Aus dem Zentrum für Muskel und Knochenforschung
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DISSERTATION

Atrophy of the postero-lateral hip and lower-limb musculature during bed-rest and the influence of two different resistive countermeasure exercises

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Contents

Abstract (English)

As part of the Berlin Bed Rest Study (BBR) and 2nd Berlin Bed Rest Study (BBR2-2), this work investigated the pattern of muscle atrophy of the postero-lateral hip and lower limb musculature during prolonged bed-rest and the effectiveness of two different exercise countermeasures- resistive exercise alone and resistive exercise with whole body vibration, in preventing muscle atrophy.

For the BBR, 20 healthy male subjects underwent 56 days of horizontal bed-rest and were assigned to either an inactive control group (CTR) or resistive vibration exercise group (VRE). For BBR2-2, 24 healthy male subjects underwent 60 days of 6 degrees head-down-tilt (HDT) bed-rest and were assigned to a control group (CTR), resistive vibration exercise group (RVE) or resistive exercise alone group (RE). Magentic resonance imaging (MRI) of the hip and thigh were taken prior to, during and at the end of bed-rest. Volume of the postero-lateral hip and lower limb musculature were calculated and the rate of muscle atrophy and the effect of countermeasure exercises examined.

During bed-rest, the CTR group demonstrated differential rates of muscle volume loss ($p<0.0001$). The fastest rates of atrophy were seen in the triceps surae (medial gastrocnemius, lateral gastrocnemius, soleus), mono-articular knee extensors (vastii), mono-articular hip extensors and quadratus femoris, an external rotator of the hip and the hamstring muscles. The other postero-medial and postero-lateral muscles of the foot/ankle in addition to the adductor magnus were also strongly affected during bed-rest. The antero-medial hip muscles and anterior tibial muscles were comparatively less affected by bed-rest. Different rates of atrophy were also seen amongst some muscle synergists. For example, the heads of triceps surae and heads of the hamstring muscle group demonstrated different rates of atrophy, the vastii demonstrated a faster rate of atrophy than rectus femoris and upper gluteus maximus atrophied faster than lower gluteus maximus and gluteus medius.

Countermeasure exercise reduced/prevented muscle atrophy in the triceps surae, vastii and gluteal muscles but was less effective for the hamstring muscles, postero-medial and postero-lateral muscles of the foot/ankle and the adductor muscles. The addition of whole body vibration to resistive exercise did not have an additional effect for the variables examined in this work.

In conclusion, a short-duration resistive exercise program with or without whole-body vibration can be effective in reducing the impact of prolonged bed-rest on postero-lateral hip and lower extremity muscle volume loss during bed-rest.

Abstrakt (Deutsch)

Als Teil der Berlin Bed Rest Study (BBR) und 2. Berlin Bed Rest Study (BBR2-2), wurden die Charakteristika der Muskel Atrophie der postero-lateralen Hüft -und Beinmuskulatur untersucht. Außerdem, wurde die Wirksamkeit von zwei verschiedenen Gegenmaßnahmen - Widerstandstraining alleine und Widerstandstraining mit Ganzkörpervibration, bei der Verhinderung von Muskelschwund bei längerer Bettruhe, untersucht.

Für die BBR-Studie, wurden 20 gesunde männliche Probanden 56 Tage in horizontaler Bettruhe entweder einer inaktiven Kontrollgruppe (CTR) oder einer Widerstands-vibrationstrainingsgruppe (VRE) zugeordnet. Für BBR2-2, wurden 24 gesunde männliche Probanden 60 Tage, in 6 Grad Kopftieflage (Head-down-tilt = HDT) einer Kontrollgruppe (CTR-Gruppe), einer Widerstandstrainingsgruppe (RE) oder einer Widerstand-Vibrationstrainingsgruppe (RVE) zugeordnet. Das Muskelvolumen der postero-lateralen Hüft- und Beinmuskulatur wurde mit MRT-Untersuchungen dargestellt und die Geschwindigkeiten der Muskelatrophie und die Wirkung der Gegenmaßnahmen berechnet.

Die Geschwindigkeiten der Muskelatrophie Entwicklung der postero-laterale Hüft- und Beinmuskulatur waren unterschiedlich ($p<0.0001$). Die größte Atrophie fand sich beim Triceps surae (Medialer gastrocnemius, Lateraler gastrocnemius, Soleus), den Kniestreckern (Vastii), bei den Hüftextensoren, dem Quadratus femoris, einem Muskel, der die Hüfte nach außen dreht, und den Oberschenkelmuskeln. Die anderen postero-medialen und postero-lateralen Muskeln des Fußes/Knöchel und der Adduktor wurden während der Bettruhe auch stark betroffen. Die antero-mediale Hüftmuskulatur und der vorderen Schienbeinmuskeln waren vergleichsweise wenig während der Bettruhe betroffen. Unterschiedliche Atrophie-Geschwindigkeiten wurden auch zwischen synergistische Muskeln gesehen. Die verschiedenen Köpfe der Triceps surae und hinteren Oberschenkel Muskel-Gruppe demonstrierte unterschiedliche Geschwindigkeiten der Atrophie; die Vastii wiesen eine schnellere Geschwindigkeit der Atrophie auf als der Rectus femoris; die oberen Gesäßmuskeln wiesen eine schnellere Geschwindigkeit der Atrophie auf als der untere Gesäßmuskel und der Gluteus medius.

Zusammenfassend vermindern die Gegenmaßnahmen die Muskelatrophie im Triceps surae, in den Vastii und in der Gesäßmuskulatur, waren aber weniger wirksam für die

hinteren Oberschenkelmuskeln, die postero-mediale und postero-laterale Muskeln des Fußes / Knöchel und der Adduktoren während längerer Bettruhe. Die zusätzliche Ganzkörpervibration beim Widerstandstraining hat keinen zusätzlichen Effekt auf die untersuchten Muskelgruppen.

Introduction

Earth's gravitational force exerts a continuous and unique loading stimulus on the human musculoskeletal system. The muscles of the human hip and lower limb have evolved to resist these gravitational forces thereby enabling us to mobilise and function in an upright, bipedal position. What happens to these muscles however, when this gravitational loading stimulus is removed or reduced? This is an important question for astronauts/cosmonauts who are exposed to extended periods of weightlessness during spaceflight and for medical patients required to undergo prolonged levels of unloading during bed-rest, immobilisation or injury. Bed rest studies provide an ideal Earth based model to simulate the effects of reduced gravitational loading on the human body (9) and understand the subsequent changes which occur in the musculoskeletal system of the hip and lower limb**.**

Previous bed-rest and spaceflight studies have documented some of these changes to include muscle atrophy (1, 3, 6, 12, 14), muscle weakness (3, 14, 15) and alterations in muscle function. There is some evidence to suggest that muscle atrophy is greatest in the anti-gravity muscles of the lower limb responsible for maintaining upright stance, i.e. the plantarflexors and knee extensors (1, 3, 6, 12), with evidence to suggest that the hip extensors are also affected but to a lesser extent (3, 14). This information is however limited to a select number of muscles with little data available on the effects of prolonged unloading on the muscles of the hip. Understanding the effects reduced gravitational loading has on individual muscles of the hip and lower limb will not only help us better understand the pattern of muscle atrophy which occurs in such an environment, but is also essential for the development of appropriate countermeasure exercise programs for at risk populations. Thus, the first aim of this research is to investigate the effect of bed rest on the muscles of the lower limb and hip regions.

