CHAPTER 4

Knee extensor fatigability after 8 weeks of bed rest with and without countermeasure

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ABSTRACT

The changes in knee extensor fatigability as a consequence of eight weeks of horizontal bed rest with or without daily resistive vibration exercise were evaluated in 17 healthy male volunteers. Bed rest increased fatigability (% decrease in maximal voluntary isometric torque per minute exercise) from –7.2 ± 0.5 to –10.2 ± 1.0 %/min ($P < 0.05$), which was accompanied by a decline (of 52.0 ± 3.7%, $P < 0.05$) in muscle blood flow. Daily resistive vibration exercise training during bed rest prevented increases in fatigability (from –10.8 ± 1.8 to –8.4 ± 1.6 %/min, $P < 0.05$), and mitigated the reduction in blood flow (decline of 26.1 ± 5.1%, $P < 0.05$). In conclusion, daily resistive exercise may be suggested as an effective countermeasure during spaceflight and illness-related prolonged bed rest to combat the detrimental changes in muscle endurance that result from gravitational unloading.

INTRODUCTION

The adaptation of skeletal muscle due to gravitational unloading extends beyond a mere downsizing of the contractile apparatus. Shifts in myosin phenotype [1;39] and metabolic enzyme activity [37] point towards the conversion of fiber properties to faster and less oxidative characteristics in response to unloading. Gravitational unloading has also been shown to significantly affect the cardiovascular system [7]. Hence, both reduced oxygen delivery and oxygen utilization may impair the capacity for prolonged exercise following unloading. Moreover, such exercise tolerance may be further influenced by impaired muscle activation after gravitational unloading [13;17]. It seems, therefore, reasonable to expect a deteriorated exercise tolerance as a consequence of simulated or actual spaceflight. However, the results of previous reports remain inconclusive. Various investigators have observed increased local muscle fatigability following gravitational unloading [14;26;27], while others have reported unchanged [5;40] or even reduced [10;34] muscle fatigability. These inconsistencies might be partly related to methodological differences between studies, such as the model and duration of gravitational unloading, differences in gender, species (humans vs. rats), or muscles tested, as well as to the individual fatigue protocols used (e.g. sub-maximal vs. maximal and electrically evoked vs. voluntary contractions). Information about possible underlying mechanisms is vital for the understanding of e.g. bed rest-induced changes in fatigability, which may help to develop effective preventative measures.

The primary purpose of the present study was to test the hypothesis that the fatigability of the human quadriceps femoris muscle would be significantly impaired after 56 days of strict horizontal bed rest. This hypothesis was evaluated by means of a 5-min intermittent sub-maximal isometric knee extension fatigue protocol. To locate causes of altered fatigability both at the central and the peripheral level, we recorded surface electromyographic (sEMG) signals from the lateral vastus muscle during the fatiguing exercise. In addition, near-infrared spectroscopy was used to determine whether bed rest-induced changes in fatigability were related to changes in local muscle oxygenation and blood flow.

The major preventative measure to offset the muscle-deconditioning effects of microgravity is physical exercise [6]. Resistive vibration exercise, which comprises both resistance exercise and vibration exercise, has recently been proposed as a promising training modality to preserve bone mass and to maintain muscle mass and strength [29]. Importantly, because resistive vibration exercise training includes a high number of (voluntary and reflexive) muscle contractions it might also attenuate changes in muscle microvasculature and metabolism and thereby preserve muscle endurance capacity [29]. The second major objective of the present study, therefore, was to determine whether changes in fatigability of the knee extensor could be effectively counteracted by daily resistive vibration exercise.

METHODS

Subjects

Twenty male volunteers participated in the study. At the start of the study the subjects were randomly assigned to a training group or an inactive control group. The training group (RVE, n = 10; mean age, height and body mass ± SD: 32.7 ± 4.8 yr, 186.3 ± 8.0 cm and 86.5 ± 16.5 kg) participated in a progressive resistive vibration exercise program during the bed rest. The subjects of the control group (Ctrl, n = 10; mean age, height and body mass ± SD: 33.4 ± 6.6 yr, 185.4 ± 7.7 cm and 79.7 ± 10.9 kg) were restricted to bed rest without countermeasure. Subjects did not participate in any specific training/exercise program prior to the start of the study and the average exercise activity (hrs/wk) prior to the start of the study was similar for the RVE (2.6 ± 2.4) and Ctrl (2.4 ± 3.6) group. The study received approval of the local ethics committee and all participants gave their written informed consent.

General design

All subjects were confined to 56 days of strict horizontal bed rest at the Benjamin Franklin Hospital of the Charité – Universitätsmedizin Berlin, Germany. During this period, the subjects were not allowed to stand up, to lift their upper body in bed more than to 30° of trunk flexion, to move their legs briskly, or to elicit large forces with their leg muscles other than during testing or vibration training sessions. Adherence to this protocol was controlled for by continuous video surveillance and by force transducers in the frames of the bed. The diet was balanced with regard to caloric intake, using the Harris-Benedict equation with an adjustment by an activity factor of 1.2 for bed rest [29]. Daily diet plans were prepared, using the nutrition-software EBISpro (Dr. Erhardt, University of Hohenheim, Germany). All meal components were weighed, and their nutritional contents were taken from prepared meal charts. Calcium input was set at 100mg per day. Ingestion of alcohol or nicotine, as well as the regular intake of medication was prohibited.

Exercise-based countermeasures

RVE subjects performed resistive exercise on a vibration system that was specifically developed for application under microgravity and bed rest conditions (Galileo Space, Novotec, Pforzheim, Germany). The applied equipment and protocol for countermeasure exercise are described in detail elsewhere [29]. In short, the training device consists of a vibration platform, which is vertically suspended on a trolley (Fig. 1). Elastic springs were attached to the trolley for the subjects to attach themselves through belts with their shoulders, hips, and hands. During bed rest, RVE subjects trained two times each day, for 6 days per week. In each training session, four resistive exercises were performed in the following order: squats, heel raises, toe raises and explosive squats. During the squat exercise the knees where extended from 90° to almost complete extension in cycles of 6 seconds for each squat. The heel and toe raises were performed with the knees almost extended.

