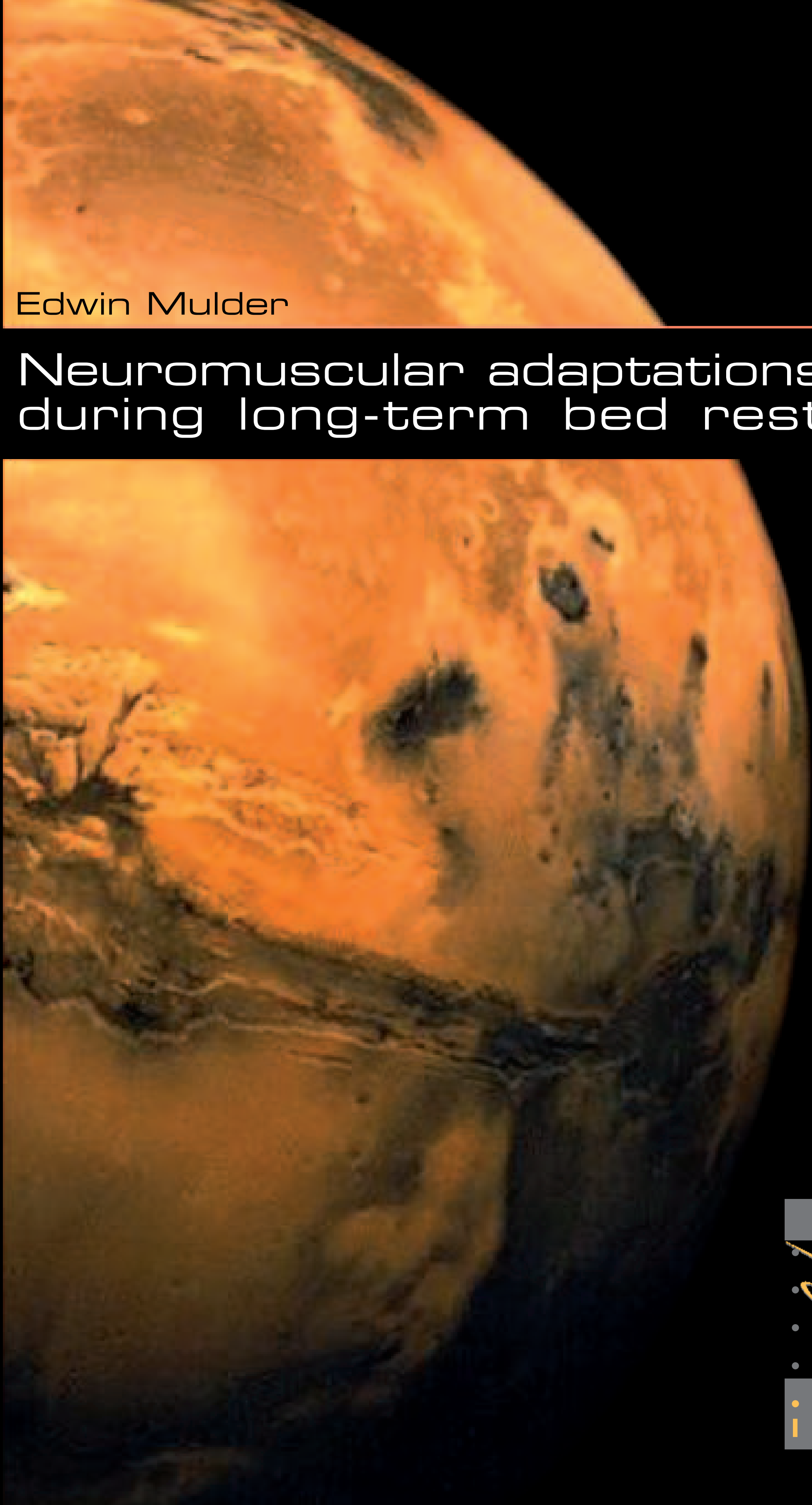
Edwin Mulder

Neuromuscular adaptations during long-term bed rest



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The work presented in this thesis is part of the research program of the Institute of Fundamental and Clinical Human Movements Sciences and was carried out at the Benjamin Franklin Hospital of the Charité – Universitätsmedizin in Berlin, Germany.

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Neuromuscular adaptations during long-term bed rest

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Edwin Richard Mulder

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Voor Moniek, Lisa en Esmée

The Earth is the cradle of humanity, but mankind cannot stay in the cradle forever

Konstantin Tsiolkovsky

CONTENTS

CHAPTER 1

General introduction and outline of the thesis

INTRODUCTION

Human (manned) spaceflight became a reality on April 12, 1961 when cosmonaut Yuri Gagarin from the former Soviet Union became the first human to orbit Earth. By doing so, Russia embarked the beginning of a new era, that of the "Space Race". With only two competitors involved, the race was ultimately won by the United States when the Lunar Module "The Eagle" (Apollo 11) landed on the Moon on July 20, 1969. Hours later, American astronaut Neil Armstrong was the first human to set foot on another celestial body. With the Moon conquered and NASA at its glory days, it seemed only a matter of time before the next (and only accessible) alien body would be visited by human explorers: the planet Mars. Now, somewhat 40 years later, Mars has only been visited by several unmanned robots, but renewed interest in the planet has also reawakened the planning for a human mission to Mars. There are, however, medical concerns related to such missions that must be addressed first before humans can start such an endeavor.

Physiological consequences of spaceflight

Although many factors affect human health during spaceflight, the dominant and single most important one is the vastly different environment encountered by human space travelers: the persisting condition of microgravity, where the weight of an object (such as the human body) is reduced to negligible values. It is this lack of weight that initiates a cascade of interrelated physiological responses that ultimately affects the whole human body, from brain to bones. This thesis outlines one biological system that is influenced by spaceflight: the neuromuscular system, which comprises skeletal muscle and the central nervous system controlling these muscles. On Earth, muscles evolved to support our upright posture and to move body parts against the pull of gravity. But in space, such antigravity functions are no longer needed for that purpose [4;7]. The dramatic interruption in load-bearing activity leads to muscular atrophy through changes in protein content with associated weakening, whereas changes in myosin phenotype result in altered contractile and biochemical characteristics and reduced endurance capacity [11;18;19;27;29]. In addition, there is compelling evidence that the central nervous system is also subjected to deconditioning by spaceflight [17]. None of these changes presents an acute problem to space travelers as long as they perform only light work, but they may very well endanger survival capabilities in emergency situations, such as fire on board a space vehicle during, or immediately post-landing on Earth (or Mars) after a long duration space mission. Unassisted emergency egress from such a craft would require immediate full strength and endurance capabilities of the anti-gravity muscles, as well as adequate neural control. As humans plan to stay in space for increasing periods of time, it will be essential to have a thorough understanding what effects, both reversible and irreversible, prolonged microgravity will have on the various constituents of the neuromuscular system, and equally important, how such adaptations can be prevented .

Bed rest as an Earth-bound simulation model

Because of the complexity and limited opportunity to study humans in space, we used a spaceflight analogue, i.e. an Earth-bound model that simulates the condition of reduced muscle usage during spaceflight, to address questions related to the alterations in the neuromuscular system and the development and testing of potential preventative measures. Several simulation models exist, but the bed rest model has been generally accepted as the most applicable one, because confinement to bed rest not only results in physiological alterations in the neuromuscular system similar to spaceflight, it also reproduces physiological alterations related to human spaceflight in many other biological systems [1]. Although quite a substantial number of bed rest campaigns have been performed over the past years, ranging from 3 up to 120 days in duration [16;25], and a substantial body of knowledge about the effects of physical inactivity on neuromuscular function has been gathered from these studies, part of the information that could be obtained is consistently lacking. In order to obtain uncompromised results, the assessment of changes in neuromuscular function as a consequence of bed rest has generally been limited to pre – post comparisons, i.e. in most bed rest campaigns functional measurements are not conducted *during* the bed rest period. The main disadvantage of such an approach is the inability to accurately assess the time course of changes as a function of bed rest duration. As such, it remains largely unresolved to what extent and in what timely manner the various subsystems within the neuromuscular system contribute to the overall deconditioning of neuromuscular integrity. This information is imperative because (1) it may identify dominant processes underlying the various manifestations of muscle weakness, which may improve the development of effective therapeutic interventions, and (2) it allows the extrapolation of gathered data to longer periods of disuse, such as during actual long-term spaceflight. Of course, cross-sectional comparisons of different studies with various study durations provide some insight in the time course of changes, but interpretations have been largely restricted by a lack of methodological standardization between different studies. The experiments described in this thesis were aimed to bridge this gap in knowledge by longitudinally studying various components of the neuromuscular system under bed rest conditions.

Another major goal of experimental bed rest studies is to develop efficient countermeasures that prevent the adaptive physiological responses in the human body as a consequence of spaceflight, i.e. nutritional, pharmaceutical and/or exercise-based procedures are first tested an refined on Earth before being applied during space missions. [13;14;21;24;26]. With respect to the neuromuscular system, it appears that conventional resistance training is effective to maintain or at least to minimize structural and functional changes in muscle mass and to preserve muscle strength during bed rest [2;12;15]. Although promising, the majority of studies that have incorporated resistance training as a countermeasure have yet only shown the potential of this training modality. Because most of the adopted training paradigms make use of the presence of gravity (for instance when lifting weights), they cannot be directly implemented during actual spaceflight, where gravity is virtually absent. To be effective in such an environment, the loading of skeletal muscles should thus arise from another source than gravitational pull. Recent developments involve devices that make use of elastomer or mass-inertia to load the skeleton and muscles [5;23]. Unfortunately these methodologies proved only partly successful to preserve mass and strength of bone and muscle [3;21]. Although the main rationale for organizing the Berlin Bed Rest study as a whole was to test resistive vibration exercise as a novel, and potentially more efficient training paradigm for both bone and muscle preservation, the focus of this thesis is on the efficacy resistive vibration exercise to preserve neuromuscular integrity of the quadriceps femoris muscle during bed rest. Resistive vibration exercise combines classical resistive exercise with vibration exercise. It is assumed that the applied vibrations to the feet evoke muscle contractions via stretch reflexes, initiated by the activation of muscle spindle (Ia) fibers [22]. This feedback loop would increase the alpha-motoneuron activity and may thus result into a greater facilitation of the muscle drive during training.

Methodological outline of the Berlin Bed Rest study

Healthy men between 20 and 45 years with a modest to active lifestyle and a high motivation for this study were recruited via various German media. Out of the 694 individuals who applied, 20 subjects were included in the study. These subjects were subsequently randomly assigned to either an inactive control group (Ctrl), or to an resistive vibration exercise (RVE) training group. The Berlin Bed Rest Study was conducted between February 2003 and June 2004 in the Charité Benjamin Franklin Hospital [20]. The study was organized in five campaigns, each comprising four subjects. In all campaigns, subjects from the training group were living in one room and the subjects from the control group in a different room. All subjects were confined to 56 days of strict horizontal bed rest, during which the subjects were by no means allowed to sit or stand up. Subjects were further instructed to limit lower leg muscular activity during the BR to an absolute minimum. As far as possible, adherence to this protocol was controlled by continuous video recordings and by force transducers in the frames of the bed. Subjects of the RVE group participated in a resistance-type strength training program that consisted of twice-daily, five days per week resistance vibration exercises in the supine position (Fig. 1), using a dedicated vibration device (Galileo Space, Novotec, Pforzheim, Germany).

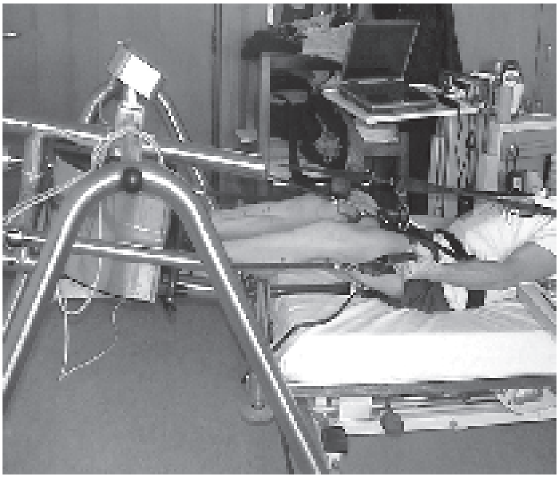

Fig. 1. The Galileo Space Device used for resistive vibration exercise at supine position during 56 days of strict bed rest.

Each training session comprised of four exercises (squats, heel and toe raises and 'kicks') that specifically targeted weight-bearing structures (muscles, tendons, bones). During morning sessions, exercises were performed for > 60 seconds (approximately ten repetitions). If 100 seconds were exceeded, vibration frequency was increased. During afternoon sessions, subjects exercised at a reduced workload (60-80% of the morning session), but performed the exercise as many times as possible for 60 seconds each without rest between successive repetitions. On Wednesday mornings, subjects were asked to exert themselves maximally by exercising each unit as long as possible. No training sessions were scheduled at Sundays.

Aim and outline of this thesis

The general aim of the present thesis was to longitudinally study the changes in the structural, contractile and electrophysiological properties of the human quadriceps muscle during prolonged bed rest. In addition, the effect of bed rest on the fatigability and associated metabolic and circulatory properties of the quadriceps femoris muscle was studied before and shortly following reambulation. Finally, this thesis further aims to evaluate the efficacy of resistive vibration exercise training to preserve neuromuscular integrity and endurance capacity under bed rest conditions. To investigate the time course of changes in neuromuscular function, a supine dynamometer was developed that allowed for the quantification of mechanical properties of the quadriceps muscle during the course of the bed rest. A pre-existing high density surface electromyography (HD-sEMG) system [6] was used to measure electrophysiological muscle characteristics during voluntary activation. The number and frequency of tests were carefully chosen to allow the assessment of an accurate time frame of changes in the neuromuscular system, whereby we anticipated that the testing regime itself would not significantly interfere with the quality or quantity of deconditioning of the neuromuscular system.

All studies reported in this thesis were conducted on the quadriceps femoris muscle group, which, because of its role as an antigravity muscle, would be significantly subjected to bed rest-induced neuromuscular deconditioning. In addition, part of the countermeasure was designed to particularly target the knee extensor group. The functional measurements during bed rest were conducted on the right leg, using the abovementioned two groups of subjects.

Chapter 2 describes a study which aimed to determine the time course of changes in muscle size, strength and voluntary activation of the quadriceps femoris muscle during bed rest in the presence and absence of a countermeasure. To assess the time course of changes in knee extensor size, measurements were conducted five times during bed rest period (in two-week intervals) by means of magnetic resonance imaging. Maximal voluntary isometric knee extensor strength was recorded prior to, and seven times during the bed rest at the optimal knee flexion angle. Associated maximal voluntary activation levels were measured by means of a superimposed stimulation technique. In addition, pre-post bed rest experiments were conducted to determine (a) whether changes in maximal muscle strength were dependent on knee flexion angle, and (b) to elucidate whether the physical testing procedure performed during bed rest had influenced the changes in neuromuscular function.

Chapter 3 deals with an elaborate analysis of high-density surface electromyography (HD-sEMG) signals that were recorded from the vastus lateralis muscle during isometric knee extensions at a range of sub-maximal contraction intensities, as well as during maximal effort. Measurements were conducted prior to and repeatedly during bed rest. Surface EMG signals were analyzed for amplitude, median frequency and muscle fiber conduction velocity and were subsequently related to isometric muscle strength to assess whether alterations in neuromuscular control strategies existed that were not, or could not have been detected by the methodology described in Chapter 2. For instance, mean muscle fiber conduction velocity is mainly related to the (changing) size of the muscle fibers, whereas the sEMG amplitude and median frequency are related both to this velocity and to alterations in neuromuscular drive to the muscle. The absolute and relative relation between sEMG variables and muscle force were used to discover potential changing muscle activation strategies.

The requisites of successful spaceflight missions aboard the International Space Station, to the Moon and to Mars with respect to muscle integrity encompass more than the preservation of muscle mass, muscle strength and adequate neural control. Importantly, there is also the need to maintain functional capacity for tasks that may require prolonged work output. In Chapter 4 we describe a study aimed to elucidate whether the fatigability of the quadriceps femoris muscle increased as a consequence of the 56-day bed rest intervention. Before and after bed rest, subjects performed a 5-min sub-maximal intermittent fatigue task in the supine position. To increase our understanding of the mechanisms that relate to changes in quadriceps femoris fatigability following bed rest, we simultaneously recorded voluntary isometric knee extensor torque and HD-sEMG signals of the vastus lateralis muscle. In addition, because of the potential effect of bed rest-induced vascular deconditioning on muscle fatigability, local blood flow and oxygenation indices were obtained at rest and during the second minute of fatiguing exercise by means of near-infrared spectroscopy (NIRS).

Apart from the capacities to produce short ‘steady-state’ (sub-)maximal muscle force (Chapters 2 and 3), and prolonged muscle force (Chapter 4), another equally important muscle functionality to be maintained during spaceflight, is the rate at which muscle force develops at the start of a forceful voluntary contraction. Because much higher activation levels are needed to reach maximal rates of force development than required for maximal isometric strength [8;9] we hypothesized that explosive muscle strength would be more affected by bed rest-induced neural deconditioning than maximal isometric ‘steady-state’ muscle strength. The study described in Chapter 5 was conducted to test this hypothesis. Although it is acknowledged that the rate of fore rise during fast voluntary contractions is predominantly determined by neural activation characteristics [10], the intrinsic contractile properties are also important. In fact, it was shown that in previously unloaded muscles the *in-vivo* muscle function is influenced by alterations in contractile properties of single fibers [28]. Therefore, to more clearly understand the processes involved in bed rest induced deconditioning, we also investigated the contractile properties of electrically evoked isometric contractions. The combination of these results with the recordings of voluntary contractions and associated HD-sEMG would allow disentangling alterations in intrinsic (peripheral) muscle properties from changes in (central) activation strategies.

Finally, main and auxiliary results of our experiments conducted during the Berlin Bed Rest study are given in Chapter 6. These findings are discussed, not only with respect to their implications for human spaceflight, but also how they, in more clinical terms, could also improve the healthcare for the elderly, or for individuals afflicted with muscle and bone-wasting diseases on Earth. Likewise, also the treatment and recovery of those that are only transiently hospitalized, bedridden or otherwise physically inactive, may be improved by studies as presented in this thesis.

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, Imai M, Kanehisa H, Suzuki Y, Gunji A, Fukunaga T. Inactivity and muscle: effect of resistance training during bed rest on muscle size in the lower limb. Acta Physiol Scand 2001; 172: 269-278.

[3] 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.

[4] Baldwin KM, White TP, Arnaud SB, Edgerton VR, Kraemer WJ, Kram R, Raab-Cullen D, Snow CM. Musculoskeletal adaptations to weightlessness and development of effective countermeasures. Med Sci Sports Exerc 1996; 28: 1247-1253.

[5] Berg HE, Tesch PA. Force and power characteristics of a resistive exercise device for use in space. Acta Astronaut 1998; 42: 219-230.

[6] Blok JH, van Dijk JG, Drost G, Zwarts MJ, Stegeman DF. A high-density multichannel surface electromyography system for the characterization of single motor units. Review of Scientific Instruments 2002; 73: 1887-1897.

[7] Convertino VA. Exercise as a countermeasure for physiological adaptation to prolonged spaceflight. Med Sci Sports Exerc 1996; 28: 999-1014.

[8] de Haan A. The influence of stimulation frequency on force-velocity characteristics of in situ rat medial gastrocnemius muscle. Exp Physiol 1998; 83: 77-84.

[9] de Ruiter CJ, Jones DA, Sargeant AJ, de Haan A. Temperature effect on the rates of isometric force development and relaxation in the fresh and fatigued human adductor pollicis muscle. Exp Physiol 1999; 84: 1137-1150.

[10] de Ruiter CJ, Kooistra RD, Paalman MI, de Haan A. Initial phase of maximal voluntary and electrically stimulated knee extension torque development at different knee angles. J Appl Physiol 2004; 97: 1693-1701.

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

[12] 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.

[13] Germain P, Guell A, Marini JF. Muscle strength during bedrest with and without muscle exercise as a countermeasure. Eur J Appl Physiol Occup Physiol 1995; 71: 342-348.

[14] Hesse C, Siedler H, Luntz SP, Arendt BM, Goerlich R, Fricker R, Heer M, Haefeli WE. Modulation of endothelial and smooth muscle function by bed rest and hypoenergetic, low-fat nutrition. J Appl Physiol 2005; 99: 2196-2203.

[15] 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.

[16] Koryak YA. Influence of 120-days 6 degrees head-down tilt bed rest on the functional properties of the neuromuscular system in man. Aviat Space Environ Med 1998; 69: 766-770.

