CHAPTER 2

Strength, size and activation of knee extensors followed during 8 weeks of horizontal bed rest and the influence of a countermeasure

Edwin R Mulder
Dick F Stegeman
Karin HL Gerrits
Micha I Paalman
Joern Rittweger
Dieter Felsenberg
Arnold de Haan

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ABSTRACT

Changes in the quadriceps femoris muscle with respect to anatomical cross sectional area (CSA), neural activation level and muscle strength were determined in 18 healthy men subjected to eight weeks of horizontal bed rest with (n = 9) and without (n = 9) resistive vibration exercise (RVE). CSA of the knee extensor muscle group was measured with magnetic resonance imaging every two weeks during bed rest. In the control subjects (Ctrl), quadriceps femoris CSA decreased linearly over the eight weeks of bed rest to -14.1 ± 5.2% ($P < 0.05$). This reduction was significantly ($P < 0.001$) mitigated by the exercise paradigm (-3.5 ± 4.2%; $P < 0.05$). Prior to and seven times during bed rest, maximal unilateral voluntary torque (MVT) values of the right leg were measured together with neural activation levels by means of a superimposed stimulation technique. For Ctrl, MVT decreased also linearly over time to –16.8 ± 7.4% after eight weeks of bed rest ($P < 0.01$), whereas the exercise paradigm fully maintained MVT during bed rest. In contrast to previous reports, the maximal voluntary activation remained unaltered for both groups throughout the study. For Ctrl, the absence of deterioration of the activation level might have been related to the repeated testing of muscle function during the bed rest. This notion was supported by the observation that for a subset of Ctrl subjects (n = 5) the MVT of the left leg, which was not tested during bed rest, was reduced by 20.5 ± 10.1%, ($P < 0.01$) which was for those five subjects significantly ($P < 0.05$) more than the 11.1 ± 9.2% ($P < 0.01$) reduction for the right, regularly tested leg.

INTRODUCTION

It is well known that spaceflight, as well as clinical and experimental conditions such as bed rest (BR) and unilateral lower limb suspension result in the effective unloading of skeletal muscles. Removal of daily-life postural weight-bearing muscular activity initiates numerous physiological and structural changes, of which muscle atrophy and weakness are frequently recognized [8;11;14;24]. Based on the strong correlation between the muscle anatomical cross sectional area (CSA) and its force-generating capacity [20], it could be expected that any difference in muscle size would be reflected in a proportional change in force-generating capacity. However, it was reported that the decrements in muscle output, e.g. force or power, often exceed those in mass or CSA after unloading [4;8;21;22;40], indicating that other factors must have contributed to the decrement. One such a factor is an adaptation in the central nervous system that results in a reduced level of activation during maximal voluntary effort following unloading [13;18;21].

As previous studies are mostly limited to pre versus post measurements, longitudinal changes, i.e. the time course of changes in muscle size, strength and voluntary activation during unloading are not well documented. Important in the present study was, therefore, to additionally assess the time course of size, strength and maximal voluntary activation level of the quadriceps femoris muscle during eight weeks of horizontal bed rest. We hypothesized that bed rest would induce a decrement in voluntary quadriceps femoris strength that exceeds the reduction in muscle size. We further expected this to be primarily associated with a reduction in maximal voluntary activation level. Unloading can also induce changes in muscle fiber length [28]. As this could influence the optimum angle for maximal muscle strength, voluntary torque-angle relationships were obtained prior to and following bed rest.

Exercise training has been frequently tested as a countermeasure to prevent deconditioning of the neuromuscular and skeletal system during simulated weightlessness [2;3;7;15;21]. Yet, all these studies used resistance training that relied on the presence of gravity. As such, these training paradigms cannot be directly implemented during actual spaceflight. Attempts to use a gravity-independent form of resistance exercise training were only partially successful in maintaining muscle and bone mass during unloading [4;31].

With Rittweger et al. [29] we speculated that vibration in combination with relatively short lasting resistive exercise might be a suitable alternative gravity-independent training modality to prevent bone loss and at the same time preserve neuromuscular function during simulated microgravity. Under ambulant conditions, vibration has an anabolic effect on trabecular and cortical bone in animals and humans [33;39] but elicits only a comparatively small amount of energy turnover [30] and was found to have no training effect on voluntary muscle strength indices in young healthy subjects [10]. Therefore, in the present study, vibration was combined with a resistive force component [29]. A second aim of the present study was to evaluate specifically the effectiveness of this training modality to prevent neuromuscular adaptations during bed rest.

METHODS

Subjects

The twenty males that volunteered to participate in the Berlin Bed Rest (BBR) study were selected from a large group of actively recruited males. All subjects were in good health conditions and were randomly assigned to either a resistance vibration exercise group, RVE (n = 10) or to a control group, Ctrl (n = 10). The mean ± SD age, height and body mass were 32.7 ± 4.8 yr, 186.3 ± 8.0 cm and 86.5 ± 16.5 kg for RVE and 33.4 ± 6.6 yr, 185.4 ± 7.7 cm and 79.7 ± 10.9 kg for Ctrl. A weekly activity history indicated that some subjects did not exercise on a regular basis prior to the start of the study, whereas others were moderately or highly active. The exercise intensity prior to the start of the study was, however, similar for the RVE (2.6 ± 2.4 hrs/wk) and the Ctrl (2.4 ± 3.6 hrs/wk) group. The subjects were familiarized with the concepts of the experiments, procedures, and the equipment during a familiarization session that was scheduled 3 days prior to the start of bed rest (Fig. 1). The study was approved by the local ethics committee and all participants gave their written informed consent.