During the heel raise exercise, the heels were raised to fatigue. Only then, brief rest periods (< 5 s) were allowed with the entire foot on the vibrating platform in order to recover, and subjects started to raise heels again. For the toe raise exercise a similar protocol was used, but toes were raised instead of heels. During the explosive squatting exercises 'kicks', knees were extended as quickly and forcefully as possible. The platform was struck with the balls of the feet, and legs rested on the Galileo framework between the kicks. This was done ten times with 10 s or rest inserted between each kick. During morning sessions, a static force, equivalent to approximately 2 times the body weight was generated with the legs in full extension. All exercises were performed while the platform was vibrated at a frequency of 19Hz. According to the overload principle in exercise physiology, vibration frequency and thus the applied force [30] was progressively increased to ~ 26Hz at the end of bed rest [29], so that time to exhaustion during the squat exercise remained between 60-100s (i.e. between 10 and 17 repetitions) during the morning sessions. Only on Wednesday mornings, subjects were asked to maximally exert themselves and do each exercise unit as long as possible. During afternoon sessions, subjects exercised at about 1.4 times the body weight (i.e. about 70 % of the static force used in the morning sessions), but to run through the squat, heel an toe raise exercises for 60 seconds each as many times as possible, without rest. The four resistive exercises were performed during vibration, with both legs simultaneously and with the feet equally distant of either side of the rotation axis at the vibration platform. No training sessions were scheduled on Sundays. Trained staff supervised all training sessions.

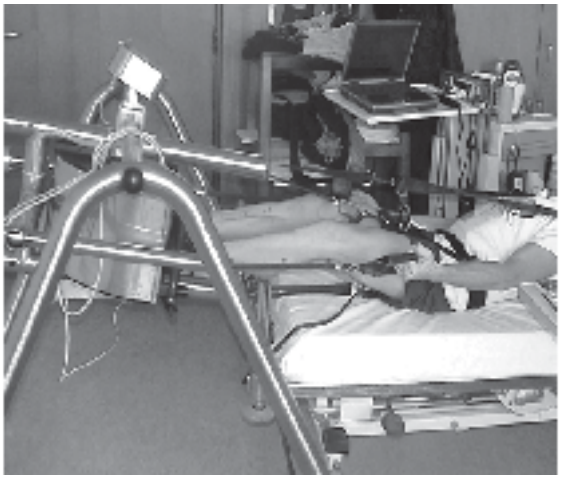

Fig. 1. Resistive vibration training device for usage in bed rest. The subjects attach themselves to a vibrating platform by belts with their waist, their shoulders and their hands. Voluntary resistive exercises are performed whilst vibration of the platform is generated by means of eccentrically rotating masses.

Measurement procedures
Subjects were tested on two occasions: baseline measurements were performed one day prior to the start of bed rest. The post bed rest measurement was scheduled on the third day of re-ambulation. The design of the measurements, which were performed in supine position, has been previously described [25]. In short, the hips were flexed to approximately 115°. The knee pits were supported by a padded rigid horizontal bar, and the subject's left and right feet were strapped in custom-built padded cuffs, with the ankle joints in neutral positions. The cuff of the right leg was connected to a force transducer (KAP-E/2kN, A.S.T. GmbH, Dresden, Germany) that was mounted on a rigid horizontal bar and oriented perpendicularly to the line of pull of the lower leg. The distance between the transducer and the axis of the knee joint (moment arm) was adjusted for each subject and was kept constant between experiments. The knee flexion angle was set at the individually determined optimal angle (either 60 or 70°), as described previously [25] and remained constant between experiments. The pelvis and upper body were securely fixed to the dynamometer by belts. Isometric force was recorded during voluntary contractions of the quadriceps femoris muscle of the right leg. Force signals were digitized using a sampling rate of 1ksamples/s and stored to disc for off-line analysis. Knee extension torque was calculated as the product of force and moment arm.

Subjects started each experimental session by performing a specific warm-up set that consisted of eight sub maximal isometric contractions of the right leg at ~40% of the individual maximal voluntary contraction. The isometric contractions were sustained for 2 s, with 4 s of rest in between. A horizontal line, displayed on the force acquisition monitor that was placed in front of the subjects, presented the target force. A second line presented the current force level generated by the subject to provide visual feedback. An audible signal was provided for the duration of the contractions. Following the warm-up, the subjects were asked to maximally exert isometric torque for 2 - 4 s. Three to five maximal attempts were made, interposed with a minimum of 2 min of rest each. The highest force was taken to calculate the maximal voluntary torque (MVT).

Fatigue task
We used an intermittent sub-maximal isometric protocol to induce volitional muscular fatigue. Such a protocol was considered more appropriate to detect changes in skeletal muscle fatigability following disuse than a sustained test protocol. First, intermittent exercise is metabolically more demanding than continuous exercise, because more energy is consumed for a given amount of isometric work [32]. Secondly, as compared to intermittent exercise, moderate or high intensity sustained exercise may rely predominantly on anaerobic energy supply because it partly or completely occludes blood vessels [35]. Because oxygen delivery is a critical factor in relative muscle fatigability, a sustained testing protocol would likely underestimate the effects of gravitational unloading on muscle fatigability.

