, that muscle is more susceptible to inactivity/training, and other works have shown strongly significant findings, including the effect of countermeasure exercise, in sample sizes half than that in the current study¹². The bed-rest protocol, as well as inclusion and exclusion criteria, is discussed in detail elsewhere²⁴. In brief, however, subjects were randomly allocated to either an inactive control group (CTRL) or a group that underwent a whole body resistive vibration exercise countermeasure programme (RVE group) using the Galileo Space exercise device (Novotec Medical, Pforzheim, Germany). The mean (SD) baseline age, height and weight of the RVE group were: 32.6(4.8) years, 183(9) cm and

81.7(14.4) kg respectively and in the CTRL group: 33.4(6.6) years, 185(7) cm and 79.4(9.7) kg.

Horizontal bed-rest was employed, though subjects were permitted to be positioned in up to thirty degrees head-up tilt for recreational activities during daylight hours (such as watching television). Subjects performed all hygiene in the supine position and were discouraged from moving excessively or unnecessarily. Force sensors placed in the bed supports, 24-hour nursing care and video surveillance permitted monitoring of subjects’ activities. The institutional ethics committee approved this study and subjects gave their informed written consent. Subjects were aware that their participation in the study was voluntary and that they were permitted to withdraw from the study at any time.

Countermeasure exercise

Resistive vibration exercise (RVE) was performed using a dedicated prototype (Galileo Space) of a commercially available vibration platform (Novotec Medical, Pforzheim, Germany). The exercise device permitted exercise in the supine position throughout bed rest. The exercise regime targeted the lower leg muscle groups with resistive loading and neuromuscular stimuli via whole-body vibration applied at the feet^{25,26}. The countermeasure exercise protocol is described in detail elsewhere²⁴. In brief, the subjects were placed in a supine position (Figure 1), with their feet resting on the vibration platform and an applied vibration amplitude between 3.5–4 mm. An axial force between 1.0 and 1.8 times body weight was placed through the subjects’ trunk and spine via elastic shoulder straps (targeted to be approximately 75–85% of the subject’s 1-repetition maximum). With the exception of Sundays and Wednesday afternoons, two exercise sessions per day, of 30 min duration (between 4–6 min pure exercise time) were performed. A total of 89 exercise sessions were scheduled for each subject.

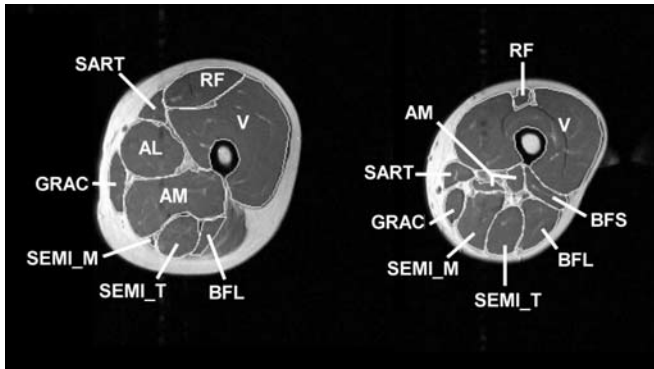


Figure 2. Thigh muscle image measurements. Example images from the upper (left) and lower (right) thigh. Thirty-five images were acquired from the superior aspect of the femoral head to the knee joint line. The cross-sectional area (when present) of the muscles rectus femoris (RF), vastii (V), sartorius (SART), gracilis (GRAC), adductor magnus (AM), adductor longus (AL), biceps femoris long head (BFL), biceps femoris short head (BFS), semitendinosus (SEMI_T) and semimembranosus (SEMI_M) were measured in each image.

Trained staff supervised all training sessions, and subjects were given feedback and encouragement. For each morning session, four resistive exercises were performed once in the following order:

- squats: knees were straightened from 90° to full extension in cycles of 6 s for 60 s whilst the vibration frequency was set to 18 Hz in the first training sessions and progressed in subsequent sessions up to a maximum of 24 Hz.
- heel raises: in almost full knee extension, the heels were raised into plantar flexion as long as the subjects could sustain this (up to 40 seconds). The vibration frequency was retained at 26 Hz.
- toe raises: with knees in full extension, the forefoot was raised into dorsiflexion up to 40 seconds. The vibration frequency was retained at 26 Hz.
- explosive kicks: 10 explosive pushes from a flexed knee and hip position against the vibrating platform were performed. These “kicks” were targeted at generating peak forces to stimulate bone formation²⁷ rather than muscle *per se*.

In the afternoon session, subjects were asked to exercise with a lower resting platform reaction force (60-80% of the value achieved in the morning). Only one exercise was performed and subjects kept their feet on the platform with their knees in a nearly extended position and performed no movement. Vibration frequency was retained at 19 Hz and the exercise performed between 4 and 6 minutes (depending on the physical ability of subject).

MRI protocol

Baseline MR scanning was conducted on the first day of bed-rest (BR1) and then at two week intervals (BR14, BR28, BR42 and BR56) through to the end of the bed-rest period.