General design

The study took place in 2003-2004 at the Charité – Campus Benjamin Franklin Hospital in Berlin, Germany. Bed rest is commonly used as a ground-based simulation model for spaceflight. Previous studies have frequently used head-down tilt in addition to the bed rest, because this model is suggested to reproduce phenomena associated with weightlessness during actual spaceflight [1]. Because horizontal bed rest was considered a practical and a clinically sufficiently relevant model for the purposes of the study, subjects in the Berlin Bed Rest study were restricted to 56 days of horizontal bed rest, i.e. without the addition of a head-down tilt. We thereby hypothesized that the absence of weight-bearing activity would be the major contributor to reductions muscle mass and function following unloading. During the 56 days of horizontal bed rest, the subjects were not allowed to stand up, lift their trunk in bed more than to 30° of trunk flexion, move their legs briskly, or elicit large forces with their leg muscles other than during testing sessions or during training sessions (the latter RVE group only). 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 respect to caloric intake and ingestion of alcohol or nicotine, excessive doses of caffeine, as well as the regular intake of any drug or medication was prohibited.

Exercised-based countermeasure

Exercises were performed on a specific vibration system that was developed for application under microgravity and bed rest conditions (Galileo Space, Novotec, Pforzheim, Germany). The construction was derived from a commercially available device for vibration exercise in standing position (Galileo 2000, Novotec, Pforzheim, Germany). The used equipment and countermeasure exercise are described in detail elsewhere [29]. In short, the vibration device consists of a vertically oriented vibration platform suspended on a trolley suited to be used in supine position. Elastic springs were attached to the trolley for the subjects to attach themselves through belts with their shoulders, hips, and hands.

This generated a static force equivalent to approximately 2 times the body weight. The RVE group started a progressive resistance exercise-training program on the 4th day of bed rest. RVE subjects trained two times each day. No resistive training exercises were performed the first three days during bed rest due to the scheduling of experiments (muscle biopsies and collection of blood samples for bone resorption markers) that required the absence of physical exercise, including testing. In each training session, four exercises were performed in the following order: squatting, heel and toe raises and explosive squatting. During morning sessions, all exercises were performed for > 60 seconds. If subjects were able to exceed 100 seconds, vibration frequency was increased. 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 only 60-80 % of the static force used in the morning sessions, but to run through the first three exercises for 60 seconds each as many times as possible. No training sessions were scheduled on Sundays. Each exercise was performed during whole body vibration, in the supine position, with both legs simultaneously and with the feet equally distant of either side of the rotation axis at the vibration platform. Trained staff supervised all training sessions, and subjects were frequently encouraged.

Anatomical CSA of the quadriceps muscles

During bed rest, magnetic resonance imaging (MRI) data were obtained from both thighs at BR1, BR14, BR28, BR42, and BR56 (Fig.1). No experiments involving muscular exercises were undertaken immediately prior (< 1 hour) to the MRI experiments, because of its potential influence on fluid distribution, which would affect the measurements. The subjects, while supine, were transported to the MRI room by hospital staff. With the subjects in supine position and the lower limbs extended and relaxed, series of transverse scans of both thighs were made with a 1.5 Tesla MRI (Vision, Siemens, Erlangen, Germany). Transverse scans were carried out with a slice thickness of 10 mm, and inter-slice gaps of 5 mm. Field of view and matrix dimension were set at 48.0 by 48.0 cm and 512 by 512 pixels, respectively. For each subject, a total of 35 images (displaying both left and right leg) were obtained per session. For each session, approximately 8 consecutive images around mid-thigh (where quadriceps CSA was expected to be highest) were selected for further analysis. From these images the quadriceps muscles were manually outlined and CSAs were calculated using the software package Photopaint (version 9.397, Corel, Ottawa, Canada). The same operator repeated this procedure on a non-consecutive day. The measurement errors due to the manual outlining appeared to be negligible considering the intraclass correlation coefficient (ICC), which was 0.993. For all the selected images each set of two measurements were averaged for left, right, and total (sum of left and right) CSA. Finally, the mean of the three highest values calculated were used for statistical analysis.

Muscle function

The subjects participated in nine experimental sessions. A schematic representation of the time line of all experiments performed prior to, during and post bed rest is provided in Fig. 1.

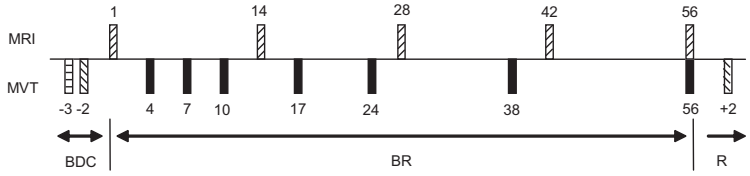

Fig. 1. Schematic representation of the timeline of experiments performed prior to, during and post bed rest. Identified are three phases: a 3-day baseline data collection phase (BDC), a 56-day bed rest phase (BR) and a 2-day early recovery phase (R). The familiarization session was scheduled at BDC-3 (bar with horizontal lines). The maximal voluntary torque (MVT)-knee flexion angle relationships were obtained at BDC-2 and R+2 (bars with descending diagonal lines). MVT and maximal activation levels prior to the start of bed rest (BR) were obtained at BDC-2, and during BR at BR4, 7, 10, 17, 24, 38 and 56 (filled bars). At BR1, 14, 28, 42 and 56 magnetic resonance images (MRI) were obtained (bars with ascending diagonal lines).

Supine dynamometer

To test subjects under bed rest conditions, a supine dynamometer was custom-built by the mechanical workshop of the Faculty of Human Movement Sciences, *Vrije* Universiteit, Amsterdam, the Netherlands (Fig. 2). In the supine position (subject's torso parallel to the bed), 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 isometric knee extension strength of the right leg was measured by connecting the cuff of the right leg 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 determined on the basis of leg length and comfort for each subject and was thereafter kept constant throughout the study. Bony landmarks on the Femur served to determine knee and hip flexion angles by using a hand-held goniometer. The dynamometer was built such that it allowed the alteration of the knee flexion angle while keeping the hip flexion angle constant. At each knee flexion angle, the rotation of the knee joint was aligned with the axis of the dynamometer and care was taken that the force transducer remained perpendicular to the line of pull of the lower leg. The pelvis and upper body were securely fixed to the dynamometer by belts. Force signals were digitized at a sampling rate of 1kHz and stored to disc for immediate and off-line analysis. Unilateral isometric knee extension torque was calculated as the product of force and moment arm.