[17] Lambertz D, Perot C, Kaspranski R, Goubel F. Effects of long-term spaceflight on mechanical properties of muscles in humans. J Appl Physiol 2001; 90: 179-188.

[18] 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.

[19] Narici M, Kayser B, Barattini P, Cerretelli P. Effects of 17-day spaceflight on electrically evoked torque and cross-sectional area of the human triceps surae. Eur J Appl Physiol 2003; 90: 275-282.

[20] Rittweger J, Belavy D, Hunek P, Gast U, Boerst H, Feilcke B, Armbrecht G, Mulder E, Schubert H, Richardson C, de Haan A, Stegeman DF, Schiessl H, Felsenberg D. Highly Demanding Resistive Vibration Exercise Program is Tolerated During 56 Days of Strict Bed-Rest. Int J Sports Med 2006; 27: 553-559.

[21] 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.

[22] 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.

[23] 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.

[24] 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.

[25] Smorawinski J, Nazar K, Kaciuba-Uscilko H, Kaminska E, Cybulski G, Kodrzycka A, Bicz B, Greenleaf JE. Effects of 3-day bed rest on physiological responses to graded exercise in athletes and sedentary men. J Appl Physiol 2001; 91: 249-257.

[26] Stein TP, Schluter MD, Leskiw MJ, Boden G. Attenuation of the protein wasting associated with bed rest by branched-chain amino acids. Nutrition 1999; 15: 656-660.

[27] Stein TP, Wade CE. Metabolic consequences of muscle disuse atrophy. J Nutr 2005; 135: 1824S-1828S.

[28] Widrick JJ, Norenberg KM, Romatowski JG, Blaser CA, Karhanek M, Sherwood J, Trappe SW, Trappe TA, Costill DL, Fitts RH. Force-velocity-power and force-pCa relationships of human soleus fibers after 17 days of bed rest. J Appl Physiol 1998; 85: 1949-1956.

[29] Widrick JJ, Romatowski JG, Norenberg KM, Knuth ST, Bain JL, Riley DA, Trappe SW, Trappe TA, Costill DL, Fitts RH. Functional properties of slow and fast gastrocnemius muscle fibers after a 17-day spaceflight. J Appl Physiol 2001; 90: 2203-2211.

CHAPTER 2

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

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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.

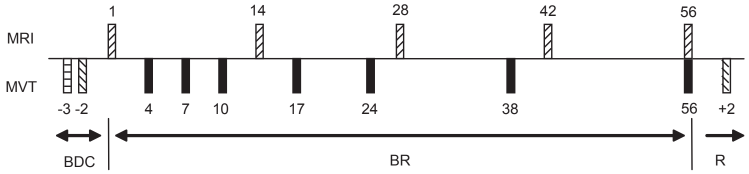

Fig. 1. Schematic representation of the timeline of experiments performed prior to, during and post bed rest. Identified are three phases: a 3-day baseline data collection phase (BDC), a 56-day bed rest phase (BR) and a 2-day early recovery phase (R). The familiarization session was scheduled at BDC-3 (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.

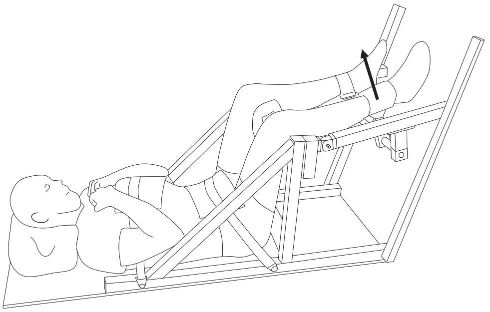

Fig. 2. Sketch of the dynamometer used to test the subjects under supine conditions. The arrow indicates the direction of the exerted isometric force. See methods for detailed information.

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 ($y = c \cdot \exp(-t/\tau)$]. 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.

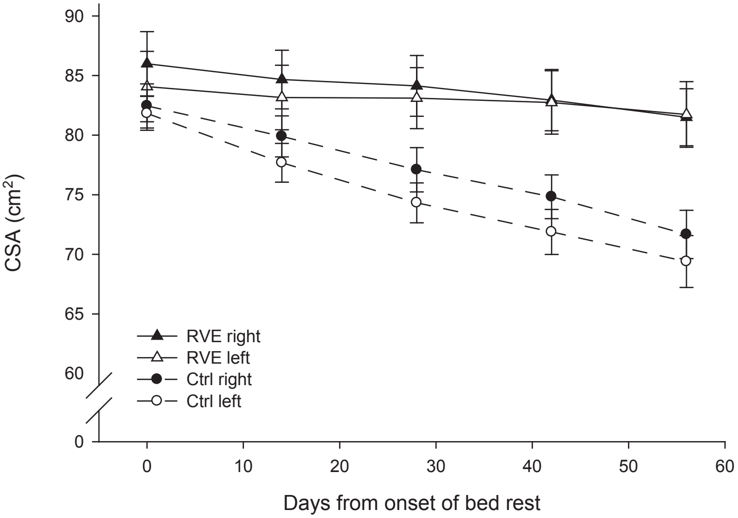

Fig. 3. Quadriceps femoris cross sectional area (CSA; mean ± SEM) during bed rest for the right (filled symbols) and left (open symbols) leg, for RVE (n = 9, triangles and solid lines) and Ctrl (n = 9, circles and dashed lines).

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.

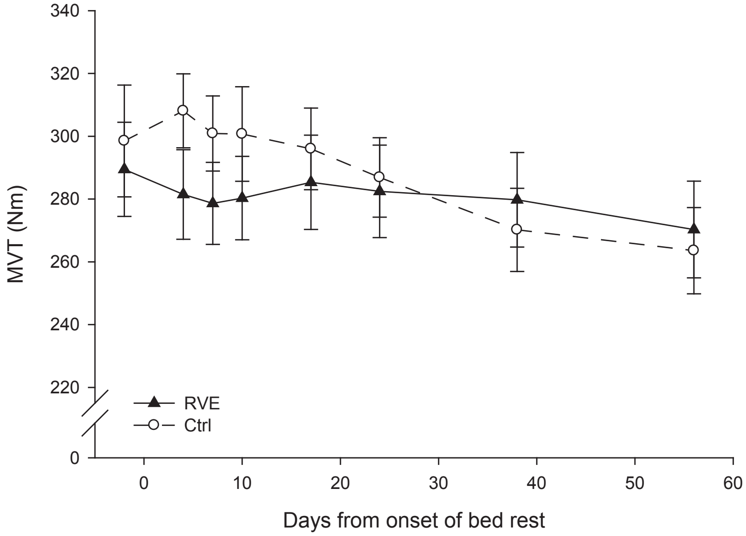

Fig. 4. Maximal voluntary torque (MVT; mean ± SEM) values obtained pre (0) and during bed rest, for RVE (n= 9, triangles, solid line) and Ctrl (n =9, circles, dashed line).

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.

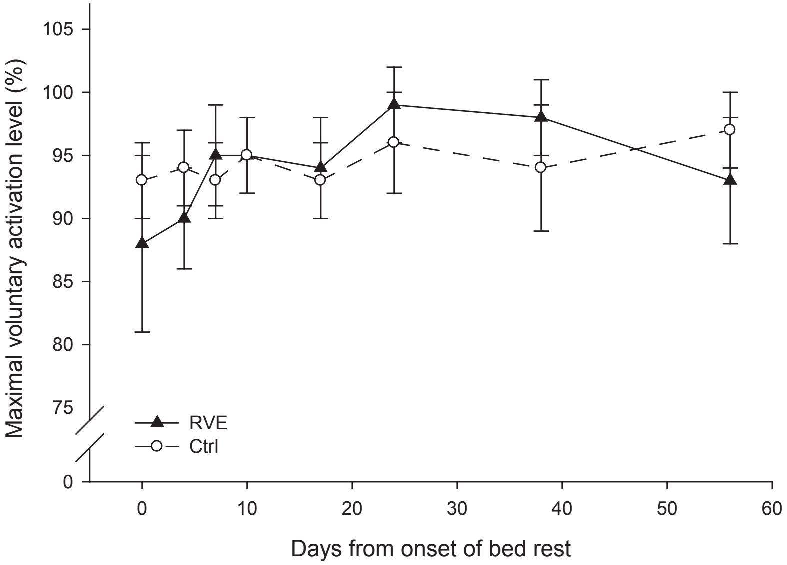

Fig. 5. Maximal voluntary activation level (mean ± SEM) values obtained pre (0) and during bed rest for RVE (n = 9, triangles, solid line) and Ctrl (n = 9, circles, dashed line).

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 (≤ 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.

CHAPTER 3

High-density surface EMG study on the time course of central nervous and peripheral neuromuscular changes during 8 weeks of bed rest with or without resistive vibration exercise

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ABSTRACT

The aim of the present study was to assess the time course and the origin of adaptations in neuromuscular function as a consequence of prolonged bed rest with or without countermeasure. Twenty healthy males volunteered to participate in the present study and were randomly assigned to either an inactive control group (Ctrl) or to a resistive vibration exercise (RVE) group. Prior to, and seven times during bed rest, we recorded high-density surface electromyogram (sEMG) signals from the vastus lateralis muscle during isometric knee extension exercise at a range of contraction intensities (5-100% of maximal voluntary isometric torque). The high density sEMG signals were analyzed for amplitude (root mean square, RMS), frequency content (median frequency, Fmed) and muscle fiber conduction velocity (MFCV) in an attempt to describe bed rest-induced changes in neural activation properties at the levels of the motor control and muscle fibers. Without countermeasures, bed rest resulted in a significant progressive decline in maximal isometric knee extension strength, whereas RMS remained unaltered throughout the bed rest period. In line with observed muscle atrophy, both Fmed and MFCV declined during bed rest. RVE training during bed rest resulted in maintained maximal isometric knee extension strength, and a strong increase (~30%) in maximal sEMG amplitude, from 10 days of bed rest on. Exclusion of other factors led to the conclusion that the RVE training increased motor unit firing rates as a consequence of an increased excitability of motor neurons. An increased firing rate might have been essential under training sessions, but it did not affect isometric voluntary torque capacity.

INTRODUCTION

Body deconditioning by bed rest immobilization is an accepted Earth-based model to study neuromuscular effects of prolonged spaceflight missions [1]. The changing neuromuscular performance of individuals after a period of motor inactivity can have several causes along the chain of intention-to-move until the activation and response of the contractile machinery in the muscle. Thus, factors within the muscle itself [45], but also factors in the motor control system may contribute to the decrement in muscle performance [22;27]. Previously [33], we studied voluntary activation at maximal voluntary effort by means of the twitch interpolation technique. In spite of the above mentioned literature, we found maximal voluntary knee extensor muscle activation to be maintained during bed rest, regardless of whether the subjects participated in an exercise countermeasure program that consisted of resistive vibration exercise training (RVE, [38]). With respect to the control subjects, we previously explained this finding as the counteracting effect of the test battery performed during bed rest [33].

Although the twitch interpolation technique provides an indication of maximal voluntary force generating capacity, it presents no information regarding the underlying activation processes of motor units. Bed rest may therefore alter recruitment of motor units and/or their firing rate modulation without being detected by the twitch interpolation technique, as used in Mulder et al. [33]. In contrast, such information is contained in sEMG signals. Motor unit recruitment and firing rate both determine the amplitude of the sEMG signal. A changed relative contribution of both processes [32] can, however, be tracked down by assessing systemic differences in the relation between sEMG amplitude and voluntary muscle force. Moreover, additional information on changes in central motor drive, such as synchronization between motor units, can be obtained by analyzing the sEMG interference pattern in terms of its spectral content. For instance, there is evidence from comparative analysis of experimental and modelling data that synchronization increases the amplitude of the sEMG signal, and that it decreases the sEMG median frequency [19;28]. Diverging responses of these sEMG variables may thus be helpful to determine relative changes in neural activation more precisely. At the peripheral level, sEMG median frequency is also influenced by the velocity of propagation of action potentials along the sarcolemma, measured as mean muscle fiber conduction velocity (MFCV, [30]). To allow the separation of central factors from this peripheral influence on Fmed, it is thus necessary to also obtain MFCV estimates. To facilitate a flexible, robust derivation of the sEMG variables, we used high-density sEMG [10] with a large number of electrodes distributed over a large part of the vastus lateralis muscle.

The aim of the present study was to detect changes in motor control by assessing sEMG amplitude and median frequency during incremental isometric knee extensions in the same subjects as described in Mulder et al. [33]. Since the field of view of the surface electrodes will be smaller than the large vastus lateralis muscle studied [14], we hypothesized from our previous study that absolute sEMG amplitudes at maximal effort would remain unchanged as a consequence of bed rest, regardless whether the subjects participated in the countermeasure program. In contrast, based on previous findings following unilateral lower limb suspension and bed rest, the relation between sEMG and sub-maximal voluntary torque might be expected to change, as the required sEMG amplitude to maintain a fixed target torque increased disproportional to what may be expected based on atrophy [7;8]. It is further hypothesized that the median frequency in the control group decreases with bed rest in proportion with atrophy (i.e. muscle fiber diameter) and MFCV. Daily resistive vibration exercise training during bed rest is expected to counteract these changes. Apart from voluntary muscle activation, resistive vibration exercise is thought also to elicit muscle contractions via the stretch reflex [39]. The indirect way of muscle activation during RVE training was one of the reasons for a more detailed analysis of its effects on motor control by observing sEMG variables in this group.

METHODS

Subjects

The twenty males that volunteered to participate in the Berlin Bed Rest study were selected from a large group of applicants. All subjects were in good health conditions and were randomly assigned to either a resistance vibration exercise group, RVE (n = 10) or to an inactive 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. The Freiburg questionnaire [20], which assesses the metabolic units spent per week in exercise, indicated a wide range of exercise habits prior to the start of the study. There were, however, no differences in exercise intensity between the RVE (2.6 ± 2.4 hrs/wk) and the Ctrl (2.4 ± 3.6 hrs/wk) group. 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 Benjamin Franklin Hospital of the Charité – Universitätsmedizin Berlin, Germany approved the study and all participants gave their written informed consent.

General design

The study took place at the Benjamin Franklin Hospital of the Charité – Universitätsmedizin Berlin. The subjects were restricted to 56 days of horizontal bed rest, during which they 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 or training (RVE only) sessions. Adherence to this protocol was controlled for by continuous video surveillance and by force transducers in the frames of the bed. The subjects received an individually balanced diet (details in [38]) and ingestion of alcohol, nicotine, and drugs were prohibited.

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 [38]. 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 the morning sessions. The exercises were performed with both legs simultaneously, and were carried out in 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 extended as quickly and forcefully as possible. Ten such 'explosive' squats were done with a rest insertion of 10 seconds in each exercise session. All exercises were performed while the platform was vibrated at a frequency of 18Hz. According to the overload principle in exercise physiology, vibration frequency and thus the applied force [39] 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.

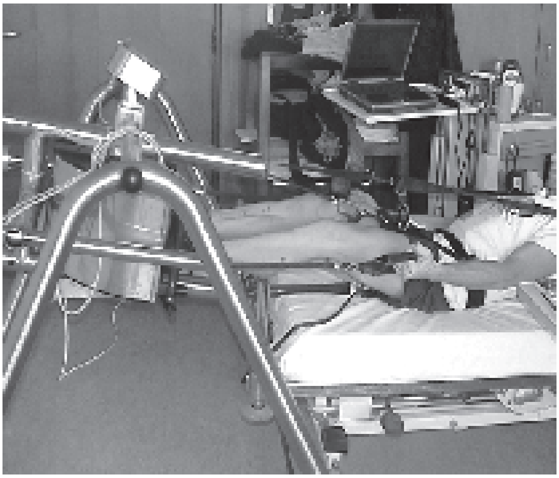

Fig. 1. The Galileo Space Device used for resistive vibration exercise (RVE) at supine position during 56 days of bed rest

Isometric knee extension

To measure the subjects' isometric knee extension torque under supine conditions, we used a custom-built supine dynamometer [33]. For each subject the optimal knee angle (either 60° or 70°, [33]) was measured at baseline and kept constant throughout the rest of the study. The hip flexion angle was set at 115° for all subjects. The subjects participated in 8 sessions. The first session was scheduled prior to the start of bed rest, but will be referred to as BR0. The remaining sessions were scheduled during bed rest at days 4, 7, 10, 17, 24, 38 and 56 (= BR4, BR7, etc.). The measurements were on the same time of the day and always before the RVE training session. This was done to exclude acute effects of vibration [34;40]. The right leg was tested.

Maximal voluntary contraction task. Following a standardized warm-up procedure [33], consisting of sub-maximal isometric contractions, the maximal voluntary torque (MVT) was assessed as the peak torque of three maximal attempts of ~3-4s in duration with a minimum of 2 min of rest in between. During the MVT attempts, the subjects were given strong verbal encouragement to achieve maximal effort. Post-contraction visual feedback was provided to achieve a higher maximal voluntary effort in a subsequent attempt.

Sub-maximal voluntary contraction task. The subjects performed in total 8 sub-maximal sustained contractions based on the MVT at the day of testing. First, contractions with low target levels (5%, 10%, 15%, 20% and 30% of MVT) were sustained for 20 s and interposed with 1 min of rest. Subsequently, contractions with high target levels (40%, 60% and 80% of MVT) were sustained for only 6 s and were interposed with 2 min of rest to minimize fatigue. The order of the trials was randomized. The large number of low contraction levels was chosen to quantify changes in motor unit recruitment or rate coding. Both the target torque and the current torque level were displayed on the computer monitor in order to provide feedback. The subjects were instructed to trace the target torque as steady as possible for the assigned durations. Display gain was the same for all torque levels.

sEMG acquisition

Concurrently with isometric knee extensor torque, sEMG signals were recorded from the vastus lateralis muscle by means of a two-dimensional, high-density sEMG system [10]. In short, the system (Active One, BioSemi, Amsterdam, The Netherlands) consists of an electrode grid of 130 gold-coated, densely spaced skin-surface electrodes. The electrodes are arranged in a 10 by 13 rectangular matrix (4.5 by 6.0 cm) with an inter-electrode distance of 5mm. The signal from each of the electrodes was recorded against a common reference electrode positioned on the patella of the tested leg (i.e. a monopolar montage). During a low intensity isometric contraction, high density sEMG signals were visualized and the electrode grid was adjusted such that the columns ran parallel to the fiber orientation. This was accomplished by utilizing the criterion that the peaks in the sEMG signal in the rows needed to be shifted in time, but had constant amplitude [37]. A representative example of 2 s of high density-sEMG data from a 20 s contraction at 20% MVT is presented in Fig. 2. The electrode grid was placed over the distal, anterio-lateral part of the vastus lateralis, such that the motor endplate zone was visible approximately halfway the columns (Fig. 2C, D). Electrode grid and amplifier were then secured in place by means of Velcro straps. The 130 pre-amplified monopolar signals were band-pass filtered (0.16-400 Hz) and simultaneously AD-converted (16 bits with a resolution of 1 μV/bit at a rate of 2048 samples/channel), and stored on hard disk. Before off-line analysis, data were high-pass filtered at 15 Hz in software.

sEMG processing

Off-line analysis was performed with customized Matlab software (Mathworks, v6.5, Natick, MA, USA). The selection of the high density sEMG variables was done on the basis of a 'go where the action is' principle [10]. Unlike a common bipolar electrode montage, the high density sEMG grid allows the spatial selection of the electrodes with the highest mean (averaged over all electrodes within a column, example of signals in a column in Fig. 2B) signal amplitude. Such spatial selection, which was separately performed for each session, assured a compensation of small variations in the placement of the electrode grid between sessions. The sEMG signals obtained during the tasks were analyzed for amplitude (root mean square, RMS) and median frequency (Fmed) from the monopolar electrode montage. RMS values were expressed as a percentage of the maximal value at the day of testing, and as a percentage of the maximal value at BR0. We used a double electrode distance (10 mm) with a single electrode pair shift distance (5mm) and the phase difference technique [6]. From the time shift away from the endplate region (e.g. between electrodes 63 and 73 in Fig. 2C and 2D) when observing subsequent bipolar recordings, muscle fiber conduction velocity (MFCV) was estimated. Estimates were obtained from all bipolar derivations for the selected column, but were accepted only when the cross-correlation between signal pairs was above 0.7 and the MFCV value below 6.5 m/s. These methodological criterions were used to reject MFCV estimates around the motor endplate zone and musculotendineous junction. For the MVC task, all sEMG variables were analyzed during one 1s epoch that yielded the highest mean torque. For each sub-maximal contraction, the sEMG variables were analyzed during one 2s signal segment (e.g. Fig. 2A). The selected segments had the lowest level of torque fluctuation (standard deviation) within the contractions.

