CHAPTER 5

Characteristics of fast voluntary and electrically evoked isometric knee extensions during 56 days of bed rest with and without countermeasure

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ABSTRACT

The contractile characteristics of fast voluntary and electrically evoked unilateral isometric knee extensions were followed in 16 healthy men during 56 days of horizontal bed rest and assessed at bed rest days 4, 7, 10, 17, 24, 38 and 56. Subjects were randomized to either an inactive control (Ctrl, n = 8) or a resistive vibration exercise countermeasure group (RVE, n = 8). No changes were observed in voluntary muscle activation, indicated by the amplitude of the surface electromyogram (EMG), or the initial rate of voluntary torque development in either group during bed rest. In contrast, for Ctrl, the force oscillation amplitude (FOA) at 10Hz stimulation increased by 48% ($P < 0.01$), the time to reach peak torque at 300Hz stimulation (TPT_{300}) decreased by 7% ($P < 0.01$), and the half relaxation time at 150Hz stimulation (HRT_{150}) tended to be slightly reduced by 3% ($P = 0.056$) after 56 days of bed rest. No changes were observed for RVE. Torque production at 10Hz stimulation relative to maximal (150Hz) stimulation was increased after bed rest for both Ctrl (15%; $P < 0.05$) and RVE (41%; $P < 0.05$). In conclusion, bed rest without countermeasures resulted in intrinsic contractile properties of a faster knee extensor group. The preserved ability to perform fast voluntary contractions suggests a protective effect of the repeated testing sequence on voluntary motor performance. The changes in intrinsic contractile properties were prevented by resistive vibration exercise, and voluntary motor performance remained unaltered for RVE subjects as well.

INTRODUCTION

Previous research has shown that exposure to actual or simulated spaceflight leads to pronounced muscle atrophy in humans. The associated muscle weakness significantly impairs the performance of various motor tasks (for reviews see [2;16-18]). Muscle function is often further impaired by adaptations in the central motor control system, as indicated by reductions in the amplitude of the surface electromyogram (EMG) during maximal voluntary steady-state contractions [4;15;22;33].

From muscle stimulation studies it is known that compared to the activation levels needed to reach maximal steady-state isometric force, reaching peak rates of muscle force development requires much higher activation levels [11;13]. Likewise, the voluntary activation level is also regarded the major determinant for the rate at which muscle force can be developed at the very start of a voluntarily contraction [14]. In daily life, elderly individuals who lack sufficient motor speed or possess poor lower extremity strength have an increased risk of fall-related bone fractures [35]. The same may hold for astronauts suffering from muscle atrophy, neural deconditioning and increased bone fragility following space missions [36]. The rate at which muscle force can be developed voluntarily is also determined by intrinsic contractile muscle speed [3]. Interestingly, exposure to actual or simulated spaceflight has been associated with enhanced contractile characteristics of individual muscle fibers [37], which according to Widrick and co-workers could partly or fully compensate for the effect of atrophy on power output of single muscle fibers [41]. However, as central neural factors are considered more important [14], the rate of voluntary knee extension torque development is likely to be reduced by muscle unloading.

The main purpose of the present study was therefore to test the hypothesis that the maximal rate of voluntary isometric torque development would decline during 56 days of bed rest. To focus specifically on the reduced ability to maximally activate the quadriceps muscle during the fast voluntary maximal isometric contractions and its effect on the maximal rate of voluntary torque development, contractile and EMG variables were normalized to the steady state maximal isometric knee extension condition at the day of testing, as previously documented for the same subjects [27]. To assess the influence of peripheral factors on voluntary rate of torque development, changes in intrinsic muscle characteristics as a consequence of bed rest were investigated by applying muscle stimulation. This is a frequently used methodology to assess muscles properties irrespective of central neural influences [12;21;23]. We hypothesized that the knee extensor group would acquire the intrinsic contractile properties of a faster muscle, which should oppose, at least in part, the potential changes in the rate of voluntary muscle torque development brought about by neural deconditioning. The third and final aim of the present study was to test the hypothesis that daily resistive vibration exercise training during bed rest [31] would prevent changes in fast voluntary isometric knee extensions, as a consequence of both preserved voluntary activation properties, and intrinsic contractile muscle characteristics.