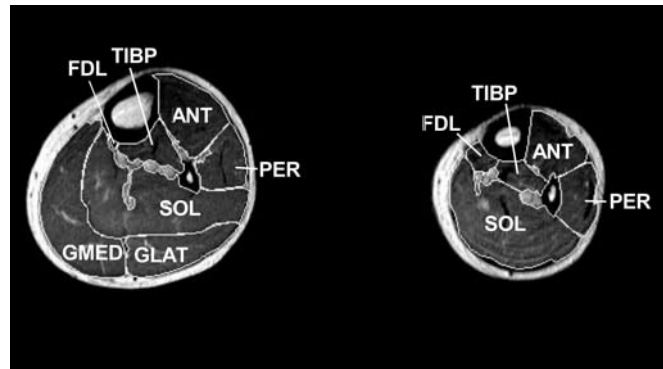


Figure 3. Lower leg muscle image measurements. Example images from the upper (left) and lower (right) calf. Thirty images were acquired from the knee joint line to the lateral malleolus. The cross-sectional area (when present) of the muscles gastrocnemius lateralis (GLAT), gastrocnemius medialis (GMED), soleus with flexor hallucis longus (SOL), tibialis posterior (TIBP), flexor digitorum longus (FDL), peroneals (PER; peroneus longus, brevis and tertius), anterior tibial (ANT; tibialis anterior, extensor digitorum longus, extensor hallucis longus) were measured in each image.

Subjects were positioned on the scanning bed in supine with their knees and hips supported in slight flexion by a pillow under the knees. Transverse MR images were acquired from the lower-limbs using a 1.5 Tesla Magnetom Vision system (Siemens, Erlangen, Germany). Typically, 35 images of the thigh (from the superior aspect of the head of femur to the knee joint line; thickness=10 mm, inter-slice distance=5 mm, TR=6000 ms, TE=15 ms, FA=180 degrees, field of view: 480 x 480 mm interpolated to 512 x 512 pixels) and 30 images of the lower leg (knee joint line to the distal most portion of the lateral malleolus; thickness=10 mm; inter-slice distance=5 mm, TR=4800 ms, TE=15 ms, FA=180 degrees, field of view: 340 x 340 mm interpolated to 512 x 512 pixels) were acquired, though for taller subjects, additional images were added to ensure the region of interest was captured. Images were stored for offline analysis.

Image measurements

One operator (TM) performed all image measurements. To ensure operator blinding to study time-point and subject group, each image was assigned a random number (www.random.org). ImageJ (Ver. 1.38x, <http://rsb.info.nih.gov/ij/>) was used for MR image analysis. The cross sectional area (CSA) of the following muscles in the thigh (Figure 2) was measured in each image: rectus femoris (RF), vastii (V; vastus medialis, lateralis and intermedius), sartorius (SART), gracilis (GRAC), adductor magnus (AM), adductor longus (AL), biceps femoris long head (BFL), biceps femoris short head (BFS), semitendinosus (SEMI_T) and semimembranosus (SEMI_M). Whilst AM and AL could be readily differentiated from adductor brevis, this adductor muscle could not easily be differentiated from pectineus and hence was not measured. In the lower leg (Figure 3) the following muscles were measured: gastrocnemius lateralis

Study-date	Lower leg musculature		Thigh musculature	
	CTRL	RVE	CTRL	RVE
BR1	8 ^a	9 ^b	6 ^{a,b}	9 ^c
BR14	10	8 ^b	10	9 ^b
BR28	10	8 ^b	9 ^b	10
BR42	10	8 ^b	10	9 ^b
BR56	10	9 ^b	10	9 ^b

^aTwo data sets missing due to MRI scanner failure
^bScanning performed but data not appropriate for analysis (e.g. movement artefacts)
^cData set missing due to MRI scanner failure
CTRL: control group; RVE: resistive vibration exercise group.
BR=day of bed-rest.

Table 1. Number of data sets available for analysis on each study-date.

(GLAT), gastrocnemius medialis (GMED), soleus with flexor hallucis longus (SOL; due to a lack of consistent anatomical landmarks (e.g. fascia) on MRI, soleus was difficult to separate from flexor hallucis longus in a number of subjects. The results of the current study do not change when soleus is considered separately from flexor hallucis longus, thus the data presented is pooled from both muscles), tibialis posterior (TIBP), flexor digitorum longus (FDL), peroneal group (PER; peroneus longus, brevis and tertius), anterior tibial muscles (ANT; tibialis anterior, extensor digitorum longus, extensor hallucis longus). Due to experiments conducted during bed-rest on the right lower-limb^{28,29}, all image measurements were performed on the left lower-limb only. 33,073 individual manual CSA measurements comprised the final data set, requiring approximately 830 person-hours of image analysis. To enable assessment of changes in entire muscle volume, individual CSA measurements for each muscle were interpolated given an image thickness of 1.0 cm and inter-image distance 0.5 cm. The resulting muscle volume data was used in further analyses.

Data processing and statistical analyses

Linear mixed-effects models³⁰ were used for each muscle, to fit fixed-effects for study-date, training-group and a study-date×training-group interaction. Baseline subject age, height and weight were included in the model as linear covariates. Random effects for each subject were permitted. Where necessary, allowances were made for heterogeneity of variance (such as due to training-group and/or study-date). Subsequent analysis of variance (ANOVA) examined the significance of each of the factors and the interaction term. An α of 0.05 was taken for statistical significance. Where significant effects were seen, subsequent post-hoc analyses determined which study-days differed from baseline (BDC). As multiple imaging sessions were undertaken on the same subjects, we looked for consistent significant differences across time points. All analyses were performed in the “R” statistical environment (version 2.4.1, www.r-project.org).