We designed a 5 min intermittent endurance test that was anticipated to be difficult, but not impossible to complete after 56 days of bed rest. Based on pilot studies, the target torque during the main measurements was set at 45% of the actual MVT, i.e. at the same relative contraction

intensity, pre and post bed rest. Setting the exercise intensity at a relative target torque level allowed the investigation of qualitative changes in fatigue characteristics, irrespective of changes in maximal torque generating capacity due to muscle atrophy. The subjects performed five consecutive exercise blocks of 1 min duration each. Each block consisted of 24 repeated isometric contractions that were sustained for 1.5 s with 1 s of rest in between (Fig. 2A). During the sub maximal contractions, both visual and auditory feedback was identical to the warm-up procedures. Within 2 s after each exercise block the subjects were instructed to perform one single MVC of 2 – 4 s in duration. In cases where subjects failed to reach the target torque during the sub-maximal contractions in 3 consecutive attempts, due to exhaustion, the subjects finished the ongoing exercise block, performed one final MVC, where after the fatigue test was ended.

sEMG acquisition

In parallel with isometric knee extensor torque, sEMG signals were recorded from the distal one-third, anterio-lateral part of the right vastus lateralis muscle by means of a high-density sEMG system (Active One, BioSemi Inc., Amsterdam, The Netherlands). The system consisted of 130 densely spaced skin-surface electrodes (5 mm inter electrode distance), arranged in a rectangular 10 x 13 matrix. The columns of 13 electrodes were aligned parallel to the muscle fiber orientation of the vastus lateralis with the motor endplate zone around the center of the columns. Before mounting the matrix, the skin was shaved, scrubbed with alcoholic pads and slightly rubbed with electrode paste. Prior to each test, the skin-electrode impedance was checked and, if necessary, the site was re-prepared. Because of the small inter-electrode distance on the high-density electrode grid, any superfluous electrode gel was removed in order to avoid short-circuiting between neighboring electrodes. The pre-amplified 130 monopolar signals (referenced to a remote electrode positioned over the patella) were band pass filtered (0.16-400Hz) and simultaneously AD-converted (16 bits with a resolution of 1 μV/bit) at a rate of 2048 samples/s/channel. Data were stored on hard disk for subsequent off-line processing.

Voluntary torque and sEMG data processing

Voluntary torque and sEMG data obtained during sub-maximal contractions where processed only when the peak voluntary torque during these attempts exceeded 30% of the initial MVT. Unexpectedly, not all subjects were able to perform all 120 sub-maximal contractions (5 exercise blocks times 24 contractions) according to this criterion before bed rest. In addition, the number of completed exercise blocks and hence number of performed sub-maximal contractions also varied between sessions (see Table 1 for mean group values). Hence, a complete data set consisting of 120 sub-maximal and 6 maximal voluntary contractions (i.e. initial and following minutes 1-5), for both pre and post bed rest, was obtained only for five Ctrl and five RVE subjects. To overcome difficulties in statistical analyses due to the differences in exercise time between subjects and conditions, torque and sEMG data were analyzed by means of linear regression. Changes in voluntary torque and sEMG variables during the fatigue test were expressed in terms of percentage rate of change of initial value per minute of exercise [27]. Regression of MVT data (torque and RMS) was thus derived from 4-6 data points, whereas regression of torque and sEMG variables during sub-maximal contractions was derived from 72-120 data points (see Fig. 2).

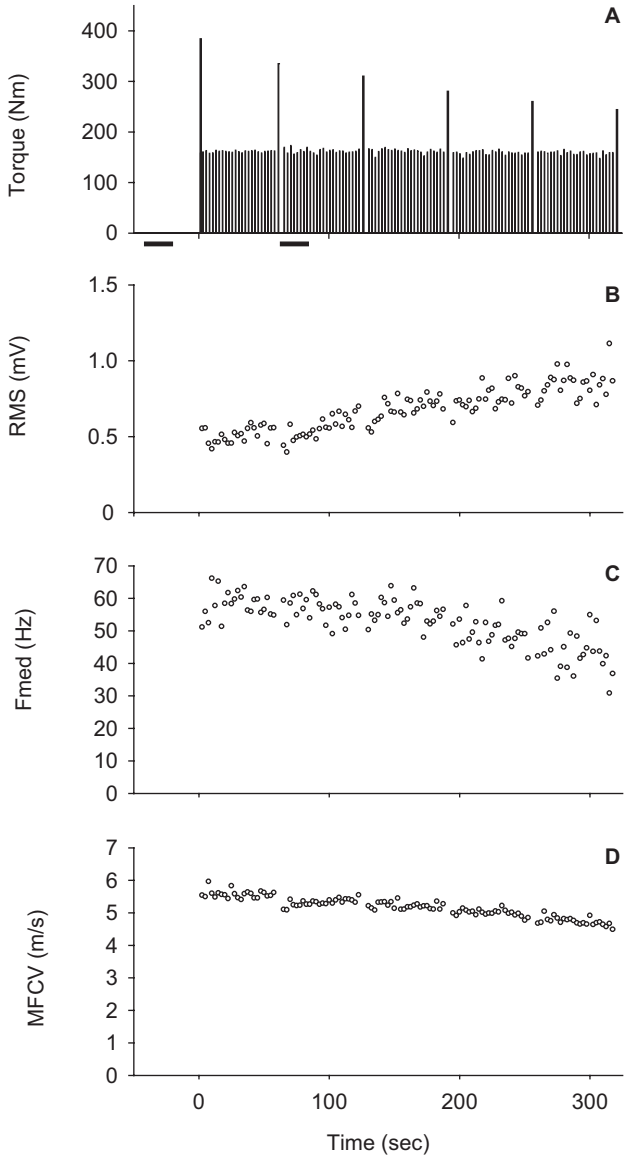

Fig. 2. Representative torque and sEMG profiles of one subject during the fatigue task. The vertical bars in panel A represent the voluntary torque, the open circles in panels B, C and D represent the different sEMG values (respectively RMS, root means square; Fmed, median frequency; MFCV, muscle fiber conduction velocity) obtained during the intermittent sub-maximal isometric contractions. Near-infrared spectroscopy recordings were obtained at rest and during the first 20 sec of the second consecutive exercise block (horizontal bars in panel A).

