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Human skeletal muscle structure and function preserved by vibration muscle exercise following 55 days of bed rest

Abstract Prolonged immobilization of the human body results in functional impairments and musculoskeletal system deconditioning that may be attenuated by adequate muscle exercise. In a 56-day horizontal bed rest campaign involving voluntary males we investigated the effects of vibration muscle exercise (RVE, 2×6 min daily) on the lower limb skeletal muscles using a newly designed foot plantar trainer (Galileo Space) for use at supine position during bed rest. The maximally voluntary isometric plantar flexion force was maintained following regular RVE bouts during bed rest (controls -18.6 %, $P < 0.05$). At the start (BR2) and end of bed rest (BR55) muscle biopsies were taken from both mixed fast/slow-type vastus lateralis (VL) and mainly slow-type soleus muscle (SOL), each having $n = 10$. RVE group: the size of myofiber types I and II was largely unchanged in VL, and increased in SOL. Ctrl group: the SOL depicted a disrupted pattern of myofibers I/II profiles (i.e., type II > 140 % vs. preBR) suggesting a slow-to-fast muscle phenotype shift. In RVE-trained SOL, however, an overall conserved myofiber I/II pattern was documented. RVE training increased the activity-dependent expression of nitric oxide synthase type 1 immunofluorescence at SOL and VL myofiber membranes. These data provide evidence for the beneficial effects of RVE training on the deconditioned structure and function of the lower limb skeletal muscle. Daily short RVE should be employed as an effective atrophy countermeasure co-protocol preferentially addressing postural calf muscles during prolonged clinical immobilization or long-term human space missions.

Keywords Skeletal muscle atrophy · Neuromuscular disorders · Countermeasure · Rehabilitation · Spaceflight

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Introduction

Activity-dependent processes addressing the brain and spinal cord locomotor units or peripheral neuromuscular/musculoskeletal structures are critical for the maintenance of human performance (Baldwin and Haddad 2001). Prolonged body immobilization results in disuse-induced malfunctions and atrophy of these systems with impaired motor tasks and performance control (Fitts et al. 2001; Roy et al. 2000). The adverse structural and functional adaptations of the deconditioned neuromuscular and musculoskeletal system should be minimized through adequate physiological stimuli such as exercise in order to support performance control following clinical immobilization, in rehabilitation, or during extended spaceflight missions (Booth and Criswell 1997; Dietz 2002; Ohira et al. 1999; Shackleford et al. 2004).

Bed rest immobilization is a well-accepted analogue of inactivity-induced body deconditioning which is normally encountered by bedridden patients in clinical settings as well as by astronauts in extended spaceflight missions (Akima et al. 2000; Berg and Tesch 1994, 1998). Resistive exercise based on low repetition/maximally force-induced coupled concentric and eccentric muscle actions has been successfully applied as a countermeasure to skeletal muscle unloading and atrophy using the fly wheel device in a bed rest study (Alkner and Tesch 2004; Berg and Tesch 1994; Rudnick et al. 2004).

Resistive fly wheel training thus maintained myofiber architecture and sarcolemma membrane expression of nitric oxide synthases (NOS) suggesting altered NO-signaling pathways at functional skeletal muscle compartments (Rudnick et al. 2004). Nevertheless, we sought to test alternative modalities of muscle exercise comprising load-induced reflexive muscle actions in a short and simple regular countermeasure protocol against atrophy of lower limb postural muscle groups following prolonged immobilization.

Resistive-like muscle actions can be also initiated by vibration forces on muscle imposed by high frequency electrical or mechanical stimuli thereby supporting muscular strength as shown in animals (Schüler and Pette 1996) and humans (Adamo et al. 2002; Delecluse et al. 2003; Griffin et al. 2001; Maffiuletti et al. 2002; Roelants et al. 2004). Frequency-dependent vibration forces were successfully used as interventions to increase muscle power and force in physical training protocols or in rehabilitation (Cardinale and Bosco 2003; McBride et al. 2004). The beneficial effects of low frequency vibration forces (< 30 Hz) on, e.g., limb muscle functions are thought to occur through adequate stimulation of the neuromuscular reflex loop activity (Rittweger et al. 2000, 2003) and the recruitment of high threshold motor units (Martin and Park 1997; McBride et al. 2004). We assumed that reflexive muscle contractions by adequate vibration stimuli might generate sufficient amounts of muscle cycles of contraction and relaxation exerting both ambient neuronal stimuli and mechanical strain. Resistive-like, i.e., load-induced reflexive muscle actions initiated by daily plantar vibration stimuli should at least partially mitigate atrophy of the lower limb muscles as an effective countermeasure to hypokinesia-induced atrophy.

We here report on the effects of a simple resistive-like vibration muscle exercise (RVE) training on ten healthy male volunteers (plus non-exercise control group, $n = 10$) using a newly designed Galileo Space device based on a vibrating foot platform for use in supine position (Novotec Inc., Pforzheim, Germany) during 56 days of voluntary bed rest immobilization (Berlin Bed Rest 2003). The main purpose of this study was to test whether short bouts of RVE training (e.g., 2×3 min per day) preserved myofiber size and phenotype distribution, and the force production of thigh and calf skeletal muscle groups during bed rest. We further hypothesized that regular daily exercise with the Galileo Space vibrating foot platform during bed rest might stimulate activity-induced NOS expression in vastus lateralis (VL) and soleus (SOL) myofibers as recently shown with the maximally force-induced resistance exercise countermeasure during the 90-day Toulouse bed rest study (Rudnik et al. 2004). If so, short daily bouts with the Galileo Space trainer should be able to preserve muscle fiber morphology, molecular architecture and force production of the leg muscles. Thus, RVE training could be simpler than other types of exercise and should be easily implemented to future countermeasure protocols

to preserve human performance control following prolonged deloading conditions on Earth or in Space.

Methods

The Berlin bed rest study (BBR) and ethical policies

A 56-day horizontal bed rest sponsored mainly by the European Space Agency (ESA) was organized at the Benjamin Franklin Hospital of the Charité University Medicine Berlin, Berlin, Germany, in 2003 and 2004. A total of twenty healthy male volunteers ($n = 20$) were included in the study [mean age (years) 33 ± 5.6; mean BMI = body mass index (kg$\times$m^{-2}) 23 ± 1.51, SD, $P < 0.01$] according to defined subject characteristics (e.g., moderately active, no regular aerobic or resistance training regimen, no clinical musculoskeletal parameters, non-smokers). The candidates were randomly assigned to one of two groups, one control (Ctrl) group i.e., non-exercise, and one vibration muscle exercise (RVE) group, each having $n = 10$. Five different campaigns including four subjects each (RVE and Ctrl, $n = 2 \times 2$) were conducted to balance any seasonal effects. During bed rest the candidates were not allowed to stand up (routine video control and force transducers in bed frames), and were asked to strictly adhere to their supine position with no trunk lifting to more than $45°$, and no brisk leg movements with large muscle force production other than during controlled exercise units. Physiotherapy in bed rest included passive ankle mobilization and gentle muscle massage (without stretching) twice a week to improve venous flow, and to help minimize any joint or muscle pain. Subjects were under the observations of a medical doctor daily. The diet was controlled with regards to caloric intake according to the Harris–Benedict equation with an adjustment factor of 1.2 (Harris and Benedict 1919).