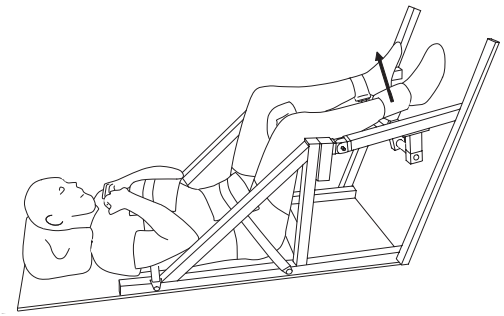

Fig. 2. Sketch of the dynamometer used to test the subjects under supine conditions. The arrow indicates the direction of the exerted isometric force. See methods for detailed information.

Warm-up procedure
All subjects performed one warm-up set prior to each testing session. A warm-up set comprised a series of maximally 10 unloaded unilateral dynamic knee extensions of the right leg, followed by 8 sub maximal isometric contractions at 40% of the individual maximal voluntary contraction (MVC). The isometric contractions were sustained for 2 s, interposed with 4 s of rest as guided in time by an audible signal. A horizontal line displayed on the force acquisition monitor presented the target force. A second line presented the current force generated by the subject in order to provide visual feedback. During the warm-up, the knee flexion angle was set at 70° (0° corresponds to full knee extension). For all sessions (except the familiarization session) the individual MVC of the preceding session was used to determine the target force.

Maximal voluntary torque-angle relationships
To determine possible changes in the optimum position for torque production, which would influence the results on muscle strength, maximal voluntary torque-angle relationships were obtained from the right leg two days prior to the start of bed rest and at the second day of reambulation (see Fig. 1). Subjects were asked to perform two MVCs of approximately 3 - 4 s in duration, separated by at least 2 min rest, at seven randomly assigned knee flexion angles of 30°, 40°, 50°, 60°, 70°, 80° and 90°. For maximal performance subjects were verbally encouraged and visual feedback was provided. The higher of the two values was used to calculate the MVT. The optimal knee flexion angle was defined as the tested angle at which MVT was highest. In the course of the study we suspected an influence of the functional testing regime on the effects of bed rest on the reduction in muscle strength for Ctrl. We therefore also obtained the MVT of the contra lateral (left) leg for six RVE and five Ctrl subjects prior to and following bed rest. For this purpose, the cuff of the other (left) leg was connected to the transducer, while all other dynamometer settings were unchanged. After warm-up procedures, three MVC attempts were allowed for the left leg at the optimum knee flexion angle of the right leg. The highest obtained value was used to calculate the MVT of the left leg.

Maximal voluntary torque and maximal voluntary activation level
Maximal voluntary torque and maximal voluntary activation level were measured prior to and repeatedly (7 times) during the bed rest (Fig. 1). After the warm-up, the subjects started by performing two to three MVCs. After this procedure, two self-adhesive surface electrodes (model 283100, Schwa-Medico, Nieuw Leusden, The Netherlands) of 80 mm x 130 mm were positioned over the quadriceps femoris muscle. The cathode was positioned over the proximal anterior thigh just distal to the inguinal ligament, and the anode was placed with its distal edge, approximately 30mm proximal to the superior border of the patella. Prior to applying the electrodes to the skin, the skin was shaved and subsequently scrubbed with alcoholic pads. The procedure to obtain the maximal activation level involved several steps. First, the electrical stimulation current from a constant current stimulator (model DS7AH; Digitimer Ltd, Welwyn Garden City, Herts, U.K.) was set such that ~40% of the total muscle mass was stimulated (concluded from a 150Hz, 700ms tetanic stimulation leading to 40% of MVC). With this stimulation intensity, the resting muscle was stimulated by a triplet stimulus at 300Hz, which elicited a torque of ~15 - 20% of MVT. Such level of muscular activity evoked minimal subject discomfort and is comparable

with rather painful supra maximal single pulse stimulation, previously used to study changes in voluntary activation due to bed rest deconditioning [12]. This was followed by superimposition of the same triplet during maximal effort. This was repeated maximally three times, separated by at least 2 min rest. The force enhancement as a result of the stimulus during maximal effort was expressed as a percentage of the force production of the same stimulus applied to the fully relaxed muscle. This procedure yielded the activation level of the quadriceps femoris muscle during the superimposed contraction as follows: activation level = (1-(triplet torque at maximal effort / resting triplet torque))•100 [21]. Next, the maximal torque generating capacity (MTGC) was calculated using the highest obtained activation level: MTGC = ((100/activation level)•torque just before moment of stimulation). Due to anticipation to the stimulus during the superimposed contractions the maximal activation level may be underestimated. Therefore, the maximal activation level was calculated by dividing MVT by MTGC: maximal activation level = (MVT / MTGC)•100. MVT was determined as the highest voluntary torque obtained during maximal effort without or with superimposed stimulation. In the latter case, the MVT was assessed prior to the administration of the triplet. All subjects were tested at the same time of day and subjects from the RVE group were always tested before their morning training sessions.

Time course of changes in CSA and MVT

The relative changes (%) over time in left, right and total quadriceps femoris muscle CSA, as well as in the MVT of the right leg were parameterized using two models: (i) a linear decay model [$y = -at + b$) and (ii) a single exponential decay model ($y = c \bullet \exp(-t/\tau)$]. The latter model was specifically hypothesized for the MVT changes over time as a consequence of the predicted rapid changes in maximal voluntary activation levels upon bed rest. This hypothesis was also the reason for the short measuring intervals in muscle function in the early stage of the bed rest. First, all individual values of MVT and CSA were normalized for the pre bed rest value (BR0 = 100%). Subsequently, the normalized data were curve-fitted by applying both the linear and the single-exponential model. The correlation coefficient (R^2) was used to determine how well each model fitted the individual data. The individual parameters of the optimal fits from both models were also used to assess the relative change in CSA and MVT resulting from eight weeks of bed rest deconditioning, thereby incorporating all available data points.