Voluntary torque. The fatigability of the quadriceps muscle was defined as the percentage decline in MVT from initial value per minute exercise. Although the peak relative torque was set at 45% of initial MVT and contractions were timed by a metronome, the actual relative stimulus to induce fatigue may vary somewhat between conditions. To check for such possible differences, the normalized time torque integral (TTI) was calculated for each sub-maximal contraction, which was defined as the area under the normalized (for the initial MVT) torque-time curve [32]. Regression analysis on these data was thus used to check for systemic changes during the fatigue test.

sEMG. For each maximal voluntary contraction during the fatigue tasks, the RMS was calculated over a 1s time interval. sEMG signals were also analyzed for each sub-maximal contraction for the period that the voluntary torque exceeded 30% of the baseline MVT. For each sub-maximal segment the sEMG was quantified for signal amplitude (RMS) and median frequency (Fmed) from a monopolar recording. Muscle fiber conduction velocity (MFCV) estimates were derived from bipolar recordings and calculated from the time delay between two differential signals, spaced 10mm apart (i.e. a double inter-electrode distance [2]). MFCV values were calculated only when correlation coefficients between consecutive bipolar signals exceeded 0.8 and the MFCV values obtained were less than 8.0 m/s. We were able to obtain MFCV estimates for 7 Ctrl subjects and 7 RVE subjects. All sEMG variables were subsequently averaged for each column. The column with the highest mean RMS was selected and the mean sEMG values of this column were used in the regressions. This spatial selection was performed both pre and post bed rest.

Near-infrared spectroscopy

Local oxygenation and hemodynamics of the vastus lateralis were monitored by near-infrared spectroscopy, using a triple-wavelength continuous wave spectrophotometer (NIRO 300, Hamamatsu Photonics, Japan). The probe consisted of a near-infrared light emitter optode and a corresponding receiver optode with 3 closely placed photodiodes. The probe, emitter and receiver optode were positioned in a soft black probe receptacle (Probe Holder S: A 7928; Hamamatsu Photonics, Japan) providing an inter optode distance of 5.0 cm and fixed to the skin without tension. The receptacle was placed directly proximal to the sEMG grid on the skin over the right vastus lateralis muscle.

In the NIRO 300, three pulsed laser diodes provide light in the near-infrared range at the wavelengths λ = 778, 813, and 853 nm. Attenuation of scattered light was detected for each near-infrared wavelength at a sampling rate of 6 samples/s. Hemoglobin oxygenation within the scanned tissue section was measured by spatially resolved reflectance spectroscopy [38]. In brief, spatial resolution measures the light attenuation gradient as a function of source-detector separation that is achieved by simultaneous measurement at the three differentially spaced photodiodes. From the relative concentrations of oxygenated and deoxygenated hemoglobin, a tissue oxygenation index (TOI) reflecting hemoglobin oxygen saturation in the scanned tissue section can be calculated as

$$\text{TOI} = \text{oxygenated hemoglobin} / (\text{oxygenated} + \text{deoxygenated hemoglobin}) \bullet 100\ \%$$

Local muscle blood flow was estimated from the kinetics of an intravenous bolus of indocyanine green (Pulsion Medical Systems, Munich, Germany) [20]. In brief, 0.02ml/kg body weight of a 5 mg/ml solution of the tracer dye were rapidly (< 1 s) injected into an antecubital vein of the left arm. Indocyanine green concentration within the scanned tissue section was calculated by the modified Beer-Lambert law [20]. From the kinetics obtained during the tracer bolus passage, a relative blood flow index (BFI) was calculated by dividing the maximum indocyanine green concentration of the indicator dilution curve by the rise time, defined as the time interval between 10 and 90% of the maximum concentration [20].

Hemoglobin oxygenation and hemodynamics were assessed at rest and during the first 20 seconds of the second consecutive block (see Fig. 2A) of the fatigue exercise protocol. Rest measurements were conducted after the subjects had been in the supine position for a minimum of 30 min. To avoid potential crosstalk between the indocyanine green bolus tracking and spatially resolved spectroscopic signals, TOI was assessed prior to the administration of the tracer. TOI was thereby calculated as the mean value over 5 s.

Statistical analysis

Data are presented as means ± SEM. Differences in the response to bed rest between the RVE and the Ctrl group with respect to voluntary torque, sEMG and near-infrared spectroscopy variables were tested with repeated measures ANOVA, with time as within-subject factor and group as between subjects factor (Statistical Package for Social Sciences, SPSS 12.0). The time factor represents the overall effect of bed rest. The time-by-group factor was used to test the effect of the RVE countermeasure. If a time-by-group interaction was found, further analysis consisted of a paired-samples *t*-test between pre- and post bed rest data within each group. Unpaired samples *t*-tests were performed to test for differences between groups, pre and post bed rest. Pearson's correlation coefficients were calculated to test for correlations between changes in muscle blood flow, tissue oxygenation and fatigability. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

In one single subject of the Ctrl group, sEMG recordings were not obtained during the post bed rest session due to an improper fixation of the sEMG grid. Another Ctrl subject encountered muscle cramps during the post bed rest measurements, which prevented this subject to complete the protocol. A third subject (RVE group) could not participate in either session because of patellar discomforts during the performance of maximal isometric contractions. The data of these subjects were discarded from the final statistical analyses. Results are thus presented for 17 subjects (8 Ctrl subjects and 9 RVE subjects), except for MFCV estimates, which were based on 14 subjects, as mentioned.