Previous works have shown that during removal of gravitational stimulus, not all muscles within a region are affected to the same extent. During unloading, muscle atrophy has been not only been shown to differ between individual muscles but also between muscle synergists. For example, it has been reported that the vastii atrophy to a greater extent than its synergist, rectus femoris (1, 15) during unloading. This has the potential to lead to muscle imbalances on return to full loading conditions, which may be associated with pain and injury in the hip and lower limb. As muscles do not work in an isolated manner to stabilize joint segments or produce movement, but rather in synergy with other muscles,

this is an important point to investigate. No studies to date have specifically examined the relative amount of muscle atrophy 'between' individual muscles of the lower limb and hip. Therefore, another aim of this research is to examine whether individual muscles and furthermore, muscle synergists in the lower limb and hip atrophy to different extents.

A further important consideration is the development of appropriate exercise countermeasures to counteract changes in muscle volume during prolonged periods of inactivity. According to physiological principles, in order to maintain its size, muscle requires high-load, resistance exercise. This is in agreement with findings from several bed-rest studies that have investigated the effects of different countermeasure exercises on lower limb muscle atrophy. Bed-rest works that incorporated low-load, low-resistance exercises (4, 13) have been shown to be ineffective at reducing lower limb muscle atrophy. In comparison, bed-rest studies that incorporated higher-load, resistive exercises as part of their countermeasures reported positive results in reducing/preventing atrophy of the lower limb musculature (1, 12, 15). These effects have however been inconsistent and often limited to a select number of muscles. To help guide effective exercise selection for populations at risk of becoming deconditioned due to decreased gravitational loading, it is essential to test the success of different countermeasures at counteracting changes in the lower limb and hip region. Thus, the second aim of this research is to evaluate the effectiveness of two different countermeasure exercises, resistive exercise alone and resistive exercise combined with whole body vibration, in protecting the lower limb and hip musculature against atrophy during unloading.

Whole body vibration (WBV) is thought to provide additional stimulus to muscles during training. Reported effects of WBV training include increased muscle strength, power and flexibility (10). It has been proposed that vibration applied directly to muscle or tendon facilitates muscle contraction via the excitation of primary and secondary muscle spindle endings. The increased excitation of these muscle spindles leads to a greater recruitment of motor neuron pools, which produces increased muscle activation and/or contraction via the tonic vibration reflex. WBV applied at the feet is transmitted through the lower limbs to the lumbar spine during loading. Hence, it is possible that WBV could be more effective in reducing lower limb and hip muscle atrophy than resistive exercise alone via increased stimulus to the muscle during exercise. As no studies to date have investigated this theory, this research wishes to examine this as part of our exercise countermeasure protocol.

Therefore, the primary aim of this work is to examine the effects of prolonged unloading (i.e. bed rest) on the relative amount and rate of atrophy in muscles of the postero-lateral hip, thigh and leg. Furthermore, this work assesses the effectiveness of two different countermeasure exercises: resistive exercise and resistive vibration exercise, in reducing and/or preventing muscle atrophy in the hip, thigh and leg musculature. This information will help guide exercise choice for preventative, maintenance and rehabilitative programs for populations at risk of becoming deconditioned such as astronauts/cosmonauts and the injured and/or immobilised medical patient.

Methods

Subjects and Bed-rest protocol

The Berlin Bed Rest Study (BBR) and 2nd Berlin Bed Rest Study (BBR2-2) were conducted at the Charité - Campus Benjamin Franklin, University Hospital in Berlin. For BBR, twenty male subjects underwent 8 weeks of horizontal bed-rest. Subjects were randomly assigned to either an inactive control (CTR) group or a resistive vibration countermeasure (RVE) group. For BBR2-2, twenty-four male subjects underwent 60 days of 6-degree head-down tilt bed-rest. Subjects were randomly assigned to a control (CTR) group, a resistive exercise countermeasure (RE) group or a resistive vibration exercise (VRE) group. Whole body resistive vibration exercise was performed using the Galileo Space exercise device (Novotec Medical, Pforzheim, Germany). More detailed information on the study protocols can be found in the following papers: BBR*:* **Rittweger J, Belavy DL, Hunek P, Gast U, Boerst H, Feilcke B, Armbrecht G, Blenk T, Mulder E, Schubert H, Richardson C, de Haan A, Stegeman DF, Schiessl H, and Felsenberg D**. *Highly demanding resistive vibration exercise program is tolerated during 56 days of strict bed-rest.* Int J Sport Med 27: 553-559, 2006; BBR2-2: **Belavý DL, Bock O, Börst H, Armbrecht G, Gast U, Degner C, Beller G, Heer M, de Haan A, Stegeman DF, Ceretteli P, Blottner D, Rittweger J, Gelfi C, Kornak U, and Felsenberg D**. *The 2nd Berlin BedRest Study: protocol and implementation.* J Musculoskelet Neuronal Interact 10: 207-219, 2010.

Figure 1: BBR resistive vibration exercise

Exercise countermeasures

For BBR, RVE subjects underwent eleven exercise sessions per week during bed-rest of 4-6 minutes exercise time (two exercise sessions daily, morning and afternoon; no afternoon session on Wednesdays, no training on Sundays). For BBR2-2, RE and VRE subjects underwent three exercise sessions per week during bed-rest of 5-6 minutes exercise time.

In a supine position, subjects placed their feet on a foot plate Galileo Space exercise device (see Figure 1) with side-alternating vibration (BBR: frequency range: 18-26Hz, amplitude=3.5-4 mm; BBR2-2: frequency range: 16-26Hz, amplitude=3.5-4 mm). An axial force (1.0-1.8 times body weight) was placed through the subjects' trunk and spine via padded elastic shoulder straps attached to the foot plate. A belt was also attached around the pelvis and hand-grips attached to the frame from which the vibrating platform was suspended. For BBR, the exercises performed were: leg press, heel raises and toe raises against the platform. Each exercise was performed for more than sixty seconds. In morning sessions, subjects also performed ten repetitions of 'explosive kicks' at intervals of ten seconds. Vibration frequency was increased if the subject could perform an exercise for more than 100 seconds. For BBR2-2, both RE and VRE subjects performed the same exercises with the exception being that for the RE group, vibration was switched off. The exercises performed were: double leg heel raises, single leg heel raises, leg press and back extensions with forefoot raise. Loading levels were increased by 5% per exercise when the subject could perform 12 exercise repetitions in two consecutive sessions.

MRI scanning protocol

For both studies, transverse MR images were acquired from the lower limbs using a 1.5 Tesla Magnetom Vision system (Siemens, Erlangen, Germany), with the hip region also being included in scanning for BBR2-2. Subjects were positioned on the scanning bed in supine with their knees and hips supported in slight flexion by a pillow under the knee. For BBR, baseline MR scanning was conducted on the first day of bed-rest (BR1) and then at two week intervals (BR14, BR28, BR42 and BR56) until the end of the bed-rest period. Typically, 35 images of the thigh (from the superior aspect of the head of femur to the knee joint line) and 30 images of the leg (knee joint line to the end of the lateral malleolus) were acquired. For BBR2-2, baseline MR scanning was conducted 9 or 8 days prior to the beginning of bed-rest (BDC-9/-8) and subsequently on days 27/28 (HDT27/28) and 55/56 (HDT55/56) of head-down tilt bed-rest. Typically, 170 images were collected from the iliac crest to the ankle joint.