Statistical analysis

For each subject, changes over time in both left and right quadriceps femoris muscle CSA as well as MVT were parameterized using the two above models. The effects of eight weeks of bed rest on CSA and MVT were then computed using the individual model parameters. All data were statistically analyzed using the SPSS (version 12.0.1) statistical software package (SPSS Inc., Chicago, IL, USA). Differences in the response to bed rest between the RVE and the Ctrl group were tested with repeated measures ANOVA, with time (and leg, for CSA) as within-subject factor(s) and group as between-subjects factor. 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 over time. If a time-by-group interaction was found, further analysis consisted of a paired- samples t-test to test for differences between legs within each group. To determine whether the reductions

in CSA and MVT were significant, one-sample t-tests were performed on the normalized changes within each group. All values in the text are presented as means ± SD. For clarity, the values in the figures are presented as means ± SEM. The level of significance was set at $P < 0.05$.

RESULTS

Maximal torque levels could not be obtained for one subject (RVE), because of experienced patellar discomfort during the performance of the isometric contractions. Another subject (Ctrl) did not receive electrical stimulation during the study. Data from these two subjects were therefore discarded from the final analyses. Data from a third subject (Ctrl) were not analyzed with respect to MVT-angle relationship, because of patellar discomfort at the more extended knee flexion angles. Finally, as already mentioned, maximal voluntary torque data from the contra-lateral (left) leg were obtained for six RVE subjects and five Ctrl subjects. This resulted in a different number of observations for some parameters.

Cross sectional area of the quadriceps femoris muscle

For Ctrl, the linear and exponential model described the time course of change in total CSA equally well (R^2 values of 0.95 ± 0.06 and 0.96 ± 0.06, respectively). This can be understood from the fact that for most subjects the time constant (τ) of the exponential model, i.e. the time needed for the model outcome to decay to about one third (exp^{-1}) of the initial value, was much longer (mean 429 ± 167 days) than the actual bed rest period. Consequently, the change in total CSA after eight weeks was not dependent on the model used (CSA decays of 14.1 ± 5.2% and 14.1± 5.1%, for, respectively, the linear and the exponential model). Similarly, the change in right CSA was equal between the linear (-12.9 ± 4.7%) and exponential (–12.8 ± 4.7%) model and no difference was found between the respective correlation coefficients of both models (0.95 ± 0.08 and 0.95 ± 0.09). Finally, although the amplitude of change in left CSA after eight weeks of bed rest was not dependent on the model, i.e. both models revealed a reduction in CSA of 15.2 ± 6.2%, the exponential model fitted the individual data slightly, yet significantly ($P < 0.05$), better ($R^2 = 0.943 \pm 0.062$) than the linear model ($R^2 = 0.938 \pm 0.064$). For RVE, the exponential model also showed long time constants. However, it could not be used for the group as a whole because CSA increased for three subjects (an exponential decay model then becomes meaningless).

Considering the above, the reported loss values after eight weeks of bed rest, used in subsequent statistical analysis (for comparison between groups and between legs) were derived from the linear regression parameters for all cases. Analysis of variance on relative changes in CSA after eight weeks of bed rest revealed that the reduction in total quadriceps CSA (-14.1 ± 5.2%; $P < 0.001$) was significantly ($P < 0.001$) larger for Ctrl when compared to RVE (-3.5 ± 4.2%; $P < 0.05$). The ANOVA further indicated a significant time by leg interaction ($P < 0.01$), which suggested a difference in atrophic response in the left and right quadriceps femoris muscle between groups. Post hoc testing (paired-samples and one-sample *t*-tests within groups) revealed

a modest tendency ($P = 0.091$) for Ctrl towards a greater reduction in CSA of the left quadriceps (-15.2 ± 6.2%; $P < 0.001$) compared to the right leg (-12.9 ± 4.7%; $P < 0.001$). For RVE the CSA of the right quadriceps (-4.8 ± 5.3%; $P < 0.05$) muscle was significantly reduced, whereas the CSA of the left leg only showed a tendency ($P = 0.077$) to reduce (-2.3 ± 3.5%). The difference between legs was significant ($P < 0.05$). Fig. 3 depicts the time course of absolute quadriceps CSA during bed rest for the left and right leg, for both groups.

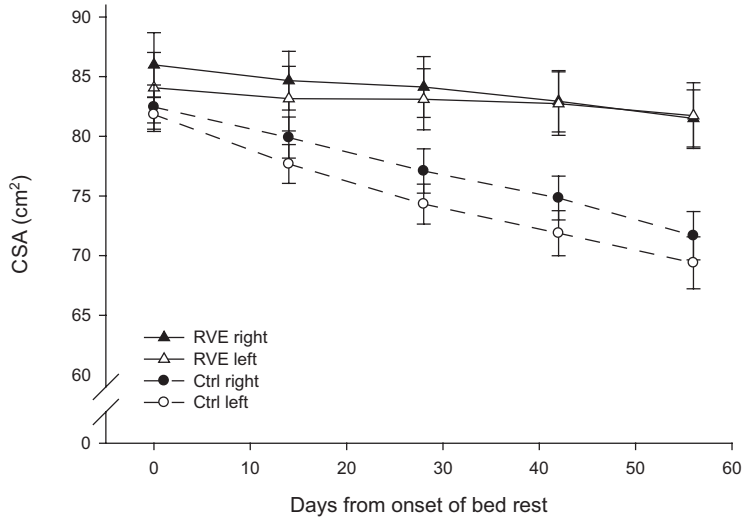

Fig. 3. Quadriceps femoris cross sectional area (CSA; mean ± SEM) during bed rest for the right (filled symbols) and left (open symbols) leg, for RVE (n = 9, triangles and solid lines) and Ctrl (n = 9, circles and dashed lines).

Maximal voluntary torque

MVT was significantly dependent on knee flexion angle both pre and post bed rest. However, bed rest did not significantly alter the angle-dependency for torque development in either group. The optimum knee flexion angle did not change as a consequence of bed rest for RVE (62 ± 4° pre bed rest versus 61 ± 3° post bed rest), nor for Ctrl (63 ± 5°, for both pre and post bed rest). Furthermore, for both experimental groups, the changes in MVT as a consequence of bed rest were not different between joint angles.