METHODS

Subjects
A total of sixteen subjects participated in the present study. All subjects were in good health and were involved in normal physical activity before participation in the study. At the start of the study the subjects were randomly assigned to an experimental group or a control group. The experimental group (RVE, n = 8; mean age, height and body mass ± SD: 33.0 ± 1.9 yr, 1.84 ± 0.03 cm and 79.5 ± 3.8 kg) participated in a progressive resistive vibration exercise (RVE) training program during the bed rest. The subjects of the control group (Ctrl, n = 8; mean age, height and body mass ± SD: 34.3 ± 2.5 yr, 1.82 ± 0.02 cm and 76.8 ± 1.8 kg, respectively) were restricted to bed rest without countermeasure. All 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. The local Ethics committee of the Charité – Campus Benjamin Franklin Berlin approved the study and all participants gave their written informed consent.

General design
All subjects underwent 56 days of strict horizontal bed rest at the Charité Benjamin Franklin Hospital, Berlin, Germany. During the bed rest, the subjects were not allowed to stand up, to lift their trunk in bed more than to 30° of trunk flexion, to move their legs briskly, or to elicit large forces with their legs muscles other than during testing sessions or during 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 using the Harris-Benedict equation and ingestion of alcohol or nicotine, excessive doses of caffeine, as well as the regular intake of any drug or medication was prohibited (details in [31]).

Exercised-based countermeasure
Exercises were performed in the supine position on a vibration system that was specifically developed for application under bed rest and microgravity conditions (Galileo Space, Novotec, Pforzheim, Germany; Fig. 1). The used equipment and the exercise protocol are described in full detail elsewhere [31]. In short, the vibration device consists of a vibration platform, which is vertically suspended on a trolley. Subjects remained in a supine position with feet resting on the vibration platform. Belts were attached to shoulders, hips and hands and via a spring system to the vibration platform (Fig. 1). The static force was individually adjusted to an equivalent of 2x body weight with the legs in the fully extended position. During bed rest, RVE subjects trained 6 days per week, two times each day (morning and afternoon sessions). Four dynamic exercises were performed in each session. The exercises were performed with both legs simultaneously, and were carried out the following order: squats, heel raises, toe raises and explosive squats. During the squat exercise the knees where extended from 90º 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, the knees were ten times extended as quickly and forcefully as possible. In order to make the training effect more intense and to recruit agonist and antagonist muscles in the same exercise, the platform was vibrated at a frequency of 18Hz. According to the overload principle in exercise physiology, vibration frequency and thus the applied force [32] was individually adjusted in weekly intervals, such that time to exhaustion during the squat exercise in the morning sessions remained between 60-100s (i.e. between 10 and 17 repetitions). During the afternoon sessions, the subjects exercised at a reduced intensity (70 % of the static force used in the morning sessions), but ran through the squat, heel and toe raise exercises for 60 seconds each, thereby performing as many repetitions as possible, without rest. No explosive squats were performed in the afternoon sessions. Trained staff supervised all training sessions.

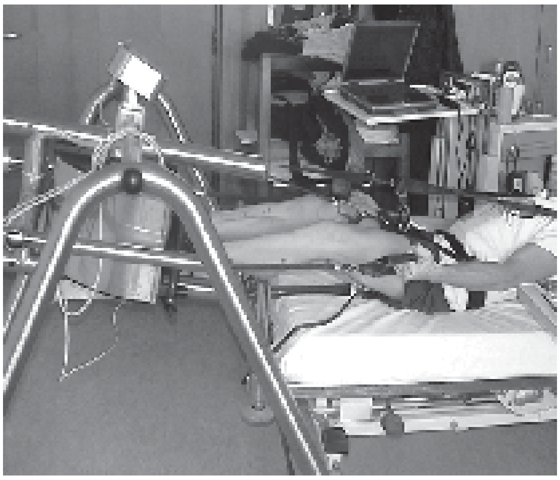

Fig. 1. Resistive vibration training device used during bed rest. The subjects attached themselves to the vibrating platform by belts at their waist, their shoulders and their hands. Voluntary resistive exercises were performed whilst vibration of the platform was generated by means of eccentrically rotating masses.

Experimental set-up

Isometric force recordings were made from voluntary and electrically evoked contractions of the knee extensor group of the right leg. Subjects were tested in the supine position using the same equipment as previously described [27]. In short, 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 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 (external moment arm) was adjusted for each subject and was thereafter kept constant for all experiments. The pelvis and upper body were securely fixed to the dynamometer with belts. Force signals were digitized using a sampling rate of 1kHz and stored on disc for immediate and off-line analysis. Torque (Nm) was off-line calculated as the product of force and external moment arm. Isometric force recordings were obtained at an individually determined pre-bed rest optimal knee flexion angle (either 60° or 70°).