Results

Due to issues such as movement artefacts or scanner failure, data sets were not available for analysis for all subjects from every scanning session. Table 1 lists the number of data sets available for analysis in the current work. No differences existed in baseline muscle volume between the groups at baseline scanning (BR1) for any of the muscles (F all < 2.73, p all > 0.12). Tables 2, 3 and 4 show, respectively, the baseline (BR1) volume of each muscle group in the lower leg and thigh.

Effect of resistive vibration exercise countermeasure – lower leg musculature

For ease of interpretation, changes in muscle volume during bed-rest in the lower leg muscles are expressed as percentage change compared to baseline in Table 2 from BR14 and beyond. ANOVA showed very strong effects for changes in volume in all muscles of the lower leg over the course of the study (study-date: $F_{4,63}$ all ≥ 3.8 , p all ≤ 0.0075). Very strong statistical evidence for an effect of the countermeasure exercise on muscle volume were seen in the gastrocnemius medialis (group: $F_{1,14}=5.9$, $p=.029$, group×study-date: $F_{4,63}=6.3$, $p=.0003$) and soleus muscles (group: $F_{1,14}=1.5$, $p=.24$, group×study-date: $F_{4,63}=5.9$, $p=.0004$), with the RVE group showing preservation of soleus muscle volume up to the BR42 scanning session with some losses thereafter. Losses in gastrocnemius medialis muscle volume in the RVE group also occurred later (BR28 and beyond) than in the CTRL group but to a lesser extent. A moderate statistical effect (group: $F_{1,14}=8.3$, $p=.012$, group×study-date: $F_{4,63}=3.0$, $p=.0247$) was also seen in the gastrocnemius lateralis for an influence of the countermeasure on muscle volume, with no significant changes in muscle volume in the RVE group over the course of the study, but with significant losses in the CTRL group.

In the peroneal (group: $F_{1,14}=0.9$, $p=.35$, group×study-date: $F_{4,63}=1.1$, $p=.36$) and tibialis posterior (group: $F_{1,14}=0.5$, $p=.49$, group×study-date: $F_{4,63}=2.3$, $p=.071$) muscle groups, little or no evidence existed in ANOVA for an effect of the countermeasure exercise. Inspection of the data in Table 2 shows, however, significant losses of volume in both muscles in the CTRL group, but no significant losses of muscle volume in tibialis posterior in the RVE group. The peroneal muscles showed a much later (BR56 in the RVE group as compared to BR28 in the CTRL group) appearance of statistically significant muscle volume loss. Evidently, a greater number of subjects would be needed to more precisely examine the extent of effectiveness of the countermeasure in these two muscle groups.

In the anterior tibial muscles, no evidence (group: $F_{1,14}=0.3$, $p=.60$, group×study-date: $F_{4,63}=0.6$, $p=.66$) existed for an influence of the countermeasure, despite a decrease in muscle volume, with loss of muscle marginally greater in the RVE group. The toe flexor, flexor digitorum longus also showed little evidence of a different response in the RVE group (group: $F_{1,14}=2.0$, $p=.18$, group×study-date: $F_{4,63}=1.8$, $p=.13$), though some loss in muscle volume occurred in the CTRL group by the end of bed-rest, with no significant change in the RVE group. The RVE group showed a marginal increase in muscle volume early in bed-rest (BR14) and subsequently no losses in muscle volume with respect to baseline thereafter.

Subject-group	Study-date				
	BR1 (cm ³)	BR14	BR28	BR42	BR56
Anterior Tibial Muscles					
CTRL	256.1(5.1)	-0.7 (1.5)%	-0.8 (1.4)%	-1.2 (1.5)%	-5.1 (1.7)%†
RVE	263.5(4.7)	-1.3 (0.8)%	-3.6 (0.6)%‡	-4.3 (1.4)%†	-6.5 (1.7)%‡
Flexor Digitorum Longus					
CTRL	30.7(2.5)	+2.9 (1.3)%*	-4.1 (2.1)%	-2.3 (1.6)%	-8.7 (1.8)%‡
RVE	34.8(2.8)	+7.8 (4.2)%	+4.2 (3.1)%	+3.6 (3.4)%	+0.7 (3.4)%
Gastrocnemius Lateralis					
CTRL	150.5(9.5)	-7.7 (3.8)%*	-11.2 (2.9)%‡	-10.5 (1.8)%‡	-14.4 (2.8)%‡
RVE	177.7(10.3)	+2.7 (2.3)%	-3.3 (2.7)%	-0.1 (2.4)%	-0.2 (3.4)%
Gastrocnemius Medialis					
CTRL	229.7(16.8)	-9.4 (1.5)%‡	-13.8 (1.6)%‡	-18.1 (1.1)%‡	-22.3 (1.5)%‡
RVE	276.8(18.1)	-1.4 (1.8)%	-6.1 (2.6)%*	-4.6 (1.5)%†	-8.7 (2.1)%‡
Peroneals					
CTRL	143.5(9.4)	-1.4 (1.6)%	-4.3 (2.0)%*	-7.5 (1.9)%‡	-10.8 (2.2)%‡
RVE	153.4(9.9)	+0.6 (1.3)%	-2.7 (1.5)%	-3.8 (1.8)%*	-5.0 (2.1)%*
Soleus with Flexor Hallucis Longus					
CTRL	589.9(22.2)	-6.2 (1.8)%†	-9.1 (1.8)%‡	-12.3 (1.8)%‡	-16.5 (1.8)%‡
RVE	588.6(22.9)	+0.9 (1.7)%	-1.5 (1.7)%	-1.1 (1.7)%	-7.2 (1.6)%‡
Tibialis Posterior					
CTRL	112.9(7.4)	-4.2 (1.5)%†	-6.1 (1.7)%‡	-6.2 (1.5)%‡	-10.2 (1.7)%‡
RVE	115.9(7.7)	+0.8 (1.2)%	-1.0 (1.1)%	-1.5 (1.8)%	-3.2 (1.8)%