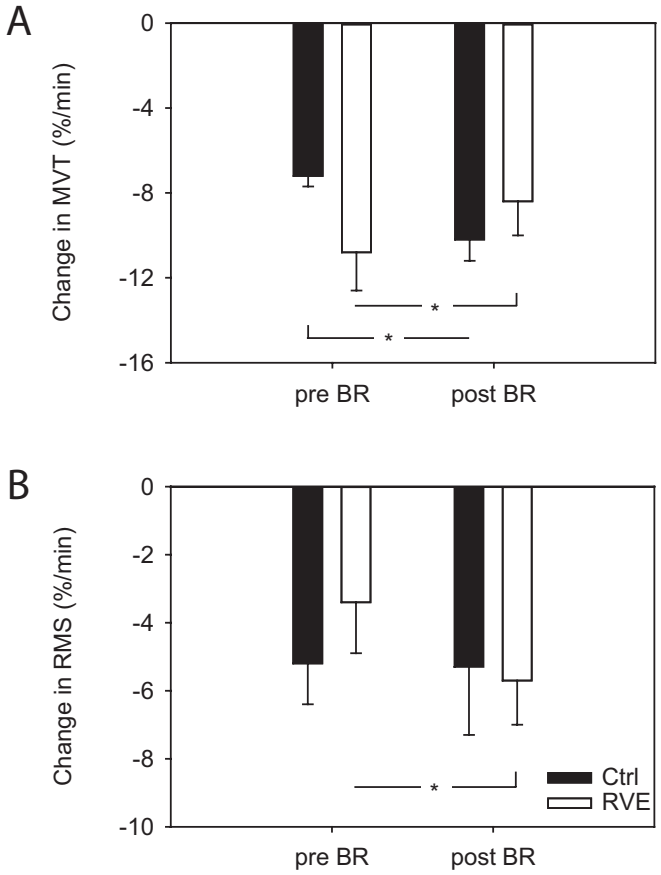

Fig 3. Fatigability (A) of the Ctrl and RVE group expressed as the percent change of initial maximal voluntary torque (MVT) per minute exercise and (B) the percent change per minute exercise from the initial maximal root mean square (RMS) of the sEMG obtained during the maximal contractions, pre and post bed rest (BR). Values are expressed as mean ± SEM. * significant difference ($P < 0.05$) between pre and post bed rest (BR).

Voluntary torque

The initial (non-fatigued) MVT decreased as a consequence of bed rest for both groups (both $P < 0.01$). For Ctrl it decreased from 317 ± 17 to 267 ± 18 Nm and for the RVE group, it decreased from 300 ± 14 to 268 ± 17 Nm. The change in MVT between the days of testing was not significantly different between groups. Fatigability was enhanced following bed rest without countermeasures, but RVE training not only prevented this effect, but even induced a reduction in fatigability ($P < 0.05$, Fig. 3A). TTI, calculated for each sub-maximal contraction [32] and

used to check for changes in the relative stimulus to induce fatigue, declined somewhat during the repeated contractions in all conditions (Table 1). For Ctrl the rate of decline was similar during pre- and post bed rest conditions, whereas for RVE, a slightly smaller decline of TTI was observed, i.e. an attenuated reduction in relative workload during the fatiguing protocol, following bed rest. The groups also responded oppositely ($P < 0.05$) with respect to the number of blocks that the subjects performed prior to and after bed rest. Two Ctrl subjects performed 1 block less following bed rest, as compared to before, whereas in contrast 3 RVE subject performed 1 or 2 blocks more following bed rest. However, the changes within the groups did not reach statistical significance at the group level (Table 1).

Table 1. Mean values of changes in Time Torque Integral and the number of exercise blocks completed, pre and post bed rest

	Ctrl	**RVE**	**Significant group by time interaction**
Change in TTI, %/min			
Pre BR	-4.4±1.4	-6.1±2.2	
Post BR	-6.0±1.3	-3.3±1.8*	†
Completed exercise blocks			
Pre BR	4.9±0.1	4.3±0.3	
Post BR	4.6±0.2	4.8±0.1	†

Values are mean ± SEM. * significant difference between pre and post bed rest (BR). † significant time course difference between Ctrl and RVE group ($P < 0.05$). TTI, time torque integral.

Surface electromyography

Representative changes in sEMG variables as a consequence of the fatiguing sub-maximal contractions are given for one subject in Fig. 2B – D.

sEMG amplitude. The sEMG amplitude (RMS) decreased during the maximal voluntary contractions (Fig 3B, all $P < 0.05$) and increased during sub-maximal contractions for each fatigue protocol in both groups (Fig 4A, all $P < 0.05$). The rate of decrease in maximal RMS was significantly greater in RVE after as compared to before bed rest ($P < 0.05$), whereas no changes were seen for Ctrl. The rate of increase in sub-maximal RMS was similar among groups during both sessions and no changes were observed as a consequence of bed rest. Mindful of the changes in maximal RMS during the fatigue protocol, we quantified the mean RMS of the last 10 sub-maximal contractions performed during each fatigue protocol, which were expressed as percentage of the RMS obtained during the final maximal voluntary contraction of the fatigue test. No significant differences were observed between Ctrl and RVE prior to (66.7 ± 5.7 vs. 63.4 ± 5.3%) or after bed rest (76.1 ± 5.6 vs. 74.7 ± 7.5%). In addition, no changes were observed between sessions.

sEMG median frequency. The median frequency (Fmed) of the sEMG decreased during the sub-maximal contractions for both groups, both pre and post bed rest (Fig. 4B, all $P < 0.01$). In the absence of countermeasures performed during bed rest, the decline in median frequency was greater post bed rest, when compared to pre bed rest ($P < 0.05$). RVE training prevented such a change.

sEMG muscle fiber conduction velocity. For Ctrl, mean muscle fiber conduction velocity (MFCV) decreased as a consequence of the repeated sub-maximal contractions, both pre and post bed rest ($P < 0.05$), and the rate of decline was enhanced ($P < 0.05$) post bed rest (Fig. 4C). In contrast, for RVE mean muscle fiber conduction velocity was unaffected by the test protocol, both pre and post bed rest

Near-infrared spectroscopy

At rest. BFI and TOI did not differ between groups prior to the start of bed rest. BFI was lower ($P < 0.05$) after bed rest in both groups, whereas no significant change was observed in muscle TOI at rest (Table 2).

During exercise. Compared to resting conditions, BFI was elevated during exercise, whereas TOI was reduced (Table 2). In Ctrl, BFI and TOI were markedly lower after as compared to before bed rest ($P < 0.05$ each). BFI was also reduced after bed rest for RVE ($P < 0.05$), yet the reduction was attenuated when compared to Ctrl ($P < 0.01$). No changes were seen in TOI during exercise for RVE. As such, post bed rest values for BFI and TOI were higher for RVE than those obtained for Ctrl subjects ($P < 0.001$ each). As shown in Fig 5, significant negative correlations were found between the relative pre to post bed rest change (i.e. the percent change from baseline) in BFI and fatigability ($P < 0.01$, $r = 0.68$, $n = 17$, Fig. 5A) as well as between the relative changes in TOI and fatigability ($P < 0.01$, $r = 0.65$, $n = 17$, Fig. 5B).