Exclusion of data

Maximal voluntary torque levels could not be obtained for one RVE subject, because of experienced patellar discomfort during the performance of the isometric contractions. From another subject (Ctrl) we obtained unreliable baseline values, because this subject did not attend the familiarization session. Data from these two subjects were therefore discarded from the statistical analyses. MFCV values according to our criteria could not be obtained for four subjects (2 from each group). Examination of their magnetic resonance imaging data used in [33] indicated a thicker subcutaneous fat layer at baseline .

Statistical analysis

All values are expressed as means ± SEM (standard error of the mean). Statistical tests were performed with the Statistical Package for the Social Sciences software program (version 11.0, SPSS, Inc., Chicago, IL, USA). Analysis of variance (ANOVA) with a repeated measure design on bed rest duration and/or torque level with group as a co-factor was used to determine the effect of bed rest and the effect of the countermeasure. In cases where significant interactions or strong tendencies were found, post hoc analysis consisted of conducting separate ANOVAs for each group with simple contrasts. Statistical significance was set at $P < 0.05$.

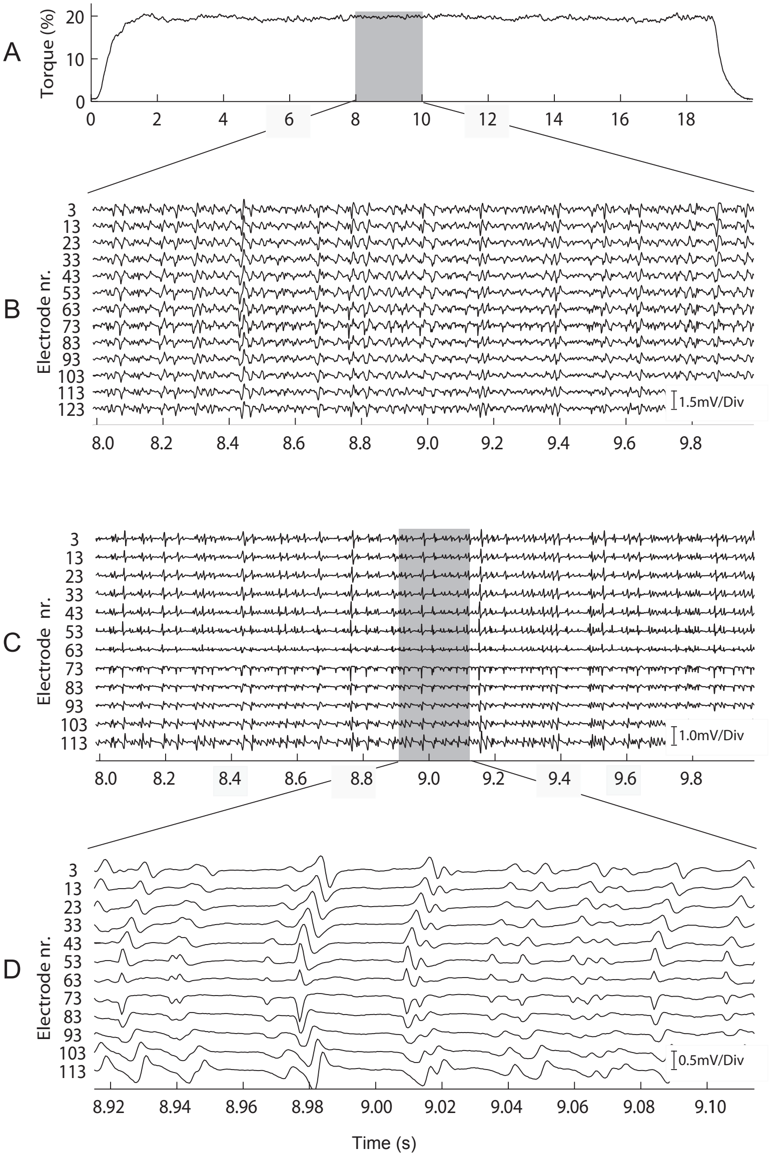

Fig. 2. Representative example of voluntary torque (A) and sEMG signals (B – D) during a sub-maximal contraction performed at 20% of actual MVT at BR0. The shaded area in A represents the 2-sec window during which torque fluctuations were minimal. During this epoch, amplitude (RMS) and median frequency (Fmed) were calculated from the monopolar sEMG signals (B). RMS was averaged over all channels within one column. The column with maximum (mean) RMS was used to average Fmed and to estimate MFCV. The latter was obtained from the bipolarly derived signal (C, D). The shaded area in C is enlarged in D. This part of the figure clearly shows the localization of the endplate region, which is characterized by the propagation of excitation in two opposing directions and a reversal of signal polarity.

RESULTS

Maximal voluntary torque

Mean group values for MVT during bed rest are provided in Fig. 3A. Bed rest resulted in a significant ($P < 0.001$) reduction in MVT for both groups. The percent reduction in MVT at BR56 was greater ($P < 0.05$) for Ctrl than for RVE, with respective values of -17.9 ± 2.5% and -9.9 ± 2.0%. Post hoc analysis revealed, however, an absence of changes in MVT from BR04 onwards for RVE, whereas a significant ($P < 0.001$) reduction in MVT (to -15.5 ± 2.7 at BR56) persisted for Ctrl.

Changes in high density sEMG signals during bed rest

sEMG amplitude. In contrast to the expected absence of changes in RMS at maximal torque level for both groups during the bed rest period, the RVE group showed a significant and strong RMS increase ($P < 0.001$) during bed rest to 130 ± 22% of the baseline value following 56 days (Fig. 3B). Post hoc analysis revealed a significant elevated RMS at BR10, 17, 24, and BR56, whereas a strong tendency towards an elevated RMS was observed at BR38 ($P = 0.054$).

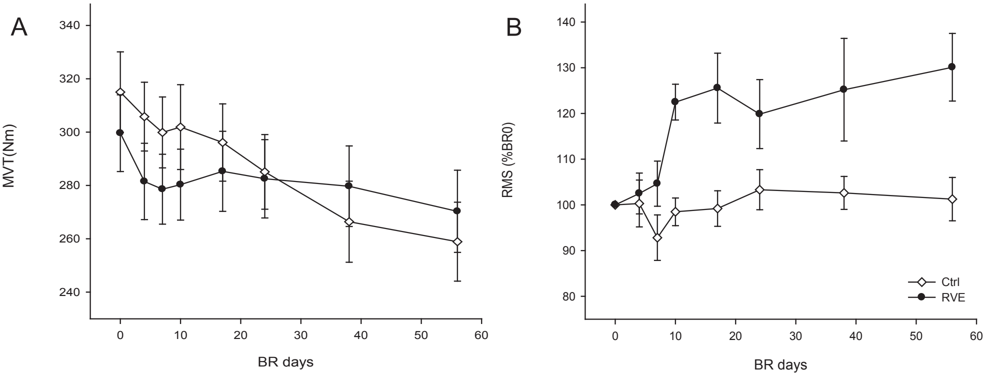

Fig. 3. Time course of maximal voluntary torque (MVT; A) and sEMG amplitude (RMS; B) at MVT during 56 days of bed rest. Data (mean ± SEM) are shown for Ctrl and RVE. RMS values are normalized for BR0. Without countermeasures, bed rest caused a significant reduction in MVT, and maintained RMS. RVE training during bed rest maintained MVT from BR04 onwards and showed an increased RMS.

In the RVE group the increase in RMS was also present at sub-maximal forces, but was most impressive at high forces (Fig. 4). To analyze the force-sEMG relationship for that group in more detail, the RMS at BR56 was also expressed as percentage of baseline RMS (BR0) for each force level (Fig. 5). The RMS at BR56 was significantly ($P < 0.05$) elevated for 15%, 30%, 40%, 60% and 100% MVT. The change in RMS at 5% and 10% MVT were significantly ($P < 0.05$) different from the increase in RMS at 100%MVT. For Ctrl, changes in RMS were also absent at the sub-maximal force levels.

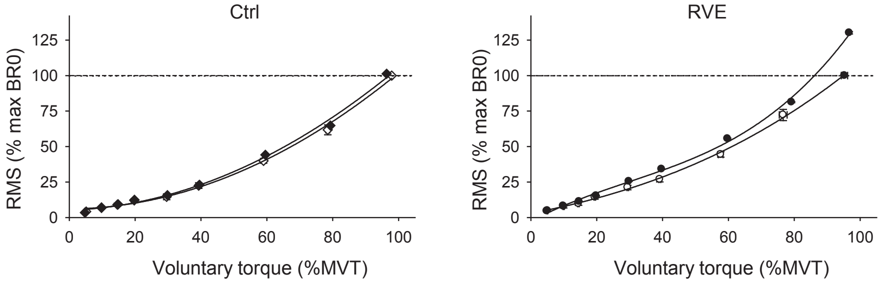

Fig. 4. Relationship between voluntary torque and sEMG amplitude for Ctrl (left panel) and RVE (right panel), pre (BR0, open symbols) and post (BR56, closed symbols) bed rest. Voluntary torque data are normalized to MVT on the day of testing. RMS data are normalized to the maximal baseline value at BR0. Each symbol represents the mean value of torque and RMS ± SEM of the corresponding group. For Ctrl, the normalized relationship remained unaltered during bed rest, whereas RVE training during bed rest resulted in a significant upward shift of this relationship. The mean relationships between RMS and voluntary torque have been fitted with a cubic equation, for clarification purposes. RMS, root mean square; MVT, maximal voluntary torque.

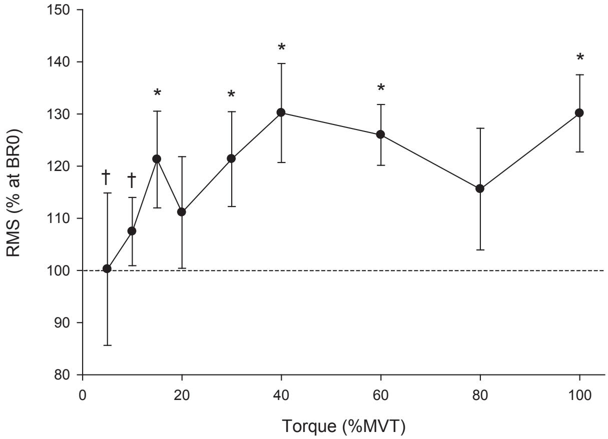

Fig. 5. Mean sEMG amplitude (RMS) values (mean ± SEM) for the RVE group at BR56, as a percentage of RMS at baseline (BR0) for the different force levels. * indicates a significantly ($P < 0.05$) increased RMS at BR56 with respect to baseline. † indicates a significant ($P < 0.05$) difference in the percentage increase in RMS with respect to the MVT level (100%) following 56 days of bed rest with RVE training.

sEMG median frequency and muscle fiber conduction velocity. In Fig. 6, the relationship between voluntary torque as a percentage of actual MVT and absolute Fmed (panels A, B) and MFCV (panels C, D) are shown for both groups. Bed rest resulted in a significant decline in both Fmed (-7.5 ± 2.3%; $P < 0.05$) and MFCV (-8.2 ± 2.6%; $P < 0.001$) across the different torque levels for Ctrl, but not for RVE.

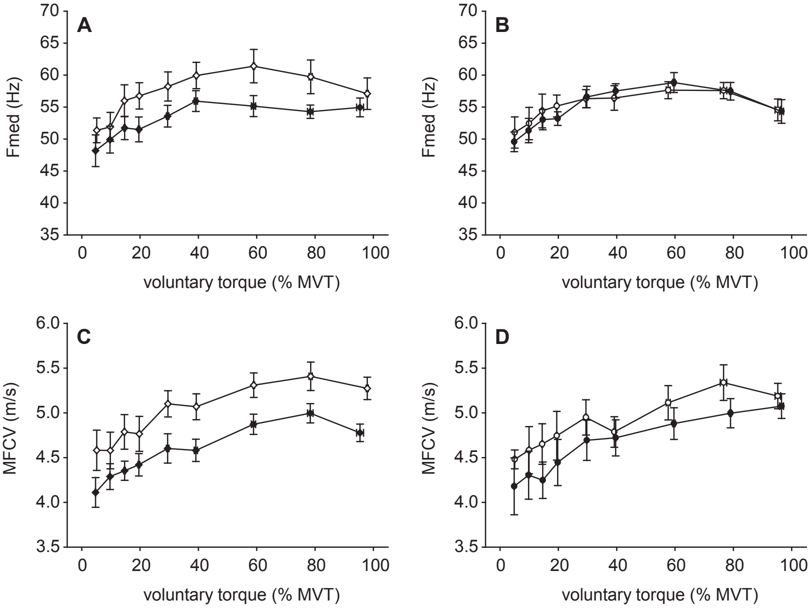

Fig. 6. Shown are the relationships between voluntary torque and sEMG median frequency (Fmed, top panels) and muscle fiber conduction velocity (MFCV, bottom panel) for Ctrl (A and C) and RVE (D and F). Depicted data (mean ± SEM) were obtained pre (open symbols) and post bed rest (filled symbols). In all panels, the voluntary torque data are normalized for the actual MVT value, i.e. the MVT of the day of testing. Without countermeasure, bed rest resulted in a significant reduction in Fmed and MFCV across the different torque levels, which was prevented by RVE training (B and D).

DISCUSSION

The effect of bed rest without countermeasures

In the Ctrl group no changes in RMS were found during the entire bed rest period. Neither did the relation between RMS and torque level change (Fig. 4). This observation indicates not only that motor unit recruitment was maintained at a maximal level [33], but also that, contrasting with initial expectations, the balance between motor unit recruitment and rate coding remained unchanged during bed rest. MFCV and Fmed (Fig. 6) declined for all force levels and by about the same percentage. Changes in Fmed are, at least in the absence of changes in motor unit firing patterns, correlated to changes in MFCV [30]. Since the MFCV is directly related to muscle fiber diameters [9], our results are well in line with quadriceps femoris muscle atrophy, measured as cross-sectional area in magnetic resonance images from the same subjects [33;43]. In contrast to previous studies [7;16;18;22;26;41], but in agreement with our previous observation that maximal voluntary torque loss could be explained by muscle atrophy alone [33], we found no evidence of change in maximum recruitment or recruitment and/or rate coding strategies, i.e. loss of neural drive capacity, as a consequence of 56 days of bed rest in the present study.

Subjects in the present study were repeatedly re-tested during the bed rest period, whereas in most previous bed rest campaigns measurements are limited to pre-post comparisons. Because of this methodological difference, we can not exclude the possibility that our testing protocol alone prevented any alteration in neural activation capacity during maximal and sub-maximal isometric knee extension exercise.

The effect of RVE training during bed rest on the peripheral level

As expected at the outset of the Berlin Bed Rest study [38], RVE was an effective countermeasure, as MVT was preserved from the fourth day of bed rest. Elsewhere, the protective effect of RVE as to muscle atrophy, measured by magnetic resonance imaging, has been described [33]. In line with these findings, no changes in the sEMG variables that could reflect muscle fiber diameter decrease (MFCV and Fmed, Fig. 6 B, D) were observed. The RVE training applied in the preset study consisted of voluntary resistive-type exercises together with externally applied vibrations to the feet. Vibration of muscles, tendons or the whole body evokes reflex contractions via the activation of primary muscle spindle afferents [36]. As said in the introduction, the indirect way of muscle activation during such training was one of the reasons for a more detailed analysis of its effects on motor control under bed rest conditions.

The effect of RVE training during bed rest on voluntary motor control

An unexpected finding was a large and rather abrupt change in RMS at maximal voluntary effort in the RVE group, which was not seen in Ctrl subjects (Fig. 3B) and has not been described in other bed rest studies that incorporated resistive exercise-based countermeasures [2;5;27]. An increase in sEMG amplitude is well recognized in resistance training under ambulatory conditions [24;29;44], though, but occurs more gradually in the course of weeks. The short time span in which the adaptations occurred in the present study might be related to the number of training sessions performed each week. In the studies cited above, subjects trained three times per week, whereas the subjects in the present study trained two times per day, for 6 days per week [38]. Furthermore, the addition of vibrations results in a large number of excitation cycles in a short time period [11]. Hence, the rapid increase in RMS during bed rest may be specific to the training regime based on RVE.

In general, an increase of RMS during a voluntary contraction could be caused by an increased number of motor unit (MU) firings per unit time, by a reduced amount of phase cancellation due to synchronization, or by changes in the MU action potential [19;31;46]. In the RVE group, MFCV and Fmed (Fig. 6) remained constant, whereas muscle cross-sectional area declined only marginally during the 56 days of bed rest [33]. Thus, changes at the muscle level can be excluded. Synchronization of MU firings is also excluded, as an increase of RMS caused by synchronization [46] would be accompanied by a decrease in Fmed [28]. The increase in RMS after about 10 days of bed rest (Fig. 3B) must therefore reflect an increased MU activation during isometric knee extension. Either more MUs were active at the same mean firing rate (recruitment), the same MUs were activated at a higher frequency (rate coding), or both. On the basis of the twitch interpolation technique, we reported also for the RVE group a high voluntary activation during maximal voluntary contractions throughout the 56 days of bed rest [33].

So, also additional recruitment of MUs can be excluded as an explanation for the increase in RMS at maximal voluntary effort in the present study.

In our view, the only remaining explanation for the increase in RMS for RVE is an increase in mean MU firing rate. In line with simulation studies of Herbert and Gandevia [25], the twitch interpolation technique as used in [33] may not have been sensitive for firing rate changes at high forces. The notion of an increased rate coding is further consistent with findings after resistance training under ambulatory conditions [17;35]. In the present study, the increase in RMS was most pronounced at the higher torque levels (Fig. 4 and 5). As mentioned in the introduction, the change in the sEMG-force relationship at high forces is compatible with a change in the maximal discharge rate of active motor units [21;32].

The details of RMS increase, in time and with torque level

Simulations of the sEMG-force relationship for a muscle with rate coding as the dominant gradation mechanism for increasing force above 50% of the maximum force predicts a curvilinear pattern, i.e., for higher forces the sEMG increases steeper than force [21]. This would explain the increase in RMS being more pronounced at the higher torque levels (Fig. 4 and 5). To explain this issue, the effects of vibration on the stretch reflex and MU firing must be considered. As mentioned, muscle, tendon or whole body vibration is believed to activate muscle spindle primary endings, which in turn, induces sustained reflex contractions in muscles at rest, known as the tonic vibration reflex [12]. When superimposed during a voluntary contraction, vibration can reinforce the excitation of motor neurons [23;42]. This may explain the increased EMG amplitude during vibration exercise [13;15]. In addition, after one single session of vibration exercise, an acute increase in stretch reflex excitability has been observed [34;40]. The increased excitability is restricted to the short-latency components [42], indicating that the plasticity is localized to the spinal level. The increase in RMS for the RVE group during voluntary isometric contractions during bed rest suggests therefore a persevering increase in excitability of the stretch-reflex loop as a consequence of eight weeks of six days per week, twice daily RVE training, even under conditions of bed rest. Although our findings are compatible with a modulation of the stretch reflex loop, modulation at higher (cortical) levels cannot be excluded. To explore the changes in excitability in more detail, other methods such as transcranial magnetic stimulation are required.

The increase in RMS had no effect on maximal voluntary knee extension strength under the isometric testing conditions. In comparison, muscle strength did increase for the same subjects during bed rest under the dynamic conditions of RVE training [38]. This can be deduced from the progressive increase in the intensity of RVE exercise during bed rest by means of augmenting vibration frequency [38]. Because the quadriceps femoris muscle atrophied slightly (up to ~5%) during the course of bed rest for the RVE group [33], the increase in dynamic knee extension strength (squat) must result from an improved neural control. In agreement with previous reports on countermeasure efficacy during bed rest [2-4], the dissociation between effects under training and testing conditions suggests that neural adaptations were task-specific and beneficial only during the RVE training itself. During isometric knee extension, the elevated

MU firing rate appears beyond the level of a substantial force increase. This may be explained by the saturating effect of firing rate on steady MU force output at high frequencies [32]. However, additional factors than little influence of increasing discharge rate on muscle force should be considered, in particular for the sub-maximal contractions. For these contractions voluntary torque should increase with increased discharge rates or motor unit recruitment. The indifferent torque responses also at sub-maximal forces might indicate that twice-daily resistive vibration exercise training altered the recruitment pattern among the different quadriceps components. The increase in activation of the vastus lateralis muscle might have been paralleled by a lesser activation of one or more of the agonist muscles. In addition, it is also conceivable that resistive vibration exercise enhanced co-activation of antagonist muscles during the isometric contractions. This would also explain why the increase in RMS in Fig. 5 in not gradual.