The intrinsically higher variability in MVT as compared to the CSA measurements resulted in lower correlation coefficients for both the linear (0.69 ± 0.25) and the exponential model (0.68 ± 0.27). Here also the mean time constant of the exponential decay model was much longer than the period of observation (mean 414 ± 241 days). Consequently, the relative changes in MVT after eight weeks of bed rest based on the linear and the exponential model, were not significantly different (-16.8 ± 7.4% and –15.6 ± 6.8% respectively). For RVE, again only the

linear model could be analyzed as three subjects showed an increase in MVT during the bed rest. The derived relative change in MVT of RVE amounted to –4.2 ± 8.7% after eight weeks of bed rest.

Considering the finding that the exponential model was not a better fit for most subjects and because the time course of changes in MVT could not be parameterized by this model in one Ctrl and three RVE subjects, the reported change values after eight weeks of bed rest, used in subsequent statistical analysis (for comparison between groups) were derived from the linear regression parameters for all cases. Analysis of variance on these normalized changes after eight weeks of bed rest revealed a significant time by group interaction ($P < 0.01$), which indicated that bed rest had a significantly different effect on the changes in MVT between the two groups (absolute MVT values (see Fig.4) did not differ at any time during the study). When groups were subsequently analyzed separately (one sample *t*-test on normalized changes in MVT), the MVT of the RVE group was maintained, whereas MVT of the Ctrl group was significantly reduced ($P < 0.001$) by 16.8 ± 7.4% after eight weeks of bed rest. For part of the Ctrl group (n = 5) the reduction in MVT of the contra lateral (left) leg (-20.5 ± 10.1%; $P < 0.01$, paired *t*-test), which was tested only before and after bed rest, was significantly greater ($P < 0.05$) compared to the more frequently tested (right) leg (-11.1 ± 9.2%; $P < 0.01$, paired *t*-test). For RVE (n = 6), no differences were observed in maximal strength between legs.

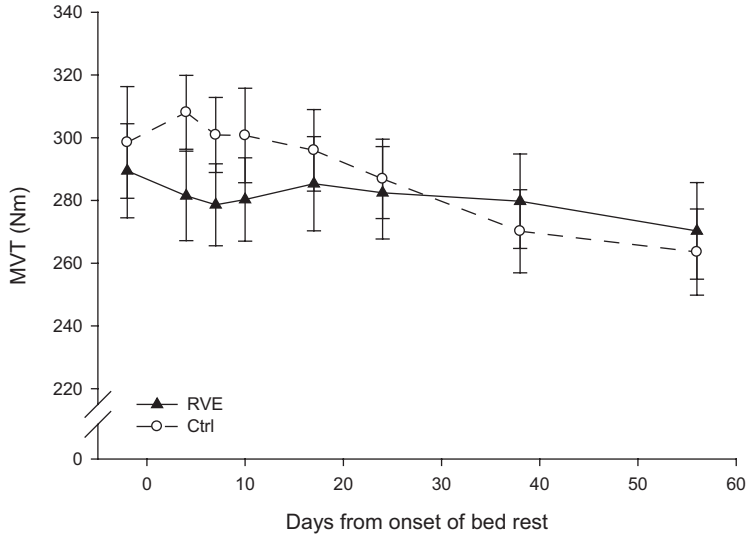

Fig. 4. Maximal voluntary torque (MVT; mean ± SEM) values obtained pre (0) and during bed rest, for RVE (n= 9, triangles, solid line) and Ctrl (n =9, circles, dashed line).

Maximal voluntary activation level
No changes over time occurred with respect to the maximal voluntary activation level in either group (Fig. 5). The mean (averaged over all sessions) maximal voluntary activation level was 94.1 ± 10.5% for RVE and 94.4 ± 8.5% for Ctrl.

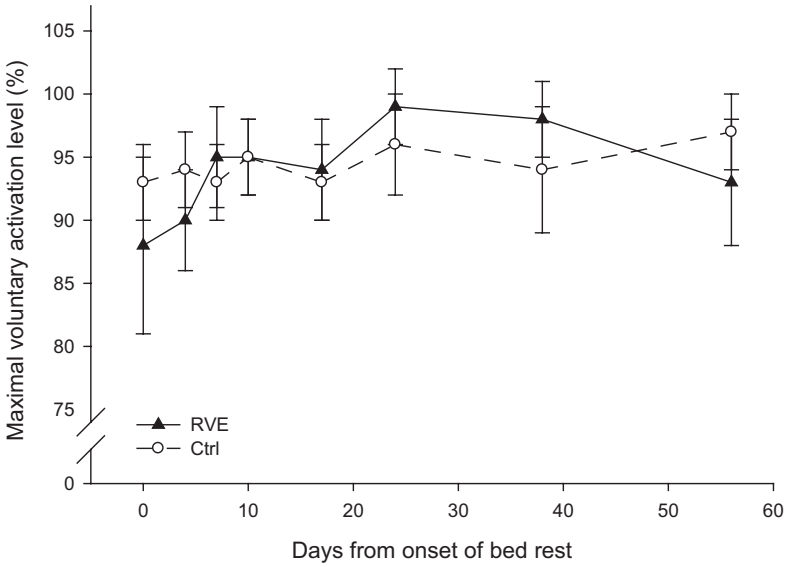

Fig. 5. Maximal voluntary activation level (mean ± SEM) values obtained pre (0) and during bed rest for RVE (n = 9, triangles, solid line) and Ctrl (n = 9, circles, dashed line).

DISCUSSION

One of the most important findings of the present study was that both knee extensor size and strength reduced linearly during eight weeks of horizontal bed rest without any countermeasure. In contrast to our expectations, the loss of quadriceps femoris muscle strength did not exceed that of quadriceps femoris muscle size, most likely because maximal voluntary activation levels were unaltered. In the group participating in the specific training program the loss of muscle strength was prevented by the used countermeasure. In this group, reductions in quadriceps femoris muscle size were mitigated as well.