Experimental procedures

During bed rest, all subjects participated in seven experimental sessions, which were scheduled at days: 4, 7, 10, 17, 24, 38 and 56, the latter being the last day of the bed rest period. The baseline experiment was conducted on the fourth day of bed rest (BR4) for logistical reasons. Although this prevents us to address rapid initial changes (< 4 days) in strength and contractile characteristics of the quadriceps by bed rest deconditioning, it does not compromise the comparison between groups during bed rest, because the start of RVE training was initiated after the measurements conducted at BR4. In addition, in this way all experiments were conducted under methodologically identical conditions. That is, at the baseline experiment subjects were already minimally 72 hours bedridden, each subject was tested at the same time of day, and subjects of the RVE group were always tested before their morning training session.

Subjects started each experimental session by performing a warm-up set that consisted of 8 to 10 unloaded dynamic contractions (right leg not connected to the force transducer), followed by 8 sub-maximal isometric contractions at a knee joint angle of 70° (0° = full knee extension). The isometric contractions were sustained for 2 s, with 4 s of rest in between, as guided in time by an audible signal and performed at approximately 40% of the maximum, based on previous attempts at maximal voluntary effort. Following the warm-up, the subjects were asked to perform a maximal voluntary contraction (MVC) of 2 - 4 s in duration at their optimal knee flexion angle. Two to three of these maximal attempts were made, interposed with 2 min of rest.

After this procedure, the quadriceps muscle was stimulated through two self-adhesive surface electrodes (model 283100, Schwa-Medico, Nieuw Leusden, The Netherlands) of 80 mm x 130mm. 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. Electrical square-wave pulses (0.2ms in duration) were generated by a constant current stimulator (model DS7AH; Digitimer Ltd, Welwyn Garden City, Herts, U.K.). The frequency and number of pulses were controlled by custom-made software. The stimulation intensity was progressively increased until 40% of the MVC torque was obtained during a 700ms tetanic contraction at 150Hz. Pilot experiments had indicated that contractile properties were not significantly influenced by the intensity of stimulation, provided that a minimum of ~30% of MVC was used (see also [5]). So, with 40% of the muscle being stimulated, we can expect reliable and representative results of the contractile characteristics of the quadriceps muscle. Thereafter, the quadriceps muscle was electrically stimulated with trains of the same single pulse intensity at low (10Hz) and high (300Hz) stimulation frequency, which lasted 700 and 80ms, respectively. The trains were applied in this order and interposed with 1 minute of rest.

Following the electrically evoked contractions, the surface electrodes were removed and the skin over the lateral vastus muscle was re-prepared for the positioning of a high-density surface EMG system (HD-sEMG, Active One, BioSemi Inc., Amsterdam, The Netherlands). The system consisted of 130 densely spaced skin-surface electrodes, arranged in a rectangular 10 by 13 matrix with 5 mm inter-electrode distance [6]. Before mounting the grid to the skin, the skin was scrubbed with alcoholic pads and slightly rubbed with electrode paste. Because of the small inter-electrode distance on the HD-sEMG electrode grid, any superfluous electrode gel was removed in order to avoid short-circuiting between neighboring electrodes. Prior to each test, the skin-electrode impedance was checked and, if necessary, the skin was re-prepared. The grid was positioned over the distal (third), anterio-lateral part of the right vastus lateralis muscle (VL), such that the columns of 13 electrodes were aligned parallel to the muscle fiber orientation of the VL and with the motor endplate zone around the center of the columns of the grid. The pre-amplified 130 monopolar signals (referenced to the patella) were bandpass filtered (0.16-400Hz) and simultaneously AD-converted (16 bits with a resolution of 1μV/bit at a rate of 2kHz/channel). Data were stored on hard disk for subsequent off-line processing.

With the sEMG system properly positioned, each subject performed another MVC of 2 – 4 s, as described before, in order to provide reference sEMG amplitude values for the subsequent fast voluntary contractions at maximal effort. If the torque deviated more than 5% from the highest torque attained during the previous MVC-tasks, i.e. the highest value of the attempts before stimulation, another attempt was made. The maximal knee extension torque (MVT) of the right leg was defined as the highest torque obtained during the entire experiment.

Subsequently, the subjects performed a total of three fast voluntary isometric knee extensions at maximal effort. Subjects were instructed to contract "as fast and forcefully as possible" on a given signal from the test leader (3-2-1 "Go"). Subjects were required to reach a minimum of 80% of the current MVT and to maintain torque at the highest attained level for approximately one sec, i.e. so-called 'kicks' were disqualified. Attempts with an initial countermovement (identified by a visible drop in the torque signal just before the onset of torque development) were also always disqualified [1;14]. The fast isometric knee extensions were interposed with 2 min of rest.