All candidates gave written informed consent to participate voluntarily in the BBR study, to the risks and benefits of the study, and to the muscle biopsies. Approval was given by the local ethics committee of the Charité Universitätsmedizin Berlin, Germany. The study was conducted in accordance with the Helsinki Declaration for the Protection of Human Subjects. More detailed descriptions of the BBR study protocol including functional muscle parameters have been published elsewhere (Bleeker et al. 2005). Re-ambulation was performed on the fifty-seventh day after the onset of bed rest, between 8 a.m. and 11 a.m. Muscle function was tested immediately after re-ambulation, and in the evening of the same day (see below).

Resistive-like vibration exercise

Resistive-like vibration exercise was performed using a special Galileo Space device (Novotech Inc., Pforzheim, Germany) feasible for a daily muscle training program at the supine condition throughout bed rest immobili-

zation. Briefly, the Galileo Space device consists of a vibrating foot platform (20–30 Hz), to which the subjects can attach themselves in supine position via elastic belts with their hips, their shoulders and their hands (see Fig. 1). The static force upon the vibration platform generated was about two times the body weight under resting conditions. The Galileo Space device generates platform vibration by means of the eccentric, anti-phase rotation of two masses under each foot. Hence, the left side and the right side of the platform accelerate alternately, i.e., when the left leg is accelerated towards the head, then the right leg is extending. As the frequency of the vibration is preset, this acceleration increases with vibration frequency, and so does the resistive-like force elicited by the leg extensor and flexor muscles. The amplitude of the vibration therefore results from the acceleration of the platform and the resistive force of the leg extension (usually in the range of 0.5–1 cm). During bed rest the vibration protocol consisted of two daily bouts (6 min each) at preset vibration frequencies of 19–25 Hz with a total of 89 exercise sessions scheduled for each subject between days BR0 and BR56 (Wednesday afternoon and Sunday off).

Calf muscle size and function

Maximum voluntary isometric plantar flexion force (MIPF) was measured in the left leg before the bed rest period (BDC-2 and BDC-1), and immediately (< 1 h) after re-ambulation (BDC-56) in the morning (R1m), and in the evening of the same day (R1e). This was done

Fig. 1 The Galileo Space device used for resistive-like vibration exercise (RVE) protocols (RVE group) at supine position during 56 days of strict bed rest. During training, the bedridden candidate pushes the foot plantar region onto the vibrating Galileo platform (*left*) using elastic shoulder and hip straps and short elastic cords held in both hands (daily bouts 2×6 min at a.m./p.m.). During exercise, however, short but high repetition mechanical load (i.e., leg extension vs. acceleration) is transmitted alternately to each leg via the vibrating platform. We therefore defined this protocol RVE

with a split ground reaction force platform (Novotec, Pforzheim, Germany) and a custom-built restraint device. The subjects were tested in a seated position, with the ankle and knee joints at an angle of 90°. They placed their forefoot and the heel on either side of the split ground reaction platform. During each test, the subjects wore the same shoes, and markers were placed on the shoes in order to exactly reposition the foot on the force plate in the subsequent tests. The restraint 'clamped' the lower leg between the foot and the upper aspect of the knee, and the arms were behind the subjects' back. MIPF was then assessed as the change in ground reaction force under the forefoot. The best in three trials was taken from each testing session. During the contractions, strong verbal encouragement was given, and time was given to prevent fatigue. Also, subjects were carefully observed to maintain their body posture during the contractions. Goniometers were used to check possible flexion or extension movements within the ankle or knee joint. Data were digitized and analyzed with a Power-Lab 16s analog–digital converting system and the integrated Chart software in its version 5.0 (AD Instruments, Sydney) at a sampling rate of 2,000 Hz. The reproducibility of this method to measure maximum plantar flexion force, as assessed over all subjects on days BDC-2 and BDC-1, turned out to be 3.4% of the mean.

Muscle biopsies

Skeletal muscle biopsies were taken on the second day (BR2) and close to the end (BR55) of the 56-day BBR, from the VL of the right hip flexor/knee extensor quadriceps muscle, and from the right calf soleus muscle (plantar flexor) of each volunteer according to a well-established method (Bergström 1962) and full medical care was thereafter provided according to a previously approved protocol (Rudnick et al. 2004). Because of potential anatomical variations (e.g., fiber size or type distribution), attempts were made to extract the samples from each individual at approximately the same location. All needle biopsy samples were immediately embedded in small silicone casts filled with Tissue-Tek (Sakura Fine Tek Europe B.V., The Netherlands), and immediately frozen in liquid nitrogen, and stored at −80°C until further analysis.

Myofiber structure and phenotype analysis

For histology and morphometry, samples were cryosectioned at 8-μm thickness (Leica CM 2800, Germany), mounted on glass slides (SuperFrost® Plus, Menzel-Glaser, Germany) and subjected to immunohistochemistry protocols. Muscle fiber typing was carried out with a monoclonal antibody (clone My-32, diluted 1:1500, Sigma Inc.) against fast myosin heavy chain (fMyHC) protein which also cross-reacts with the antigens IIA,B

and IIC/X preferentially coexpressed in fast-type II myofibers, followed by application of the Cy-5 conjugated AffiniPure™ goat anti-mouse IgG secondary antibody (Dianova, Hamburg, Germany). In cross-sectioned profiles, only distinctly My-32 immunopositive fibers were designated as myofibers II (fast-type). The My-32 immunonegative fibers were always designated as myofibers I (slow-type). A small amount of myofibers revealing only faint My-32 immunostaining (i.e., hydrid fibers) was omitted from the analysis. Classification of myofiber types I and II into subtypes was not considered as adaptive responses of muscle fibers including time-dependent fiber transition (Pette and Staron 2001) may have occurred during the first weeks of bed rest. In addition, only very limited amounts of human biopsy tissue were available mainly due to ethical reasons (Rudnick et al. 2004).