Table 2. Blood flow and tissue oxygenation measured by Near-infrared spectroscopy during rest and exercise, pre and post bed rest

	Ctrl	**RVE**
BFI (nmol/L/s)		
Pre BR		
Rest	8.2±0.8	8.4±0.9
Exercise	23.4±0.7	23.3±1.7
Post BR		
Rest	5.6±0.7*	7.3±0.6*
Exercise	11.4±0.9*†	17.0±1.3*
TOI (%)		
Pre BR		
Rest	73.7±0.6	73.3±0.5
Exercise	54.1±1.2	53.2±1.3
Post BR		
Rest	73.4±1.0	72.2±2.1
Exercise	42.1±1.2*†	54.9±1.7

Values are means ± SEM. * significantly lower post bed rest (BR) as compared to pre BR values ($P < 0.05$). † significantly lower as compared to RVE ($P < 0.05$). Compared to resting conditions, BFI was significantly elevated during exercise, whereas TOI was significantly reduced in both groups. BFI, blood flow index; TOI, tissue oxygenation index.

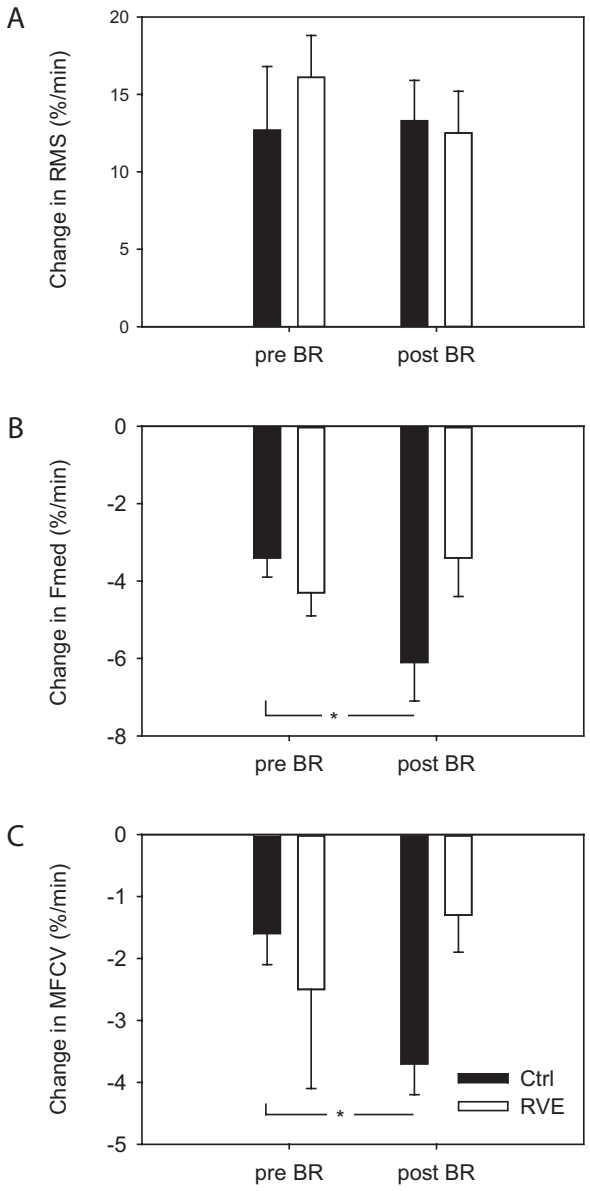

Fig. 4. Changes in sEMG amplitude; RMS (A), median frequency; Fmed (B) and muscle fiber conduction velocity; MFCV (C) for the Ctrl and RVE group as a consequence of the fatiguing sub-maximal contractions, pre and post bed rest (BR). Values (mean ± SEM) are expressed in percent change of initial value per minute exercise. * significant difference ($P < 0.05$) between pre and post bed rest (BR).