Data analysis

Fast voluntary isometric knee extensions. The neural activation of muscle fibers at the start of a contraction greatly determines the performance of specific voluntary motor functions, such as the rate at which muscle force develops during fast and forceful voluntary isomeric contractions [14]. Even so, de Ruiter et al. [14] also showed that the maximal rate of isometric torque development achieved during a forceful contraction did not differentiate subjects according to their ability to generate high neural activation levels at the very start of the contraction. Instead, the time torque integral, calculated as the area under the time torque curve over the first 40ms after the onset of torque development, showed to be more sensitive to the initial level of voluntary activation [14]. Based on these findings, we calculated the voluntary time torque integral ($vTTI_{40}$) as a measure of maximal isometric tension development under voluntary command (Fig. 2). The onset of torque development was thereby defined as the point at which the force curve exceeded baseline force by more than three standard deviations. To correct for absolute torque differences among the subjects and to correct for the changes in absolute torque across the different experimental retesting sessions as a consequence of atrophy [27], absolute $vTTI_{40}$ was subsequently expressed relative to the MVT at each session (i.e. expressed as %MVT•s).

Voluntary neural activation during the fast isometric knee extensions was assessed by averaging the amplitude (based on root mean square, RMS) of the monopolarly recorded sEMG signals (Fig. 2) over 40ms before the onset of torque development (RMS_{-40-0}). Unlike a single bipolar recording, the HD-sEMG system allowed for the assessment of monopolar recordings, and allowed for the spatial selection of the grid column with the highest amplitude (based on the mean of all electrodes within one column). To indicate the level of voluntary activation at the very start of the fast contractions in relation to that during maximal steady-state contraction, RMS_{-40-0} was expressed as a percentage of that obtained at the plateau of the MVC [14]. To investigate how the relative neural activation related to the relative maximal rate of torque development for each individual, $vTTI_{40}$ values were corresponded to RMS_{-40-0} data. For this purpose, each contraction of each session was included (see Fig. 4). For statistical comparison of both RMS_{-40-0} and $vTTI_{40}$ over time, i.e. to detect differences between groups and changes as a function of bed rest duration, for each session, the data of one single contraction was incorporated into the analyses. The contraction with highest RMS_{-40-0} value was selected for this purpose.

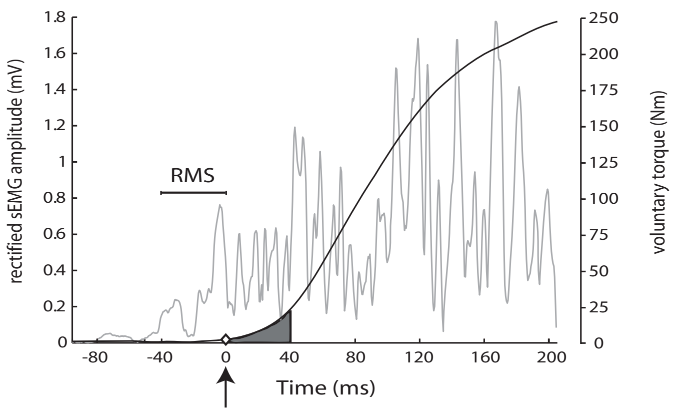

Fig. 2. Voluntary torque (thick line) and rectified surface EMG of the vastus lateralis muscle (thin line) time traces, obtained from a representative subject during an isometric voluntary knee extension performed as fast and forcefully as possible. The arrow and diamond at time 0ms indicate the start of voluntary torque development. The shaded area under the voluntary torque trace reflects the $vTTI_{40}$, calculated the first 40ms after onset of torque development. The horizontal bar indicates the 40ms immediately preceding the onset of torque development (i.e. from -40 – 0ms) for which the root mean square (RMS) of the surface EMG was calculated. Subsequently, both $vTTI_{40}$ and RMS-40-0 were normalized to the steady state maximal isometric knee extension condition at the day of testing.

Electrically evoked contractions. For each experimental session, the peak torque (T) attained during the 10Hz (T10) train was expressed relative to the maximal tetanic torque reached during the 150Hz tetanus (T150), i.e. expressed as T10/T150 ratios, respectively. The force profiles of the 10Hz tetanus showed clear oscillations (Fig. 3). The force oscillation amplitude (FOA) relative to the mean force was calculated and used as a measure of the degree of force-fusion [19;40].

The contractile characteristics at high pulse frequency (300Hz) stimulation were quantified by assessing the time to peak tension at 300Hz stimulation (TPT_{300}) from the start of the evoked contraction. The latter was defined as the instant the first pulse of the 80ms train was delivered. Half-relaxation time was determined for each session as the time needed for the elicited torque to decay to half the maximal value following the last pulse of the 150Hz tetanus (HRT150). Force data were filtered using a fourth-order, 50Hz low-pass filter. This filter was found not to affect the course of torque development; it only removed high-frequency noise from the signal.