The pattern of slow and fast-type myofiber profiles was determined by quantification of the amount of immunostained myofibers I and II in cryosections. Selective counts of My-32 immunonegative versus immunopositive fibers were made in arbitrarily chosen cohorts of 50 cross-sectioned myofibers found each in cryosections of VL and SOL biopsies from all subjects of the Ctrl and RVE group at the start (BR2) or end of bed rest (BR55) followed by subject-matched analysis (triple determination).

The size of muscle fibers was determined by mean myofiber cross-sectional area (CSA) determination in NOS1/My-32 double-immunostained cryosections (Rudnick et al. 2004). The standardized area profiles thus generated represented the square microns (μm^2) myofiber CSA. A total of 100 myofibers I and II was thus measured in double-immunostained VL or SOL cryosections of either groups at the start (pre) and end of bed rest (post).

NOS immunofluorescence intensity

The expression of NOS1 was determined by measuring the relative fluorescence intensity of immunostained sarcolemma membrane structures according to a well-established NOS1 immunostaining protocol optimized for human skeletal muscle cryosections (Rudnick et al. 2004). Briefly, the area pixel intensity of the defined regions of interest (ROI) selected from the immunostained sarcolemma structures (total 1,000 μm^2) of each myofiber type I or II was measured in digital confocal image scans and expressed as arbitrary units (a.u.) by the Leica software (in the range of 0–255 a.u.). At least ten type I and/or type II myofibers were thus measured from each cryosection. Changes of NOS1 intensity at myofiber sarcolemma membranes determined by area-based pixel intensity measurements between individual candidates and groups were calculated as percent changing of a.u. of postBR ($\Delta\%$) versus preBR (set as zero %).

In all immunostaining protocols we used either green fluorescent anti-mouse ALEXA 488-conjugated and/or red fluorescent anti-mouse ALEXA 555-conjugated affinity-purified secondary antibodies (Molecular Probes, OR) diluted at final concentrations of 1:3,000–1:5,000, respectively. Immunohistochemical staining was applied on subject-matched cryosections from the Ctrl and RVE group in one and the same incubation protocol in order to achieve identical immunostaining conditions for comparison analysis. Immunofluorescence images were scanned with a three channel confocal laser scanning microscope (Leica TCS SP-2, Leica Microsystems, Bensheim, Germany) at standardized image settings, and all digitalized images were analyzed using the Leica confocal software.

Biochemical analysis

The relative NOS 1 protein content in skeletal muscle biopsies was determined in electrophoresed subject-matched lysates of muscle biopsies, immunoblotted (Protean mini-system, BioRad Inc.), and quantified by densitometric scanning of immunostained protein bands (GS-800 device, Quantity-One™ software, BioRad Inc., Munich, Germany). Because the total amount of biopsy material was very limited only samples of three subjects were immunoblotted for each group (i.e., $2 \times n = 3$) in triplicate according to previously described methods (Rudnick et al. 2004). Mean optical density (OD) values were expressed as the relative percent difference of postBR versus subject-matched preBR samples (preBR arbitrarily set as the zero percent baseline).

Data analysis and statistics

MVC data of the two baseline testing sessions (BDC-2 and BDC-1) were averaged to yield a single baseline data collection (BDC) value. The SPSS software package (http://www.spss.com) was used to perform a t test for group differences at baseline, and a repeated measures ANOVA for time effects (BDC, R1 morning, and R1 evening) and group interactions were carried out with simple contrasts referring to BDC. Post hoc tests and t tests were performed in order to further analyze group time interactions for the different days. Muscle biopsy data were analyzed using the SigmaPlot software and are given as mean ± SEM. The significance of differences of data was analyzed with the Student's test. Differences were regarded to be statistically significant at $P < 0.05$. Values represent triple determination from each immunostained cell structure or biopsy sample.

Results

RVE training by the Galileo Space device

Figure 1 illustrates the experimental set-up of the Galileo Space device during an active training session

(i.e., before start of bed rest) at supine position in bed rest. In general, RVE was well tolerated by the subjects. During the 8 weeks of bed rest, exercise progression was achieved mainly through increases in vibration frequency, which was set to 19 Hz at the beginning in all subjects, and to 25.9 Hz (SD 1.9) toward the end of bed rest on average at the end (60) (Rittweger et al. 2003). During BR, lower limb pain was slightly more frequent in the RVE subjects than in Ctrl ($P = 0.035$). However, this led to the canceling of only 12 out of 770 exercise sessions because of pain.

Calf muscle force (maximum voluntary isometric plantar flexion force)

Repeated measures using ANOVA revealed significant changes in the MIPF ($P < 0.001$ for both). For comparison, relative changes are illustrated (Fig. 2). Within the RVE group, no significant change was observed from BDC to the re-ambulation day 1 (R1, $P = 0.83$). Conversely, a decrease by 18.9% (SD 9.0) was observed in the Ctrl group ($P < 0.001$). In both groups, the loss in MIPF was reduced between the re-ambulation day 1 morning (R1m) and evening (R1e) measurements. In the RVE group, this reduction was 9.2% (SD 12.0, $P = 0.015$) of the BDC value, and in Ctrl it was by 32.9 (SD 10.7, $P = 0.009$).

Myofiber size measurement (cross-sectional area)

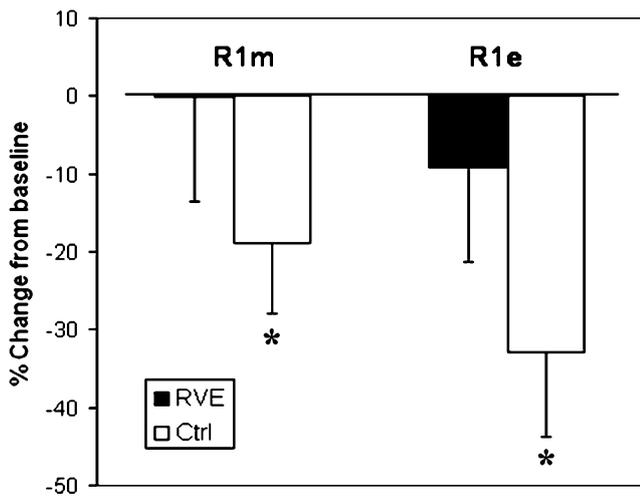We measured the myofiber CSA in VL and SOL muscles by the morphometric analysis of subject-matched pre

Fig. 2 Percent changes in maximum isometric voluntary plantar flexion force (MIPF) relative to baseline. Measurements were performed in the morning and in the evening of the re-ambulation day (R1m and R1e, respectively). Significant differences were found not only between groups ($P < 0.001$), but also between R1m and R1e (*asterisk*). For the RVE group alone, the change at R1m was non-significant ($P = 0.98$), indicating that force generation was unimpaired in this group directly after re-ambulation

and post biopsy samples. In VL, significant changes of the myofiber size were not apparent in both the Ctrl and RVE group. In non-trained SOL, CSA values, however, decreased in myofibers I and II (Fig. 3). Following RVE, the CSA increased in both myofibers I and II at the end of the bed rest period. The latter results documented an increase in SOL myofiber I and II size possibly due to fiber hypertrophy induced by the RVE training during bed rest.