Image measurement protocol

Each dataset was coded with a random number to blind the operator to study time-point. The same experienced operator used ImageJ (http://rsb.info.nih.gov/ij/) to measure the cross-sectional area of muscles of the hip and lower-limb on the left and right sides of the

body in all images. In the hip (Figure 2), CSA of the following muscles was measured: Gluteus Maximus (Upper Gluteus Maximus (UGM), Lower Gluteus Maximus (LGM)), Gluteus Medius (GMED), Gluteus Minimus (GMIN)), Piriformis (PIRI), Quadratus Femoris (QUAD_FEM), Obturator Externus (OBT_EXT) and Obturator Internus (OBT_INT); IC: Ischial tuberosity; FH: Femoral Head

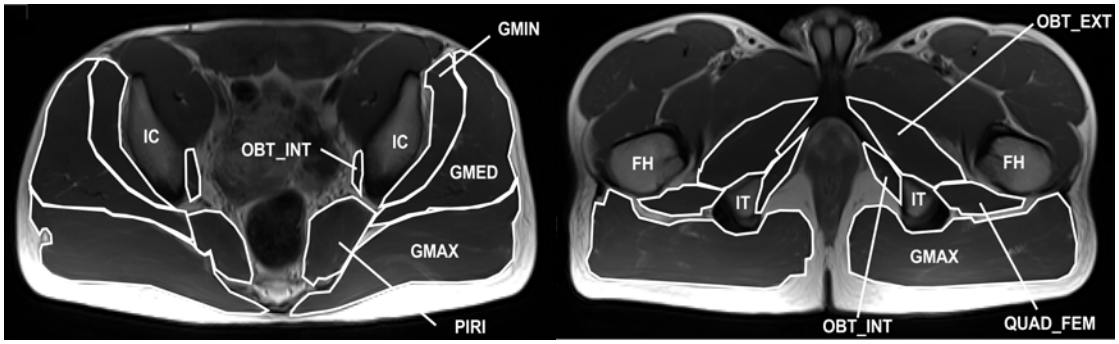

Figure 2: Cross-section of the hip; Left: proximal hip; Right: distal hip

In the thigh (Figure 3), the CSA of the following muscles was measured: Rectus Femoris (RF), Vastii (V Vastus Lateralis, Vastus Medialis, Vastus Intermedialis), Semimembranosus (SEMI_M), Semitendinosus (SEMI_T), Biceps Femoris Long Head (BFL), Biceps Femoris Short Head (BFS), Gracilis (GRAC), Sartorius (SART), Adductor Magnus (AM), Adductor Longus (AL) and Adductor Brevis (not shown)

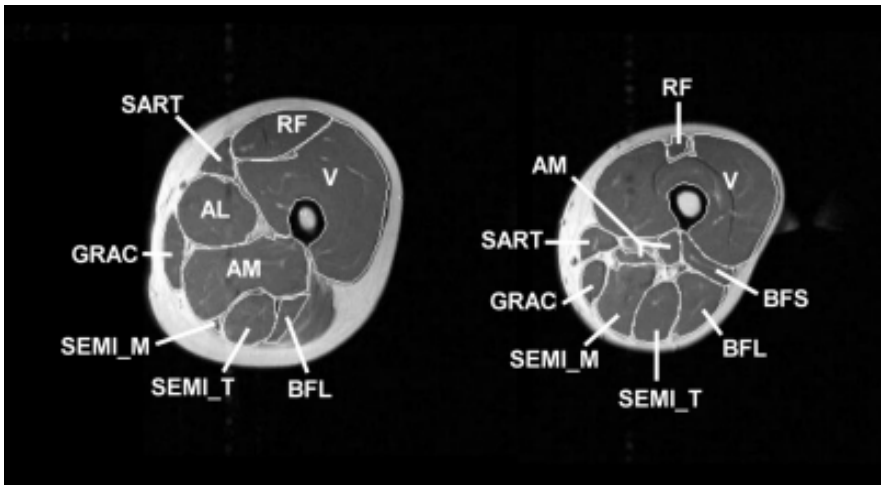

Figure 3: Cross section of the thigh; Left: proximal thigh; Right: distal thigh

In the leg (Figure 4) the CSA of the following muscles was measured: Gastrocnemius Lateralis (GLAT), Gastrocnemius Medialis (GMED), Soleus (SOL), Flexor Hallicus

Longus (FHL)-BBR2-2 only, Peroneals (PER; peroneus longus, brevis and tertius), Anterior tibial muscles (ANT; tibialis anterior, extensor digitorum longus, extensor hallicus longus -for BBR the anterior tibial muscles were grouped together and for BBR2-2, they were seperated into tibialias anterior and extensor muscle groups), Tibialis Posterior (TIBP), Flexor Digitorum Longus (FDL).

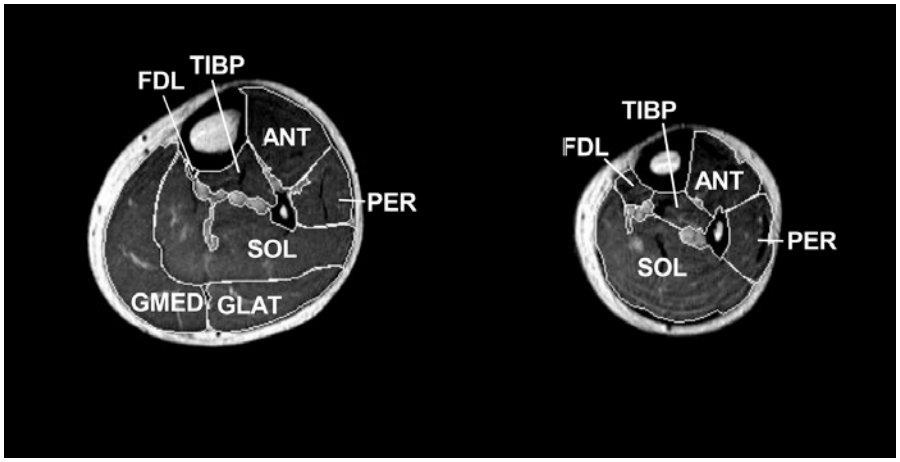

Figure 4: Cross-section of the leg; Left: proximal calf; Right: distal calf

Further data processing and statistical analysis

After measurement of a subject's entire data, the data were plotted and checked visually by the operator and another author, also blinded to study time point and subject group, to screen for any errors. Muscle volume was estimated via interpolation of cross-sectional area measurements for each muscle using slice thickness (BBR-1: 10mm; BBR-2: 6.6mm) and inter-slice distance (BBR-1: 5mm; BBR-2: 6mm). Statistical analysis focused on two aspects: the effect of bed-rest in the inactive control group only and the impact of countermeasure on changes in muscle volume during the bed-rest phase. Change in muscle volume compared to baseline muscle volume and relative amount (percentage) of muscle atrophy was calculated for each muscle. Subsequently, to facilitate comparisons between muscles and to determine which muscles atrophied the most/fastest in the CTR group, rate of muscle atrophy was calculated and the compared across the muscles examined. The effect of the countermeasure was examined in addition to whether or not the response between the two countermeasure groups RVE and RE (BBR2-2 only) differed. A p-value of ≤ 0.05 was chosen to denote statistical significance. Unless otherwise specified, results are presented as mean (SD). The "R" statistical environment (version 2.10.1, www.r-project.org) was used for all analyses.