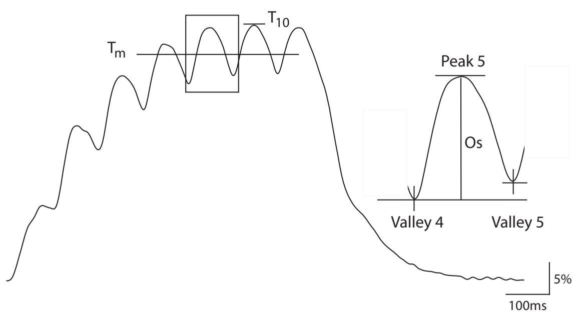

Fig. 3. Methods used for evaluation of contractile properties of the quadriceps femoris muscle evoked by electrically evoked muscle stimulation at 10Hz. The torque elicited at 10Hz stimulation was first expressed as a percentage of the maximal torque evoked at 150Hz (T150). T10 was the peak value of the of the 10Hz torque trace. The force oscillation amplitude (FOA) was determined by expressing the mean amplitude of the torque oscillation (Os) between the 4th and 7th stimulus as a fraction of the mean torque (Tm) during this time.

Statistical analysis

Values are expressed as means ± SEM (standard error of the mean). Independent-samples t-tests were used to determine whether the absolute values of variables related to muscle strength, voluntary activation and contractile properties of the quadriceps femoris muscle differed at baseline (BR4). Changes in muscle strength and contractile properties after 56 days of bed rest were assessed by means of linear regression, and expressed as a percentage change with respect to the value at BR4. One-sample t-tests were used to determine whether the normalized slope (slope/intercept on y-axis) of the linear regression was significantly different from zero. Independent-sample t-tests were used to determine whether the groups differed in their response to bed rest. Pearson's correlation coefficients were calculated to establish significance of correlation. The level of significance was set at $P < 0.05$.

RESULTS

Baseline values of voluntary activation and contractile properties of voluntary and electrically elicited isometric unilateral knee extensions were similar between groups except for the FOA and T_{10}/T_{150} ratio (see Fig. 6).

Fast voluntary isometric knee extensions

Data of steady state isometric strength are presented elsewhere [27]. Briefly, for Ctrl, maximal voluntary isometric strength significantly declined by ~17% after 56 days of bed rest, whereas maximal voluntary torque was maintained in the RVE group. The ability to perform fast voluntary contractions showed a substantial variability during bed rest. There was considerable variation in $vTTI_{40}$ as well as inRMS_{-40-0} during these contractions, both within one session as well as across sessions. Nonetheless, significant positive linear relationships ($P < 0.01$) between RMS_{-40-0} and the $vTTI_{40}$ were obtained for 12 of the 16 subjects in the present study (significant Pearson's correlation coefficients (*r*) ranged from 0.52 – 0.90; with a median value of 0.74). A representative example is shown in Fig. 4 for one of the 12 subjects. There was, however, no clear trend of data points from the right upper corner towards the left lower corner with increasing bed rest duration for this (Fig. 4), or any other subject. Accordingly, RMS_{-40-0} and $vTTI_{40}$ remained unaltered during the present study for both groups (Fig. 5).

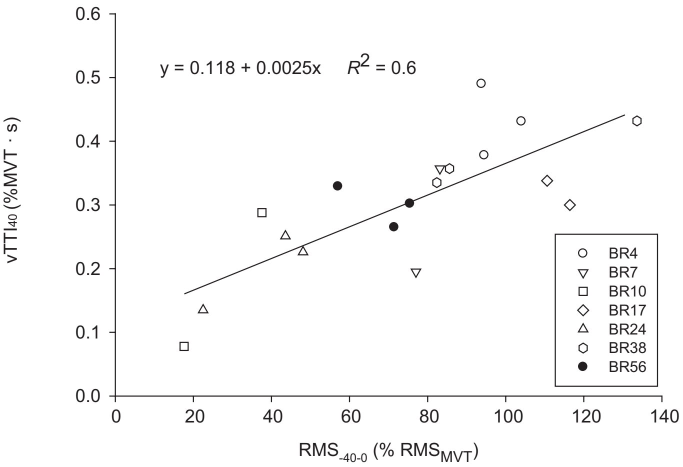

Fig. 4. A representative example of the positive correlation ($P < 0.01$, R2 = 0.6) between and the time torque integral over the first 40 ms ($vTTI_{40}$) after torque development. Positive individual relationships were obtained for 12 of the 16 subjects. To obtain the individual relationships, for each session, both RMS_{-40-0} and $vTTI_{40}$ were expressed as a percentage of the corresponding values at the steady state maximal voluntary torque (MVT) of that session.