Cross sectional area of the quadriceps muscle during bed rest without countermeasure
One of the most common findings in literature is that bed rest and spaceflight induce a significant atrophy of the knee extensor muscles [3-5;7;8;14;24]. It was not very clear, however, how atrophy progresses over time. Bamman et al. [7] suggested that the time course of myofiber atrophy during unloading follows a rapid onset, but that with continuous unloading the process

of atrophy reaches a plateau. The results of the present study partly agree with this notion. Of the two models tested, the exponential model slightly better parameterized the change in left (not frequently tested) quadriceps femoris muscle CSA, indicating a slight leveling off, which did not occur for the right leg. This observation might be related to the finding that the quadriceps femoris muscle of the right leg also showed a tendency to atrophy slightly less than the quadriceps muscle of left leg. A plausible explanation relates to the testing regime during the bed rest, which involved exclusively the right leg. Similar to what is reported by Trappe et a. [37], it is possible that our measurements slightly altered both the magnitude and time course of the atrophic response as a consequence of unloading, despite the fact that the experiments were performed relatively infrequent towards the end of the eight weeks bed rest period. The absence of a difference with a linear approach in almost all cases resulted from the fact that the time constant (τ) of the exponential models was much longer than the 56 days of bed rest, which suggests either the lack of an initial rapid reduction in CSA, or alternatively, the absence of a leveling off towards the end of the bed rest. In the period studied, the differences existed between the two used models were in effect insignificant. It is, however, noteworthy that on the basis of physiological considerations an exponential type of decay would have been found can be expected for all parameters in a longer bed rest period. When the presented data of the non-tested left leg are compared with the literature, the reduction of ~ 6-11% in CSA between 3 to 6 weeks of bed rest compares quite well with the ~7-13% reduction in muscle size after similar durations of bed rest unloading reported by others [2;3;9;21;23], but appears somewhat less than the ~16% reduction in knee extensor size reported by Ferretti et al. [16] and Hather et al. [19] after 6 weeks of unloading. After eight weeks of bed rest the loss of CSA of the left leg further increased to ~15%, which approaches the reductions (~15-18%) found after more prolonged periods of unloading (i.e. after 90-120 days) [4;25]. Taking our data together with earlier research, it is suggested that muscle atrophy deviates only slightly from linear up to durations of about 6-8 weeks of unloading, but that a significant leveling off occurs beyond those 6-8 weeks.

Time course of maximal voluntary torque and maximal voluntary activation level without countermeasure

The ~17% reduction in MVT after eight weeks of bed rest appears to deviate substantially from previous results where similar reductions in knee extensor strength were seen after much shorter ($\leq$ 20 days) duration of unloading [2;7;11;22]. Unlike most studies, where the knee flexion angle is chosen to be the same for all subjects, e.g. 90° [2;21], our tests were performed with individually determined optimal knee angle. Since there was no change in the optimum angle for torque production and the strength loss was not significantly different across knee-flexion angles, other factors than those related to altered torque-angle relation must underlie the differences in strength loss between our study and the literature. The differences in strength reduction might be related to a maintained ability of the central nervous system to near-maximally (~94%) drive the quadriceps muscle in our subjects (Fig. 5). In accordance with these observations, we found similar relative reductions in muscle strength and size after eight weeks of bed rest. These results contrast with reports of significant impairments in neural activation after unloading [13;18;21], which would (at least partly) account for the greater reductions in muscle performance than in muscle mass [3;6;8;21;22;40]. We suggest that the repeated testing during bed rest resulted in

habituation to the task, such that neuronal deconditioning was prevented in the present study. This is supported by the observation that the loss of maximal voluntary isometric strength of the left leg, which was not tested during the bed rest, was twice as large as that of the right leg (-20.5 vs. -11.1%) and did not compare to the loss of CSA between left and right (-11 vs. –9%) in the subgroup which was bilaterally tested. If repeated testing were indeed the major determinant, our results imply that only little motor activity is needed to counteract changes in neural control.

Yet, it has to be mentioned, that the majority of previous studies reporting a discrepancy between reduction in muscle mass and reduction in muscle strength, measured muscle function after re-ambulation, whereas in the present study, the post bed rest MVT was obtained at the last day of the bed rest period, i.e. prior to re-ambulation. After a period of unloading, re-ambulated muscles appear more susceptible to muscle damage [17;26;27]. Indeed, in the present study, virtually all our subjects (both Ctrl and RVE) suffered from pain in the lower limb muscles (albeit predominantly in the calf muscles) upon re-ambulation [29]. It is therefore important to consider that re-ambulation-inducing muscle soreness may partly account for the discrepancy frequently found between muscle strength and muscle mass after unloading conditions.

The effects of eight weeks of horizontal bed rest on muscle size and strength with resistive vibration as a countermeasure

The rationale behind resistance exercise to counteract changes in the neuromuscular system during bed rest is based on the notion that bed rest and strength training display opposite physiological effects [7]. Although proven effective to maintain or mitigate changes in the neuromuscular system [2;7;15;21] and the skeletal system [35] during unloading, conventional resistance training is dependent on the gravitational pull. In a weightless environment, this gravitational component must be replaced by an alternative force or power source. Previously described suggestions include the use of an elastomer-based [34] or a mass inertia-based exercise device [3;4]. Elastomer training under ambulant conditions was as effective as free weight training with respect to muscle, but it was found to be not effective in stimulating bone [34]. Flywheel training during 12 weeks of bed rest was only partly effective to preserve muscle and bone at the calf [4;31]. As an alternative countermeasure, Rittweger et al. [29] suggested the use of resistive vibration exercise. Under ambulant conditions, vibration without additional training loads showed to be effective to prevent and treat osteoporosis and to improve muscle strength in post menopausal women and patients with disabling conditions [32;36;38;39]. It seems, that vibration is specifically apt to prevent bone loss, possibly even when applied with minute strains [33]. However, unloaded vibration elicits only a comparatively small amount of energy turnover [30] and was found to have no training effect on voluntary muscle strength indices in young healthy subjects [10]. Therefore, in the present study, vibration was combined with a resistive force component equivalent to twice the body weight [29]). It is obvious that, from the data obtained in this study, no statement can be made as to the differential effects of vibration and resistive exercise.