In conclusion, there was no evidence for a decrease in central neural drive in both groups Ctrl and RVE, in line with our previous report on the same subjects [33]. For Ctrl, this was possibly related to the repeated, albeit infrequently physical testing performed during the course of the bed rest, which might have preserved neural capabilities for this group. The decline in Fmed in Ctrl was in line with the concurrent changes in MFCV as a result of muscle fiber atrophy of the quadriceps femoris muscle. Resistive vibration exercise training during 56 days of bed rest, resulted in a significant increase in muscle activation during maximal and sub-maximal voluntary isometric knee extension in the trained subjects, as evidenced by the rapid and profound increase in RMS throughout bed rest. The increased excitation, which most likely resulted in an enhanced firing frequency of activated MUs, appeared ineffective to increase muscle strength under the isometric testing conditions. The presented data suggest that neural training effects of resistive vibration training during bed rest are highly task specific. Finally, it can be stated that this is the first study that demonstrates that the atrophy-preventing effect of resistive vibration exercise may come at the price of alterations in motor control, which appear inefficient for tasks dissimilar to the training mode itself.

REFRERENCES

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

[2] 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.

[3] Bamman MM, Caruso JF. Resistance exercise countermeasures for spaceflight: implications of training specificity. J Strength Cond Res 2000; 14: 45-49.

[4] 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.

[5] Bamman MM, Hunter GR, Stevens BR, Guilliams ME, Greenisen MC. Resistance exercise prevents plantar flexor deconditioning during bed rest. Med Sci Sports Exerc 1997; 29: 1462-1468.

[6] Beck RB, Houtman CJ, O'Malley MJ, Lowery MM, Stegeman DF. A technique to track individual motor unit action potentials in surface EMG by monitoring their conduction velocities and amplitudes. IEEE Trans Biomed Eng 2005; 52: 622-629.

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

[8] Berg HE, Tesch PA. Changes in muscle function in response to 10 days of lower limb unloading in humans. Acta Physiol Scand 1996; 157: 63-70.

[9] Blijham PJ, ter Laak HJ, Schelhaas HJ, van Engelen BG, Stegeman DF, Zwarts MJ. Relation between muscle fiber conduction velocity and fiber size in neuromuscular disorders 2. J Appl Physiol 2006; 100: 1837-1841.

[10] Blok JH, van Dijk JG, Drost G, Zwarts MJ, Stegeman DF. A high-density multichannel surface electromyography system for the characterization of single motor units. Review of Scientific Instruments 2002; 73: 1887-1897.

[11] Blottner D, Salanova M, Puttmann B, Schiffl G, Felsenberg D, Buehring B, Rittweger J. Human skeletal muscle structure and function preserved by vibration muscle exercise following 55 days of bed rest. Eur J Appl Physiol 2006; 97: 261-271.

[12] Burke D, Schiller HH. Discharge pattern of single motor units in the tonic vibration reflex of human triceps surae. J Neurol Neurosurg Psychiatry 1976; 39: 729-741.

[13] Cardinale M, Lim J. Electromyography activity of vastus lateralis muscle during whole-body vibrations of different frequencies. J Strength Cond Res 2003; 17: 621-624.

[14] De Luca CJ. The use of surface electromyography in biomechanics. J Appl Biomech 1997; 13: 135-163.

[15] Delecluse C, Roelants M, Verschueren S. Strength increase after whole-body vibration compared with resistance training. Med Sci Sports Exerc 2003; 35: 1033-1041.

[16] 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.

[17] Duchateau J, Enoka RM. Neural adaptations with chronic activity patterns in able-bodied humans. Am J Phys Med Rehabil 2002; 81: S17-S27.

[18] Dudley GA, Duvoisin MR, Adams GR, Meyer RA, Belew AH, Buchanan P. Adaptations to unilateral lower limb suspension in humans. Aviat Space Environ Med 1992; 63: 678-683.

[19] Farina D, Merletti R, Enoka RM. The extraction of neural strategies from the surface EMG. J Appl Physiol 2004; 96: 1486-1495.

[20] Frey I, Berg A, Grathwohl D, Keul J. [Freiburg Questionnaire of physical activity--development, evaluation and application]. Soz Praventivmed 1999; 44: 55-64.

[21] Fuglevand AJ, Winter DA, Patla AE. Models of recruitment and rate coding organization in motor-unit pools. J Neurophysiol 1993; 70: 2470-2488.

[22] 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.

[23] Griffin L, Garland SJ, Ivanova T, Gossen ER. Muscle vibration sustains motor unit firing rate during submaximal isometric fatigue in humans. J Physiol 2001; 535: 929-936.

[24] Hakkinen K, Alen M, Kallinen M, Newton RU, Kraemer WJ. Neuromuscular adaptation during prolonged strength training, detraining and re-strength-training in middle-aged and elderly people. Eur J Appl Physiol 2000; 83: 51-62.

[25] Herbert RD, Gandevia SC. Twitch interpolation in human muscles: mechanisms and implications for measurement of voluntary activation. J Neurophysiol 1999; 82: 2271-2283.

[26] Jaweed MM, Grana EA, Glennon TP, Monga TN, Mirabi B. Neuromuscular adaptations during 30 days of cast-immobilization and head-down bedrest. J Gravit Physiol 1995; 2: 72-73.

[27] 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.

[28] Kleine BU, Stegeman DF, Mund D, Anders C. Influence of motoneuron firing synchronization on SEMG characteristics in dependence of electrode position. J Appl Physiol 2001; 91: 1588-1599.

[29] Lindeman E, Spaans F, Reulen JP, Leffers P, Drukker J. Surface EMG of proximal leg muscles in neuromuscular patients and in healthy controls. Relations to force and fatigue. J Electromyogr Kinesiol 1999; 9: 299-307.

[30] Linssen WH, Stegeman DF, Joosten EM, van't Hof MA, Binkhorst RA, Notermans SL. Variability and interrelationships of surface EMG parameters during local muscle fatigue. Muscle Nerve 1993; 16: 849-856.

[31] Merletti R, Lo Conte LR. Surface EMG signal processing during isometric contractions. J Electromyogr Kinesiol 1997; 7: 241-250.

[32] Milner-Brown HS, Stein RB. The relation between the surface electromyogram and muscular force. J Physiol 1975; 246: 549-569.

[33] Mulder ER, Stegeman DF, Gerrits KH, Paalman MI, Rittweger J, Felsenberg D, de Haan A. Strength, size and activation of knee extensors followed during 8 weeks of horizontal bed rest and the influence of a countermeasure. Eur J Appl Physiol 2006; 97: 706-715.

[34] Nishihira Y, Iwasaki T, Hatta A, Wasaka T, Kaneda T, Kuroiwa K, Akiyama S, Kida T, Ryol KS. Effect of whole body vibration stimulus and voluntary contraction on motoneuron pool. Adv Exerc Sports Physiol 2002; 8: 83-86.

[35] Patten C, Kamen G, Rowland DM. Adaptations in maximal motor unit discharge rate to strength training in young and older adults. Muscle Nerve 2001; 24: 542-550.

[36] Proske U. What is the role of muscle receptors in proprioception? Muscle Nerve 2005; 31: 780-787.

[37] Rau G, Disselhorst-Klug C. Principles of high-spatial-resolution surface EMG (HSR-EMG): single motor unit detection and application in the diagnosis of neuromuscular disorders. J Electromyogr Kinesiol 1997; 7: 233-239.

[38] Rittweger J, Belavy D, Hunek P, Gast U, Boerst H, Feilcke B, Armbrecht G, Mulder E, Schubert H, Richardson C, de Haan A, Stegeman DF, Schiessl H, Felsenberg D. Highly Demanding Resistive Vibration Exercise Program is Tolerated During 56 Days of Strict Bed-Rest1. Int J Sports Med 2006; 27: 553-559.

[39] Rittweger J, Ehrig J, Just K, Mutschelknauss M, Kirsch KA, Felsenberg D. Oxygen uptake in whole-body vibration exercise: influence of vibration frequency, amplitude, and external load. Int J Sports Med 2002; 23: 428-432.

[40] Rittweger J, Mutschelknauss M, Felsenberg D. Acute changes in neuromuscular excitability after exhaustive whole body vibration exercise as compared to exhaustion by squatting exercise. Clin Physiol Funct Imaging 2003; 23: 81-86.

[41] Schulze K, Gallagher P, Trappe S. Resistance training preserves skeletal muscle function during unloading in humans. Med Sci Sports Exerc 2002; 34: 303-313.

[42] Shinohara M, Moritz CT, Pascoe MA, Enoka RM. Prolonged muscle vibration increases stretch reflex amplitude, motor unit discharge rate, and force fluctuations in a hand muscle. J Appl Physiol 2005; 99: 1835-1842.

[43] Stegeman DF, Mulder ER, Gerrits KH, Rittweger J, Felsenberg D, de Haan A. Peripherally and centrally induced electromyographic changes in 8 weeks of horizontal bed rest with and without resistance exercise with concurrent whole body vibration. J Gravit Physiol 2005; 12: 155-156.

[44] Suetta C, Aagaard P, Rosted A, Jakobsen AK, Duus B, Kjaer M, Magnusson SP. Training-induced changes in muscle CSA, muscle strength, EMG, and rate of force development in elderly subjects after long-term unilateral disuse. J Appl Physiol 2004; 97: 1954-1961.

[45] Widrick JJ, Norenberg KM, Romatowski JG, Blaser CA, Karhanek M, Sherwood J, Trappe SW, Trappe TA, Costill DL, Fitts RH. Force-velocity-power and force-pCa relationships of human soleus fibers after 17 days of bed rest. J Appl Physiol 1998; 85: 1949-1956.

[46] Yao W, Fuglevand RJ, Enoka RM. Motor-unit synchronization increases EMG amplitude and decreases force steadiness of simulated contractions. J Neurophysiol 2000; 83: 441-452.

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 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 '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.

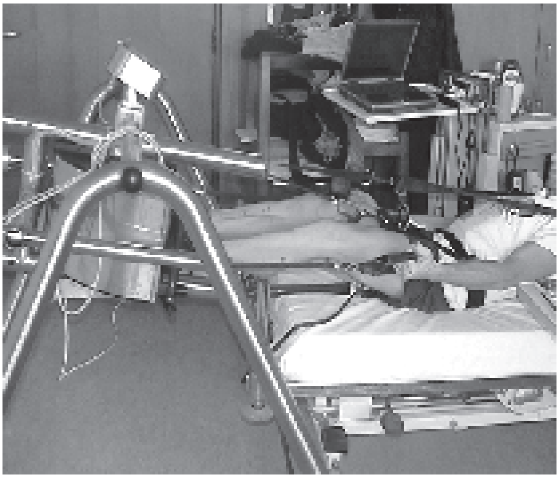

Fig. 1. Resistive vibration training device for usage in bed rest. The subjects attach themselves to a vibrating platform by belts with their waist, their shoulders and their hands. Voluntary resistive exercises are performed whilst vibration of the platform is generated by means of eccentrically rotating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

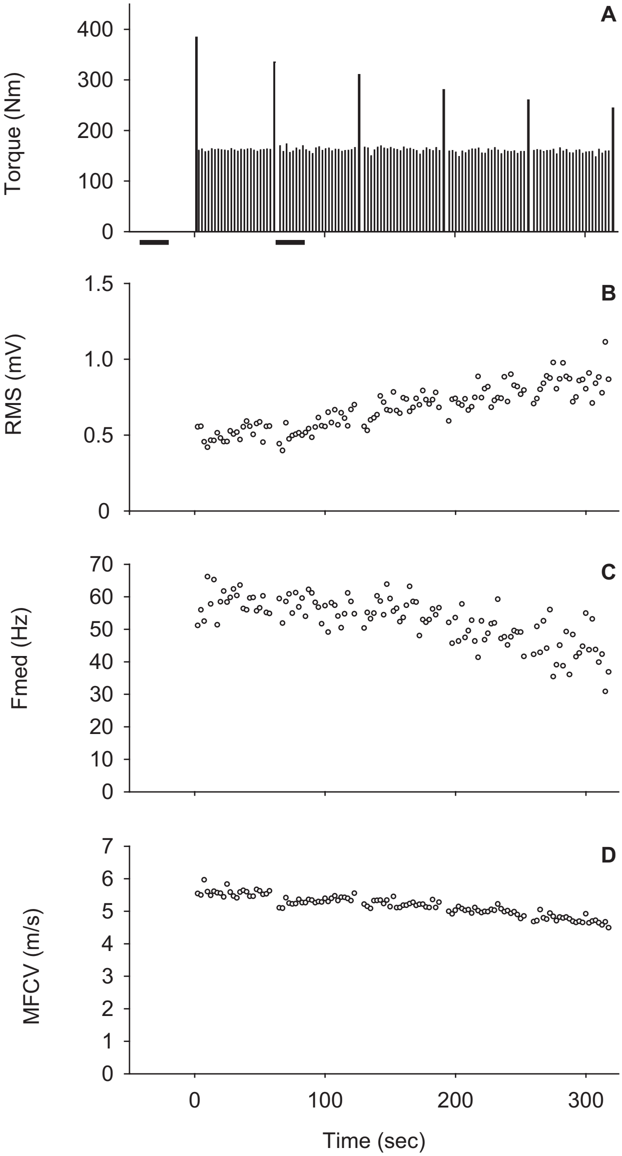

Fig. 2. Representative torque and sEMG profiles of one subject during the fatigue task. The vertical bars in panel A represent the voluntary torque, the open circles in panels B, C and D represent the different sEMG values (respectively RMS, root means square; Fmed, median frequency; MFCV, muscle fiber 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 Phototonics, 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 Phototonics, 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.

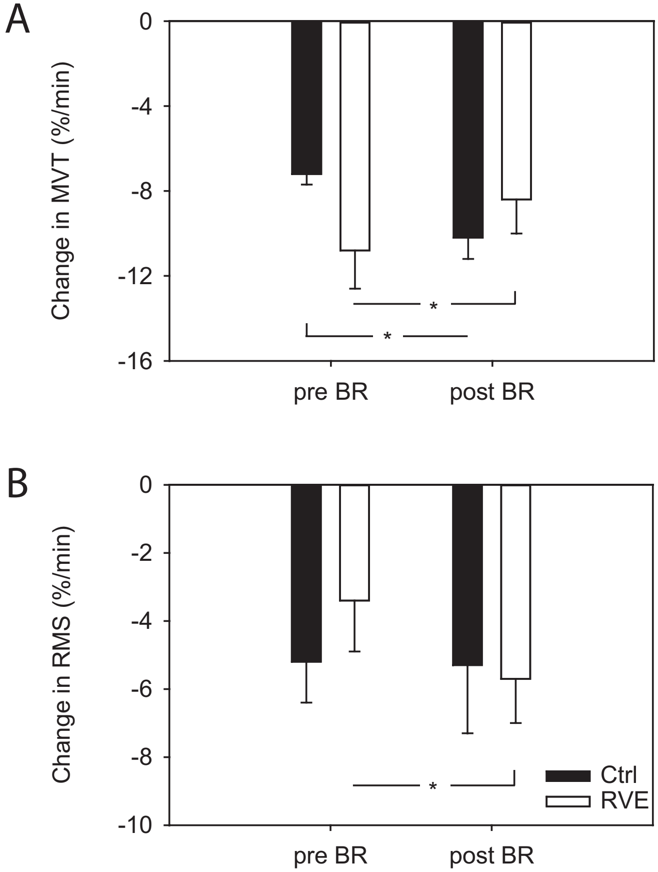

Fig 3. Fatigability (A) of the Ctrl and RVE group expressed as the percent change of initial maximal voluntary torque (MVT) per minute exercise and (B) the percent change per minute exercise from the initial maximal root mean square (RMS) of the sEMG obtained during the maximal contractions, pre and 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.

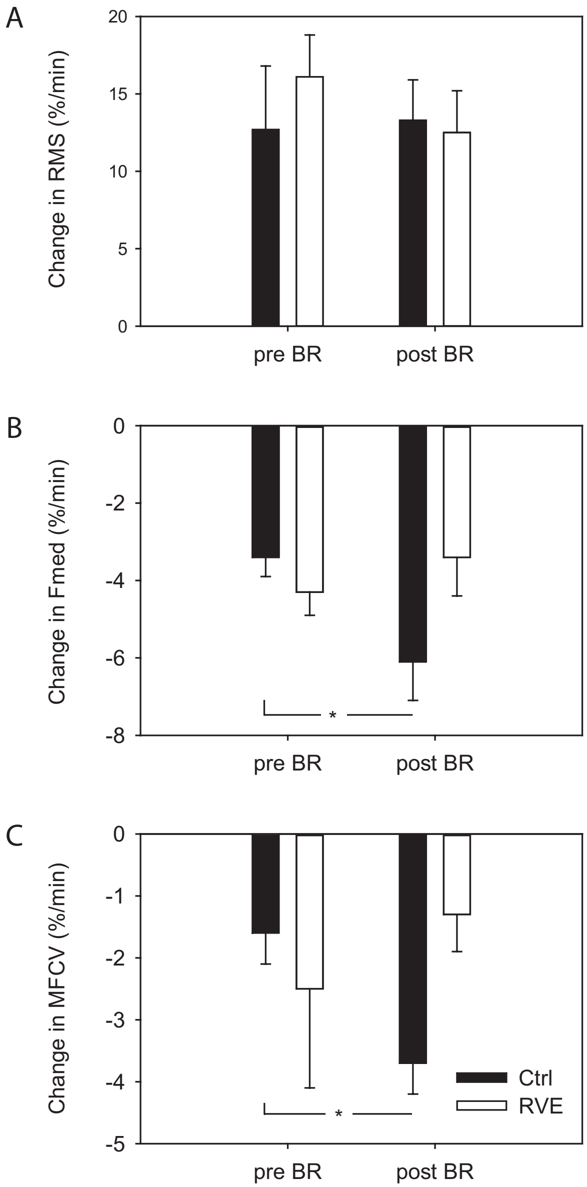

Fig. 4. Changes in sEMG amplitude; RMS (A), median frequency; Fmed (B) and muscle fiber conduction velocity; MFCV (C) for the Ctrl and RVE group as a consequence of the fatiguing sub-maximal contractions, pre and post bed rest (BR). Values (mean ± SEM) are expressed in percent change of initial value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

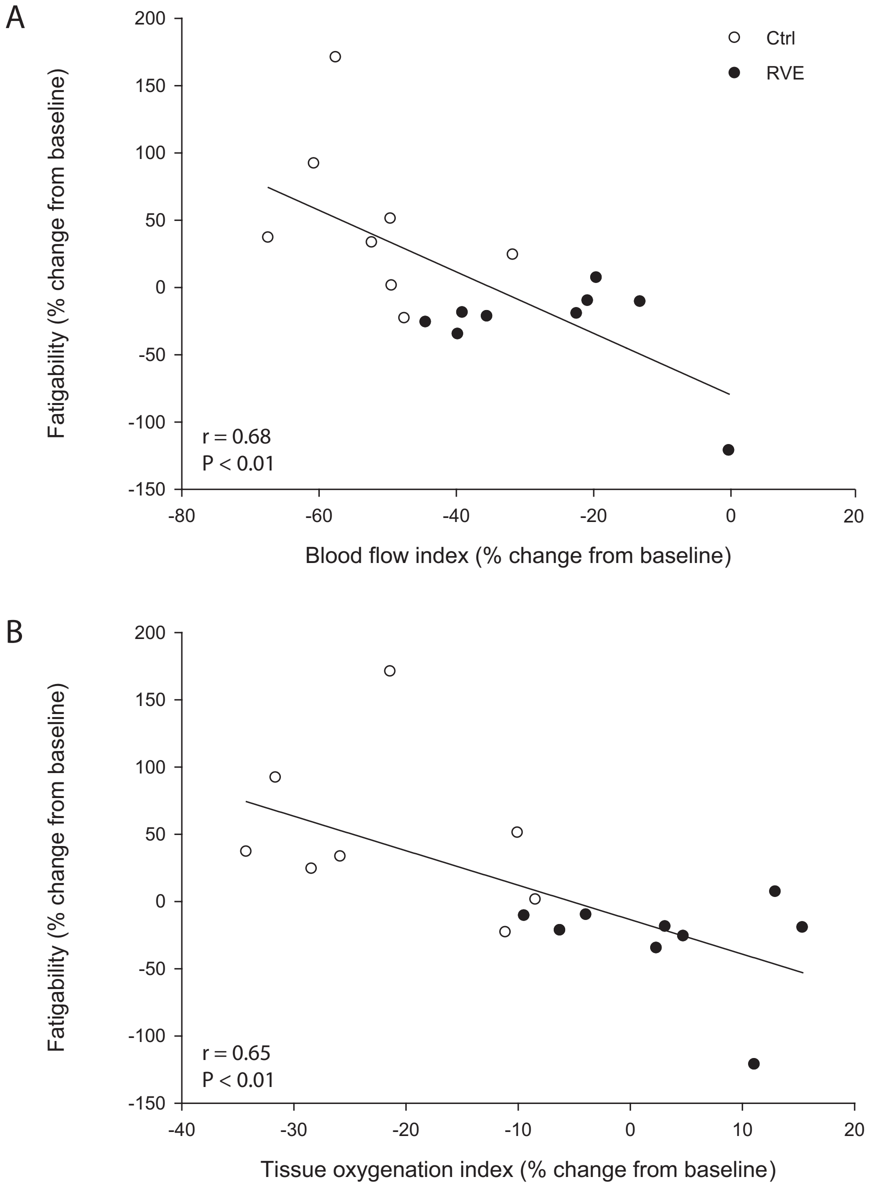

Fig. 5. Correlation between bed rest-induced changes in muscle blood flow and fatigability (A) and correlation between bed rest-induced changes in tissue oxygenation index and fatigability (B). Individual values (n = 17) are expressed as percent changes from baseline. Both linear regressions were significant.