At 1st day of bed-rest (BR1) values are mean(SEM) volume in cm³, beyond BR1 values are mean and standard error of the mean percentage change compared to BR1. *: $p < .05$; †: $p < .01$; ‡: $p < .001$ and indicate significance of difference to baseline value. BR= day of bed-rest. Anterior tibial muscles comprise the tibialis anterior, extensor digitorum longus and extensor hallucis longus muscles. No differences between groups existed at baseline (BR1; $F_{1,12}$ all < 2.73 , p all > 0.12)

Table 2. Changes in lower leg muscle volume during bed-rest and effect of countermeasure.

Subject-group	Study-date				
	BR1 (cm ³)	BR14	BR28	BR42	BR56
Anterolateral Tibial Muscles					
CTRL	400.1(12.1)	-1.0 (1.5)%	-2.0 (1.5)%	-3.4 (1.5)%*	-7.2 (1.8)%‡
RVE	416.3(12.0)	-0.6 (0.4)%	-3.4 (0.7)%‡	-4.2 (1.0)%‡	-5.9 (1.8)%†
Triceps Surae					
CTRL	908.6(38.2)	-7.8 (1.9)%‡	-11.2 (1.8)%‡	-14.4 (1.8)%‡	-18.3 (2.0)%‡
RVE	971.9(37.6)	+0.7 (1.0)%	-3.0 (1.0)%†	-1.9 (0.7)%†	-6.6 (1.8)%‡
Quadriceps Femoris					
CTRL	2235.1(92.4)	-6.4 (3.5)%	-9.1 (3.4)%*	-12.0 (3.4)%‡	-14.4 (3.5)%‡
RVE	2133.3(63.0)	-1.0 (1.6)%	-1.4 (1.4)%	-1.7 (1.5)%	-3.3 (1.6)%*
Hamstrings					
CTRL	880.6(42.6)	-6.0 (3.3)%	-6.4 (3.2)%*	-9.3 (3.2)%†	-11.3 (3.1)%‡
RVE	868.1(38.6)	-6.5 (2.4)%†	-8.3 (2.0)%‡	-10.9 (2.0)%‡	-10.9 (2.2)%‡
Knee Flexors					
CTRL	1176.1(55.7)	-5.3 (3.0)%	-5.0 (3.0)%	-7.6 (3.0)%*	-9.6 (3.0)%†
RVE	1146.1(52.7)	-6.0 (2.5)%*	-7.3 (2.2)%†	-9.5 (2.1)%‡	-9.7 (2.3)%‡
Adductors					
CTRL	769.6(27.1)	-3.2 (2.4)%	-3.8 (3.0)%	-4.5 (1.8)%*	-5.1 (2.1)%*
RVE	780.6(30.6)	-2.4 (2.7)%	-5.8 (2.4)%*	-5.4 (2.7)%*	-6.9 (2.5)%†

At 1st day of bed-rest (BR1) values are mean(SEM) volume in cm³, beyond BR1 values are mean and standard error of the mean percentage change compared to BR1. *: $p < .05$; †: $p < .01$; ‡: $p < .001$ and indicate significance of difference to baseline value. BR=day of bed-rest. Anterolateral tibial muscles = tibialis anterior, extensor hallucis longus, extensor digitorum longus, peroneus longus, brevis and tertius; triceps surae= soleus, gastrocnemius medialis and lateralis; quadriceps= vastii, rectus femoris, hamstrings= biceps femoris (short and long heads), semitendinosus, semimembranosus; knee flexors= hamstrings with gracilis and sartorius; Adductors= adductor magnus and longus.

Table 3. Changes in muscle volume in different muscle groups.