RESULTS

The Berlin Bed Rest Study (BBR)

Effect of bed-rest on muscle volume loss in the lower limb musculature

No differences existed in baseline muscle volume for any muscles between groups. Strong evidence existed for changes in volume of all leg muscles during bed-rest (p all <0.0075). The plantarflexors of the ankle (soleus, gastrocnemius lateralis, gastrocnemius medialis) and tibialis posterior all showed evidence for reductions in volume within 2-weeks of bed-rest. The peroneal muscles showed decreases in volume somewhat later (from BR28) and continued to decrease in volume through to the end of bed-rest. The anterior tibial muscles and flexor digitorum longus also showed decreases in muscle volume, but this first reached statistical significance at the end of bed-rest (BR56).

In the thigh, the single joint knee extensors (vastii), the medial hamstrings (semimembranosus, semitendinosus) and lateral hamstrings (long and short head of biceps femoris), all showed strong effects for changes in volume during bed-rest (p all $\leq .0013$). The vastii were decreased in volume after 14-days of bed-rest, however, this first reached statistical significance after 28-days of bed-rest. Semimembranosus showed decreases in volume within 2-weeks of bed-rest, whereas significant decreases in volume first occurred in its synergists, semitendinosus and biceps femoris long head, at the end of bed-rest (BR56), with effect of bed-rest weaker for the short-head of biceps femoris. For the medial thigh muscles (adductor longus, adductor magnus, gracilis, sartorious), little statistical evidence existed for changes in muscle volume during bed-rest, though closer inspection of the data showed that adductor magnus decreased in muscle volume in the last 4 weeks of bed-rest (BR42 and BR56) and gracilis underwent reductions in muscle volume at the end of bed-rest. Rectus femoris showed no change in volume throughout the bed-rest phase.

Effect of countermeasure exercise on the lower limb musculature

Strong evidence existed for an effect of the countermeasure exercise on soleus (p=0.004) and gastrocnemius medialis (p=0.029) muscles, with preservation of muscle volume until the BR28 scanning date for gastrocnemius medialis and BR42 scanning date for soleus, with some losses thereafter. The lateral gastrocnemius showed evidence (p=0.0247) for an influence of the countermeasure on muscle volume, with no significant changes occurring in muscle volume over the course of the study in the RVE compared to the CTR group. Little or no evidence existed for an effect of the countermeasure exercise in the peroneal or

tibialis posterior muscle groups. On closer inspection of the data, however, no significant losses of muscle volume were seen in the RVE versus CTR group of the tibialis posterior and muscle volume of the peroneals, appeared to be preserved in the RVE group up until BR42. Of the thigh muscles, an effect of the countermeasure was only seen in the vastii muscle group ($p < 0.0001$) where losses in muscle volume first reached statistical significance at BR56 compared to BR28 in the CTR group.

Effect of bed-rest on rate of muscle volume loss in the lower limb

Rate of muscle atrophy differed strongly between muscles of the lower limb during bed-rest ($p<0.0001$). The greatest rate of atrophy was seen in the medial gastrocnemius, which atrophied at a slightly faster rate than the vastii and soleus muscles. The medial hamstrings, biceps femoris long head and lateral gastrocnemius all demonstrated similar rates of atrophy, which were marginally but not significantly slower than the vastii and soleus. The tibialis posterior, peroneal muscle group and adductor magnus also demonstrated significant rates of atrophy during bed-rest. No significant rates of atrophy were seen in the adductor longus, gracilis, sartorius, rectus femoris, flexor digitorum longus or in the anterior tibial muscle group. Different rates of atrophy were also seen amongst synergistic muscles. Adductor magnus atrophied at a significantly faster rate than adductor longus ($p=0.009$), medial gastrocnemius atrophied at a greater rate than lateral gastrocnemius ($p=0.002$) and soleus ($p=0.0305$) and vastii atrophied faster than the rectus femoris muscle ($p=0.0002$).

The 2nd Berlin Bed Rest Study (BBR2-2)

Effect of bed-rest on muscle volume loss in the postero-lateral hip musculature

No differences existed in baseline muscle volume for any muscles between groups. With the exception of obturator internus, obturator externus and piriformis, strong evidence existed for changes in volume of the postero-lateral hip musculature during the course of the study ($p \leq 0.004$). At the end of bed-rest, greatest losses in muscle volume were seen in quadratus femoris, which lost an estimated 18% of its muscle volume, semimembranosus and biceps femoris long head. Gluteus minimus, upper and lower gluteus maximus, gluteus medius and semitendinosus also demonstrated significant losses in muscle volume, although to a lesser extent (Table 1). Non-significant changes in muscle volume were observed in the biceps femoris short head, obturator internus, obturator externus and piriformis during bed-rest.

Table 1: Percentage change in gluteal and hamstring muscle volume during bed-rest

Group	Study time					
	BDC	HDT27/28	HDT55/56	BDC	HDT27/28	HDT55/56
	Gluteus Minimus			*Biceps Femoris Long Head*		
CTR	124.8(16.0)	-11.3(1.5)%‡	-11.2(1.4)%‡	235.1(26.2)	-9.6(2.0)%‡	-17.7(1.9)%‡
RE	112.5(21.6)	-3.7(1.9)%	-5.4(1.5)%‡	245.6(54.3)	-9.3(1.4)%‡	-13.1(1.5)%‡
RVE	112.6(19.5)	-9.4(2.6)%‡	-5.6(2.4)%*	209.9(24.0)	-10.2(2.2)%‡	-13.6(2.0)%‡
	Gluteus Medius			*Biceps Femoris Short Head*		
CTR	373.1(41.9)	-2.2(0.9)%*	-3.7(0.9)%‡	140.1(29.7)	0.4(3.4)%	-6.5(3.7)%
RE	363.0(75.4)	-1.9(1.1)%	0.0(1.2)%	134.8(29.9)	0.6(2.3)%	-2.7(2.6)%
RVE	373.6(43.3)	-4.8(1.9)%*	-3.6(2.3)%	128.8(35.4)	0.8(3.4)%	-2.7(3.4)%
	Gluteus Maximus (Lower)			*Semimembranosus*		
CTR	573.3(128.6)	-1.9(1.8)%	-5.7(1.6)%‡	300.4(42.8)	-10.6(1.8)%‡	-18.1(1.6)%‡
RE	609.5(113.3)	6.0(2.0)%†	8.2(2.1)%‡	292.1(56.0)	-10.0(1.4)%‡	-12.8(1.5)%‡
RVE	510.5(58.2)	1.2(2.1)%	7.0(2.3)%†	256.1(43.9)	-11.2(2.4)%‡	-14.1(2.3)%‡
	Gluteus Maximus (Upper)			*Semitendinosus*		
CTR	397.4(37.9)	-8.5(2.3)%‡	-9.9(2.4)%‡	220.9(30.0)	-2.0(1.9)%	-5.2(1.9)%†
RE	398.2(87.8)	-0.9(2.2)%	2.5(2.9)%	236.1(55.9)	-2.0(1.9)%	-5.3(1.9)%†
RVE	397.4(52.2)	-1.6(2.1)%	-4.3(2.0)%*	207.7(59.5)	-0.4(3.7)%	-2.4(3.6)%

CTR: inactive control group, RE: resistive exercise only group, RVE: resistive exercise with whole-body vibration group. BDC: baseline data, HDT27/28: head-down-tilt bed-rest day 27/28, HDT55/56: head-down-tilt bed-rest day 55/56. At BDC values are means (SD) muscle volume in cm3. Subsequent to BDC, values are means (SD) percentage change compared with BDC. *: $p<0.05$; †: $p<0.01$; ‡: $p<0.001$ and indicate significance of difference to baseline value.