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 ± 5.7% vs. 76.1 ± 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 ± 5.3% vs. 74.7 ± 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.

REFERENCES

[1] 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.

[2] Beck RB, Houtman CJ, O'Malley MJ, Lowery MM, Stegeman DF. A technique to track individual motor unit action potentials in surface EMG by monitoring their conduction velocities and amplitudes. IEEE Trans Biomed Eng 2005; 52: 622-629.

[3] Bleeker MW, De Groot PC, Rongen GA, Rittweger J, Felsenberg D, Smits P, Hopman MT. Vascular adaptation to deconditioning and the effect of an exercise countermeasure: results of the Berlin Bed Rest study. J Appl Physiol 2005; 99: 1293-1300.

[4] Boushel R, Langberg H, Olesen J, Nowak M, Simonsen L, Bulow J, Kjaer M. Regional blood flow during exercise in humans measured by near-infrared spectroscopy and indocyanine green. J Appl Physiol 2000; 89: 1868-1878.

[5] Brown M, Hasser EM. Weight-bearing effects on skeletal muscle during and after simulated bed rest. Arch Phys Med Rehabil 1995; 76: 541-546.

[6] Convertino VA. Exercise as a countermeasure for physiological adaptation to prolonged spaceflight. Med Sci Sports Exerc 1996; 28: 999-1014.

[7] Convertino VA. Cardiovascular consequences of bed rest: effect on maximal oxygen uptake. Med Sci Sports Exerc 1997; 29: 191-196.

[8] Convertino VA, Bloomfield SA, Greenleaf JE. An overview of the issues: physiological effects of bed rest and restricted physical activity. Med Sci Sports Exerc 1997; 29: 187-190.

[9] Delp MD, Colleran PN, Wilkerson MK, McCurdy MR, Muller-Delp J. Structural and functional remodeling of skeletal muscle microvasculature is induced by simulated microgravity. Am J Physiol Heart Circ Physiol 2000; 278: H1866-H1873.

[10] 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.

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

[12] Ferretti G, Girardis M, Moia C, Antonutto G. Effects of prolonged bed rest on cardiovascular oxygen transport during submaximal exercise in humans. Eur J Appl Physiol Occup Physiol 1998; 78: 398-402.

[13] 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.

[14] Grichko VP, Heywood-Cooksey A, Kidd KR, Fitts RH. Substrate profile in rat soleus muscle fibers after hindlimb unloading and fatigue. J Appl Physiol 2000; 88: 473-478.

[15] Hunter SK, Enoka RM. Changes in muscle activation can prolong the endurance time of a submaximal isometric contraction in humans. J Appl Physiol 2003; 94: 108-118.

[16] Jubrias SA, Crowther GJ, Shankland EG, Gronka RK, Conley KE. Acidosis inhibits oxidative phosphorylation in contracting human skeletal muscle in vivo. J Physiol 2003; 553: 589-599.

[17] 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.

[18] Keenan KG, Farina D, Maluf KS, Merletti R, Enoka RM. Influence of amplitude cancellation on the simulated surface electromyogram. J Appl Physiol 2005; 98: 120-131.

[19] Krogh-Lund C, Jorgensen K. Myo-electric fatigue manifestations revisited: power spectrum, conduction velocity, and amplitude of human elbow flexor muscles during isolated and repetitive endurance contractions at 30% maximal voluntary contraction. Eur J Appl Physiol Occup Physiol 1993; 66: 161-173.

[20] Kuebler WM, Sckell A, Habler O, Kleen M, Kuhnle GE, Welte M, Messmer K, Goetz AE. Noninvasive measurement of regional cerebral blood flow by near-infrared spectroscopy and indocyanine green. J Cereb Blood Flow Metab 1998; 18: 445-456.

[21] Laaksonen MS, Kalliokoski KK, Kyrolainen H, Kemppainen J, Teras M, Sipila H, Nuutila P, Knuuti J. Skeletal muscle blood flow and flow heterogeneity during dynamic and isometric exercise in humans. Am J Physiol Heart Circ Physiol 2003; 284: H979-H986.

[22] Linssen WH, Jacobs M, Stegeman DF, Joosten EM, Moleman J. Muscle fatigue in McArdle's disease. Muscle fibre conduction velocity and surface EMG frequency spectrum during ischaemic exercise. Brain 1990; 113 (Pt 6): 1779-1793.

[23] Marsden CD, Meadows JC, Merton PA. "Muscular wisdom" that minimizes fatigue during prolonged effort in man: peak rates of motoneuron discharge and slowing of discharge during fatigue. Adv Neurol 1983; 39: 169-211.

[24] Masuda K, Masuda T, Sadoyama T, Inaki M, Katsuta S. Changes in surface EMG parameters during static and dynamic fatiguing contractions. J Electromyogr Kinesiol 1999; 9: 39-46.

[25] Mulder ER, Stegeman DF, Gerrits KH, Paalman MI, Rittweger J, Felsenberg D, de Haan A. Strength, size and activation of knee extensors followed during 8 weeks of horizontal bed rest and the influence of a countermeasure. Eur J Appl Physiol 2006; 97: 706-716.

[26] Narici M, Kayser B, Barattini P, Cerretelli P. Effects of 17-day spaceflight on electrically evoked torque and cross-sectional area of the human triceps surae. Eur J Appl Physiol 2003; 90: 275-282.

[27] Portero P, Vanhoutte C, Goubel F. Surface electromyogram power spectrum changes in human leg muscles following 4 weeks of simulated microgravity. Eur J Appl Physiol Occup Physiol 1996; 73: 340-345.

[28] Redlin M, Boettcher W, Huebler M, Berger F, Hetzer R, Koster A, Kuebler WM. Detection of lower torso ischemia by near-infrared spectroscopy during cardiopulmonary bypass in a 6.8-kg infant with complex aortic anatomy. Ann Thorac Surg 2006; 82: 323-325.

[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 DF, Schiessl H, Felsenberg D. Highly Demanding Resistive Vibration Exercise Program is Tolerated During 56 Days of Strict Bed-Rest1. Int J Sports Med 2006; 27: 553-559.

[30] Rittweger J, Ehrig J, Just K, Mutschelknauss M, Kirsch KA, Felsenberg D. Oxygen uptake in whole-body vibration exercise: influence of vibration frequency, amplitude, and external load. Int J Sports Med 2002; 23: 428-432.

[31] Rochette L, Hunter SK, Place N, Lepers R. Activation varies among the knee extensor muscles during a submaximal fatiguing contraction in the seated and supine postures. J Appl Physiol 2003; 95: 1515-1522.

[32] Russ DW, Elliott MA, Vandenborne K, Walter GA, Binder-Macleod SA. Metabolic costs of isometric force generation and maintenance of human skeletal muscle. Am J Physiol Endocrinol Metab 2002; 282: E448-E457.

[33] Schillings ML, Stegeman DF, Zwarts MJ. Determining central activation failure and peripheral fatigue in the course of sustained maximal voluntary contractions: a model-based approach. J Appl Physiol 2005; 98: 2292-2297.

[34] Semmler JG, Kutzscher DV, Enoka RM. Limb immobilization alters muscle activation patterns during a fatiguing isometric contraction. Muscle Nerve 2000; 23: 1381-1392.

[35] Sjogaard G, Savard G, Juel C. Muscle blood flow during isometric activity and its relation to muscle fatigue. Eur J Appl Physiol Occup Physiol 1988; 57: 327-335.

[36] Stegeman DF, Linssen WH. Muscle fiber action potential changes and surface EMG: A simulation study. J Electromyogr Kinesiol 1992; 2: 130-140.

[37] Stein TP, Wade CE. Metabolic consequences of muscle disuse atrophy. J Nutr 2005; 135: 1824S-1828S.

[38] Suzuki S, Takasaki S, Ozaki T, Kobayashi Y. A tissue oxygenation monitor using NIR spatially resolved spectroscopy. J Biomed Opt : Proceedings of SPIE 2395 1999; 124-129.

[39] Trappe S, Trappe T, Gallagher P, Harber M, Alkner B, Tesch P. Human single muscle fibre function with 84 day bed-rest and resistance exercise. J Physiol 2004; 557: 501-513.

[40] Warren GL, Stallone JL, Allen MR, Bloomfield SA. Functional recovery of the plantarflexor muscle group after hindlimb unloading in the rat. Eur J Appl Physiol 2004; 93: 130-138.

[41] Zhang LF. Vascular adaptation to microgravity: what have we learned? J Appl Physiol 2001; 91: 2415-2430.

[42] Zwarts MJ, Arendt-Nielsen L. The influence of force and circulation on average muscle fibre conduction velocity during local muscle fatigue. Eur J Appl Physiol Occup Physiol 1988; 58: 278-283.

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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Submitted

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.

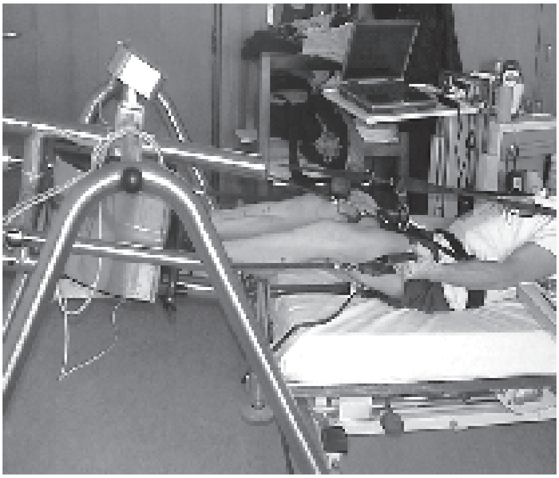

Fig. 1. Resistive vibration training device used during bed rest. The subjects attached themselves to the vibrating platform by belts at their waist, their shoulders and their hands. Voluntary resistive exercises were performed whilst vibration of the platform was generated by means of eccentrically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

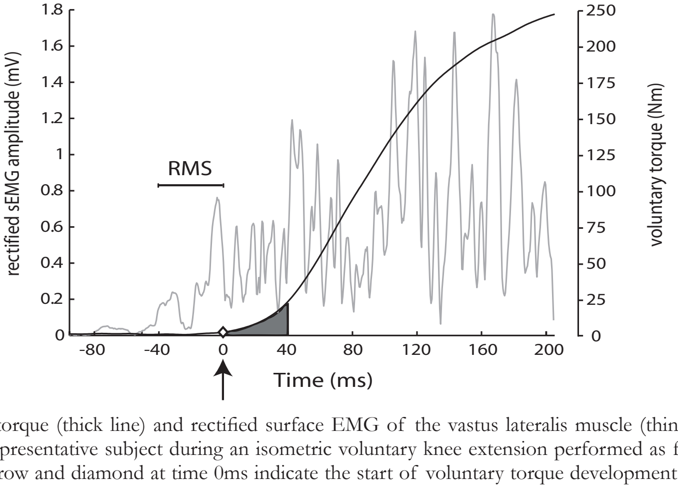

Fig. 2. Voluntary torque (thick line) and rectified surface EMG of the vastus lateralis muscle (thin line) time traces, obtained from a representative subject during an isometric voluntary knee extension performed as fast and forcefully as possible. The arrow and diamond at time 0ms indicate the start 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.

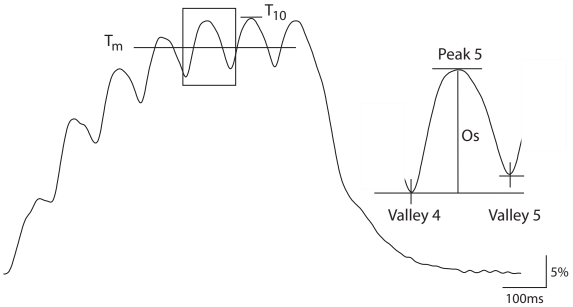

Fig. 3. Methods used for evaluation of contractile properties of the quadriceps femoris muscle evoked by electrically evoked muscle stimulation at 10Hz. The torque elicited at 10Hz stimulation was first expressed as a percentage of the maximal torque evoked at 150Hz (T150). T10 was the peak value of 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).

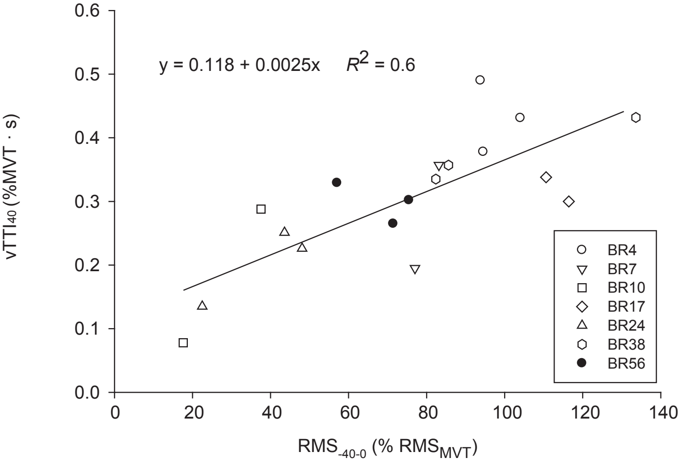

Fig. 4. A representative example of the positive correlation ($P < 0.01$, R2 = 0.6) between and the time torque integral over the first 40 ms ($vTTI_{40}$) after torque development. Positive individual relationships were obtained for 12 of the 16 subjects. To obtain the individual relationships, for 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.

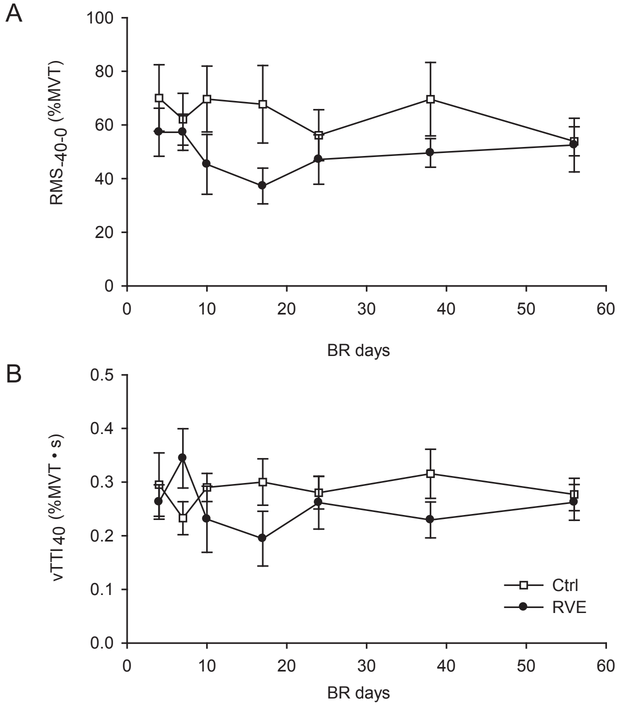

Fig. 5. Mean values (± SEM) of the voluntary time torque integral over the first 40 ms (TTI40) after torque development ($vTTI_{40}$; B) and the sEMG amplitude 40 ms before the onset of torque development (RMS_{-40-0}; A) obtained during 56 days of bed rest (BR). For each session, both $vTTI_{40}$ and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

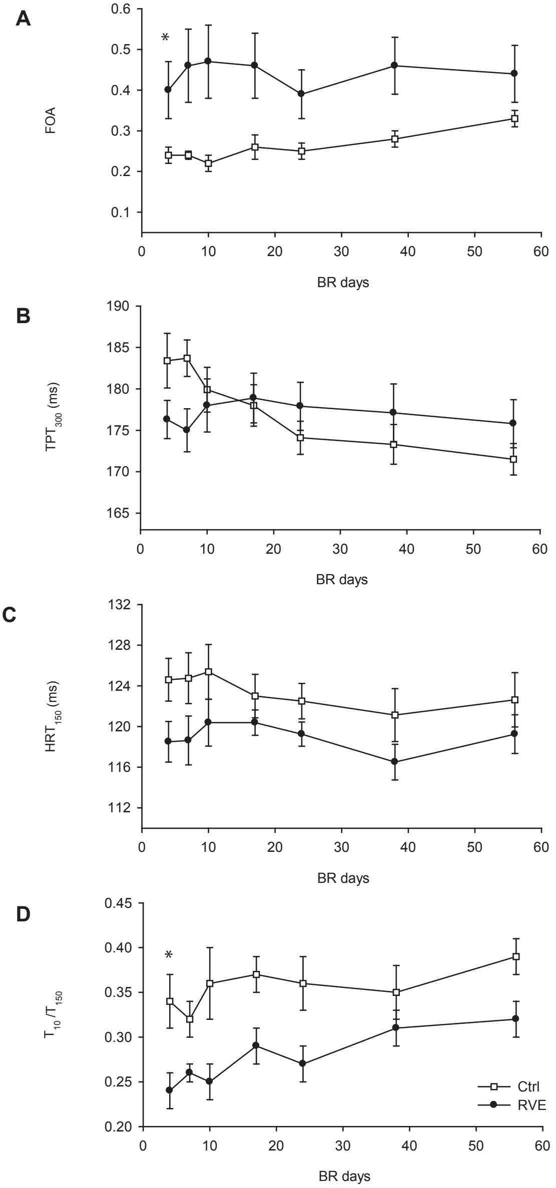

Fig. 6. Mean values (± SEM) of the force oscillation amplitude (FOA; A) time to peak tension at 300Hz stimulation (TPT300: B), half relaxation time at 150Hz stimulation (HRT150), and peak torque at 10Hz stimulation expressed as a fraction of the maximal torque obtained during tetanic stimulation at 150Hz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

REFERENCES

[1] Aagaard P, Simonsen EB, Andersen JL, Magnusson P, Dyhre-Poulsen P. Increased rate of force development and neural drive of human skeletal muscle following resistance training. J Appl Physiol 2002; 93: 1318-1326.

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

[3] Andersen LL, Aagaard P. Influence of maximal muscle strength and intrinsic muscle contractile properties on contractile rate of force development. Eur J Appl Physiol 2006; 96: 46-52.

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

[5] Binder-Macleod SA, Halden EE, Jungles KA. Effects of stimulation intensity on the physiological responses of human motor units. Med Sci Sports Exerc 1995; 27: 556-565.

[6] Blok JH, van Dijk JG, Drost G, Zwarts MJ, Stegeman DF. A high-density multichannel surface electromyography system for the characterization of single motor units. Review of Scientific Instruments 2002; 73: 1887-1897.

[7] Blottner D, Salanova M, Puttmann B, Schiffl G, Felsenberg D, Buehring B, Rittweger J. Human skeletal muscle structure and function preserved by vibration muscle exercise following 55 days of bed rest. Eur J Appl Physiol 2006; 97: 261-271.

[8] Bojsen-Moller J, Magnusson SP, Rasmussen LR, Kjaer M, Aagaard P. Muscle performance during maximal isometric and dynamic contractions is influenced by the stiffness of the tendinous structures. J Appl Physiol 2005; 99: 986-994.

[9] Caiozzo VJ, Baker MJ, Herrick RE, Tao M, Baldwin KM. Effect of spaceflight on skeletal muscle: mechanical properties and myosin isoform content of a slow muscle. J Appl Physiol 1994; 76: 1764-1773.

[10] Davies CT, Rutherford IC, Thomas DO. Electrically evoked contractions of the triceps surae during and following 21 days of voluntary leg immobilization. Eur J Appl Physiol Occup Physiol 1987; 56: 306-312.

[11] de Haan A. The influence of stimulation frequency on force-velocity characteristics of in situ rat medial gastrocnemius muscle. Exp Physiol 1998; 83: 77-84.