Myofiber type and distribution pattern

Robust changes in the myofiber type I and II distribution pattern in subject-matched biopsy samples were found in SOL but not in the VL muscle using fast MyHC immunohistochemistry based on high-resolution confocal laser microscopy (Fig. 4). In the Ctrl group, the relative amount of myofibers II in a defined population of SOL myofibers increased significantly ($> 140\%$ vs. preBR control) while the relative amount of myofibers I in the same population remained largely unchanged. In the RVE-trained SOL (Fig. 4), significant changes were not detectable between the relative amounts of slow and fast-type myofibers I and II. Therefore, the normal fiber
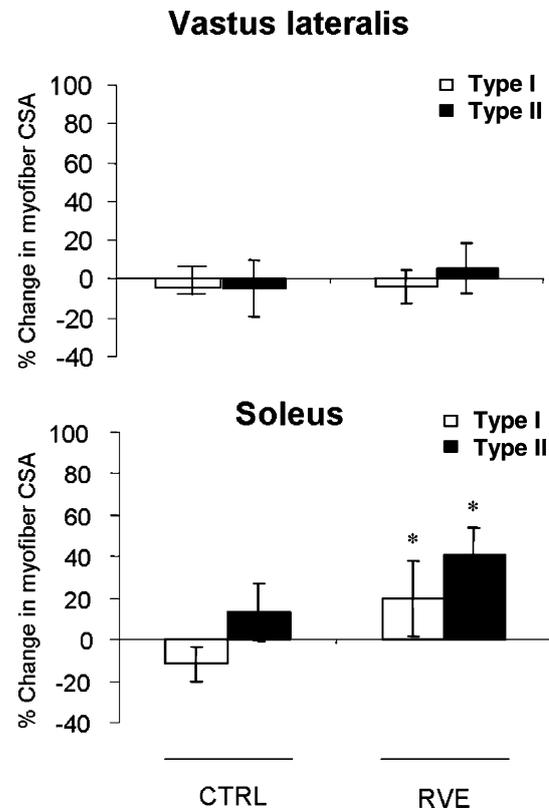

Fig. 3 Bar graphs with myofiber size measurements by cross-sectional area (CSA) determination. **a** In VL and SOL, myofiber I and II CSA changes at postBR were not significantly different from the preBR values in either of the groups. **b** In trained SOL, CSA values of myofibers I and II were significantly increased (*asterisk*) suggesting that hypertrophy was induced by RVE muscle activity
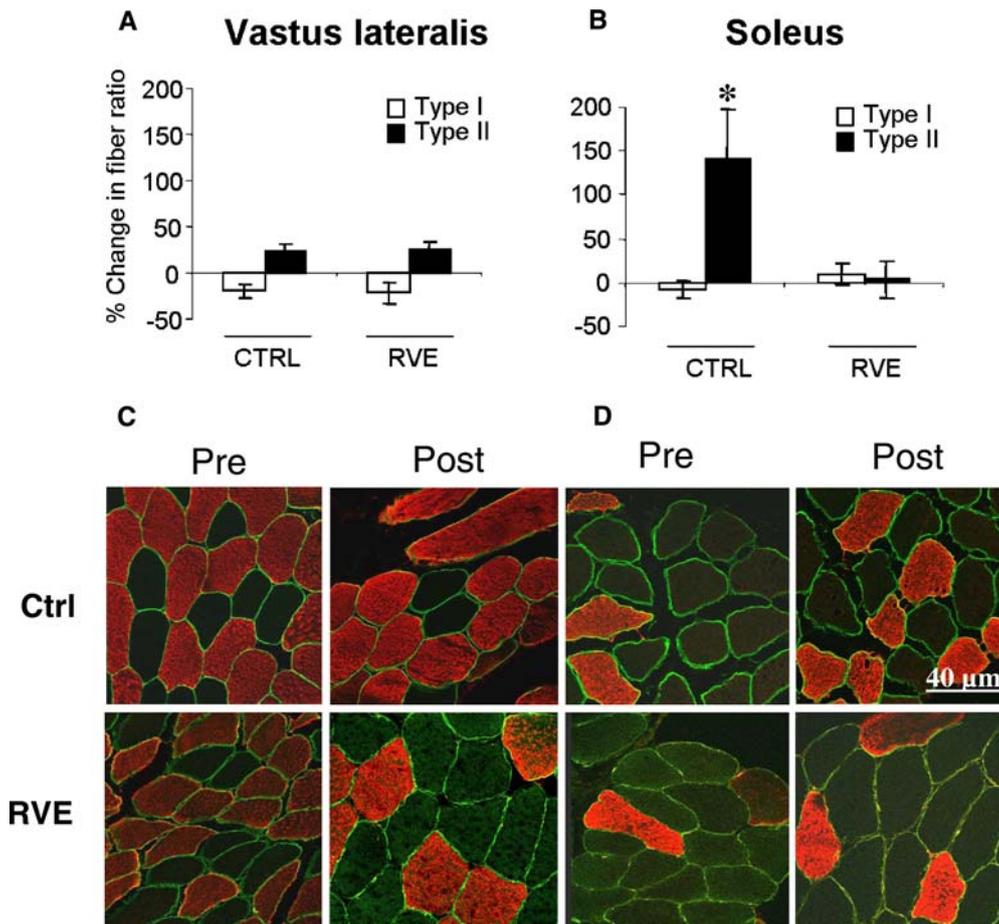
Fig. 4 Determination of myofiber ratio (type I vs. II) in VL and SOL biopsies of the control versus RVE group. **a** In VL muscle, the amount of myofibers I and II did not change significantly between the preBR and postBR samples of either groups. **b** In SOL muscle, significantly more myofibers II were detectable in the control group (+140% baseline) with bed rest only. Notably, this dramatic change was absent from the SOL trained by RVE during bed rest suggesting maintenance of muscle phenotype. **c, d** Representative pairs of merged confocal images (NOS1/fMyHC) that show marked changes in the presence of myofibers I (My-32 negative) and II (My-32 positive, red). *Bar* 40 μm (C, D only top)

type I > type II distribution pattern determined in SOL at the start of bed rest was clearly disrupted in untrained SOL (without RVE) at the end of bed rest period. However, similar changes were not observed in RVE-trained SOL at the end of bed rest. Given the fact that subject-matched analysis has been performed in this study, RVE training prevented the SOL from phenotype shifting toward a morphological "fast-type" muscle that is likely to contain more myofibers II (fast-type) than normally found in such a slow-type postural calf muscle.