Effect of countermeasure exercise on the postero-lateral hip musculature

There was strong evidence for an effect of the countermeasure exercise on the volume of all of the gluteal muscles ($P\leq0.02$) with no significant differences between the two training groups. Countermeasure exercise produced significant increases in muscle volume (i.e. muscle hypertrophy) in the lower gluteus maximus by end bed-rest (HDT 55/56) and reduced muscle volume loss in both training groups in the upper gluteus maximus up to mid-bedrest (HDT27/28) and in the gluteus minimus throughout bed-rest. Countermeasure exercise had an effect on muscle volume for semimembranosus and biceps femoris long head ($p\leq 0.0005$), with a partial reduction in atrophy of these muscles evident in the training versus CTR group during bed-rest (Table 1). There was no significant difference

between the two training groups for these muscles. Quadratus femoris was the only hip rotator muscle to demonstrate a significant loss of volume throughout bed-rest, however, weak statistical evidence existed for an effect of the countermeasure on this muscle.

Effect of bed-rest on rate of muscle volume loss in the postero-lateral hip

Rates of muscle atrophy differed between muscles of the postero-lateral hip ($p \leq 0.0001$) with the fastest rates of muscle volume loss observed in the semimembranosus and quadratus femoris, followed by the other hamstring and gluteal muscles (Figure 5). Non-significant rates of atrophy were seen in the gluteus medius, obturator internus, obturator externus, and piriformis during bed-rest. Rates of muscle atrophy differed also between some muscle synergists. For example, in the hamstring muscle group, semimembranosus atrophied at a significantly faster rate than biceps femoris short head ($p<0.0001$) and semi-tendinosus ($p<0.0001$), which in turn atrophied faster than biceps femoris short head ($p<0.0001$). In the gluteal muscle group, gluteus minimus atrophied at a faster rate than gluteus medius ($p=0.0005$) and lower gluteus maximus ($p=0.008$) whilst upper gluteus maximus atrophied faster than lower gluteus maximus ($p=0.04$) and gluteus medius (0.004). Different synergistic muscle groups also atrophied at different rates, for example, the bi-articular hip and knee extensors biceps femoris long head and semimembranosus, demonstrated faster atrophy than the mono-articular hip extensors, the gluteal muscles.

Effect of bed-rest on muscle volume loss in the lower limb musculature

With the exception of gracilis, pectinues, adductor brevis and rectus femoris, significant changes in muscle volume were seen in all muscles during the course of the study ($p<0.0098$). In the lower limb, greatest losses in muscle volume occurred in the medial and lateral gastrocnemius and soleus, tibialis posterior, flexor hallicus longus, flexor digitorum longus, vastii and in the hamstrings, with biceps femoris short head less affected (Table 2).

Effect of countermeasure exercise on the lower limb musculature

Atrophy of the medial and lateral gastrocnemius, soleus, vastii, tibialis posterior, flexor hallucis longus and flexor digitorum longus was reduced in the RE group compared to control. Atrophy of the medial and lateral gastrocnemius and tibialis posterior was reduced in the RVE group compared to control ($p \leq 0.044$). The countermeasure had no significant effect on muscle volume loss in the hamstrings, extensor digitorum longus and tibialis anterior. There were no

significantly different responses in muscle volume between the RE and RVE groups during bed-rest.

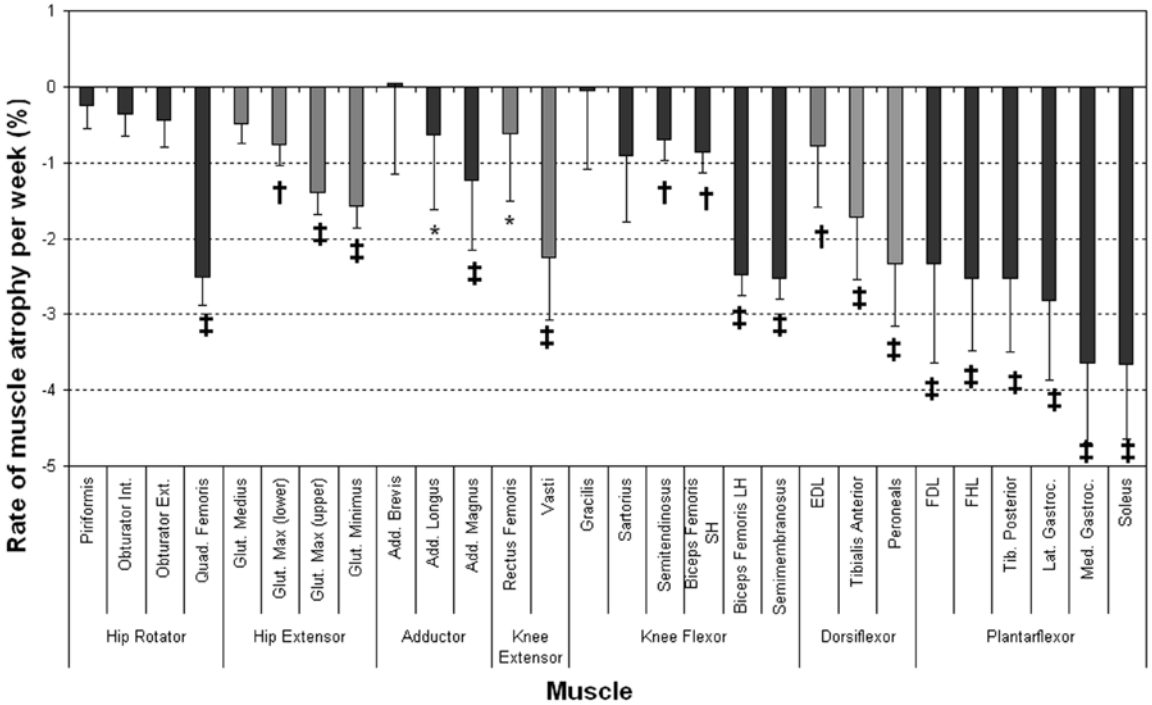

Figure 5. Estimates of rates of volume loss in the hip and lower limb muscles during bed-rest

Values are mean (error bars: SD) estimates of the time constant *k* in the fitted exponential decay model $e^{(k * (BRx-1))}$ (where BR*x* is the xth day of bed-rest). More negative time constants indicate faster loss of muscle volume during bed-rest. * p<0.05; † p<0.01 and ‡ p<0.001 indicate significance of the percentage change in muscle volume compared to zero. Otherwise p>0.05. FHL: Flexor hallicus longus, FDL: Flexor digitorum longus, EDL: Extensor digitorum longus

Effect of bed-rest on rate of muscle volume loss in the lower limb

Rates of muscle atrophy differed between individual muscles ($p \leq 0.0001$) and between some synergistic muscles (Figure 5). In the adductor muscle group, adductor magnus demonstrated faster atrophy than adductor brevis ($p=0.0008$), but this did not differ to the rate observed in adductor longus. In the knee extensors, the vastii atrophied faster than its synergist, rectus femoris ($p<0.0001$). For the plantarflexor muscles, soleus and medial gastrocnemius atrophied at a faster rate (*p for* both <0.028) than the lateral gastrocnemius. The other postero-lateral muscles of the ankle and foot arch (tibialis posterior, peroneals, flexor hallucis longus, flexor digitorum longus) all atrophied at similar rates and in turn demonstrated a faster rate of atrophy than the tibialis anterior muscle ($p \leq 0.014$).