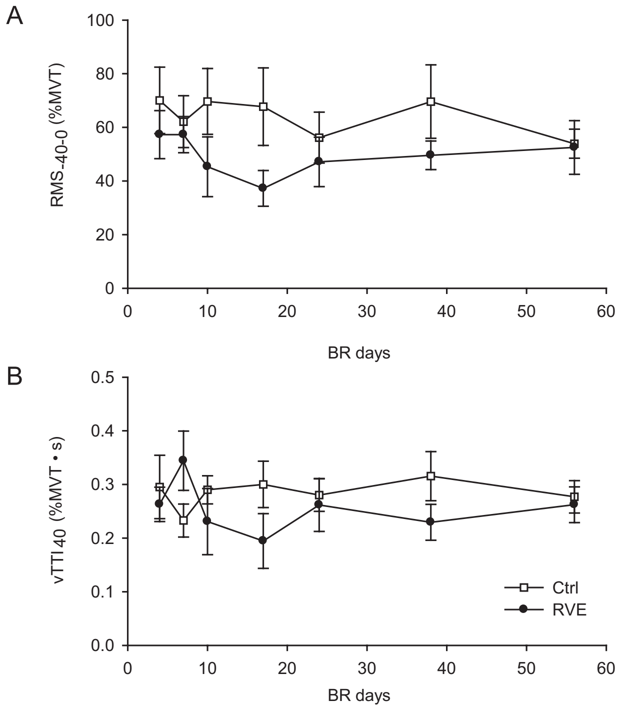

Fig. 5. Mean values (± SEM) of the voluntary time torque integral over the first 40 ms (TTI40) after torque development ($vTTI_{40}$; B) and the sEMG amplitude 40 ms before the onset of torque development (RMS_{-40-0}; A) obtained during 56 days of bed rest (BR). For each session, both $vTTI_{40}$ and RMS_{-40-0} are expressed as a percentage of the corresponding values at the steady state maximal voluntary torque (MVT) of that session

Electrically evoked knee extensions

The contractile properties obtained from electrically evoked contractions during bed rest are shown in figure 6. The course of FOA during the bed rest period was significantly different for Ctrl and RVE subjects ($P < 0.05$). For Ctrl, the FOA increased substantially (by 47.5 ± 10.0%, $P < 0.01$) during the bed rest study (Fig. 6A). In contrast, no changes in FOA were observed for RVE during the study. The groups also differed in their response to bed rest with respect to contraction time as indicated by TPT_{300} ($P < 0.01$). TPT_{300} declined significantly by 6.8 ± 0.9% ($P < 0.001$) for Ctrl, whereas no changes were observed for RVE (Fig. 6B). Furthermore, in the Ctrl group the HRT_{150} tended to be somewhat shortened by 2.5 ± 1.1% ($P = 0.056$) after 56 days of bed rest, whereas for RVE it remained unaltered (Fig. 6C). Group differences in HRT_{150} did, however, not reach significance. Interestingly, the T_{10}/T_{150} ratio increased for both Ctrl (by 15.0 ± 5.5%; $P < 0.05$) RVE (by 40.6 ± 17.1%; $P < 0.05$) and these changes were not significantly different between groups (Fig. 6D).