NOS1 immunohistochemistry

In skeletal muscle, NOS1 immunofluorescence signals are concentrated at sarcolemma membrane structures that may be altered by muscle activity. We therefore used relative fluorescence intensity measurements of NOS1 protein patterns by confocal microscopy and the ROI-dependent pixel analysis as morphological indices of the functional sarcolemma membrane integrity in skeletal myofibers of both VL and SOL. In the Ctrl group, the sarcolemmal NOS1 immunofluorescence intensity significantly decreased in both myofibers I and II of both VL and SOL (Figs. 5, 6). In the RVE group, both VL and SOL showed increased relative fluorescence intensity values of sarcolemma NOS1 independently of the myofiber type I or II identified by fast MyHC in double-staining. Confocal analysis confirmed that in both VL and SOL muscle RVE training maintained or even upregulated the NOS1 immunofluorescence intensity at the myofiber sarcolemma membrane.

NOS 1 immunoblot analysis

We also determined the relative changes of NOS 1 protein concentration in VL and SOL in three subject-matched skeletal muscle biopsy samples by the quantitative immunoblot analysis of pre versus post samples of equal protein amounts in both the Ctrl and RVE groups (Fig. 7). In samples from the Ctrl group ($n = 3$), the post

Fig. 5 Expression of muscle fiber activity marker NOS1 in subject-matched SOL muscle biopsies. **a** Graph with percent changing of the relative immunofluorescence intensity of sarcolemmal NOS1 in myofibers I (*open bars*) and II (*black bars*) of the control and RVE group. **b** Pairs of representative confocal images showing NOS 1 immunoreactivity (*green*), and NOS1/fast MyHC double-staining (*red/green merged*) from the same subjects of the RVE group (Ctrl group not shown). Sarcolemma NOS-1 immunoreactivity as well as myofiber size are significantly increased in both myofibers I and II (RVE) due to vibration muscle exercise. *Bar* 40 μm
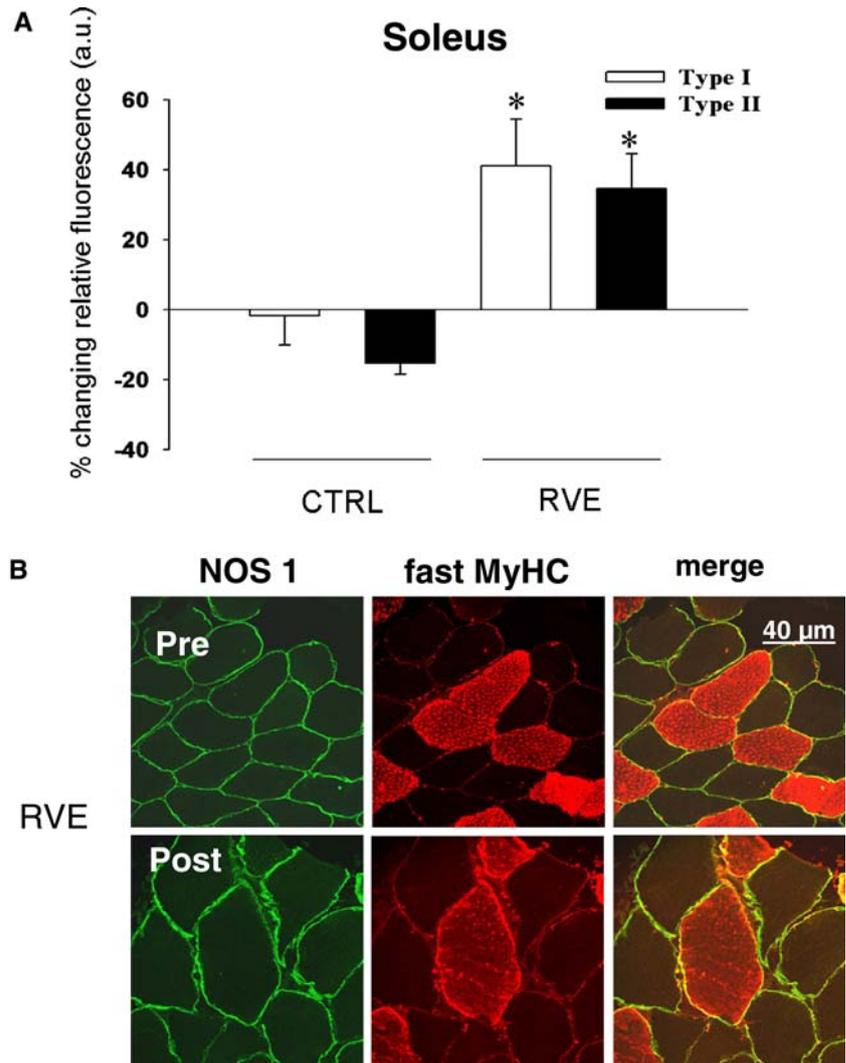
bed rest NOS1 protein level was clearly changed as compared to the pre bed rest levels set as baseline (by minus 10–30% relative to baseline). In samples from the RVE group ($n = 3$), the post bed rest NOS1 protein level was clearly increased (by plus 20–130% relative to the baseline). Similar changes were not found in VL biopsies of either the Ctrl or RVE group.

Discussion

Vibration muscle exercise in bed rest

In the present study vibration muscle exercise was applied to lower limb muscles by a vibrating footplate for use at supine position in order to test the efficacy of the Galileo Space device as a countermeasure against the loss in skeletal muscle structure and function following extended body immobilization. The device has been constructed in order to effectively transmit the vibration stimulus (i.e., controlled by Hz and amplitude) to the leg muscles (and bone) by the pressing of the subject's feet onto the vibrating footplate using a simple system of elastic straps and belts (cf. Fig. 1). As foot and leg pressing appear to be necessary for transmitting adequate vibration stimuli generated by the oscillated foot platform acceleration (cf. ergometric lower body imbalance control), the vibration exercise protocol is based on both reflexive-loop coordination (via muscle or tendon spindles) and loading as well as unloading of the leg extensor and flexor muscle chains. The RVE protocol must be therefore characterized as reflexive plus "resistive-like" vibration muscle exercise. Simple muscle vibration or strenuous shaking impulses on local muscle groups coming from different vectors or angles have been frequently reported to show multiple adverse effects (e.g., edema, nerve conductivity problems; cf. Adamo et al. 2002; Bongiovanni et al. 1990; Gauthier et al. 1981; Ivanenko et al. 2000; Martin and Park 1997; Rittweger et al. 2003; Roelants et al. 2004). Therefore, inclusion of a second control group subjected to shaking/vibration impulses of the calf or thigh region without