[12] de Haan A, de Ruiter CJ, van der Woude LH, Jongen PJ. Contractile properties and fatigue of quadriceps muscles in multiple sclerosis. Muscle Nerve 2000; 23: 1534-1541.

[13] de Ruiter CJ, Jones DA, Sargeant AJ, de Haan A. Temperature effect on the rates of isometric force development and relaxation in the fresh and fatigued human adductor pollicis muscle. Exp Physiol 1999; 84: 1137-1150.

[14] de Ruiter CJ, Kooistra RD, Paalman MI, de Haan A. Initial phase of maximal voluntary and electrically stimulated knee extension torque development at different knee angles. J Appl Physiol 2004; 97: 1693-1701.

[15] 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.

[16] Desplanches D. Structural and functional adaptations of skeletal muscle to weightlessness. Int J Sports Med 1997; 18 Suppl 4: S259-S264.

[17] Edgerton VR, Roy RR. Invited review: gravitational biology of the neuromotor systems: a perspective to the next era. J Appl Physiol 2000; 89: 1224-1231.

[18] Fitts RH, Riley DR, Widrick JJ. Physiology of a microgravity environment invited review: microgravity and skeletal muscle. J Appl Physiol 2000; 89: 823-839.

[19] Gerrits HL, de Haan A, Hopman MT, van der Woude LH, Jones DA, Sargeant AJ. Contractile properties of the quadriceps muscle in individuals with spinal cord injury. Muscle Nerve 1999; 22: 1249-1256.

[20] Gerrits HL, Hopman MT, Offringa C, Engelen BG, Sargeant AJ, Jones DA, de Haan A. Variability in fibre properties in paralysed human quadriceps muscles and effects of training. Pflugers Arch 2003; 445: 734-740.

[21] Gerrits HL, Hopman MT, Sargeant AJ, de Haan A. Reproducibility of contractile properties of the human paralysed and non-paralysed quadriceps muscle. Clin Physiol 2001; 21: 105-113.

[22] 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.

[23] Harridge SD, Bottinelli R, Canepari M, Pellegrino MA, Reggiani C, Esbjornsson M, Saltin B. Whole-muscle and single-fibre contractile properties and myosin heavy chain isoforms in humans. Pflugers Arch 1996; 432: 913-920.

[24] Koryak Y. Effect of 120 days of bed-rest with and without countermeasures on the mechanical properties of the triceps surae muscle in young women. Eur J Appl Physiol Occup Physiol 1998; 78: 128-135.

[25] 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.

[26] Lambertz D, Goubel F, Kaspranski R, Perot C. Influence of long-term spaceflight on neuromechanical properties of muscles in humans. J Appl Physiol 2003; 94: 490-498.

[27] Mulder ER, Stegeman DF, Gerrits KH, Paalman MI, Rittweger J, Felsenberg D, de Haan A. Strength, size and activation of knee extensors followed during 8 weeks of horizontal bed rest and the influence of a countermeasure. Eur J Appl Physiol 2006.

[28] Narici M, Kayser B, Barattini P, Cerretelli P. Effects of 17-day spaceflight on electrically evoked torque and cross-sectional area of the human triceps surae. Eur J Appl Physiol 2003; 90: 275-282.

[29] Ohira Y, Yoshinaga T, Ohara M, Nonaka I, Yoshioka T, Yamashita-Goto K, Shenkman BS, Kozlovskaya IB, Roy RR, Edgerton VR. Myonuclear domain and myosin phenotype in human soleus after bed rest with or without loading. J Appl Physiol 1999; 87: 1776-1785.

[30] Reeves ND. Adaptation of the tendon to mechanical usage. J Musculoskelet Neuronal Interact 2006; 6: 174-180.

[31] Rittweger J, Belavy D, Hunek P, Gast U, Boerst H, Feilcke B, Armbrecht G, Mulder E, Schubert H, Richardson C, de Haan A, Stegeman DF, Schiessl H, Felsenberg D. Highly Demanding Resistive Vibration Exercise Program is Tolerated During 56 Days of Strict Bed-Rest. Int J Sports Med 2006; 27: 553-559.

[32] Rittweger J, Ehrig J, Just K, Mutschelknauss M, Kirsch KA, Felsenberg D. Oxygen uptake in whole-body vibration exercise: influence of vibration frequency, amplitude, and external load. Int J Sports Med 2002; 23: 428-432.

[33] Schulze K, Gallagher P, Trappe S. Resistance training preserves skeletal muscle function during unloading in humans. Med Sci Sports Exerc 2002; 34: 303-313.

[34] Seki K, Taniguchi Y, Narusawa M. Alterations in contractile properties of human skeletal muscle induced by joint immobilization. J Physiol 2001; 530: 521-532.

[35] Shigematsu R, Rantanen T, Saari P, Sakari-Rantala R, Kauppinen M, Sipila S, Heikkinen E. Motor speed and lower extremity strength as predictors of fall-related bone fractures in elderly individuals. Aging Clin Exp Res 2006; 18: 320-324.

[36] Smith SM, Heer M. Calcium and bone metabolism during space flight. Nutrition 2002; 18: 849-852.

[37] Talmadge RJ. Myosin heavy chain isoform expression following reduced neuromuscular activity: potential regulatory mechanisms. Muscle Nerve 2000; 23: 661-679.

[38] Trappe S, Trappe T, Gallagher P, Harber M, Alkner B, Tesch P. Human single muscle fibre function with 84 day bed-rest and resistance exercise. J Physiol 2004; 557: 501-513.

[39] Unsworth BR, Witzmann FA, Fitts RH. A comparison of rat myosin from fast and slow skeletal muscle and the effect of disuse. J Biol Chem 1982; 257: 15129-15136.

[40] Vøllestad NK, Sejersted I, Saugen E. Mechanical behavior of skeletal muscle during intermittent voluntary isometric contractions in humans. J Appl Physiol 1997; 83: 1557-1565.

[41] Widrick JJ, Norenberg KM, Romatowski JG, Blaser CA, Karhanek M, Sherwood J, Trappe SW, Trappe TA, Costill DL, Fitts RH. Force-velocity-power and force-pCa relationships of human soleus fibers after 17 days of bed rest. J Appl Physiol 1998; 85: 1949-1956.

[42] Widrick JJ, Romatowski JG, Norenberg KM, Knuth ST, Bain JL, Riley DA, Trappe SW, Trappe TA, Costill DL, Fitts RH. Functional properties of slow and fast gastrocnemius muscle fibers after a 17-day spaceflight. J Appl Physiol 2001; 90: 2203-2211.

[43] Yamashita-Goto K, Okuyama R, Honda M, Kawasaki K, Fujita K, Yamada T, Nonaka I, Ohira Y, Yoshioka T. Maximal and submaximal forces of slow fibers in human soleus after bed rest. J Appl Physiol 2001; 91: 417-424.

CHAPTER 6

General discussion

INTRODUCTION

The first aim of this thesis was to obtain insight into the changes in neuromuscular integrity of an antigravity muscle as a consequence of 56 days of bed rest immobilization. We were particularly interested in the time course of changes in knee extensor muscle size, strength, contractile characteristics and neural activation processes during bed rest, because such information might increase the understanding of dominant processes underlying the various manifestations of muscle weakness following muscle unloading. In addition, another equally important aim of this thesis was to assess whether such changes could be effectively prevented by resistive vibration exercise training during bed rest. In this chapter, the main results of, and conclusions drawn from the studies performed will be revisited and supplemented by noteworthy findings that have not been addressed in the main chapters in this thesis. The findings are placed into perspective with respect to their implications for human spaceflight, as well as their clinical relevance.

NEUROMUSCULAR ADAPTATIONS TO BED REST

Exposure to microgravity conditions initiates a cascade of interrelated events that result in the adaptation of virtually the entire human body. Because of the complexity and limited opportunity to study humans in space, we used the bed rest model to simulate conditions of microgravity. For the neuromuscular system, the most important aspect of bed rest immobilization is the interruption of the weight-bearing activity of skeletal muscles whose primary task it is to stabilize body posture and to move body parts against gravity. Such reduced muscle usage has been demonstrated to cause unambiguous adaptations in muscle function, but not much is known about the time course in which these adaptations occur.

The lack of longitudinal information on time course of changes in neuromuscular function, despite a wealth of bed rest campaigns, is not without reason. The assessment of the time course of changing muscle function presents a scientific challenge, because repeated functional testing, which is needed to allow an accurate time course evaluation, may oppose the very effects of the bed rest intervention [33]. Mindful of this consideration, we tried to prevent such an effect by two means. First, we limited the number of high-force contractions for each test session, especially during bed rest, because these were expected to elicit the most potent training response. Secondly, we substantially reduced the frequency of testing during the course of the bed rest. Based on cross-sectional comparison of previous reports, the most rapid changes were foreseen for the first 10 days of bed rest, such that we expected an accurate estimate of changes with longer testing intervals after this time period.

Despite the abovementioned two precautions, a consistent observation over subjects was an absence of deconditioning of neural control in the frequently tested right leg, whether assessed by means of the twitch interpolation technique (Chapter 2), or assessed by the analysis of vastus lateralis high density surface electromyography (sEMG) amplitude during steady-state and ballistic voluntary contractions (Chapters 3 and 5). The most likely explanation for these findings was that indeed the repeated functional testing during bed rest caused a habituation to the performed tasks. For steady-state contractions, this notion was supported by the findings for the left leg, which was only tested pre- and post bed rest. For this leg, the loss in muscle strength exceeded the loss in muscle size by almost a factor two (Chapter 2). From a scientific point of view these findings illustrate that longitudinal study designs may not be appropriate to address questions related to the time course of changes in neural capacity, and that such information should be obtained by cross-sectional comparison of studies with varying study durations, instead. However, at present there is no international agreement on a common procedure to measure and evaluate the effects of bed rest. Strict standardization of methods in future bed rest campaigns is therefore an absolute necessity. In practical terms, the abovementioned findings for the tested leg, although not intended, are significant and encouraging because they seem to demonstrate that relatively little effort is needed to preserve neural integrity. Such conclusions may direct future space medicine research to address the minimal requirements of preventative interventions to combat the effects of prolonged spaceflight.

Eight weeks of bed rest resulted in a significant reduction in the anatomical cross-sectional area (CSA) of the knee extensor group of about 14% as measured with magnetic resonance imaging (MRI). Interestingly, for the muscular system the repeated functional testing presented no significant stimulus, as the change in CSA following bed rest was similar between the frequently tested right leg and the non-tested left leg (Chapter 2). Apparently, more muscular activity is needed to preserve muscle size than to maintain voluntary activation processes. The observation that changes in muscle size were well described by a linear model (Chapter 2) indicated that the rate of downsizing of the quadriceps femoris muscle as a whole had not reached its nadir during the 56 days of bed rest. However, the susceptibility to atrophy was not equally shared among the four heads of the quadriceps femoris muscle. It was interesting to find that the size of the rectus femoris muscle was not influenced by bed rest confinement at all. Because both left and right leg showed this absence of change in size, the finding was apparently not related to the functional testing during bed rest. Instead, this finding may be explained by the continued use of this muscle to raise the leg under bed rest conditions. Such interpretation would be consistent with the absence of changes in size of this muscle during unilateral lower limb suspension [18]. In this model, the rectus femoris of the suspended leg is also likely to be activated to some degree, particularly during the forward swing in crutch-assisted walking. By continuously recording sEMG signals of the rectus femoris muscle, future studies might be able to determine how often and at what intensity this muscle is activated under unloading conditions. Interestingly, we did also not find a change in the angle-dependency of maximal voluntary torque production. This suggests that the length characteristics of the quadriceps femoris muscle did most likely not change as a consequence of the bed rest intervention, whereas previously significant changes in muscle fiber length following bed rest were reported for the plantar flexor [22]. Video surveillance of the subjects evidenced that the knee was frequently held in flexed positions, i.e. at a stretched quadriceps femoris muscle length. Such passive stretching may haven been sufficient to oppose changes in muscle fiber length.

In addition to the downsizing in muscle mass as a result of the reduced physiological demands during bed rest, modulation of the muscle phenotype appears to be another important adaptive capacity of skeletal muscle [32]. However, the conversion of fiber properties to faster (Chapter 5) and potentially less oxidative characteristics in response to unloading can also be referred to as deconditioning because it may substantially impair the capacity for prolonged exercise following unloading [30]. The increase in *relative* quadriceps femoris muscle fatigability as a consequence of bed rest was, based on changes in sEMG variables, most likely of a peripheral origin (Chapter 4). The assessment of vastus lateralis oxygenation and hemodynamics during exercise by near-infrared spectroscopy (NIRS) further suggested that the increased fatigability resulted predominantly from the consequences of a significant reduction in muscle blood flow during exercise and not, or at least to a lesser degree, to a reduced oxidative capacity of the quadriceps femoris muscle. In fact, with respect to the pre-bed rest condition, the relative extraction of oxygen from the blood was increased during exercise, following bed rest. This is interesting, because bed rest resulted in an impaired oxygen-carrying capacity, as red blood cell mass decreased by approximately 11% (D. Böning, personal communication). The increased oxygen extraction could only partly diminish the dramatic reduction in its delivery. Hence, it can be expected that there was an increased need for energy supply by anaerobic pathways in order to fulfill the energy demand of the required task. Although not mentioned in Chapter 4, the reduction in blood flow was further consistent with the slower rate of recovery from fatigue following bed rest. Indeed, it seems likely that the wash-out of produced metabolic waste products would also have been significantly reduced by an impaired muscle blood flow. Bleeker et al. [5] reported a ~17% reduction in the diameter of the quadriceps femoris muscle feed arteries, yet the reduction in blood flow in our study was dramatically more reduced, implying that other factors contributed to the reduction in blood flow. One such a factor might be a significant reduction in total plasma volume as a consequence of bed rest [17]. It should be mentioned that in the study presented in Chapter 5, total blood volume was not measured. In addition, blood flow was assessed during the second minute of exercise only. For future studies it would not only be desirable to measure blood flow throughout the entire fatigue protocol, but also blood flow in all superficial heads of the quadriceps femoris muscle to obtain a more comprehensive picture. Hardware limitations prevented such quantifications during the Berlin Bed Rest study, as the NIRS apparatus had a maximal storing capacity of approximately 2 minutes worth of data.

EFFICACY OF RESISTIVE VIBRATION EXERCISE

A major objective of space medicine research is to provide astronauts with time-efficient and effective countermeasures to combat the adaptive changes in the human physiological system as a consequence of spaceflight. Over the last decades, the generally accepted countermeasure during actual spaceflight has been physical exercise with a high aerobic component, such as pedaling a cycle-ergometer or exercising on a rowing machine. Although such efforts have been partly effective to maintain muscle function, these interventions are particularly ineffective to preserve the integrity of the weight-bearing skeleton [8]. In contrast, ‘conventional’ resistance training, which involves isometric and dynamic (concentric and eccentric) contractions against gravity, should be more promising, because under ambulant conditions such exercise paradigms provide the most potent stimulus for simultaneously increasing mass en strength of muscle and bone. Recent bed rest campaigns have indeed confirmed the potential of resistance training to maintain neuromuscular function as well as bone mass and strength under simulated spaceflight conditions [1;14;19;28]. However, minimal requirements with respect to training modality, types of exercises, the volume and frequency of training sessions have yet to be determined. Moreover, to be effective during actual spaceflight, the loading of weight-bearing structures cannot arise from gravity, but has to originate from other sources. Recent developments in exercise hardware that was designed according to such criteria were found insufficiently effective to preserve bone and muscle simultaneously [2;25].

This thesis addresses one of the latest developments in countermeasure design: resistive vibration exercise (RVE). In short, RVE is a neuromuscular training technique where subjects perform loaded weight-bearing exercises on a platform which applies vibrations to the two feet. It is assumed that these imposed vibrations evoke reflexive muscle contractions [24], probably via stretch reflexes, i.e. through the activation of muscle spindle (Ia) fibers [26]. This feedback loop increases the alpha-motoneuron activity and may thus result into a greater facilitation of the muscle drive during training. Under ambulant conditions, vibration training has been successfully tested for prevention and treatment of osteoporosis in postmenopausal women as well as in physically disabled children [34;35]. In contrast, the effect of vibration training to induce muscular changes in healthy adults has been discussed with much more controversy [11;12;26;31]. De Ruiter et al. [11] suggested that that young, physically fit, individuals may not necessarily benefit from such a training paradigm, unless additional loads are applied. Hence in the Berlin Bed Rest study the allocated training regime consisted of strength training exercises performed during whole body vibration. Because of this, the magnitude of stimulus due to vibration alone has yet to be quantified. From a biomechanical perspective it can be speculated, however, that the added vibration increased the efficacy of the countermeasure for two main reasons. First, without added vibration, the loading of the subject would have been generated only by means of the springs that restrained the subject to the footplate of the Galileo device. Springs have the disadvantage that they absorb energy; energy that is otherwise transferred into an impulse-type loading on the skeletal muscles and skeleton. This impulse is suggested crucial for maintenance of bone under disuse conditions [27]. In addition, the efficacy of standard weight-lifting programs is partly ascribed to the performance of eccentric contractions [13]. In the case of resistance created by means of springs or by other materials with elastic properties, this eccentric component may be greatly reduced, which means that part of the efficacy of the training program will be lost. To test these hypotheses, the most direct approach for future research would to be to incorporate two training groups; one group would perform resistive exercise in the presence of added vibrations, whereas the other group would execute an identical protocol, but without the added vibrations. It should be recognized, however, that such an approach is feasible only, when a limited number of different countermeasures are compared for their individual and combined effect(s). Since different types of countermeasures offer different degrees and even different kinds of protection, future bed rest studies will most likely integrate nutritional, pharmacological as well as exercise prescriptions in search for the most effective 'cocktail of stimuli' that simultaneously provides the best overall protection against spaceflight-induced deconditioning. For such studies, it will be practically impossible to determine single contributing effects of the various components.

When compared to the present exercise prescriptions for astronauts aboard the International Space Station, total RVE training time was very short; less than 10 minutes per day. Nonetheless, this proved effective to substantially diminish loss of muscle mass (Chapter 2), and to prevent changes in intrinsic contractile characteristics related to muscle speed (Chapter 5). Such preservations are not necessarily sufficient to maintain voluntary muscle strength. Indeed, the concept of training specificity indicates that adequate maintenance of neural control may still be compromised even when muscle mass is maintained, particularly when tasks performed during training substantially differ from those performed during testing [3;4]. In this thesis it could not be ascertained whether RVE training truly contributed to the preservation of neural capacity under bed rest conditions. The efficacy of RVE training as presented in Chapter 2 is confounded by the preservation of maximal voluntary activation for Ctrl. In Chapter 3 it is demonstrated that the amplitude of the high density sEMG substantially increased during the bed rest period for the trained individuals, suggesting that maximal neural drive was in fact rapidly intensified for RVE subjects during the course of the bed rest. Because such changes were not seen for Ctrl, it seems reasonable to expect that the difference between groups relates to the presence or absence of the countermeasure. Because the increased neural drive was not accompanied by strength gains, and could also not be attributed to motor unit synchronization, we speculated that sEMG amplitude increased during bed rest as a result of an increase in the mean firing rate of motor units.

In Chapter 4, we demonstrated that the relative resistance to fatigue increased following bed rest with RVE training. Bleeker et al. [5] showed that RVE training also significantly diminished vascular deterioration during bed rest. Nonetheless, because blood flow during the fatigue task was reduced following bed rest, and red blood cell mass was also decreased for most RVE subjects (D. Böning, personal communication), it can be expected that oxygen supply was also impaired. It remains difficult to explain why this did not present a functional limitation for the RVE subjects. Although no clear changes in fiber type in the quadriceps femoris muscle were seen following bed rest [7], it is possible that some metabolic properties of the quadriceps muscle changed as a result from adaptations to intense training [16]. For instance, Rittweger et al. [23] noted that, concomitant with increased training duration for the squat exercise, progressively higher lactate values were recorded for RVE subjects during the course of the bed rest. Such changes may indicate an increased tolerance to anaerobic conditions of training, which could have resulted in a lower relative fatigability following bed rest.