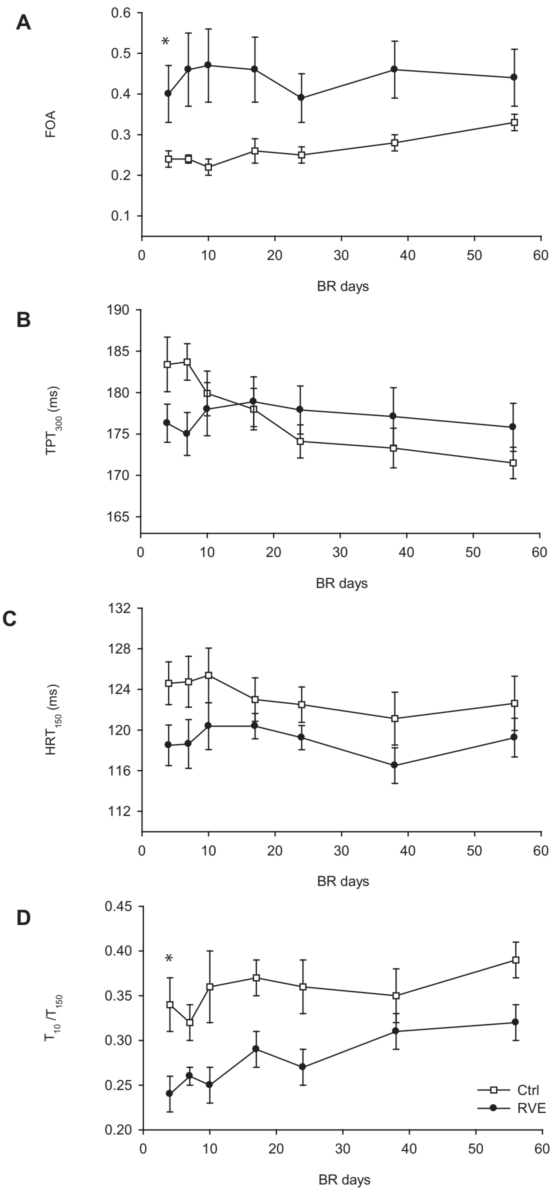

Fig. 6. Mean values (± SEM) of the force oscillation amplitude (FOA; A) time to peak tension at 300Hz stimulation (TPT300: B), half relaxation time at 150Hz stimulation (HRT150), and peak torque at 10Hz stimulation expressed as a fraction of the maximal torque obtained during tetanic stimulation at 150Hz (T10/T150) obtained during 56 days of bed rest (BR).

DISCUSSION

Contractile characteristics of electrically evoked knee extensions

An important underlying aspect behind our primary hypothesis on the rate of voluntary force development was that bed rest could induce an alteration in the contractile response of the knee extensor muscle groups irrespective of changes in neural activation. To investigate this possibility, the knee extensor muscle group was activated with different stimulation frequencies by means of percutaneous sub-maximal muscle stimulation. This is a reliable method [21], which has been used to assess intrinsic muscle characteristics in various human populations [5;12;19;23]. The conclusion to be drawn from the electrical muscle activation is that after 56 days of bed rest without countermeasure, the knee extensors exhibited characteristics of a faster muscle. Enhanced contractile speed was reflected by a reduced degree of fusion at 10Hz stimulation, a reduced time to reach peak torque at 300Hz stimulation and a tendency for faster relaxation after tetanic stimulation at 150Hz.

The musculotendinous stiffness is amongst the factors known to affect the rate of torque development [8]. Hence, it can be argued that the above mentioned changes in isometric contractile characteristics might result from a bed rest induced increased stiffness (e.g. [26]). Although not measured in the present study, from previous other studies it appears that muscle unloading often decreases the musculotendinous stiffness [25;30]. This would tend to result in a reduced rate of torque development, opposite to what we observed. In contrast, it is conceivable that the increased rate of torque development, as well as the tendency towards a faster rate of relaxation in the present study could be explained by an elevated rate of cross-bridge cycling [39]. Although at odds with some previous findings (e.g. [10;22;28]) such interpretation would be consistent with documented elevations in maximal unloaded shortening velocity [9;42;43] and shifts in muscle fiber phenotype from slow to fast as a consequence of muscle unloading [18;20;29;37;38].

An interesting finding of the present study was that, despite the faster contractile properties, torque production at 10Hz stimulation relative to maximal (150Hz) stimulation was increased after bed rest. Although consistent with previous research [34], the higher relative torque responses at low frequency stimulation did not result from a significant enhancement of twitch summation at low stimulation frequency, as previously opted [22], because the FOA substantially increased during bed rest period in the present study. Such modulation should diminish torque production, not enhance it. At present, the exact processes responsible for these anomalous findings remain unclear, but a similar phenomenon was reported in the paralyzed muscles of individuals with spinal cord injury [19], which may be considered as an extreme model for muscle unloading.

The selectivity of the countermeasure paradigm to prevent the observed changes in contractile properties to direct muscle stimulation remains inconclusive at this point. In part, our data supports the hypothesis postulated by Rittweger et al. [31] that the large number of contraction-relaxation cycles during resistive vibration exercise [7] may be effective in preserving muscle fiber contractile properties. Indeed, no changes in time to reach peak torque at 300Hz stimulation, the rate of relaxation after tetanic stimulation at 150Hz, or the level of fusion at low stimulation frequency (i.e. the FOA) were observed in the physically trained subjects. Most likely, the latter explains the greater increase (albeit not significantly) in peak torque at 10Hz stimulation compared to Ctrl after 56 days of bed rest. The significant difference between some baseline values, e.g. FOA and T_{10}/T_{150} (Fig. 6A, D), and the tendencies for TPT_{300} ($P = 0.098$) and HRT_{150} ($P = 0.057$) to be lower in RVE compared to Ctrl at baseline may point towards a difference between groups with respect to muscle fiber type at the start of the study, with RVE exhibiting a faster muscle. Nonetheless, at least for the FOA it has been demonstrated that it is still much higher (i.e. 0.65 in [19]) in paralyzed muscles of people with spinal cord injury. This makes it unlikely that the preservation FOA in RVE resulted from a ceiling effect for this group. On the other hand, as mentioned, the RVE group also displayed an increase in peak torque at 10Hz stimulation during the course of the bed rest. This observation remains difficult to explain, and warrants additional research into the efficacy of resistive vibration exercise as a countermeasure. The more, because the effect of the added vibration to the resistive exercise could not be quantified with the present countermeasure design.