Fig. 6 Expression of muscle fiber activity marker NOS1 in subject-matched VL muscle biopsies. **a** Graph with percent changing of the relative immunofluorescence intensity of sarcolemmal NOS1 in myofibers I (*open bars*) and II (*black bars*) of the control and RVE group expressed as arbitrary units (a.u.). **b** Pairs of representative confocal images showing NOS 1 immunoreactivity (*green*), and NOS1/fast MyHC double-staining (*red/green merged*) from the same subjects of the RVE group (Ctrl group not shown). NOS 1 immunoreactivity is slightly decreased in the myofibers I and significantly decreased in the myofibers II (Ctrl) group. In trained VL, both myofibers I and II revealed elevated fluorescence intensity values reflecting increased NOS 1 immunodetectable antigen expression due to RVE-dependent muscle activity. *Bar* 40 μm
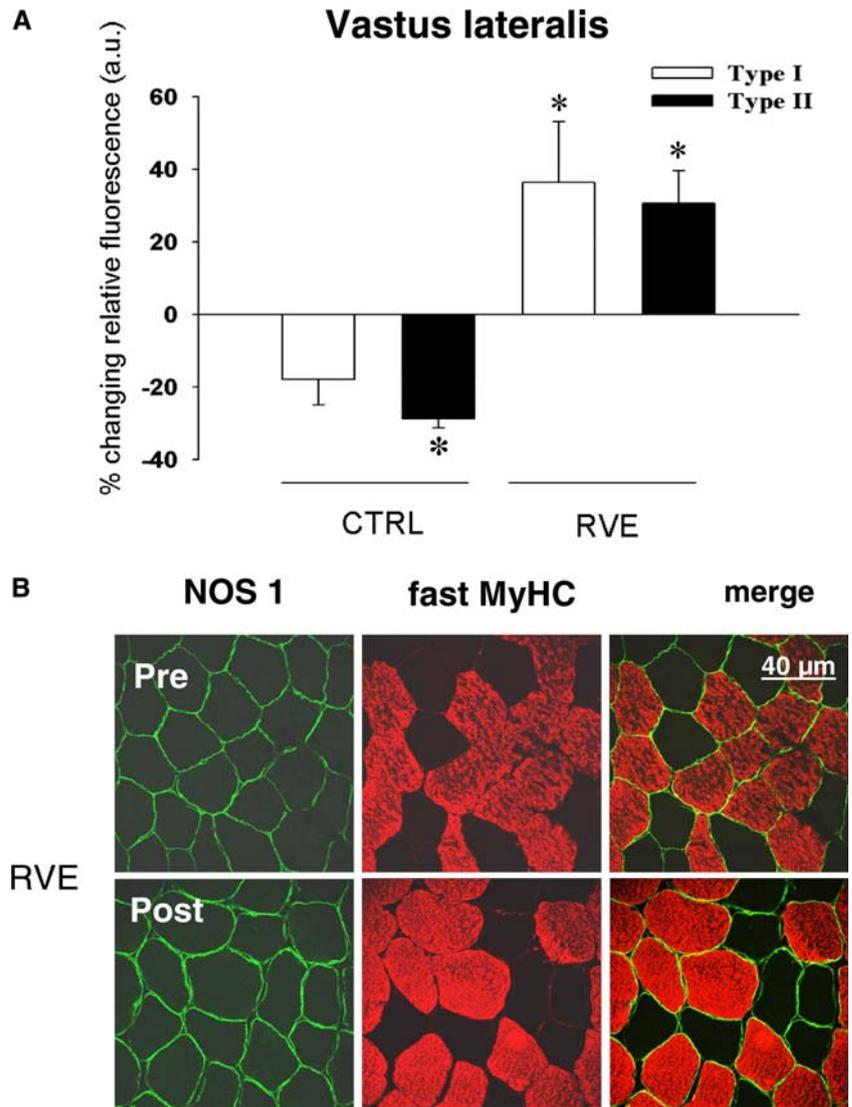
frequency-controlled RVE was not considered due to the obvious adverse effects and the overall experimental design and hypothesis tested in this study.

Multiple effects on the musculoskeletal system exposed to high frequency vibration forces were reported in animal models (Rubin et al. 2001) or in humans (Ivanenko et al. 2000). Plantar vibration exercise with the Galileo Space device (19–26 Hz) is, however, thought to be based upon reflexive muscle actions generating high numbers of muscle contraction–relaxation cycles (i.e., 26 Hz is equivalent to approximately 1,600 cycles/min). Reflexive muscle actions are likely producing mechanical strain as well as neuromuscular activation sufficient to maintain the structure and function of the musculoskeletal system (Sale 1988). The RVE protocol used in and the results from the BBR study are in support of this hypothesis. As discussed above, RVE is clearly different from the effects of whole body vibration or extremely high vibration forces to defined body regions (> 100 Hz) that might

result in adverse body effects or even detrimental effects on motor firing rates and force (Bongiovanni et al. 1990). Frequency-controlled muscle vibration therefore has been previously tested for an effective training protocol in exercise and sports performance (Cardinale and Bosco 2003; Meester et al. 1999; Roelants et al. 2004) as well as in rehabilitation (Cardinale 2004). Nevertheless, the precise neuromuscular control mechanisms remain to be determined.

Muscle fiber type and size

Muscle exercise affects the distribution of the myofiber phenotype I/II profile in terms of fiber type composition in a given muscle. In previous animal hypokinesia studies, a shift from slow-to-fast or fast-to-slow myofiber pattern was documented in lower limb muscles, a process known as fiber type transition (Pette and Staron 2001). In humans, the myofiber type pattern in SOL
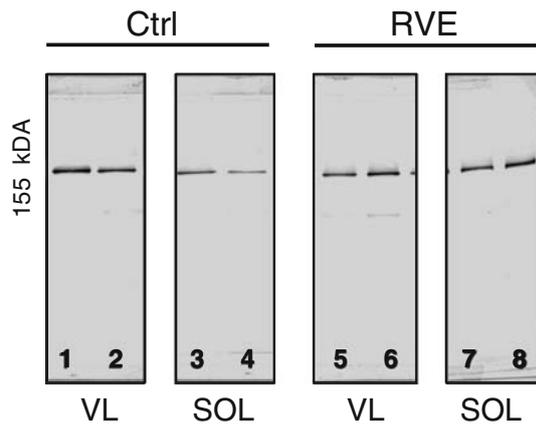
Fig. 7 NOS-1 immunoblot analysis. Subject-matched VL and SOL immunoblots showing representative pairs of pre versus post biopsy samples of the Ctrl and RVE group. Identical amounts of protein/lanes 1–8 (protein load 50 μg/ml) were electrophoresed for three subjects ($n = 3$) per group in the same gels followed by subsequent immunoblotting (triple determination). As shown, NOS-1 immunostained protein bands (155 kDa) of post samples (lanes 2, 4, 6, and 8) are either decreased (Ctrl) or increased (RVE) as compared with pre samples (lanes 1, 3, 5, and 7). For densitometric analysis of immunostained bands (cf. results)