Subject-group	Study-date				
	BR1 (cm ³)	BR14	BR28	BR42	BR56
	Adductor Longus				
CTRL	181.7(11.8)	+2.7 (3.7)%	+0.4 (3.6)%	+0.5 (3.2)%	+0.8 (3.1)%
RVE	192.9(11.5)	-1.2 (2.2)%	-3.4 (1.6)%*	-1.2 (2.0)%	-3.1 (2.6)%
	Adductor Magnus				
CTRL	588.7(22.5)	-5.1 (2.8)%	-5.0 (3.6)%	-6.2 (2.3)%*	-7.0 (2.6)%*
RVE	586.6(25.0)	-2.5 (3.2)%	-6.3 (2.9)%*	-6.5 (3.2)%*	-7.8 (2.9)%†
	Gracilis				
CTRL	118.4(7.0)	-2.9 (2.2)%	-2.7 (2.3)%	-4.0 (2.2)%	-4.4 (2.2)%*
RVE	114.2(7.2)	-3.9 (2.4)%	-3.8 (1.9)%	-4.9 (1.6)%†	-5.0 (2.0)%*
	Sartorius				
CTRL	177.7(10.1)	-3.8 (3.0)%	-0.7 (3.0)%	-2.1 (2.7)%	-4.9 (3.3)%
RVE	164.3(10.9)	-5.1 (3.5)%	-4.8 (3.4)%	-5.4 (3.4)%	-6.3 (3.5)%
	Biceps Femoris Long Head				
CTRL	232.8(16.1)	-5.2 (5.6)%	-6.7 (5.6)%	-10.2 (5.5)%	-12.5 (5.5)%*
RVE	229.0(12.0)	-6.0 (3.0)%*	-9.4 (2.5)%‡	-12.3 (2.6)%‡	-13.3 (2.7)%‡
	Biceps Femoris Short Head				
CTRL	123.7(8.4)	-3.8 (3.1)%	-2.1 (3.0)%	-3.3 (2.7)%	-7.3 (3.0)%*
RVE	119.2(8.3)	-3.6 (3.1)%	-1.8 (1.4)%	-4.8 (0.9)%‡	-3.7 (1.8)%*
	Semimembranosus				
CTRL	273.6(11.5)	-6.5 (2.4)%†	-6.5 (2.4)%†	-11.1 (1.4)%‡	-12.3 (1.3)%‡
RVE	274.2(15.8)	-7.5 (3.8)%	-10.1 (3.7)%†	-14.3 (3.7)%‡	-13.8 (4.0)%†
	Semitendinosus				
CTRL	250.1(16.8)	-6.5 (4.9)%	-7.6 (4.9)%	-8.7 (4.9)%	-10.4 (4.9)%*
RVE	240.2(12.9)	-5.5 (2.3)%*	-6.9 (2.1)%†	-7.7 (1.6)%‡	-7.8 (1.8)%‡
	Rectus Femoris				
CTRL	318.2(19.8)	-4.1 (3.5)%	-2.7 (3.5)%	-2.9 (3.4)%	-5.1 (3.5)%
RVE	311.5(18.7)	+1.8 (2.2)%	+3.7 (1.7)%*	+4.6 (2.0)%*	+4.0 (2.1)%
	Vastii				
CTRL	1914.5(83.1)	-6.7 (3.7)%	-9.9 (3.6)%†	-13.3 (3.5)%‡	-15.9 (3.7)%‡
RVE	1822.4(57.7)	-1.5 (1.6)%	-2.4 (1.5)%	-2.8 (1.5)%	-4.6 (1.6)%†

At 1st day of bed-rest (BR1) values are mean (SEM) volume in cm³, beyond BR1 values are mean and standard error of the mean percentage change compared to BR1. *: $p < .05$; †: $p < .01$; ‡: $p < .001$ and indicate significance of difference to baseline value. BR= day of bed-rest. No differences between groups existed at baseline (BR1; $F_{1,9}$ all < 1.76 , p all > 0.22).

Table 4. Changes in thigh muscle volume during bed-rest and effect of countermeasure.

To facilitate comparison to other studies, the muscles measured were grouped together into larger muscle groups. Both the triceps surae and anterolateral muscle groups showed significant losses of volume over time (study-date: $F_{4,63}$ both > 15.8 , p both $< .0001$), though this reached statistical significance in triceps surae from BR14 and in the anterolateral tibial muscles from BR42 (Table 3). The triceps surae (group: $F_{1,14}=7.9$, $p=.013$, group \times study-date: $F_{4,63}=25.9$, $p<.0001$) but not the anterolateral tibial muscles (group: $F_{1,14}=1.49$, $p=.24$, group \times study-date: $F_{4,63}=.53$, $p=.71$) showed statistical evidence for an effect of the countermeasure exercise.

Effect of resistive vibration exercise countermeasure – thigh musculature

Of the thigh musculature, the vastii, adductor magnus, semimembranosus, semitendinosus, biceps femoris (long and short

heads) and gracilis all showed strong evidence for changes in muscle volume during bed-rest (study-date: $F_{4,62}$ all > 5.08 , p all $\leq .0013$) with reductions in muscle volume seen in the CTRL group during bed-rest (Table 4). Of these muscles, only the vastii demonstrated strong statistical evidence for an different response in the RVE group (group: $F_{1,15}=2.63$, $p=.13$, group \times study-date: $F_{4,62}=30.7$, $p<.0001$), where losses in muscle volume in the RVE only reached statistical significance at the end of bed-rest (BR56), but were evident in the CTRL group from bed-rest (BR28). Of the other muscles, the RVE group appeared to show losses in muscle volume slightly earlier in the biceps femoris (long and short heads), semitendinosus, semimembranosus, adductor magnus and gracilis, though these differences were not statistically significant (group: $F_{1,15}$ all < 2.28 , p all $> .15$, group \times study-date: $F_{4,62}$ all < 1.1 , p all $> .38$).