Table 2: Percentage change in muscle volume during bed-rest of the knee extensors, thigh adductors, postero-medial and antero-lateral leg muscles

Group	Study-time					
	BDC(cm3)	HDT27/28	HDT55/56	BCD(cm3)	HDT27/28	HDT55/56
	Rectus femoris			*Tibialis posterior*		
CTR	311.8(41.8)	-1.9(7.2)%	-5.5(7.8)%	119.6(18.4)	-13.0(4.2)%‡	-15.9(6.0)%‡
RE	324.8(47.9)	0.4(4.4)%	1.8(3.9)%	100.9(20.2)	0.5(3.7)%	-2.2(5.1)%
VRE	300.7(39.3)	-2.5(5.8)%	-0.6(5.0)%	109.0(21.2)	-4.0(8.6)%	-5.7(10.1)%
	Vasti			*Peroneals*		
CTR	1948.6(147.9)	-9.1(6.9)%†	-15.2(8.5)%‡	158.5(29.5)	-11.6(6.0)%‡	-16.4(9.6)%‡
RE	2017.9(366.3)	-1.7(6.2)%	-1.4(6.1)%	143.8(9.5)	-4.3(3.0)%†	-8.2(3.6)%‡
VRE	1916.1(270.5)	-4.4(9.0)%	-6.7(10.6)%	137.1(17.0)	-6.0(3.8)%‡	-10.2(6.2)%‡
	Adductor Magnus			*Soleus*		
CTR	600.6(82.9)	-5.2(8.2)%	-9.2(8.7)%*	574.9(93.8)	-16.8(6.7)%‡	-22.7(9.5)%‡
RE	633.9(147.2)	-1.0(4.6)%	-1.3(4.6)%	527.0(96.2)	-7.3(3.8)%‡	-12.7(4.7)%‡
VRE	558.5(52.0)	-2.6(3.7)%	-4.3(4.8)%*	564.4(59.0)	-9.8(7.3)%†	-16.4(8.4)%‡
	Adductor Longus			*Medial gastrocnemius*		
CTR	185.1(17.2)	-1.6(5.6)%	-5.1(4.8)%*	297.6(50.0)	-17.0(9.0)%‡	-24.8(10.5)%‡
RE	212.4(26.7)	-3.3(5.2)%	-4.6(7.5)%	301.8(35.6)	-4.2(7.5)%	-7.9(5.9)%†
VRE	162.5(16.1)	3.2(7.3)%	1.0(8.6)%	272.1(40.3)	-5.3(6.2)%	-10.8(8.6)%†
	Flexor digitorum longus			*Lateral gastrocnmeius*		
CTR	31.3(3.4)	-11.3(8.6)%†	-15.2(10.1)%‡	188.0(32.8)	-12.3(11.7)%†	-19.5(12.5)%‡
RE	29.3(8.3)	1.7(5.0)%	1.3(8.8)%	195.8(34.1)	-2.7(8.4)%	-2.9(7.5)%
VRE	25.9(7.5)	0.4(9.9)%	-5.5(11.0)%	167.9(30.2)	0.4(10.0)%	-3.1(11.5)%
	Flexor hallicus longus			*Tibialis Anterior*		
CTR	87.8(7.2)	-10.5(4.0)%‡	-16.7(8.7)%‡	169.3(21.2)	-7.4(3.8)%‡	-12.0(4.2)%‡
RE	82.1(26.3)	2.7(4.6)%	-3.0(5.6)%	159.6(32.6)	-6.8(4.1)%‡	-10.9(3.2)%‡
VRE	75.4(11.2)	-1.3(12.6)%	-8.3(14.0)%	142.4(11.7)	-6.2(4.6)%†	-8.9(6.7)%†

CTR: inactive control group, RE: resistive exercise only group, RVE: resistive exercise with whole-body vibration group. BDC: baseline data, HDT27/28: head-down-tilt bed-rest day 27/28, HDT55/56: head-down-tilt bed-rest day 55/56. At BDC values are means (SD) muscle volume in cm3. Subsequent to BDC, values are means (SD) percentage change compared with BDC. *: $p<0.05$; †: $p<0.01$; ‡: $p<0.001$ and indicate significance of difference to baseline value.

DISCUSSION

Although previous works have investigated the effects of gravitational unloading on selected gluteal (3, 14) and lower limb muscles (1, 3, 6, 14), this is the first work to investigate the effect of bed-rest on the individual muscles of the postero-lateral hip and lower limb and to directly compare the rates of muscle atrophy between these muscles. Together, this information provides a comprehensive picture of the changes in muscle volume that occur in this region during prolonged periods of gravitational unloading.

The main finding of this research is that muscles of the postero-lateral hip and lower limb atrophy at different rates during bed-rest, with the fastest rates of atrophy observed in the ankle plantarflexors (medial gastrocnemius, soleus, lateral gastrocnemius), followed by the monoarticular knee extensors (vastii), the bi-articular hamstring muscles and the mono-articular hip extensors (gluteals). Muscles of the foot and ankle, such as tibialis posterior, the peroneal muscle group and toe flexors were also quite strongly affected, as was the quadratus femoris, an external rotator of the hip. The adductor magnus and anterior tibial muscles also showed significant losses in muscle volume although to a lesser extent. The rectus femoris and other anteromedial hip muscles (adductor brevis, adductor longus, sartorius and gracilis) were comparatively less affected by bed-rest. Rate of muscle atrophy was found to differ not only between individual muscles but also between muscle synergists.

The second main finding of this research was that high-load, muscle specific, resistive exercise (with or without whole body vibration) is effective at reducing/preventing atrophy in the ankle plantarflexors, the mono-articular knee extensors, the mono-articular hip extensors and some muscles of the foot and ankle, however, is less effective for the bi-articular hip extensor, the hamstrings. The addition of whole body vibration to resistive exercise did not have a supplementary effect over resistive exercise alone in reducing muscle atrophy in the muscles examined.

This work found a hierarchical pattern of muscle atrophy in the anti-gravity muscles of the postero-lateral hip and lower limb, with greater atrophy seen in the ankle plantarflexors, followed by the mono-articular knee extensors and the hip extensors. These findings are comparable to another work (3), which reported a similar pattern of muscle atrophy during bed-rest. The lower limb and hip are responsible for the generation of extension forces at

the ankle, knee and hip joints, which, enable us to resist gravitational forces and mobilise in an upright position. From a biomechanical perspective, greater muscular moments are required lower in the kinetic chain (e.g. ankle) to support the load above, hence, with reduction of gravitational loading stimulus, as in bed-rest, it is not surprising that the plantarflexors of the ankle undergo the greatest losses in muscle volume, followed by the extensors of the knee and the hip. The strong effect of bed-rest on the other postero-medial (tibialis posterior, flexor hallicus longus and flexor digitorum longus) and lateral (peroneal muscle group) muscles of the ankle and foot may be attributed to the important role these muscles play in support of the medial foot arch and in control of the ankle and foot during functional tasks (11).

The different rates of atrophy demonstrated amongst muscle synergists in this work can also be explained by examination of the functional role of these muscles during daily activities. In the hamstring muscles, a similar pattern of muscle atrophy to that of the current work has been described in a previous unloading study, with semitendinosus and biceps femoris short head comparatively less affected than semimembranosus and biceps femoris long head (1). On closer examination of the function of the hamstrings, it has been reported that the bi-articular semimembranosus and biceps femoris long head are active during the loading and swing phases of walking and running (7) and are thought to play an important role in providing hip extension force for propulsion. Biceps femoris short head in contrast, does not cross the hip joint and is thought to be more involved in knee flexion and control (7), which may help explain why this muscle is less affected by unloading.