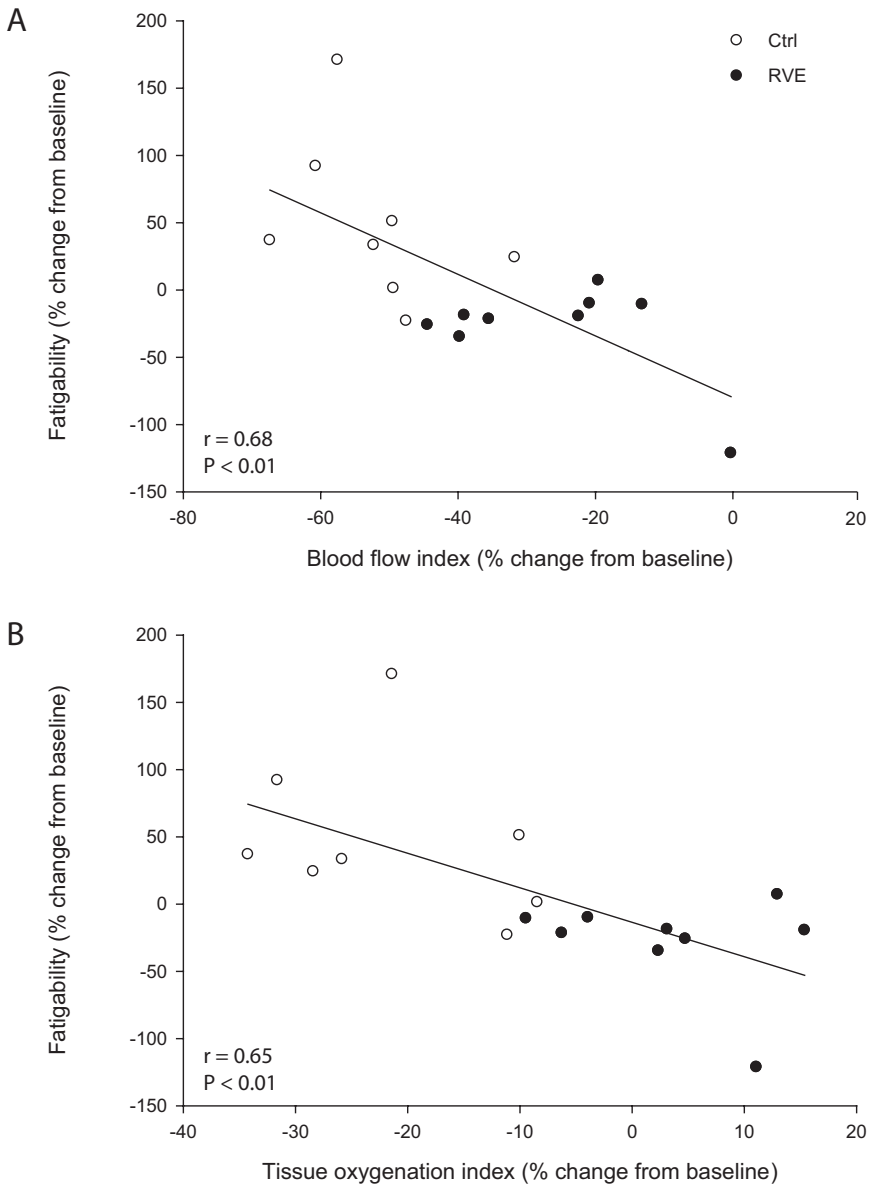

Fig. 5. Correlation between bed rest-induced changes in muscle blood flow and fatigability (A) and correlation between bed rest-induced changes in tissue oxygenation index and fatigability (B). Individual values (n = 17) are expressed as percent changes from baseline. Both linear regressions were significant.

DISCUSSION

Bed rest induced changes in fatigability

In the present study the effect of eight weeks bed rest on the resistance to fatigue of the quadriceps femoris muscle during voluntary repetitive sub-maximal isometric knee extension was assessed. While the relative target torque (45%) was kept equal across pre- and post bed rest conditions the fatigability of the quadriceps was significantly accelerated by approximately 50% after the bed period. The ability to maintain torque output during repeated voluntary contractions depends on intrinsic/metabolic properties of the muscle fibers, local blood flow providing the muscle with oxygen as well as on neural activation properties. To obtain information about neural and metabolic/circulatory properties, which may separately or in combination underlie bed rest-induced alterations in exercise tolerance, we additionally recorded sEMG and near-infrared spectroscopy signals during the fatiguing exercise.

sEMG profiles during the fatigue task

RMS declined during the maximal voluntary contractions in the fatiguing task. This finding is consistent with the loss in sEMG signal amplitude during a sustained maximal contraction [31], and may be explained by the development of central activation failure [33], the decrease in MFCV [36], or a reduced mean motor unit firing frequency [23]. In contrast, central drive was intensified during the sub-maximal contractions, as indicated by the increase in RMS during these contractions (Fig. 4A). In agreement with other studies [24;31], the increase in sub-maximal sEMG amplitude is considered to reflect compensation for the fatigue-induced loss in force output of individual motor units by the recruitment of additional motor units and some modulation of the discharge rate of the motor units [15]. In addition, a fatigue-induced increase in synchronization of motor unit firing patterns [19] may contribute to the increase in RMS. Though we cannot exclude the possibility of loss of signal for the sub-maximal contractions due to e.g. amplitude cancellation [18], no consistent changes were observed in either maximal or sub-maximal RMS profile after as compared to before bed rest. Therefore, a similar percentage of the maximal instantaneous neural capacity should be expected towards the end of each fatigue protocol. Indeed, the mean RMS of the 10 last sub-maximal contractions, expressed as percentage of the instantaneous RMS level of the last maximal voluntary contraction, was not significantly different between pre and post bed rest (66.7 $\pm$ 5.7% vs. 76.1 $\pm$ 5.6%). This suggests that changes in neural activation [34] cannot explain the increased fatigability after 56 days of bed rest.

Two additional sEMG variables that are frequently used to rate local muscle fatigue are the median frequency (Fmed) of the power density spectrum and the mean muscle fiber conduction velocity (MFCV). Consistent with previous reports [24], MFCV decreased during the pre and post bed rest fatigue protocol (Figs. 4B and C), which reflects the deteriorating metabolic status of the muscle [22]. The finding that both Fmed and MFCV declined at a faster rate following bed rest as compared to before, strongly suggests that an accelerated development of peripheral fatigue (i.e. within the muscle itself) occurred following bed rest.

Muscle blood flow and oxygenation

In skeletal muscle, approximately 70% of the obtained signal with near-infrared spectroscopy is derived from the venous compartment, whereas capillaries and arterioles contribute 20% and 10%, respectively [28]. The muscle tissue oxygenation index (TOI) reflects the balance between oxygen delivery and consumption within tissues [4]. The combined measurements of muscle blood flow and oxygenation, therefore, allow determining whether impaired muscle blood flow may have presented a functional limitation during repeated sub-maximal contractions and thus contributed to an increased peripheral fatigability following bed rest.

The present data clearly show that during the repeated isometric contractions muscle blood flow increases substantially (Table 2) as compared to resting conditions, which is in accordance with previous reported results [21]. More importantly, our data demonstrated a substantial reduction of approximately 50% in muscle blood flow during exercise, following bed rest (Table 2), thus suggesting a severe restriction in oxygen delivery. This diminished perfusion during exercise cannot be solely related to the reduction in target torque, since changes in blood flow markedly exceeded this in target torque, and since the effects of bed rest on muscle blood flow were attenuated in the RVE group, which did not differ in target torque from Ctrl subjects. Similarly to previous data published by Ferretti et al. [12] the limitation in oxygen supply was in part compensated for by a higher relative extraction of oxygen, as reflected by a diminished TOI. This finding indicates a reduced partial pressure of oxygen in the skeletal muscle during exercise following bed rest as compared to pre bed rest. The negative correlations between bed rest-induced changes in fatigability versus changes in blood flow and tissue oxygenation suggest that the greater rate of peripheral fatigue following bed rest likely reflects an increased need for anaerobic energy supply during exercise. This notion is supported by data of Grichko et al. [14], who demonstrated an increased glycolytic activity during exercise, following gravitational unloading in rats.