In both the adductor longus and sartorius muscles, little statistical evidence existed for changes in muscle volume during bed-

Day of bed-rest (BR), head-down tilt (HDT) or lower-limb suspension (ULLS)													
Muscle	14d HDT		20d HDT		20d HDT	BR28	29d HDT	30d HDT	35d ULLS	BR42	BR56	89d HDT	119d BR
	BR14	Zange 2008	Akima 2001	Akima 2007	Akima 2007		Alkner 2004	Berry 1993	Tesch 2004			Alkner 2004	Shackelford 2004
ANT	-1.3%		-9.4%	-2.6%	-3.6%‡		-7.6%*		-4.3%†	-6.5%‡			
PER	+0.6%				-2.7%				-3.8%*	-5.0%*			
FDL	+7.8%				+4.2%				+3.6%	+0.7%			
GLAT	+2.7%	-5.5%	-16.8%†	+1.3%	-3.3%		-6.1%*		-0.1%	-0.2%			-9.2%
GMED	-1.4%	-7.3%*	-12.3%†	-1.1%	-6.1%*		-8.6%*		-4.6%†	-8.7%‡			
SOL	+0.9%	-5.8%	-9.8%†	-5.4%	-1.5%		-12.6%*		-1.1%	-7.2%‡			-6.8%
TS	+0.7%	-6.4%*	-12.2%‡	-3.1%	-3.0%†	-8.0%*	-10.2%*	-11.1%*	-1.9%†	-6.6%‡	-15.0%*		
TIBP	+0.8%				-1.0%				-1.5%	-3.2%			
AL	-1.2%			-2.4%	-3.4%*				-1.2%	-3.1%			
AM	-2.5%			-0.2%	-6.3%*				-6.5%*	-7.8%†			
ADD	-2.4%			-0.7%	-5.8%*				-5.4%*	-6.9%†			-3.4%
GRAC	-3.9%		+3.4%	-1.3%	-3.8%				-4.9%†	-5.0%*			
SART	-5.1%		-3.4%	-4.5%	-4.8%				-5.4%	-6.3%			+4.8%
BFL	-6.0%*		+1.4%	-3.1%	-9.4%‡				-12.3%‡	-13.3%‡			
BFS	-3.6%		-4.6%	+0.3%	-1.8%				-4.8%‡	-3.7%*			
SEMI_M	-7.5%		-5.9%	-7.1%	-10.1%†				-14.3%‡	-13.8%†			
SEMI_T	-5.5%*		-5.9%	-0.9%	-6.9%†				-7.7%‡	-7.8%‡			
HAMS	-6.5%†	-6.1%*			-8.3%‡				-10.9%‡	-10.9%‡			-8.1%
KF	-6.0%*		-3.0%*	-3.4%	-7.3%†				-9.5%‡	-9.7%‡			
RF	+1.8%	-3.6%*	-2.2%	+3.2%	+3.7%*	NS	-12.0%*	+16.7%*	+4.6%*	+4.0%	-9.0%*		
V	-1.5%	-7.0%*	7.4%	0.6%	-2.4%	NS			-2.8%	-4.6%†	NS		
QUADS	-1.0%	-6.6%*	+6.0%‡	+1.0%	-1.4%	NS	-11.0%*	+7.7%*	-1.7%	-3.3%*	NS		+5.2%

ANT: anterior tibial muscles (tibialis anterior, extensor digitorum longus, extensor hallucis longus), PER: peroneals (peroneus longus, brevis and tertius), FDL: flexor digitorum longus, GLAT: gastrocnemius lateralis, GMED: gastrocnemius medialis, SOL: soleus (with flexor hallucis longus in current work), TS: triceps surae, TIBP: tibialis posterior, AL: adductor longus, AM: adductor magnus, ADD: adductors combined, GRAC: gracilis, SART: sartorius, BFL: biceps femoris long head, BFS: biceps femoris short head, SEMI_M: semimembranosus, SEMI_T: semitendinosus, RF: rectus femoris, V: vastii, QUADS: quadriceps femoris. *: $p < .05$; †: $p < .01$; ‡: $p < .001$ indicate significance of difference to baseline value; NS indicates non-significant difference to baseline value (percentage change not reported or volume values reported in graphical form). Vastii data from Akima 2001 and 2007 summarized from reported pre- and post volumes of individual heads of the vastii. *P*-values not reported in male training subjects by Shackelford et al (2004). Data for men and women not reported separately by Tesch et al (2004). BR: bed-rest; HDT: head-down tilt bed-rest; ULLS: unilateral lower-limb suspension.

Table 5. Percentage changes in muscle size in the countermeasure group in the current and other studies.

rest or an effect of the countermeasure (study-date: $F_{4,62}$ both < 1.7 , p both $> .15$; group: $F_{1,15}$ both < 1.8 , p both $> .20$; group \times study-date: $F_{4,62}$ both $< .66$, p both $> .62$; Table 4), but when data were pooled between groups, analysis suggested that some atrophy of sartorius did occur, though this was marginal and did not reach statistical significance until BR56 ($-8.4[3.9]\text{cm}^3$; $p=.033$). Similarly, in the rectus femoris muscle, little evidence existed for changes during bed-rest or an effect of the countermeasure (study-date: $F_{4,62}=1.5$, $p=.22$; group: $F_{1,15}=.35$, $p=.56$; group \times study-date: $F_{4,62}=1.4$, $p=.25$), but inspection of the data in Table 4 shows a subtle (but non-significant) decrease in volume of rectus femoris in the CTRL group. The RVE group, in contrast, demonstrated increased volume throughout the bed-rest phase, though this was statistically significant only at BR28 and BR42.