Fast voluntary isometric knee extensions at maximal effort

Adequate preservation of the rate at which muscle torque develops during a forceful volitional contraction is imperative for astronauts, because neuromuscular deconditioning, coupled to a weakened load-bearing skeleton increases the risk of fall-related bone fractures after prolonged space missions. Because the level of activation that is needed to obtain a muscle's maximal rate of force development is much higher than the level of activation required to reach maximal isometric force production [11;13], we argued that the ability to perform fast and forceful voluntary contractions would be more deteriorated by bed rest confinement than the ability to perform maximal steady-state contractions. Surprisingly, and at odds with the finding of e.g. [24], we found no evidence for such bed rest induced functional impairment. More precisely, the relative voluntary activation level at the initial start of an isometric contraction performed as quickly and forcefully as possible was not significantly deteriorated compared to the activation to reach maximal steady state torque for either group during the bed rest intervention.

Previously we reported the absence of neural deconditioning during bed rest in the same subjects for maximal voluntary steady-state contractions performed with the right leg, using the twitch interpolation technique. The left leg that underwent no functional testing during bed rest exhibited a reduction in maximal isometric knee extension strength that exceeded the level of atrophy by approximately a factor of two [27]. As such, we concluded that the preservation of voluntary activation of the right knee extensor group was likely associated with the repeated functional testing sequence employed during bed rest [27]. Based on the findings of the present study, we are inclined to extend this suggestion to also neural activation during the fast isometric knee extensions. Although such an effect of the testing regime was not intended at the time, this supposition is important since it may suggest that relatively little training effort might be needed to preserve motor control, even for a motor task that requires substantially higher levels of voluntary activation than maximal steady-state contractions. It can influence future space medicine research when addressing the requirements of countermeasures to oppose neural deconditioning arising from long-duration space missions. Similarly, it may also guide the development of preventative clinical strategies for patients that are bedridden or otherwise inactive on Earth.

Based on previous research, we argued that changes in voluntary muscle functionality following disuse could be partly mitigated by opposing changes in the contractile properties of previously unloaded muscle. Widrick et al. [41] reported a significant atrophy of single human soleus muscle fibers after 17 days of spaceflight. Absolute peak power of these fibers was, however, partly or fully preserved by an elevated contraction velocity. In the present study, the examined muscle group indeed acquired mechanical characteristics of a faster muscle during the course of the bed rest. As neural deconditioning could not be demonstrated, the expectation might arise of an increased rate of voluntary torque development when corrected for the atrophic response in the Ctrl group. We found no evidence for such changes during the bed rest period. In comparison with the activation of whole muscles by computer-controlled electrical stimulation or the activation of single skinned fibers, voluntary muscle activation is much more variable as is obvious from the substantial variance in RMS_{-40-0} values within and across sessions (see example in Fig. 4). This implies that larger alterations in intrinsic contractile characteristics than those observed for Ctrl would have been required to allow detectable changes in the voluntary rate of torque development during the course of bed rest at the group level. This aside, it must also be noted that in the present study we measured the activation properties of the vastus lateralis muscle only, whereas the net knee extension torque was measured from whole quadriceps muscle. However, although we cannot fully exclude the possibility that bed rest confinement altered the contribution of the vastus lateralis muscle, or that of antagonistic muscles, relative to net knee extension torque we observed a significant positive relationship between RMS_{-40-0} and $vTTI_{40}$ in most of our subjects, similar to that previously reported [14].

In conclusion, in the subjects who were confined to eight weeks of bed rest without preventative measures, the knee extensor muscle group acquired intrinsic contractile properties of a faster muscle. Resistive vibration exercise proved effective to counteract these changes at the muscle level. An unexpected finding of the present study was that in both groups no deterioration could be shown in the capacity to maximally activate the knee extensors during voluntary contractions performed as fast and forcefully as possible. It is conceivable that the multiple retesting sessions contributed to the preservation of voluntary activation during bed rest.

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