The current exercise regime strongly reduced, but did not fully offset muscle atrophy, since a small (< 5%) reduction in quadriceps CSA was still observed (Fig. 3). This level of efficacy in maintaining muscle mass was also reported by others [2-4;7;21] and suggests that the quadriceps femoris muscle was sufficiently trained during the present study. The squat exercise was most likely the prime contributor to maintain the muscle CSA of the knee extensors although the quadriceps muscles were most likely also active in stabilizing the body during the performance of the heel and toe extension exercises. It is surprising that the small reduction in quadriceps CSA was only significant for the right leg in this group, since the training exercises were performed with both legs simultaneously. The stimulus provided by the training must by far have outweighed the stimulus of the testing regime apart from the fact that the tested leg then should rather have shown a lessened instead of an elevated level of atrophy. We have no explanation for this phenomenon. However, given the small differences between the legs, these results can be regarded functionally insignificant.

The current countermeasure was further successful in maintaining maximal isometric knee extension torque during bed rest (Fig. 4). Considering the small atrophic response along with the maintenance of the maximal voluntary activation level, preservation of unilateral isometric strength might be expected. However, test-mode specificities may be important to consider when assessing the efficacy of a training program during unloading, since previous studies have shown discrepancies in maintenance of strength between test and the training modes (e.g. [4;7]). These conflicting observations were related to neuronal differences between task-specific and non-task specific contractions. It can be argued that prevention of maximal voluntary strength in the present study was a consequence of the frequently repeated testing having acted as a (neural) countermeasure. However, after eight weeks, the isometric knee extension strength of the not-regularly tested left leg was similarly preserved as that of the tested right leg in a subgroup of six RVE subjects. Considering that both legs were simultaneously trained during the vibration exercises, and since this finding contrasted with the results in the left leg in a subset of Ctrl subjects, we suggest that the current countermeasure by itself also preserved neural capacity and muscle strength.

In conclusion, eight weeks of horizontal bed rest resulted in significant atrophy and weakness of the quadriceps femoris muscle in a non-trained control group, which progressed linearly over this time period. In contrast to our hypothesis, the reduction in maximal voluntary strength did not exceed that of CSA of the quadriceps muscle, as maximal voluntary activation levels remained unaltered throughout the study. For the Ctrl group the repeated testing of muscle function during the bed rest may have influenced the capacity for voluntary muscle activation. In this light, it is concluded that neural deterioration can be fairly easily prevented by brief muscle usage, even when only infrequently practiced. The atrophic response and loss of maximal isometric muscle strength of knee extensor muscles could be prevented or substantially reduced by the applied countermeasure.

REFERENCES

[1] Adams GR, Caiozzo VJ, Baldwin KM. Skeletal muscle unweighting: spaceflight and ground-based models. J Appl Physiol 2003; 95: 2185-2201.

[2] Akima H, Kubo K, Kanehisa H, Suzuki Y, Gunji A, Fukunaga T. Leg-press resistance training during 20 days of 6 degrees head-down-tilt bed rest prevents muscle deconditioning. Eur J Appl Physiol 2000; 82: 30-38.

[3] Alkner BA, Tesch PA. Efficacy of a gravity-independent resistance exercise device as a countermeasure to muscle atrophy during 29-day bed rest. Acta Physiol Scand 2004; 181: 345-357.

[4] Alkner BA, Tesch PA. Knee extensor and plantar flexor muscle size and function following 90 days of bed rest with or without resistance exercise. Eur J Appl Physiol 2004; 93: 294-305.

[5] Andersen JL, Gruschy-Knudsen T, Sandri C, Larsson L, Schiaffino S. Bed rest increases the amount of mismatched fibers in human skeletal muscle. J Appl Physiol 1999; 86: 455-460.

[6] Antonutto G, Capelli C, Girardis M, Zamparo P, di Prampero PE. Effects of microgravity on maximal power of lower limbs during very short efforts in humans. J Appl Physiol 1999; 86: 85-92.

[7] Bamman MM, Clarke MS, Feeback DL, Talmadge RJ, Stevens BR, Lieberman SA, Greenisen MC. Impact of resistance exercise during bed rest on skeletal muscle sarcopenia and myosin isoform distribution. J Appl Physiol 1998; 84: 157-163.

[8] Berg HE, Larsson L, Tesch PA. Lower limb skeletal muscle function after 6 wk of bed rest. J Appl Physiol 1997; 82: 182-188.

[9] Convertino VA, Doerr DF, Mathes KL, Stein SL, Buchanan P. Changes in volume, muscle compartment, and compliance of the lower extremities in man following 30 days of exposure to simulated microgravity. Aviat Space Environ Med 1989; 60: 653-658.

[10] de Ruiter CJ, Van Raak SM, Schilperoort JV, Hollander AP, de Haan A. The effects of 11 weeks whole body vibration training on jump height, contractile properties and activation of human knee extensors. Eur J Appl Physiol 2003; 90: 595-600.

[11] Deschenes MR, Giles JA, McCoy RW, Volek JS, Gomez AL, Kraemer WJ. Neural factors account for strength decrements observed after short-term muscle unloading. Am J Physiol Regul Integr Comp Physiol 2002; 282: R578-R583.

[12] Duchateau J. Bed rest induces neural and contractile adaptations in triceps surae. Med Sci Sports Exerc 1995; 27: 1581-1589.

[13] Duchateau J, Hainaut K. Electrical and mechanical changes in immobilized human muscle. J Appl Physiol 1987; 62: 2168-2173.

[14] Edgerton VR, Zhou MY, Ohira Y, Klitgaard H, Jiang B, Bell G, Harris B, Saltin B, Gollnick PD, Roy RR, Kay MK, Greenisen M. Human fiber size and enzymatic properties after 5 and 11 days of spaceflight. J Appl Physiol 1995; 78: 1733-1739.

[15] Ferrando AA, Tipton KD, Bamman MM, Wolfe RR. Resistance exercise maintains skeletal muscle protein synthesis during bed rest. J Appl Physiol 1997; 82: 807-810.