Although the gluteal muscles act synergistically with the hamstring muscles, they have different functions and hence, demonstrate a different response to unloading. This work showed significantly greater atrophy in the upper gluteus maximus compared with lower gluteus maximus. These two muscle portions are embryologically and functionally different, with lower gluteus maximus thought to act primarily as a hip extensor and upper gluteus maximus as a hip abductor. Aside from these anatomical and functional differences, two alternate explanations for these findings of differential atrophy are also possible. Firstly, it cannot be excluded that, for the purpose of muscle measurements, the division of the upper and lower gluteus maximus was imperfect. Secondly, although subjects were instructed to limit load bearing through their feet during bed-rest, during changes in position, daily transfers, and use of bed pans, it is possible that one or both feet

were partially loaded. Although relatively infrequent, these hip extension movements may have activated the lower gluteus maximus sufficiently enough to maintain its muscle volume. Unfortunately, previous works in spaceflight and bed-rest, which investigated the effect of unloading on the gluteal musculature, grouped the muscles together, making comparisons to the findings in this work difficult. Interestingly, inconsistent responses of the gluteal muscles to unloading were reported in these studies, with 2.3% muscle volume loss documented after 35 days of bed rest (3) compared with 8% muscle volume after 17 days of spaceflight (14). These findings demonstrate that, although bed-rest may generally represent physical inactivity, this does not mean that all muscles are inactive, and, moreover, it may be possible for increased usage of some muscles to occur.

An interesting finding from this work was that of quadratus femoris, which appears to have a unique response among the hip external rotators to unloading. Originating on the lateral border of the ischial tuberosity and inserting on the quadrate tubercle of the femur, this deep muscle of the hip demonstrated the fastest rate of atrophy and greatest loss of muscle volume of the hip muscles examined in this study. The current understanding of quadratus femoris and its function is limited, with research on this muscle in humans largely comprising of anatomical and embryological studies. Case studies on injury of this muscle may help us to understand that it is active in load bearing, thigh adduction, and external rotation, however, further investigation is necessary to elucidate the role of quadratus femoris in control of the hip joint and function.

Regarding the knee extensors, the rate of atrophy of the vastii was significantly greater than that of the rectus femoris, which was not strongly affected during bed-rest. These findings concur with data from other unloading studies (1, 15). Not only has vastii been shown to be more vulnerable than its synergist to unloading, data from another work has shown that it is preferentially activated over the rectus femoris during closed-chain, combined hip and knee extension exercises i.e. during leg-press/ squats (5). Such combined hip-knee extension movements are frequently utilised on a daily basis during changes of position and during mobilisation. With removal and/or reduction of physical activity, it is not surprising that this muscle undergoes a significant reduction in volume.

Furthermore, in the adductor muscle group, the adductor magnus muscle also demonstrated a faster rate of atrophy than that of its synergists, adductor longus and/or adductor brevis.

Adductor magnus also plays an important role in extension of the hip and/or eccentric control of hip flexion (7) and is similarly recruited over adductor longus during combined hip and knee extension (5). The only other unloading study separating the adductors muscles during analysis (1) found the relative atrophy of the adductor longus during 20-days of bed-rest to be almost double that of adductor magnus, which is in contrast to the current work. This study, however, did not perform direct statistical comparisons. With inactivity and 'unloading' of the lower-limbs, the more predominant roles that muscles, such as the bi-articular hamstrings, vastii and adductor magnus play in 'extension' of the lower quadrant for propulsion and maintenance of upright posture during locomotion, are no longer needed and therefore, display a greater rate of atrophy during bed-rest.

The ankle plantarflexors also showed differing rates of atrophy, with the medial gastrocnemius demonstrating a faster rate of atrophy than the soleus and lateral gastrocnemius. With muscles comprising a higher percentage of slow (type I) muscle fibres suggested to be more predisposed to atrophy during prolonged inactivity, one could expect that the soleus, rich in slow, type I muscle fibres, to undergo a greater amount of muscle atrophy than its synergists, the medial and lateral gastrocnemius. Data from other studies in bed-rest have however, produced inconsistent results with greater (1) and less (4) atrophy of the gastrocnemius muscles than the soleus being reported. It should be noted that these prior studies however, did not perform any statistical tests on the relative differences of atrophy between these muscles. A consistent finding, however, amongst unloading studies, is that the plantarflexors are largely affected during bed-rest. Due to the important functional role of this muscle group during bipedal locomotion, the inclusion of exercises to counteract these changes should be a primary consideration in the development of countermeasure exercise programs.

The findings of differential atrophy between muscles and among muscle synergists in the current work have important implications for exercise countermeasure development and rehabilitation of the muscles after unloading. Muscle atrophy corresponds to changes in muscle force and power. In order to compensate for these changes, it is reasonable that increased levels of activation may occur in synergistic and antagonist muscles in the affected region/s of the postero-lateral hip and lower limb. Data from modelling studies, for example, have shown, that decreased muscle force in the gluteal muscles during hip extension results in increased superior and anterior forces on the hip joint with subsequent

substitution and increased muscle activation in muscles surrounding this region. Furthermore, research suggests that hip muscle force imbalances contribute to increased risk of injury in the hip and lower limb. Differential atrophy during bed-rest has the potential to lead to muscle imbalances, altered joint loading and subsequent increased risk of injury in the hip and lower limb on return to full loading conditions and is therefore, an important point to consider during countermeasure exercise selection.

This work supports the idea that high-load, muscle specific, resistive exercise is one of the best known current methods for reduction and/or prevention of muscle atrophy of the hip and lower limb during prolonged gravitational unloading. In contrast, previous studies that utilised low-load, aerobic based exercises (4, 13) were less effective at reducing lower limb muscle atrophy during bed-rest. Whilst aerobic countermeasures are important for maintaining cardiovascular fitness in the deconditioned patient and/or astronaut/cosmonaut during prolonged levels of inactivity, they are not an effective for muscle atrophy.

The countermeasure exercise program in this study was largely successful, however, was not effective for all muscles affected by bed-rest. Countermeasure exercise prevented/reduced muscle volume loss in the mono-articular gluteal musculature but had less effect on the bi-articular hamstring musculature. The strongest effect of the countermeasure was observed in lower gluteus maximus, which is strongly (55–60% MVIC) activated during squats/leg-press (2) and resulted in the hypertrophic response observed in both training groups by end bed-rest. The hamstring musculature, in contrast, is only moderately activated during squats (3–30% MVIC) (2), and, as a result, underwent significant atrophy in the training groups in this study. Isolated hamstring curls are reported to produce twice as much hamstring muscle activity (between 67 and 70% MVIC) as squats or leg press (2) and facilitate more equal recruitment of semimembranosus and biceps femoris (2). An effect of leg-press exercise on reducing hamstring muscle atrophy during bed-rest has, however, been shown in a previous work (1) and it is possible that the range of hip and knee flexion utilised for the exercises in the current work was not sufficient to recruit this muscle group effectively. Future countermeasure exercise programs could consider increasing range of hip and knee flexion during squats and/or including resisted isolated hamstring curls to help reduce muscle atrophy.

Furthermore, the calf musculature appears particularly difficult to maintain during bed-rest. In BBR, where training was performed eleven times a week, the soleus (RVE group) muscle still underwent a significant 7% loss in muscle volume at the end of bed-rest compared to a 15% loss in muscle volume in BBR2-2 where training was performed only three times a week. During activities of daily living, these muscles are used for a number of low load activities such as walking (8), hence, the inclusion of endurance exercises in addition to high-load, resistive plantarflexion may also be of benefit for the calf muscles.