muscle (with usually more type I than II myofibers) showed a slow-to-fast shift following mechanical deloading (Caiozzo et al. 1996) confirming a similar fiber transition as previously seen in animal studies. Surprisingly, we found that in RVE-trained SOL the relative myofiber type I/II ratio was not significantly altered when compared with subject-matched pre bed rest ratios suggesting preservation of muscle fiber phenotype. Notably, soleus and vastus muscles have different fiber patterns which may explain for the muscle-specific fiber patterns resulting from inactivity/activity or microgravity responses of slow-type versus fast-type muscles. However, similar results were not found in the RVE-trained VL muscle using identical morphometric analysis and immunostaining protocols.

As shown in previous studies, the morphometric analysis of muscle fiber size as well as protein synthesis in VL and SOL suggested a relative inability of the SOL muscle to respond to resistive exercise training protocols in bed rest or ambulatory candidates (Rudnick et al. 2004; Trappe et al. 2004). Further metabolic studies using amino acid infusion into VL and SOL of ambulatory individuals as an attempt to compensate for the ineffectiveness of SOL following resistive exercise countermeasure confirmed the presence of muscle-specific protein metabolism (Carrithers et al. 2002; Carroll et al. 2004). Our results support the idea that our RVE protocol predominantly recruited the calf muscles (local ankle plantar flexors) via the single-joint muscle kinetic chain. However, the structure and function of the VL muscle was less well preserved by our RVE protocol. Therefore, we assume that plantar vibration exercise may be less effective for recruitment of the lower limb multi-joint muscle kinetic chain including the knee extensors or thigh flexor muscles.

Nitric oxide expression

Molecular domains of the muscle membrane are considered as putative targets of mechanical stimulus transmission and should be, at least partially, affected in response to mechanical stimuli. In skeletal muscle cells, NOS1 is associated with the sarcolemmal dystrophin–glycoprotein complex via syntrophin which is linked to the subsarcolemmal actin network (Bredt 1999). Nitric oxide signals generated by NOS play important roles in normal muscle physiology. The production of NO is upregulated by muscle activity. Nitric oxide signals are released from contracting muscle cells (Balon 1999) and were shown to increase force and actomyosin ATPase activity in skeletal muscle (Perkins et al. 1997). NO appears to have important functions as local modulators of functional membrane molecules such as ion channels and receptors that are involved in various transduction processes at the outer muscle cell membrane during muscle contraction (Blottner and Lück 2001). In a previous study, we found that bed rest immobilization clearly impaired NOS1, -2 and -3 protein synthesis in human skeletal muscle and decreased the sarcolemma NOS1 expression of muscle cell fibers indicating important functional roles for activity-dependent NO-signaling (Rudnick et al. 2004). The NOS 1 protein is normally found concentrated at sarcolemma structures but can also be found in the sarcosol due to intracellular redistribution as shown in mouse development (Blottner and Lück 1998). Subcellular redistribution mechanisms of signaling proteins like NOS1 detectable by immunohistochemistry may occur during extended bed rest without affecting relative protein amounts per mg of tissue. This could explain some discrepancies between relative NOS1 protein levels immunoblotted from biopsy lysates and NOS 1 immunostaining patterns found at myofiber membranes in cryosections of pre versus post bed rest biopsies.

Effects of muscle vibration in neuro-rehabilitation or clinical muscle atrophy

Human postural reflexes play important functions following body deconditioning as well as in neuro-rehabilitation. Postural reflexes have been previously investigated in underwater simulated microgravity (Dietz et al. 1989). The effects of vibration on limb muscles may occur through the stimulation of cutaneous, muscular and articular mechanoreceptors/sensors (Gauthier et al. 1981; Park and Martin 1993) and facilitation of lower limb muscle stretch reflexes as neuromuscular compensatory response to, e.g., body imbalance or gait coordination (Ivanenko et al. 2000; Verschueren et al. 2002). More recently, the plantar

load receptor hypothesis has been discussed for proprioreceptive muscle stretch reflexes in human gait control (Dietz and Duysens 2000). Muscle fiber recruitment by vibration stimuli mechanisms may be also critical, for example, for bipedal body equilibrium in gait control. The load-induced proprioceptive reflexes for bipedal body equilibrium control activating intrafusal muscle fibers or Golgi tendon spindles via excitatory group Ia/b afferent input may be adapted by feedback stimuli programmed in the central nervous system (Dietz 2002). Finally, RVE could also serve as a countermeasure against neuromuscular atrophy in skeletal muscle disease. Therefore, RVE protocols may have implications not only for astronauts in space but also for patients in various clinical settings including neuromuscular diseases or rehabilitation.

In conclusion, the present findings provide provocative evidence that both calf muscle structure and force production can be maintained during 8 weeks of strict bed rest by RVE training. Similar finding were not confirmed for VL muscle following the RVE protocol. Our findings also indicated obvious functional correlations between NOS distribution at the sarcolemma membrane and RVE-driven muscle activity, underpinning the efficacy of the RVE training for maintenance of the soleus muscle architecture and its myofiber composition in particular. Short plantar RVE training protocols appear to be simpler than other types of exercise and may be implemented in future countermeasure protocols to offset disuse-induced atrophy of postural leg muscles during prolonged clinical bed rest, in rehabilitation, or in spaceflight.