In addition to its effect on oxygen delivery, the reduction in blood flow may have also reduced the capacity to washout metabolic waste products following bed rest. As oxidative capacity is diminished in acidic environments [16], the lack of sufficient blood flow may thus have caused a faster fatigue of the knee extensor muscle group following bed rest, because it limited oxidative metabolism both directly and indirectly. Mechanisms underlying the observed reduction in muscle blood flow remain speculative at this point, but likely involve adaptation processes within the (micro)vasculature of the skeletal muscle [3;9].

Confounding factors in experimental models of muscular fatigability

The observed deteriorated fatigue resistance is in line with other reports of increased skeletal muscle fatigability following different models of gravitational unloading, including hind limb unloading in rats [14], and bed rest [27] and spaceflight [26] in man. Yet, muscle fatigability has also been reported to remain unchanged [11;40] or even to decrease [10;34] following gravitational unloading in both humans and rats. Although it is difficult to identify the reasons for this inconsistency, it may in part be attributable to differences in the models and durations of unloading, in gender, species, and muscles tested. In addition, the methodology used to induce

muscular fatigue may in part explain differences between studies. In contrast to intermittent contractions, sustained contractions may lead to partial or complete occlusion of blood vessels [42]. Such protocols would tend to exclude the potential effects of structural and functional (cardio)vascular changes following gravitational unloading [8;41]. Furthermore, in order to accurately assess the influence of actual or simulated spaceflight on muscle fatigability it seems important to compare the performance of the muscles at equal relative target workloads (that is at a similar percentage of maximal voluntary torque). Differences in fatigability may be overestimated when the sub-maximal target torque is fixed between experiments [27]. Normalization of the target torque to the actual maximal voluntary torque does not take into account a potential reduction in neural activation following unloading, which could in turn result in underestimation of relative fatigability [10;11]. Such a bias could be excluded in the present study, because neural deconditioning was not observed during bed rest for either group [25]. Moreover, altered fatigue responses following muscle unloading not only result from differences in the peak relative target torque but may also arise when differences exist in the total relative stimulus to induce fatigue. Although we observed a reduction in the TTI during the fatiguing protocol for both pre-and post bed rest conditions, the degree of the reduction was similar, confirming that the relative workload was indeed similar between conditions (Table 1) in the present study. These results indicate that the changes in fatigability following bed rest were not affected by changes in the execution of the protocol. Finally, the subjects of the present study were all men. Because muscle fatigability following muscle unloading may depend on gender [34], the findings of this study can be applied only to men at this point.

Efficacy of resistive vibration exercise

Exercise training is used as the primary preventative measure to preserve human physiological systems that are otherwise deconditioned by spaceflight [6]. Here, we demonstrate that resistive vibration exercise not only effectively counteracted the reduction of exercise tolerance following eight weeks of bed rest, but even significantly reduced muscle fatigability following bed rest as compared to pre bed rest, as indicated by an attenuated loss of maximal knee extension torque during the exercise protocol (Fig. 3A). These changes occurred despite a small but significant attenuated reduction in TTI during exercise after bed rest. This would suggest that the RVE subjects performed slightly better in terms of relative workload, and hence, that their enhanced fatigue resistance following bed rest is in fact underestimated. The increased exercise tolerance in RVE subjects at the peripheral level was further reflected in unaltered Fmed and MFCV profiles during the sub-maximal contractions (Fig. 4). A paradoxical finding in the present study was the faster rate of decline in RMS obtained at the maximal attempts post bed rest (Fig. 3B). A faster decrease in the (maximal) discharge rate of alpha motoneurons during the post bed rest fatigue task ("muscle wisdom" [23]) could account for the faster decline in RMS at the maximal attempts without additional loss in maximal voluntary torque. Still, like in the Ctrl group, the mean RMS of the 10 last sub-maximal contractions, expressed as percentage of the instantaneous RMS level of the last maximal voluntary contraction, was similar between pre and post bed rest (63.4 $\pm$ 5.3% vs. 74.7 $\pm$ 7.5%). These findings indicate that both groups exercised at an equal percentage of their maximal neural capacity at the end of each task.

RVE training limited the reduction in muscle blood flow and increased oxygenation during exercise as compared to Ctrl subjects (Fig. 5) and thus effectively counteracted the effects of bed rest. Although RVE did not fully preserve muscle blood flow at exercise, the absence of changes in TOI suggests that tissue oxygenation and thus oxidative metabolism during exercise were not critically limited after bed rest in the RVE subjects. The previous finding that the current exercise regime mitigated the reduction in arterial femoral diameter in the trained group [3] further supports the notion that sufficient tissue perfusion was maintained in the RVE trained subjects during exercise. Thus, resistive vibration exercise may have preserved muscle endurance at least in part due to attenuation of structural and functional changes in the muscle (micro)vasculature [3]. The combined nature of the exercise intervention makes it impossible to say whether it was the vibration per se that elicited these effects. Future studies will be needed to clarify this issue.

In conclusion, the present study demonstrates that the fatigability of the quadriceps femoris muscle during voluntary intermittent sub-maximal isometric knee extension was significantly enhanced following eight weeks of bed rest. Collectively, the sEMG and near-infrared spectroscopy data suggest that the enhanced fatigability following bed rest is primarily related to impaired blood flow resulting in an impaired oxidative capacity. The resistive vibration exercise countermeasure induced a reduction in fatigability, prevented changes in fatigue-related sEMG variables, and mitigated the changes in blood flow. Such time efficient (6min/day) exercise paradigm may therefore be suggested as an effective countermeasure to combat detrimental changes as a result of gravitational unloading such as during spaceflight and possibly during illness-related prolonged bed rest.

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