METHODOLOGICAL CONSIDERATIONS

We realize that some methodological issues must be regarded in the interpretations of our results. Some are inextricably connected to this study; others could be taken care of in future studies. One factor that potentially may have influenced some of our findings was the relatively large heterogeneity in properties related to neuromuscular function in the subjects at the start of the study. Apart from genetic factors, the observed initial heterogeneity may have been related to differences in active life style of the subjects. For bed rest studies, this notion may be critically important, because the difference between the level and mode of normal physical activity and that during bed rest is one, if not the most important factor that dictates the severity of the deconditioning [9;29]. In other words, physically fit individuals are likely to decondition more than those that are less fit. For bed rest studies it may be difficult to reduce subject heterogeneity on the basis of narrowing already stringent inclusion criteria, because such effort will further limit the number of suitable volunteers. However, because initial subject differences will confound statistical interpretations of the study, it would be desirable to increase group homogeneity prior to the start of the bed rest phase as much as possible. One option would be to match groups according to subject activation history over the last months preceding the study, instead of randomly assigning subjects to one of the experimental groups upon inclusion.

Part of the initial heterogeneity might also be attributable to the short baseline data collection period of the Berlin Bed Rest study, which covered only three days immediately preceding the start of the bed rest phase. For an accurate determination of baseline muscle functionality it is imperative that the subjects are properly familiarized with the required tasks. Although a learning response was apparent for most subjects, some subjects clearly profited more from the repeated testing than did others. Indeed, some subjects reached their highest maximal voluntary strength after several days of bed rest. Needless to say, for these individuals, pre- to post bed rest comparisons would not have been very accurate to represent the effect of the bed rest intervention. The main advantage of repeated testing showed here. The measurement of variables also during the bed rest allowed us to use a statistical tool that not only reduced the effect of day-to-day variations, it also allowed the assessment of more accurate baseline values at the start of the bed rest.

In the field of kinesiology, the electrical activity of superficial muscles is commonly recorded at the skin surface by a single bipolar electrode montage. In the Berlin Bed Rest study, we used a high density sEMG recording montage instead [6], in which a large number of electrodes is distributed over the vastus lateralis muscle. This system provided multiple advantages, such as proper reproducibility, a larger field of view, and the assessment of muscle fiber conduction velocity. The main disadvantage of any sEMG recording at higher force levels is the inability to disentangle the interference pattern into its constituents: the contribution of single motor units and their firings characteristics. A complete disentanglement of high density sEMG is only feasible at force levels too low for practical relevance in our study. This is the reason that we could not ascertain the exact origin of changes in sEMG recordings in RVE subjects (Chapter 3). For future bed rest studies, it may be desirable to include the use of wire electrodes to record EMG in combination with agonist and antagonist sEMG during voluntary contractions to determine the mechanism(s) underlying neural deconditioning typically seen following bed rest. However, the invasive character combined with the time consuming process of this technique most likely limits extensive practical application.

CLINICAL IMPLICATIONS

The Berlin Bed Rest study was in part funded by the European Space Agency's Microgravity Application Program, which looks to involve universities and industry in the development of space-related research. In the case of the Berlin Bed Rest study, the main objective was to assess the changes in muscles and bones typically arising during long-duration spaceflight and to evaluate an exercise countermeasure to counteract such changes. Because spaceflight and bed rest immobilization affects virtually all biological systems, a great number of additional experiments were conducted during the study. For instance, the assessment of cardiovascular function pre and post bed rest may not only improve our understanding of the effect of strict bed rest on cardiovascular deconditioning, the experiments also help to determine how an exercise countermeasure for bone and muscle may affect other physiological systems.

Part of the commitment of space agencies to fund bed rest studies is related to the awareness that space medicine may also greatly benefit the healthcare for individuals afflicted with muscle and bone-wasting diseases, or improve the treatment and recovery of hospitalized, bedridden or otherwise immobilized individuals on Earth. Indeed, most biological disorders are quickly complicated by their subsequent effect on neuromuscular activity levels and mobility. For instance, arthritis-induced pain leads to a reduced motivation to move, which results in reduced muscle use, disrupted metabolic priorities, which can lead to diabetic and cardiovascular dysfunction. Spaceflight and experimental bed rest studies provide such excellent research opportunities because they study how all physiological systems accommodate reduced muscular demands in healthy subjects.

From a clinical viewpoint, what are then the important functional neuromuscular parameters of this thesis? Of course, maintenance of muscle size and strength of anti-gravity musculature is important because muscle strength is needed for carrying body weight and activities such as walking up and down stairs. Nonetheless, it is likely that the muscle strength requirements to perform such activities are well below the maximal isometric knee extension strength in normal healthy individuals, meaning that most individuals can cope with some reduction in maximal muscle size and strength before any significant impairment is evident during normal daily life activities. In contrast, adequate neural control of such activities is a necessity, especially in the light of the reduction in bone mineral density that accompanies inactivity. Any deterioration in the capacity to quickly and adequately avoid obstacles during gait after resumption of daily life activities may increase the incidences of fall-related bone fractures. Interestingly, one of the main observations presented in this thesis was the finding that neural deconditioning could be comparatively easily prevented. With respect to the reduction in bone mineral density, resistive vibration exercise, or at least whole body vibration training - vibration training with only body weight - might be a particularly promising training modality in geriatric and therapeutic sectors. Whole body vibration appears a safe and valid substitute for conventional strength training for those who are not attracted or able to perform this type of exercise [26]. Finally, many tasks in daily life comprise repetitive muscle contractions at low muscle forces. Based on the findings of Chapter 4, it is imperative to minimize changes in endurance capacity, because this would significantly impair a patient's ability to perform such tasks independently. In Chapter 4, the workload after bed rest was corrected for changes in absolute muscle strength, as we were interested in changes in intrinsic muscle fatigability. Of course, in daily life such normalization has no practical relevance. In fact, a similar absolute load (e.g. body mass) presents an even increased relative workload for disused muscles because of the reduction in maximal force generating capacity. Because it is the relative load that dictates the severity of muscle fatigue, it can be expected that for daily life, the changes in muscle fatigability exceed the findings in Chapter 4. Additionally, because our subjects were tested in the supine position (Fig. 2 in Chapter 2), orthostatic factors such as an increased venous compliance of the legs [10] did not contribute to the reduction in muscle blood flow in our experiments. In the upright posture, however, such factors will undoubtedly further impair blood flow and hence exercise tolerance following bed rest.

By the continued monitoring of the subjects after the cessation of the bed rest period, we aimed to determine the time course of recovery of the neuromuscular system. Such information can be helpful in improving medical rehabilitation programs for various patient populations for whom exercise prescriptions are not applicable during a period of restricted mobility. From the Berlin Bed Rest study, but also from preceding studies it is evident that previously disused skeletal muscle are highly susceptible for muscle damage due to eccentric muscle actions [15;20;21;23]. Hence, at the initiation of the rehabilitation process, such muscle activity should be limited, for instance by applying alternative training regimes such as swimming. Because the Berlin Bed Rest study did not incorporate a standardized rehabilitation program, we chose not to address the time course of recovery of the neuromuscular system in the main chapters of this thesis. However, it can be mentioned here that maximal isometric muscle strength recovered to baseline values between 45 and 90 days after reambulation, which roughly equals the time of the inactive period. Since we did not observe any reductions in neural activation during the bed rest period, the recovery of maximal muscle strength may reflect the recuperation of muscle mass. It has long been questioned whether weight-bearing bones have a similar capacity to fully recover, i.e. there has been concern that some changes in muscle structure might be irreversible damaged by periods of simulated or actual spaceflight. Preliminary data (J. Rittweger, personal communication) indicate that during reambulation, bone mineral is deposited at sites where it was previously lost, implying that also changes in weight-bearing bones are completely reversible by resumed weight-bearing activity. These are reassuring observations indeed, for patients, future bed rest volunteers, as well as for those of us who dare to venture into the cosmos.

REFERENCES

[1] 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.

[2] 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.

[3] Bamman MM, Caruso JF. Resistance exercise countermeasures for space flight: implications of training specificity. J Strength Cond Res 2000; 14: 45-49.

[4] 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.

[5] Bleeker MW, De Groot PC, Rongen GA, Rittweger J, Felsenberg D, Smits P, Hopman MT. Vascular adaptation to deconditioning and the effect of an exercise countermeasure: results of the Berlin Bed Rest study. J Appl Physiol 2005; 99: 1293-1300.

[6] Blok JH, van Dijk JG, Drost G, Zwarts MJ, Stegeman DF. A high-density multichannel surface electromyography system for the characterization of single motor units. Review of Scientific Instruments 2002; 73: 1887-1897.

[7] Blottner D, Salanova M, Puttmann B, Schiffl G, Felsenberg D, Buehring B, Rittweger J. Human skeletal muscle structure and function preserved by vibration muscle exercise following 55 days of bed rest. Eur J Appl Physiol 2006; 97: 261-271.

[8] Cavanagh PR, Licata AA, Rice AJ. Exercise and pharmacological countermeasures for bone loss during long-duration space flight. Gravit Space Biol Bull 2005; 18: 39-58.

[9] Convertino VA, Bloomfield SA, Greenleaf JE. An overview of the issues: physiological effects of bed rest and restricted physical activity. Med Sci Sports Exerc 1997; 29: 187-190.

[10] Convertino VA, Doerr DF, Stein SL. Changes in size and compliance of the calf after 30 days of simulated microgravity. J Appl Physiol 1989; 66: 1509-1512.

[11] 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.

[12] Delecluse C, Roelants M, Verschueren S. Strength increase after whole-body vibration compared with resistance training. Med Sci Sports Exerc 2003; 35: 1033-1041.

[13] Dudley GA, Tesch PA, Harris RT, Golden CL, Buchanan P. Influence of eccentric actions on the metabolic cost of resistance exercise. Aviat Space Environ Med 1991; 62: 678-682.

[14] 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.

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

[16] Goreham C, Green HJ, Ball-Burnett M, Ranney D. High-resistance training and muscle metabolism during prolonged exercise. Am J Physiol 1999; 276: E489-E496.

[17] Greenleaf JE, Bernauer EM, Ertl AC, Trowbridge TS, Wade CE. Work capacity during 30 days of bed rest with isotonic and isokinetic exercise training. J Appl Physiol 1989; 67: 1820-1826.

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

[19] 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.

[20] 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.

[21] 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.

[22] 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.

[23] Rittweger J, Belavy D, Hunek P, Gast U, Boerst H, Feilcke B, Armbrecht G, Mulder E, Schubert H, Richardson C, de Haan A, Stegeman DF, Schiessl H, Felsenberg D. Highly Demanding Resistive Vibration Exercise Program is Tolerated During 56 Days of Strict Bed-Rest. Int J Sports Med 2006; 27: 553-559.

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

[25] 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.

[26] 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.

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

[28] 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.

[29] Smorawinski J, Nazar K, Kaciuba-Uscilko H, Kaminska E, Cybulski G, Kodrzycka A, Bicz B, Greenleaf JE. Effects of 3-day bed rest on physiological responses to graded exercise in athletes and sedentary men. J Appl Physiol 2001; 91: 249-257.

[30] Stein TP, Wade CE. Metabolic consequences of muscle disuse atrophy. J Nutr 2005; 135: 1824S-1828S.

[31] 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.

[32] Trappe S, Trappe T, Gallagher P, Harber M, Alkner B, Tesch P. Human single muscle fibre function with 84 day bed-rest and resistance exercise. J Physiol 2004; 557: 501-513.

[33] 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.

[34] 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.

[35] 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.

SUMMARY

NEUROMUSCULAR ADAPTATIONS DURING LONG-TERM BED REST

The weightless environment encountered during human spaceflight virtually eliminates the mechanical loading of the human body. The accompanying physical inactivity sets in motion a cascade of changes that affects practically every physiological system in the human body. Of particular medical and operational concern are the decrements in skeletal muscle strength (force, power and endurance capacity) of the legs and the demineralization of weight-bearing bones. By now, it is acknowledged that these functional impairments may be prevented by adequate muscle exercise.

This thesis addresses the study into the neuromuscular adaptations in the quadriceps femoris muscle as a consequence of bed rest-induced physical inactivity. Bed rest is hereby used as a simulation model of human spaceflight. The nature and progression of the adaptations, but also the preventative effect of physical excersise were studied. In the Berlin Bed Rest study, the used training paradigm comprised combined resistance and vibration exercise. Strength training exercises were performed against a mechanically vibrated platform.

We were interested in the changes that occur during – particularly in the early stage of – bed rest. Hence, besides conducting experiments pre- and post bed rest, we also performed seven experiments – with an increasing time interval between experiments - during the eight weeks of bed rest. The assessment of the time course of changes in the neuromuscular system required an experimental setup that was suited for conducting functional measurements under bed rest conditions. Most notably was the development of a supine dynamometer that allowed the assessment of specific neuromuscular properties of the knee extensor muscle during contractions under isometric conditions during bed rest. To disentangle the neural activation of the muscle from more intrinsic muscle characteristics both voluntary and electrically stimulated contractions were assessed. Voluntary motor control was assessed using two procedures. In Chapter 2 we utilized electrical stimulation and a modified twitch interpolation technique to study the extra capacity of a muscle over maximal voluntary drive. In Chapters 3, 4 and 5, voluntary motor control was determined by recording the electrophysiological properties of the quadriceps muscle by means of a sophisticated high-density surface electromyography methodology (HD-sEMG).

In Chapter 2, a study is described which aimed to determine the time course of changes in muscle size, voluntary activation and maximal voluntary isometric knee extension strength during bed rest. In the inactive control subjects, the decrease in anatomical cross-sectional area (CSA) of the quadriceps femoris muscle and the decrease in maximal voluntary isometric knee extension torque both evolved linearly in time to approximately 15%. In contrast to previous research, which indicated that decrements in voluntary muscle force often exceed those in mass or CSA after unloading, for the control subjects the reduction in maximal voluntary isometric strength

was comparable to that in muscle size. By means of the twitch-interpolation technique, this finding could be explained by the absence of a decrease in maximal voluntary activation of the quadriceps femoris muscle during bed rest. In contrast, for the contra lateral leg, which was only tested pre- and post bed rest, the loss in muscle strength exceeded the reduction in muscle size by almost a factor two. Based on these observations, it was concluded that the functional testing conducted during the bed rest had preserved the integrity of motor control of the repeatedly re-tested quadriceps femoris muscle. Although unintended, these findings provide valuable information, because it was shown that neural deconditioning can be prevented without vigorous exercise regimes, even during long-term bed rest. However, the preservation of neural integrity for the repeatedly re-tested right leg of the control subjects complicated the interpretations for the subjects that underwent training. Even though the allocated countermeasure contributed substantially to diminish quadriceps femoris atrophy, the possibility that the absence of a significant change in neural activation also in this group could have resulted from the repeated functional testing during bed rest could not be entirely excluded.

In Chapter 3 we used a different approach to assess alterations in voluntary motor control. HD-sEMG signals were repetitively recorded from the vastus lateralis muscle during bed rest for a series of isometric knee extensions, which varied in intensity from low to maximal. Surface EMG signals were analyzed for amplitude, median frequency and muscle fiber conduction velocity, and were subsequently related to isometric muscle strength to obtain HD-sEMG-force relationships. The normalized relationships were used to obtain a global indication of muscle activation with increasing voluntary force. Motor control of isometric force production was robust to the effects of bed rest, as none of the normalized relations between surface EMG characteristics and muscle force changed as a consequence of bed rest. This implies that the neural strategy to increase muscle force did not change by bed rest. In addition, the amplitude of the sEMG at maximal effort remained unaltered in the control group. The latter implies also the maintenance of maximal neural capacity, which is in agreement with the findings presented in Chapter 2. The observed changes sEMG median frequency and muscle fiber conduction velocity were interpreted as a reflection of atrophy of the quadriceps femoris muscle (vastus lateralis) for these subjects (Chapter 2). The group that underwent resistive vibration exercise training displayed a substantial (around 30%) and rapid (after 10 days bed rest) increase in sEMG amplitude, with no change in maximal muscle strength. These observations not only differed from the findings of the control group, they also contrasted the findings presented in Chapter 2. We speculated that the intensified muscle activation might have resulted from an increase in the mean firing rate of motor units, as a consequence of the training regime. The discrepancy between the findings of Chapters 2 and 3 might be explained if the sEMG methodology were more sensitive than the twitch-interpolation technique to detect specific neural modulations. From the unchanged knee extension strength for this group, it appeared that the proposed altered neural strategies were functionally ineffective to influence isometric knee extension strength.

The purpose of the study presented in Chapter 4 was to test the effect of the 56 days of bed rest on the fatigability of the quadriceps femoris muscle. The experiments were conducted only prior to and immediately following bed rest. The results show that the relative fatigability increased for the control subjects, as maximal voluntary strength decreased faster during 5 minutes of voluntary repetitive sub-maximal isometric knee extensions following bed rest as compared to before. In accordance with this increased fatigability was the observation of an accelerated decline in HD-sEMG median frequency and mean muscle fiber conduction velocity following bed rest. These findings suggested that the increased fatigability was most likely of peripheral origin. Near-infrared spectroscopy measurements indicated that this was most likely the consequence of a strong reduction in local muscle blood flow and hence oxygen delivery during exercise, following bed rest. For the trained group muscle fatigability decreased. The changes in sEMG variables as seen in the inactive control subjects were effectively prevented. The reduction in muscle blood flow was also mitigated for this group. The latter findings might be related to the - also in this bed rest campaign shown - attenuation of structural and functional changes in the vascular system by the adopted training paradigm.

Apart from the capacities to produce short steady-state (sub-)maximal muscle force (Chapters 2 and 3), and prolonged repeated sub-maximal muscle force (Chapter 4), another important muscle functionality that was studied, is the rate at which muscle force develops at the start of a forceful voluntary contraction. As described in Chapter 5, also the contractile properties of the quadriceps femoris muscle were repetitively tested during bed rest for fast voluntary and electrically evoked contractions. It was clearly shown that the quadriceps femoris muscle of the control subjects acquired the intrinsic contractile properties of a faster muscle. Daily resistive vibration exercise proved effective to prevent such changes. Unexpectedly, the ability to produce a high rate of torque development during voluntary contractions was preserved and the addition of resistive vibration exercise did not influence this capacity. This maintained functionality was, simular to that described in Chapters 2 and 3, associated with an absent neural deterioration, as measured by means of HD-sEMG. It was concluded that the alterations in mechanical properties during electrically evoked contractions were of insufficient magnitude to (detectably) affect voluntary muscle functionality. This was most likely a consequence of the large inter-, but also intra-individual variability in the performance of the fast voluntary contractions. However, we could not rule out the possibility that also here the repeated functional testing conducted during bed rest sufficed to maintain adequate motor control. This could even apply to a more skilful task that requires considerably more neural activation than needed for steady-state contractions.

Finally, Chapter 6 contains a general discussion of the results presented in this thesis, their relation to auxiliary and previous findings, as well as their practical implications for both human spaceflight and physically inactive individuals on Earth. The results of the studies described in this thesis have contributed to a better understanding of the underlying mechanisms and the time course in which they contribute to the various manifestations of muscle weakness as a result of physical inactivity imposed by strict bed rest. Most notably were the findings of a linear reduction in voluntary isometric knee extension strength, and an increase in relative muscle fatigability. These adaptations were predominantly the result of a linear decay in the cross-sectional area of the quadriceps femoris muscle and a reduced blood flow as a consequence of bed rest. The changes in intrinsic contractile characteristics of the quadriceps femoris towards a faster muscle also progressed linearly in time. An unexpected finding across experiments was that the adopted longitudinal study, which included repeated functional re-testing of the quadriceps femoris muscle during 56 days of bed rest, fully prevented neural deconditioning. Vigorous resistive vibration exercise training during bed rest appeared a suitable gravity-independent countermeasure that offset or substantially mitigated most of the adaptive changes in quadriceps femoris muscle that evolved during bed rest in the absence of this countermeasure.

SAMENVATTING

NEUROMUSCULAIRE ADAPTATIES TIJDENS LANGDURIGE BEDRUST

Gewichtloosheid tijdens bemande ruimtevluchten elimineert vrijwel alle mechanische belasting op het menselijk lichaam. De fysieke inactiviteit die hiermee gepaard gaat brengt een cascade van veranderingen op gang in het lichaam. Nagenoeg alle fysiologische systemen worden daardoor beïnvloed. Vanuit medisch en operationeel oogpunt zijn de voornaamste redenen tot zorg het ontstaan van spierzwakte (verlies in kracht, vermogen en uithoudingsvermogen) in de beenspieren - vooral in de knie-extensoren - en de demineralisatie van gewichtdragende botstructuren. Het is duidelijk dat deze functionele achteruitgang mogelijk kunnen worden beperkt door gerichte fysieke training.

Dit proefschrift behandelt studies naar de neuromusculaire adaptaties in de m. quadriceps femoris als gevolg van de door bedrust opgelegde inactiviteit, waarbij bedrust geldt als een model voor bemande ruimtevaart. Daarbij werd het karakter en de voortgang van de veranderingen, maar ook het beschermende effect van fysieke training bekeken. In de Berlijnse bedrust studie werd gebruik gemaakt van gecombineerde weerstands- en vibratietraining, waarbij krachttrainingsoefeningen werden uitgevoerd tegen een platform dat mechanische trillingen - vibraties - opwekte.