To facilitate comparison to other studies, we combined muscles into differing groups (Table 3). All muscle groups (adductors, hamstrings, knee flexors, quadriceps femoris) showed significant changes over the course of the study (study-date:

$F_{4,62}$ all > 4.9 , p all $\leq .0017$), with losses in muscle volume in all muscle groups. Statistical evidence existed for a different response of the RVE group for the quadriceps muscle group only (group: $F_{1,15}=.83$, $p=.37$; group \times study-date: $F_{4,62}=8.0$, $p<.0001$), with the increases in rectus femoris volume tending to mask vastii volume loss. To further facilitate comparison to other studies, the changes in muscle volume in the current and other studies are presented in Table 5.

Discussion

The main findings of the current study were that a high-load resistive vibration countermeasure with exercises comprising heel raises, squats and toe raises was most effective in reducing atrophy of the soleus, medial gastrocnemius and vastii muscles during prolonged bed-rest, with losses in volume of these muscles occurring later and to a much lesser extent than in the control group. The exercise countermeasure also prevented

significant changes in muscle volume of the lateral gastrocnemius muscle. Additionally, muscle volume of the tibialis posterior and flexor digitorum longus was stable in the RVE group, with losses seen in the CTRL group. Reductions in peroneal muscle volume occurred later in the RVE group and the rectus femoris muscle actually increased in volume in the RVE group, with no change in the CTRL group. The effects in the tibialis posterior, flexor digitorum longus, peroneal and rectus femoris muscles were, however, statistically weak. Little evidence existed for an effect of the countermeasure on the four members of the hamstrings group, adductor magnus and gracilis, with significant losses in muscle volume occurring in both subject groups. Bed-rest itself had little or no effect on the adductor longus, sartorius and anterior tibial muscles.

The strongest effect of the countermeasure on the extensors of the ankle and knee attests to the use of squats and heel raise exercises - i.e. exercises to target these specific muscle groups. In contrast, the hip extensors (hamstrings and adductor magnus) were unaffected by the countermeasure, and no exercises were specifically performed for these muscles. In other bed-rest and related studies where countermeasure exercise comprised only squat or leg-press exercise (such as on a Cybex²³ or Flywheel device^{9,10,31}; see Table 5) and the calf muscles were not targeted directly, the retention of calf muscle volume was much less effective than in works where exercises also targeted this muscle group (such as the current study and in other works^{11,13}). In contrast to the current work, Akima and colleagues¹³ showed an effect of their leg-press and plantarflexor exercises on reducing hamstrings atrophy in bed-rest. In walking, the semimembranosus and biceps femoris typically activate to decelerate the lower-limb towards the end of the swing phase and are also active in initial load-bearing¹ (i.e. they contribute to a hip extension moment in a position of hip flexion). A squat-type or combined hip-knee extension exercise from a more flexed hip position could activate the different members of the hamstring muscles to a greater degree^{32,33}, and this may be appropriate modification to countermeasure exercise in bed-rest. It should, however, be remembered that the gluteal muscles are also important extensors of the hip (particularly the inferior portion of gluteus maximus such as at heel-strike and mid-stance in walking or in stair ascension)¹. The gluteal muscles do atrophy in bed-rest³⁴, but as yet, no work has considered the differential effect of bed-rest on the hip extensors (i.e. gluteal muscles and hamstrings) and it should also be noted that different rates of atrophy are apparent amongst the four members of the hamstrings muscle group during bed-rest²². Thus, gaining a deeper understanding the effect of bed-rest/spaceflight on the hip extensors as a whole (and not just hamstring muscles) is important for further countermeasure development.

It is worthy to note that although the countermeasure group showed a similar volume loss in the anterior tibial muscles, the exercises in the current study targeted at this muscle group (toe raises) were conducted against low resistance. Whilst the addition of more resistance to the dorsiflexion exercises may result in a greater effect of exercise, this muscle group was

also little affected by bed-rest (in the inactive group)²² and given this lesser susceptibility, the priorities of countermeasure exercise may be better set at exercises for other, more susceptible muscles (e.g. hip extensors²² or more proximal systems such as the lumbar extensors^{35,36}).

Zange and co-workers⁸ also implemented whole-body vibration as part of countermeasure exercise for the lower-limb musculature during bed-rest. Compared to the current work, after 14-days of bed-rest their countermeasure subjects exhibited a relatively greater extent of atrophy, in all of the lower-limb muscle groups except the hamstrings (see Table 5). It is noteworthy that the countermeasure by Zange and co-workers was performed in standing with only an addition of 15% of body-weight and no muscle-specific exercises being performed (i.e. the subjects purely retained their position). It is likely therefore, that this kind of training stimulus was insufficient for the lower-limb muscles. Interestingly, another work, examining vibration exercise at the lumbar spine using static loading at approximately 60% of body-weight found no effect of the countermeasure on losses of lumbar extensor muscle cross-sectional area during bed-rest³⁷. These findings^{8,37} are in line with the argument that higher-load muscle specific exercises are needed if individual muscle size is to be retained. In our opinion, whole body vibration during resistive exercise may help to provide an additional stimulus, above that of resistive exercise alone, for muscle activity and force development and retention of muscle mass during bed-rest. It is important to note that the current study was not intended to examine the extent of any additional benefit of whole-body vibration on muscle loss during bed-rest *per se*, but rather to examine the efficacy of the countermeasure as a whole. Further work is necessary to examine any additive effect of whole-body vibration during resistance exercise.