[16] Ferretti G, Berg HE, Minetti AE, Moia C, Rampichini S, Narici MV. Maximal instantaneous muscular power after prolonged bed rest in humans. J Appl Physiol 2001; 90: 431-435.

[17] Fitts RH, Riley DR, Widrick JJ. Functional and structural adaptations of skeletal muscle to microgravity. J Exp Biol 2001; 204: 3201-3208.

[18] Gondin J, Guette M, Maffiuletti NA, Martin A. Neural activation of the triceps surae is impaired following 2 weeks of immobilization. Eur J Appl Physiol 2004; 93: 359-365.

[19] Hather BM, Adams GR, Tesch PA, Dudley GA. Skeletal muscle responses to lower limb suspension in humans. J Appl Physiol 1992; 72: 1493-1498.

[20] Ikai M, Fukunaga T. Calculation of muscle strength per unit cross-sectional area of human muscle by means of ultrasonic measurement. Int Z Angew Physiol 1968; 26: 26-32.

[21] Kawakami Y, Akima H, Kubo K, Muraoka Y, Hasegawa H, Kouzaki M, Imai M, Suzuki Y, Gunji A, Kanehisa H, Fukunaga T. Changes in muscle size, architecture, and neural activation after 20 days of bed rest with and without resistance exercise. Eur J Appl Physiol 2001; 84: 7-12.

[22] Kubo K, Akima H, Kouzaki M, Ito M, Kawakami Y, Kanehisa H, Fukunaga T. Changes in the elastic properties of tendon structures following 20 days bed-rest in humans. Eur J Appl Physiol 2000; 83: 463-468.

[23] Larsson L, Li X, Berg HE, Frontera WR. Effects of removal of weight-bearing function on contractility and myosin isoform composition in single human skeletal muscle cells. Pflugers Arch 1996; 432: 320-328.

[24] LeBlanc A, Rowe R, Schneider V, Evans H, Hedrick T. Regional muscle loss after short duration spaceflight. Aviat Space Environ Med 1995; 66: 1151-1154.

[25] LeBlanc AD, Schneider VS, Evans HJ, Pientok C, Rowe R, Spector E. Regional changes in muscle mass following 17 weeks of bed rest. J Appl Physiol 1992; 73: 2172-2178.

[26] Ploutz-Snyder LL, Tesch PA, Hather BM, Dudley GA. Vulnerability to dysfunction and muscle injury after unloading. Arch Phys Med Rehabil 1996; 77: 773-777.

[27] Prou E, Marini JF. Muscle research in space--increased muscle susceptibility to exercise-induced damage after a prolonged bedrest. Int J Sports Med 1997; 18 Suppl 4: S317-S320.

[28] Reeves NJ, Maganaris CN, Ferretti G, Narici MV. Influence of simulated microgravity on human skeletal muscle architecture and function. J Gravit Physiol 2002; 9: 153-154.

[29] Rittweger J, Belavy D, Hunek P, Gast U, Boerst H, Feilcke B, Armbrecht G, Mulder E, Schubert H, Richardson C, de Haan A, Stegeman D, Schiessl H, Felsenberg D. Highly demanding Resistive Vibration Exercise program is tolerated during 56 days of strict bed-rest. Int J Sports Med 2006.

[30] Rittweger J, Beller G, Felsenberg D. Acute physiological effects of exhaustive whole-body vibration exercise in man. Clin Physiol 2000; 20: 134-142.

[31] Rittweger J, Frost HM, Schiessl H, Ohshima H, Alkner B, Tesch P, Felsenberg D. Muscle atrophy and bone loss after 90 days' bed rest and the effects of flywheel resistive exercise and pamidronate: Results from the LTBR study. Bone 2005; 36: 1019-1029.

[32] Roelants M, Delecluse C, Verschueren SM. Whole-body-vibration training increases knee-extension strength and speed of movement in older women. J Am Geriatr Soc 2004; 52: 901-908.

[33] Rubin C, Turner AS, Bain S, Mallinckrodt C, McLeod K. Anabolism. Low mechanical signals strengthen long bones. Nature 2001; 412: 603-604.

[34] Schneider SM, Amonette WE, Blazine K, Bentley J, Lee SM, Loehr JA, Moore AD, Jr., Rapley M, Mulder ER, Smith SM. Training with the International Space Station interim resistive exercise device. Med Sci Sports Exerc 2003; 35: 1935-1945.

[35] Shackelford LC, LeBlanc AD, Driscoll TB, Evans HJ, Rianon NJ, Smith SM, Spector E, Feeback DL, Lai D. Resistance exercise as a countermeasure to disuse-induced bone loss. J Appl Physiol 2004; 97: 119-129.

[36] Torvinen S, Kannus P, Sievanen H, Jarvinen TA, Pasanen M, Kontulainen S, Nenonen A, Jarvinen TL, Paakkala T, Jarvinen M, Vuori I. Effect of 8-month vertical whole body vibration on bone, muscle performance, and body balance: a randomized controlled study. J Bone Miner Res 2003; 18: 876-884.

[37] Trappe SW, Trappe TA, Lee GA, Widrick JJ, Costill DL, Fitts RH. Comparison of a space shuttle flight (STS-78) and bed rest on human muscle function. J Appl Physiol 2001; 91: 57-64.

[38] Verschueren SM, Roelants M, Delecluse C, Swinnen S, Vanderschueren D, Boonen S. Effect of 6-month whole body vibration training on hip density, muscle strength, and postural control in postmenopausal women: a randomized controlled pilot study. J Bone Miner Res 2004; 19: 352-359.

[39] Ward K, Alsop C, Caulton J, Rubin C, Adams J, Mughal Z. Low magnitude mechanical loading is osteogenic in children with disabling conditions. J Bone Miner Res 2004; 19: 360-369.

[40] Zange J, Muller K, Schuber M, Wackerhage H, Hoffmann U, Gunther RW, Adam G, Neuerburg JM, Sinitsyn VE, Bacharev AO, Belichenko OI. Changes in calf muscle performance, energy metabolism, and muscle volume caused by long-term stay on space station MIR. Int J Sports Med 1997; 18 Suppl 4: S308-S309.