We wilden veranderingen tijdens - vooral ook in het begin van - de bedrust observeren. Daarom zijn, naast experimenten voor en na de bedrust, ook tijdens de acht weken bedrust op zeven dagen - met een toenemend tijdinterval - experimenten uitgevoerd. Het vaststellen van het tijdpad waarin veranderingen plaatsvinden in het spier-zenuwstelsel (neuromusculaire systeem) vroeg om een experimentele methode die geschikt is voor functionele metingen tijdens bedrust. In het bijzonder betrof dit de ontwikkeling van een liggende ergometer die ons in staat stelde om *tijdens* bedrust specifieke neuromusculaire eigenschappen van de kniestrekkers te kwantificeren onder isometrische testcondities. Om de neurale aansturing van de spier te kunnen onderscheiden van de meer intrinsieke spierkarakteristieken, werd gebruik gemaakt van zowel vrijwillige als van elektrisch gestimuleerde spiercontracties. Vrijwillige aansturing van de m. quadriceps femoris werd op twee verschillende manieren onderzocht. In Hoofdstuk 2 gebruikten we een procedure waarbij de spier elektrisch wordt gestimuleerd tijdens het maximaal aanspannen om de capaciteit van de spier te achterhalen die niet (meer) door vrijwillige aansturing gerekruteerd kan worden. In de Hoofdstukken 3, 4 en 5 werd de aansturing van de spier door het zenuwstelsel gemeten aan de hand van het bepalen van de elektrofysiologische reacties van de spier tijdens vrijwillige aansturing. Een geavanceerde methode, ‘high density’ oppervlakte elektromyografie (HD-sEMG), werd daarbij gebruikt.

In Hoofdstuk 2 wordt een studie beschreven die tot doel had om het tijdpad van veranderingen in grootte, vrijwillige activatie en maximale vrijwillige kracht van de m. quadriceps femoris tijdens isometrische knie-extensie in kaart te brengen. In de inactieve controlegroep verminderde de anatomische dwarsdoorsnede van de spier en de maximale vrijwillige knie-extensie kracht lineair gedurende bedrust, beide met ongeveer 15%. In tegenstelling tot voorgaand onderzoek, waarin werd gevonden dat de achteruitgang in vrijwillige spierkracht vaak groter is dan het verlies in spiermassa of dwarsdoorsnede, waren zowel de grootte als ook het tijdpad van de veranderingen in dwarsdoorsnede en kracht dus vergelijkbaar. Door gebruik te maken van elektrische stimulatie en de twitch-interpolatie techniek kon deze bevinding verklaard worden. De capaciteit waarmee de spier vrijwillig maximaal geactiveerd kon worden bleef namelijk gehandhaafd. Echter, voor het linker been dat enkel getest werd voor en na bedrust, was het verschil tussen het verlies in maximale kracht en dat in dwarsdoorsnede bijna een factor twee. Uit deze observaties concludeerden wij dat de herhaalde functionele spiermetingen gedurende bedrust een beschermend effect hadden op de integriteit van vrijwillige aansturing van het, tijdens de bedrust geteste, been. Hoewel dit een onbedoeld effect betrof, verschaffen deze gegevens waardevolle informatie. Er wordt mee aangetoond dat de deconditionering van het neurale systeem, zelfs tijdens langdurige bedrust, tegengegaan kan worden zonder daar rigoureus voor te trainen. Wel bemoeilijkte de onveranderde neurale integriteit van het gemeten been van de controle proefpersonen de interpretatie van de resultaten van de proefpersonen die wel training hadden ondergaan. Hoewel de gebruikte trainingsvorm een substantiële bijdrage leverde tot het beperken van atrofie van de m. quadriceps femoris, is het mogelijk dat de afwezigheid van neurale deconditionering deels een gevolg is van het herhaaldelijk fysiek testen van ook deze proefpersonen gedurende bedrust.

In Hoofdstuk 3 werd gebruik gemaakt van een alternatieve benadering om neurale en spierfysiologische veranderingen te achterhalen. Daartoe werd de EMG activiteit van de m. vastus lateralis herhaaldelijk gemeten gedurende bedrust bij een reeks van isometrische contracties variërende in intensiteit van gering tot maximaal. De elektrofysiologische signalen werden geanalyseerd voor amplitude en mediane frequentie van het EMG signaal en de snelheid waarmee de actiepotentialen worden voortgeleid over het spiervezelmembraan. Deze variabelen werden vervolgens gerelateerd aan isometrische spierkracht om EMG-kracht relaties te bepalen. Zodoende werd een indicatie verkregen van de spieractivatie tijdens oplopend krachtniveau. De manier waarop de spier wordt geactiveerd van lage tot hoge isometrische krachten bleek ongevoelig voor het effect van bedrust. Geen van de relatieve relaties tussen oppervlakte EMG en spierkracht veranderde. Dit impliceert dat de strategie van het centrale zenuwstelsel voor krachtopbouw niet was veranderd als gevolg van bedrust. Bovendien bleef ook de amplitude van het EMG signaal tijdens maximale inspanning onveranderd in de controlegroep. Dit impliceert dat ook de capaciteit van het centrale zenuwstelsel om de m. quadriceps femoris maximaal aan te kunnen sturen onveranderd bleef. Dat komt overeen met de bevindingen zoals gepresenteerd in Hoofdstuk 2. De mediane frequentie en de snelheid van voortgeleiding van actiepotentialen over het spiervezelmembraan veranderden wel sterk in de controlegroep. Dit kan vooral worden geïnterpreteerd als een reflectie van de atrofie van de spiervezels in de deze proefpersonen (Hoofdstuk 2). De groep die deelnam aan de training toonde zonder uitzondering een substantiële (ongeveer 30%) en vrij plotselinge (na 10 dagen bedrust) verhoging in de amplitude van het EMG, bij onveranderde maximale kracht. Deze bevinding contrasteert niet alleen met die van de controlegroep, maar verschilt ook van de resultaten beschreven in Hoofdstuk 2. We speculeren dat de toegenomen spieractiviteit resulteerde uit een toegenomen vuurfrequentie van motorische eenheden als een gevolg van het trainingsregime. De discrepantie tussen de bevindingen van Hoofdstuk 2 en die van Hoofdstuk 3 zou kunnen worden verklaard wanneer het EMG gevoeliger is voor specifieke modulaties in aansturing dan de eerder gebruikte twitch-interpolatie techniek. Uit de onveranderde maximale vrijwillige kracht voor deze groep blijkt dat deze centrale aanpassing tijdens isometrische contracties geen functioneel voordeel heeft.

Het doel van de studie gepresenteerd in Hoofdstuk 4 was om het effect van de 56 dagen bedrust op de vermoeibaarheid van de m. quadriceps femoris te onderzoeken. Deze experimenten zijn alleen voor en direct na de bedrust uitgevoerd. De resultaten laten zien dat de relatieve vermoeibaarheid van de spier toeneemt voor de inactieve controlegroep. De maximale kracht gedurende vijf minuten van vrijwillige herhaalde submaximale isometrische knie-extensies nam sneller af ná bedrust dan vóór bedrust. In overeenstemming met deze toegenomen vermoeibaarheid vonden we een snellere afname in mediane frequentie van het EMG signaal en ook in de gemiddelde voortgeleidingssnelheid van actiepotentialen na bedrust. Dit suggereerde dat de toegenomen vermoeibaarheid voornamelijk perifere oorzaken heeft. Zogenaamde "near-infrared spectroscopy" (NIRS) metingen gaven aan dat bovenstaande waarschijnlijk een consequentie is van een sterk gereduceerde bloedtoevoer en daarmee een beperking in zuurstofvoorziening gedurende inspanning na bedrust. Bij de getrainde groep trad geen toegenomen, maar zelfs een verminderde spiervermoeibaarheid op. De toegenomen verandering in EMG variabelen bij de controlegroep waren dan ook afwezig bij deze groep. Training gedurende bedrust beperkte ook de achteruitgang in bloedvoorziening. Het laatste kan gerelateerd zijn aan het - ook in deze bedruststudie aangetoonde - beschermende effect van de training op structurele en functionele veranderingen in het vasculaire systeem.

Naast het vermogen om eenmalig kortdurende (sub)maximale (stabiele) krachten (Hoofdstukken 2 en 3) en langdurig herhaalde sub maximale krachten (Hoofdstuk 4) te kunnen leveren, is er een andere belangrijke functionaliteit van een spier gemeten, namelijk de snelheid waarmee spierkracht kan worden gegenereerd aan het begin van een krachtige vrijwillige contractie. In Hoofdstuk 5 staat beschreven hoe wij ook de contractiele eigenschappen van de m. quadriceps femoris herhaaldelijk hebben gemeten gedurende bedrust met behulp van zowel vrijwillige alsook elektrisch gestimuleerde contracties. Duidelijk werd dat de m. quadriceps femoris van de inactieve controle groep de intrinsieke eigenschappen kreeg van een snellere spier. Fysieke training gedurende bedrust bleek effectief om deze veranderingen tegen te gaan. Onverwacht was dat de capaciteit om snel vrijwillig een maximale kracht op te bouwen behouden bleef en dat deze capaciteit niet beïnvloed werd door de dagelijkse training. Deze onveranderde functionaliteit, wordt, zoals ook in Hoofdstukken 2 en 3, geassocieerd met een afwezigheid van de achteruitgang van de capaciteit vanuit het centrale zenuwstelsel, gemeten met behulp van EMG. We concluderen dat de veranderingen in mechanische eigenschappen gedurende elektrisch gestimuleerde contracties te gering waren om een (meetbaar) effect te hebben op vrijwillige spierfunctionaliteit. Dit is waarschijnlijk mede een gevolg van een substantiële inter-, maar ook intra-individuele variabiliteit in de uitvoer van de snelle vrijwillige contracties. Echter, we konden opnieuw niet uitsluiten dat het herhaaldelijk fysiek testen van de proefpersonen gedurende bedrust voldoende was geweest om adequate aansturing te handhaven. Dat zou dus zelfs kunnen gelden voor het uitvoeren van een taak die aanzienlijk meer neurale input vergt dan een krachtstabiele contractie.

Tot slot bevat Hoofdstuk 6 een algemene discussie van de resultaten in dit proefschrift, hun relatie met supplementaire en eerdere bevindingen en hun praktisch nut voor zowel bemande ruimtevaart als voor fysiek inactieve individuen op aarde. De resultaten hebben bijgedragen aan een verbetering van het inzicht in onderliggende mechanismen en het tijdpad waarin zij bijdragen aan de verschillende manifestaties van spierzwakte na langdurige inactiviteit. Zo vonden we een lineaire afname in knie-extensie kracht en de toegenomen relatieve vermoeibaarheid van de m. quadriceps femoris. Deze adaptaties waren voornamelijk het resultaat van een vrijwel lineaire afname van de dwarsdoorsnede van deze spier en een sterk gereduceerde spierdoorbloeding als gevolg van strikte bedrust. De veranderingen in intrinsieke eigenschappen van de m. quadriceps femoris in de richting van een snellere spier evolueerden ook lineair in de tijd. Een onverwachte bevinding was dat de gebruikte longitudinale studie opzet, waarbij herhaaldelijk functionele metingen werden verricht gedurende bedrust, het deconditioneren van het neurale systeem volledig leek tegen te gaan. Gecombineerde weerstands- en vibratietraining gedurende bedrust bleek een effectieve zwaartekrachtonafhankelijke tegenmaatregel. De meeste adaptieve veranderingen die optraden als gevolg van strikte bedrust werden effectief door de dagelijkse training bestreden, dan wel substantieel verminderd.

PUBLICATIONS

Papers in international journals

Schneider SM, Amonette WE, Blazine K, Bentley J, Lee SM, Loehr JA, Moore Jr. AD, Rapley M, **Mulder ER**, Smith SM. Training with the International Space Station interim resistive exercise device. *Medicine & Science in Sports & Exercise* 2003; 35: 1935-1945.

Stegeman DF, **Mulder ER**, Gerrits KH, Rittweger J, Felsenberg D, de Haan A. Peripherally and centrally induced electromyographic changes in 8 weeks of horizontal bed rest with and without resistance exercise with concurrent whole body vibration. *Journal of Gravitational Physiology* 2005; 12: 111-112.

Mulder ER, Stegeman DF, Gerrits KH, Rittweger J, Felsenberg D, de Haan A. Resistance exercise with concurrent whole body vibration preserves isometric knee extension strength during 8 weeks of horizontal bed rest. *Journal of Gravitational Physiology* 2005; 12: 155-156.

Mulder ER, Stegeman DF, Gerrits KH, Paalman MI, Rittweger J, Felsenberg D, de Haan A. Strength, size and activation of knee extensors followed during 8 weeks of horizontal bed rest and the influence of a countermeasure. *European Journal of Applied Physiology* 2006; 97: 706-715.

Rittweger J, Belavy D, Hunek P, Gast U, Boerst H, Feilcke B, Armbrecht G, **Mulder ER**, Schubert H, Richardson C, de Haan A, Stegeman DF, Schiessl H, Felsenberg D. Highly demanding resistive vibration exercise program is tolerated during 56 days of strict bed-rest. *International Journal of Sports Medicine* 2006; 27: 553-559.

Mulder ER, Gerrits KH, Kleine BU, Rittweger J, Felsenberg D, de Haan A, Stegeman DF. High-density surface EMG study on the time course of central nervous and peripheral neuromuscular changes during 8 weeks of bed rest with or without resistive vibration exercise. *Journal of Electromyography & Kinesiology*. In press.

Mulder ER, Kuebler WM, Gerrits KH, Rittweger J, Felsenberg D, Stegeman DF, de Haan A. Knee extensor fatigability after 8 weeks of bed rest with and without countermeasure. *Muscle & Nerve*. Under revision prior to acceptance.

Mulder ER, Gerrits KH, Rittweger J, Felsenberg D, Stegeman DF, de Haan A. Characteristics of fast voluntary and electrically evoked isometric knee extensions during 56 days of bed rest with and without countermeasure. Submitted.

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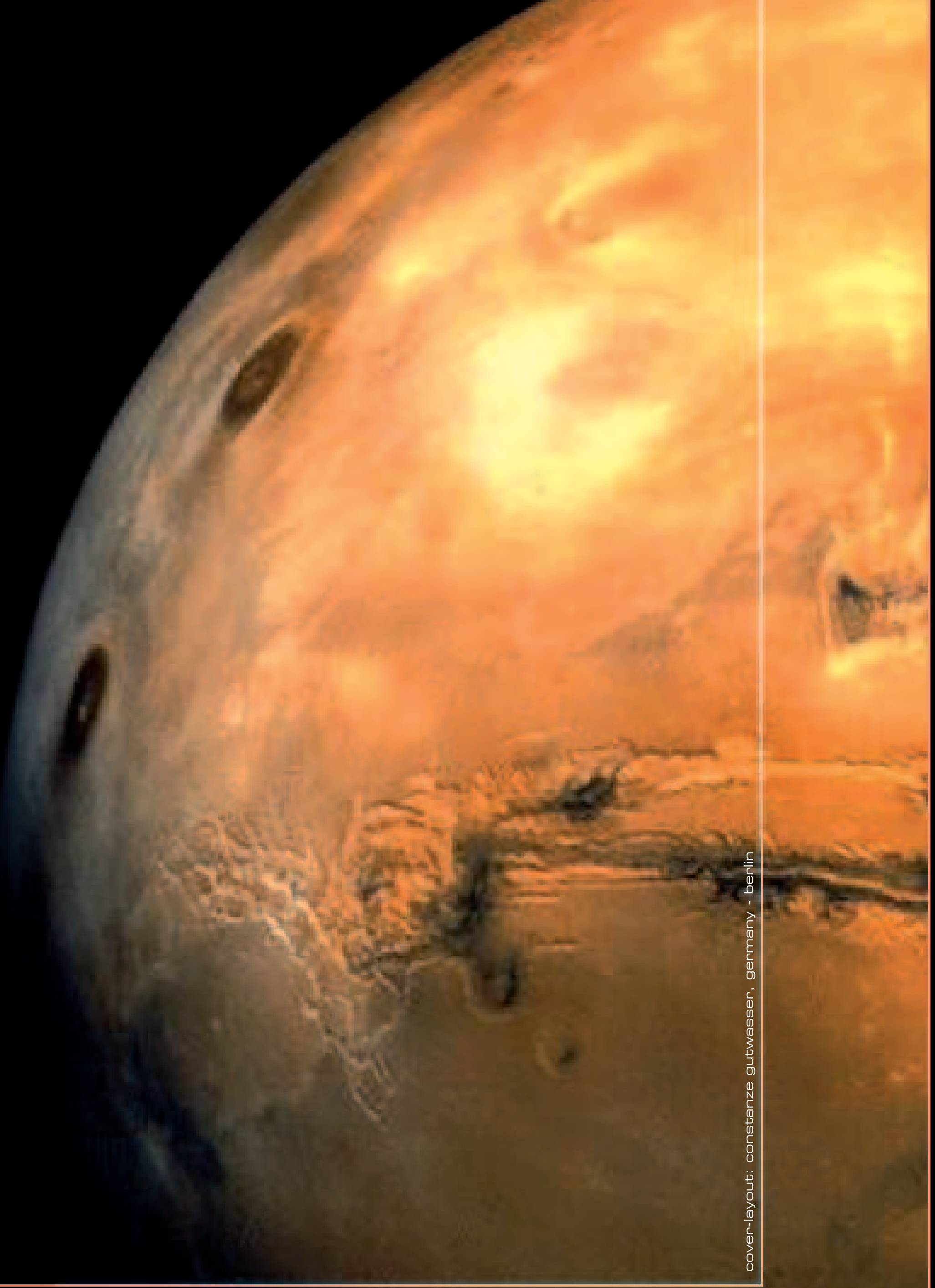
CURRICULUM VITAE

Edwin Mulder is geboren op 25 april 1975 in Schoonoord (Drenthe) waar hij opgroeide en de lagere school bezocht. In Emmen werden achtereenvolgens het MAVO, HAVO en VWO diploma behaald. In 1995 verhuisde hij naar Amsterdam en startte met de opleiding bewegingswetenschappen aan de faculteit der Bewegingswetenschappen van de Vrije Universiteit in Amsterdam. In 2001 werd deze studie afgerond. Zijn afstudeerwerk heeft hij gedaan bij het inspanningsfysiologisch laboratorium van de Amerikaanse Ruimtevaartorganisatie NASA op het Johnson Space Center in Houston, Texas, USA. In 2002 startte hij met zijn promotieonderzoek binnen het kader van het Instituut voor Fundamentele en Klinische Bewegingswetenschappen (IFKB) bij de afdeling Klinische Neurofysiologie van het Universitair Medisch Centrum St Radboud in Nijmegen. In 2004 maakte hij deel uit van het 'Dutch Investigator Support Team', opgezet door de Europese Ruimtevaartorganisatie ESA om wetenschappelijke onderzoekers te ondersteunen tijdens de DELTA missie van de Nederlandse astronaut André Kuipers. Sinds 2006 werkt Edwin op de Faculteit der Bewegingswetenschappen van de Vrije Universiteit in Amsterdam aan de voorbereidingen van de tweede Berlijnse bedrust studie, die in het najaar van 2007 zal starten. Hij is getrouwd met Moniek Landman. Samen hebben ze twee dochters, Lisa (2004) en Esmée (2006).

Edwin Mulder was born on the 25th of April 1975 in Schoonoord, the Netherlands, where he grew up and attended the primary school. In Emmen he received his pre-university education. In 1995, he started his education in human movement sciences at the Faculty of Human Movement Sciences of the VU University in Amsterdam. He finished his MSc degree in 2001. The research for his master's thesis was performed at the Exercise Physiology Laboratory at the NASA Johnson Space Center in Houston, Texas, USA. In 2002, he joined the Institute for Fundamental and Clinical Human Movement Sciences (IFKB) and started a PhD project at the Department of Clinical Neurophysiology of the Radboud University Medical Center in Nijmegen. In 2004 he was a member of the Dutch Investigator Support Team, initiated by the European Space Agency ESA to assist scientific researcher during the DELTA mission of the Dutch astronaut André Kuipers. Since 2006 Edwin works at the Faculty of Human Movement Sciences in preparation of the second Berlin Bed Rest study starting autumn 2007. He is married to Moniek Landman. Together they have two daughters, Lisa (2004) and Esmée (2006).



cover-layout: constanze gutwasser, germany - berlin

Edwin Mulder

Neuromuscular adaptations during long-term bed rest