Deconditioning during spaceflight is not localised to the musculoskeletal system and a variety of body systems (e.g. cardiovascular, vestibular) require countermeasures of their own. Aerobic exercise (such as cycling or lower-body negative pressure) is considered appropriate for the maintenance of the cardiovascular system, but such exercise is ineffective in maintaining muscle^{6,7,38} or bone³⁹⁻⁴¹. A combination of approaches is therefore needed. Astronauts, however, do not have an indefinite amount of time available for exercises and priorities of countermeasure exercise need to be set such that the available time is used most effectively.

In our opinion, there are four key aspects for countermeasure design to ensure the most effective use of exercise time for preventing muscle atrophy in the lower-limbs: 1) which muscles are indeed affected by bed-rest and/or spaceflight? 2) What exercise manoeuvres need to be implemented to target these muscles in the most time effective fashion? 3) What loading levels should be used? 4) What “dose” of exercise (number of times per day or week, duration of exercises) is necessary? In the current study, the muscles that were most affected (>10% loss of volume at the end of bed-rest in the inactive group) were all members of triceps surae, the vastii, long head biceps femoris, semitendinosus, semimembranosus, tibialis

posterior and the peroneals (see also Belavý and colleagues²²). An isolated knee extension exercise would be effective for the vastii muscles, but this would also stimulate the rectus femoris^{32,33,42}, which is much less affected in bed-rest²², and potentially lead to the development of muscle imbalances. Modified leg-press exercises (combined hip and knee extension), on the other hand, target the vastii, with less activation of the rectus femoris^{32,33,42}, but also require contribution from the hamstrings and adductor magnus^{32,33,42}, thus comprising a more time effective exercise. Calf raises, when appropriately performed, can also be effective in stimulating the peroneal and tibialis posterior muscles groups⁴³, though care needs to be taken in exercise selection for calf raises as knee position can markedly modify the contribution of the gastrocnemius and soleus muscles⁴⁴. Controlling the posture of the arch of the foot appears to be an important factor for optimal activation of the tibialis posterior muscle^{44,45}, and this should also be incorporated into exercise. Exercises to target the muscles of the hip and lumbar spine should also be considered^{34,35,37,46,47}.

Furthermore, based upon the results of the current and prior work, it appears that high (between 75-85% of a person's 1-repetition maximum) loading levels⁹⁻¹⁴ during exercise are better at maintaining muscular mass and function than low-load or endurance exercise⁶⁻⁸. This does not imply that low-load exercise has no role to play in countermeasure exercise programmes for maintenance of muscle in bed-rest/spaceflight. In the current work, rectus femoris muscle volume increased whilst vastii volume decreased in the countermeasure exercise group. This could imply the development of muscle imbalance due to exercise. Some subjects did report the development of knee pain during training²⁴, which could imply inappropriate loading and/or muscle activation. One particular benefit of including low-load exercises could be to ensure that the individual can perform the exercises using the correct technique and optimal/appropriate body posture prior to progressing the exercises to a higher-load. This may help to avoid the development of muscle imbalances due to inappropriate movement/loading patterns. However, future work will have to consider this in more detail.

Finally, the frequency (per day or per week) with which exercise countermeasures should be performed during bed-rest/spaceflight is a topic which still requires further study. Works by Alkner and Tesch^{9,10} have shown that resistive exercise on a relatively infrequent schedule (every three days) is capable of reducing lower-limb muscle atrophy during bed-rest (Table 5). Further research on exercise dose during bed-rest could provide valuable information to optimise the efficiency of countermeasure exercises.

It should be noted that in the current work we considered only muscle size when evaluating the effectiveness of the countermeasure exercises. Other aspects of muscle function, such as muscle activation, proprioception and postural control were not evaluated. Whilst it may be possible to argue that changes in muscle size do correlate with changes in muscle force production capacity^{28,48}, other aspects of muscle function, particularly postural control, are dependent upon integration of information from a number of systems (e.g. vestibular, pro-

prioceptive, visual)⁴⁹ which may need to be addressed using additional countermeasures.

In conclusion, we examined the effect of a resistive vibration exercise countermeasure comprised of squats, heel raises and toe raises during 56-days of bed-rest on muscle volume loss in the lower-limbs. We found the countermeasure to be most effective in reducing or preventing atrophy in the three heads of triceps surae and the vastii muscles. Conversely, the countermeasure was comparatively ineffective for the hamstring and thigh adductor muscles. Countermeasure design needs to consider which muscular systems are most affected by bed-rest and also ensure that other body systems (e.g. cardiovascular) are trained. Further work is necessary to evaluate the optimal exercise "dose" (per day / per week) for a time- and cost-effective intervention.

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