References

Adamo DE, Martin BJ, Johnson PW (2002) Vibration-induced muscle fatigue, a possible contribution to musculoskeletal injury. Eur J Appl Physiol 88:134–140

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

Alkner BA, Tesch PA (2004) Knee extensor and plantar flexor muscle size and function in response to 90 d bed rest with or without resistance exercise. Eur J Appl Physiol 93:294–305

Baldwin KM, Haddad F (2001) Effects of different activities and inactivity on heavy chain gene expression in striated muscle. J Appl Physiol 90:345–357

Balon TW (1999) Integrative biology of nitric oxide and exercise. Exerc Sports Sci Rev 27:219–253

Berg HE, Tesch PA (1994) A gravity-independent ergometer to be used for resistance training in space. Aviat Space Environ Med 65:752–756

Berg HE, Tesch PA (1998) Force and power characteristics of a resistance exercise device for use in space. Acta Astronaut 42:219–230

Bergström J (1962) Muscle electrolytes in man: determination by neutron activation analysis in needle biopsy specimens: a study on normal subjects, kidney patients, and patients with chronic diarrhea. Scand J Clin Lab Invest 14:1–110

Bleeker MW, De Groot PC, Rongen GA, Rittweger J, Felsenberg D, Smits P, Hopman MT (2005) Vascular adaptation to deconditioning and the effect of an exercise countermeasure: results of the Berlin Bed Rest study. J Appl Physiol 99:1923–1300

Blottner D, Lück G (2001) Just in time and place: NOS/NO system assembly in neuromuscular junction formation. Microsc Res Techn 55:171–180

Blottner D, Lück G (1998) Nitric oxide synthase (NOS) in mouse skeletal muscle development and differentiated myoblasts. Cell Tissue Res 292:293–302

Bongiovanni LG, Hagbarth KE, Stjernberg L (1990) Prolonged muscle vibration reducing motor output in maximally voluntary contracting in man. J Physiol 423:15–36

Booth FW, Criswell DS (1997) Molecular events underlying skeletal muscle atrophy and the development of effective countermeasures. Int J Sports Med 18:265–269

Bredt D (1999) Endogenous nitric oxide synthesis: biological functions and pathophysiology. Free Radical Res 31:577–596

Caiozzo VJ, Haddad F, Baker MJ, Herrick RE, Prietto N, Baldwin KM (1996) Microgravity induced transformation of myosin isoforms and contractile proteins of skeletal muscle. J Appl Physiol 81:123–132

Cardinale M, Bosco C (2003) The use of vibration as an exercise intervention. Exerc Sports Sci Rev 31:3–7

Cardinale M (2004) High-frequency vibration training able to increase muscle power in postmenopausal women. Arch Phys Med Rehabil 85:1854–1857

Carrithers JA, Tesch PA, Trieschmann J, Ekberg A, Trappe TA (2002) Skeletal muscle protein composition following 5 weeks of ULLS and resistance exercise. J Gravit Physiol 9:P155–P156

Carroll JA, Carrithers JA, Trappe TA (2004) Contractile protein concentration in human single muscle fibers. J Muscle Res Cell Motil 25:55–59

Delecluse C, Roelants M, Verschueren S (2003) Strength increase after whole body vibration compared with resistance training. Med Sci Sports Exerc 35:1033–1041

Dietz V (2002) Proprioception and locomotor disorders. Nature Rev Neurosci 3:781–790

Dietz V, Duysens J (2000) Significance of load receptor input during locomotion: a review. Gait Posture 11:102–110

Dietz V, Horstmann GA, Trippel M, Gollhofer A (1989) Human postural reflexes and gravity—an underwater simulation. Neurosci Lett 106:350–355

Fitts RH, Riley DR, Widrick J (2001) Functional and structural adaptations of skeletal muscle to microgravity. J Exp Biol 204:3201–3208

Gauthier GM, Roll JP, Martin BJ, Harley F (1981) Effects of whole body vibration on sensory motor system performance in man. Aviat Space Environ Med 52:473–479

Griffin L, Garland SJ, Ivanova T, Gossen ER (2001) Muscle vibration sustains motor firing rate during submaximal isometric fatigue in humans. J Physiol 535:929–936

Harris JA, Benedict FG (1919) A biometric study of basal metabolism in man. Carnegie Institute of Washington, Washington

Ivanenko YP, Grasso R, Lacquantini F (2000) Influence of leg muscle vibration on human walking. J Neurophysiol 84:1737–1747

Martin BJ, Park HS (1997) Analysis of tonic vibration reflex: influence of vibration variables on motor unit synchronization and fatigue. Eur J Appl Physiol 75:504–511

Maffiuletti NA, Pensini M, Martin A (2002) Activation of human plantar flexor muscles increases after electrostimulation training. J Appl Physiol 92:1383–1392

McBride JM, Porcari JP, Scheunke MD (2004) Effect of vibration during fatiguing resistance exercise on subsequent muscle activity during maximal voluntary isometric contractions. J Strength Cond Res 18:777–781

Meester J, Spitzenfeil P, Schwarzer J, Seifriz F (1999) Biological reaction to vibration-implications for sport. J Sci Med Sport 2:211–226

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

Park HS Martin BJ (1993) Contribution of the tonic vibration reflex to muscle stress and muscle fatigue. Scand J Work Environ Health 19:35–42

Perkins WJ, Han Y-S, Sieck G (1997) Skeletal muscle force and actomyosin ATPase activity reduced by nitric oxide donor. J Appl Physiol 83:1326–1332

Pette D, Staron RS (2001) Transitions of muscle fiber phenotype profiles. Histochem Cell Biol 115:359–372

Rittweger J, Beller G. Felsenberg D (2000) Acute physiological effects of exhaustive whole body vibration exercise in man. Clin Physiol 20:134–142

Rittweger J, Mutschelknauss M, Felsenberg D (2003) Acute changes in neuromuscular excitability after exhaustive whole body vibration exercise compared to exhaustion by squatting exercise. Clin Physiol Funct Imaging 23:81–86

Roelants M, Delecluse C, Goris M, Verschueren S (2004) Effects of 24 weeks of whole body vibration training on body composition and muscle strength in untrained females. Int J Sports Med 25:1–5

Rubin C, Turner AS, Bain S, Mallinckrodt C, McLeod K (2001) Anabolism. Low mechanical signals strengthen long bones. Nature 412:603–604

Rudnick J, Püttmann B, Tesch PA, Alkner B, Schoser BG, Salanova M, Kirsch K, Gunga HC, Schiffl G, Lück G, Blottner D (2004) Differential expression of nitric oxide synthases (NOS 1–3) in human skeletal muscle following exercise countermeasure during 12 weeks of bed rest. FASEB J 18:1228–30

Roy RR, Zhong H, Monti RJ, Vallance KA, Kim JA, Edgerton VR (2000) Mechanical properties and fiber type composition of chronically inactive muscles. J Gravit Physiol 7:P103–104

Sale DG (1988) Neural adaptation to resistance training. Med Sci Sports Exerc 20:S135–S145

Schuler M, Pette D (1996) Fiber transformation and replacement in low-frequency stimulated rabbit fast-twitch muscles. Cell Tissue Res 285:297–303

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

Trappe TA, Raue U, Tesch PA (2004) Human soleus muscle protein synthesis following resistance exercise. Acta Physiol Scand 182:189–196

Verschueren SMP, Swinnen SP, Desloovere K, Duysen J (2002) Effects of tendon vibration on the spatiotemporal characteristics of human locomotion. Exp Brain Res 143:231–239