There was no difference between the resistive exercise only and resistive exercise with vibration groups for the outcome parameters examined in this research, therefore, the hypothesis that less muscle atrophy would occur in the lower-limb muscles with the supplementation of vibration to resistive exercise was not able to be confirmed. Low subject numbers may have prevented accurate assessment of any potential additional effect(s) of vibration. Future studies could consider pooling findings to help resolve this issue. Another possibility is that, although resistive vibration training has been reported to have positive effects on functional muscle parameters such as muscle strength, power, and flexibility (10), muscle volume is a morphological parameter and, as such, may not be ideal to reflect additional effects of vibration.

In conclusion, this work found that the muscles most affected by bed-rest are the ankle plantarflexors, mono-articular knee extensors, mono-articular hip extensors and bi-articular hamstring muscles. During prolonged levels of unloading, different rates of atrophy occur not only between individual muscles but also between some muscle synergists. Differential atrophy may lead to muscle imbalances in the hip and lower limb during extended periods of inactivity, potentially increasing the risk of injury on return to full gravity conditions. A short duration, high load, resistive exercise program performed three days a week is sufficient to reduce/prevent muscle atrophy of the ankle plantarflexors and mono-articular knee and hip extensors, but is less effective for the hamstrings muscles. Whole body vibration does not have an additional effect over resistive exercise alone in counteracting muscle atrophy of the hip and lower limb. Future countermeasure exercise programs should focus on incorporating closed chain hip-knee-ankle extension exercises, which target those muscles most affected by bed-rest. Also, future countermeasure exercise could consider including isolated, resisted hamstring curls to better target this muscle group and endurance exercises to increase recruitment of the plantarflexor muscle group.

References

1. **Akima H, Ushiyama J, Kubo J, Fukuoka H, Kanehisa H, and Fukunaga T**. Effect of unloading on muscle volume with and without resistance training. *Acta Astronaut* 60: 728-736, 2007.
2. **Andersen LL, Magnusson SP, Nielsen M, Haleem J, Poulsen K, and Aagaard P**. Neuromuscular activation in conventional therapeutic exercises and heavy resistance exercises: implications for rehabilitation. *Phys Ther* 86: 683-697, 2006.
3. **Berg HE, Eiken O, Miklavcic L, and Mekjavic IB**. Hip, thigh and calf muscle atrophy and bone loss after 5-week bedrest inactivity. *Eur J Appl Physiol* 99: 283-289, 2007.
4. **Berry P, Berry I, and Manelfe C**. Magnetic-resonance-imaging evaluation of lower-limb muscles during bed rest - a microgravity simulation-model. *Aviat Space Environ Med* 64: 212-218, 1993.
5. **Enocson AG, Berg HE, Vargas R, Jenner G, and Tesch PA**. Signal intensity of MR-images of thigh muscles following acute open- and closed chain kinetic knee extensor exercise - index of muscle use. *Eur J Appl Physiol* 94: 357-363, 2005.
6. **Le Blanc A, Lin C, Shackelford L, Sinitsyn V, Evans H, Belichenko O, Schenkman B, Kozlovskaya I, Oganov V, Bakulin A, Hedrick T, and Feeback D**. Muscle volume, MRI relaxation times (T2), and body composition after spaceflight. *J Appl Physiol* 89: 2158-2164, 2000.
7. **Montgomery WH, 3rd, Pink M, and Perry J**. Electromyographic analysis of hip and knee musculature during running. *The American Journal of Sports Medicine* 22: 272-278, 1994.
8. **Neptune RR, Kautz SA, and Zajac FE**. Contributions of the individual ankle plantar flexors to support, forward progression and swing initiation during walking. *J Biomech* 34: 1387-1398, 2001.
9. **Pavy-Le Traon A, Heer M, Narici MV, Rittweger J, and Vernikos J**. From space to Earth: advances in human physiology from 20 years of bed-rest studies (1996-2006). *Eur J Appl Physiol* 101: 143-194, 2007.
10. **Rittweger J**. Vibration as an exercise modality: how it may work, and what its potential might be. *Eur J Appl Physiol* 108: 877-904, 2010.
11. **Segal RL, and Song AW**. Nonuniform activity of human calf muscles during an exercise task. *Arch Phys Med Rehabil* 86: 2013-2017, 2005.
12. **Shackelford LC, LeBlanc AD, Driscoll TB, Evans HJ, Rianon NJ, Smith SM, Spector E, Feeback DL, and Lai D**. Resistance exercise as a countermeasure to disuse-induced bone loss. *J Appl Physiol* 97: 119-129, 2004.
13. **Suzuki Y, Kashihara H, Takenaka K, Kawakubo K, Makita Y, Goto S, Ikawa S, and Gunji A**. Effects of daily mild supine exercise on physical performance after 20 days bed rest in young persons. *Acta Astronaut* 33: 101-111, 1994.
14. **Tesch PA, Berg HE, Bring D, Evans HJ, and LeBlanc AD**. Effects of 17-day spaceflight on knee extensor muscle function and size. *Eur J Appl Physiol* 93: 463-468, 2005.
15. **Tesch PA, Trieschmann JT, and Ekberg A**. Hypertrophy of chronically unloaded muscle subjected to resistance exercise. *J Appl Physiol* 96: 1451-1458, 2004.

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Beitrag im Einzelnen (bitte kurz ausführen):
- Image analysis
- Collection of pain data
- Interpretation of MRI data
- Interpretation of statistics
- Drafting the article
- Revising the article

Publikation 2: **Miokovic T**, Armbrecht G, Felsenberg D, Belavý DL (2012): Heterogeneous atrophy occurs within individual lower limb muscles during 60d bed-rest. *Journal of Applied Physiology* 113(10):1545-59

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- Image analysis
- Interpretation of MRI data
- Drafting the article

- Revising the article

Publikation 3: **Miokovic T**, Armbrecht G, Felsenberg D, Belavý DL (2011): Differential atrophy of the postero-lateral hip musculature during prolonged bed-rest and the influence of exercise countermeasures. *Journal of Applied Physiology* 110(4): 926-934.

Beitrag im Einzelnen (bitte kurz ausführen):
- Image analysis
- Interpretation of MRI data
- Interpretation of statistics
- Revising the article
- Drafting the article

Publikation 4: Belavý DL, **Miokovic T**, Armbrecht G, Rittweger J, Felsenberg D (2009): Resistive vibration exercise reduces lower limb muscle atrophy during 56-day bed-rest. *Journal of Musculoskeletal and Neuronal Interactions* 9(4):225-235

Beitrag im Einzelnen (bitte kurz ausführen):
- Image analysis
- Interpretation of MRI data
- Revising the article

Publikation 5: Belavý DL, **Miokovic T**, Armbrecht G, Richardson CA, Rittweger J, Felsenberg D (2009): Differential atrophy of the lower-limb musculature during prolonged bed-rest. *European Journal of Applied Physiology* 107(4):489-499.

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- Interpretation of MRI data
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Heterogeneous atrophy occurs within individual lower limb muscles during 60 days of bed rest

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Miokovic T, Armbrecht G, Felsenberg D, Belavý DL. Heterogeneous atrophy occurs within individual lower limb muscles during 60 days of bed rest. *J Appl Physiol* 113: 1545–1559, 2012. First published September 13, 2012; doi:10.1152/japplphysiol.00611.2012.—To better understand disuse muscle atrophy, via magnetic resonance imaging, we sequentially measured muscle cross-sectional area along the entire length of all individual muscles from the hip to ankle in nine male subjects participating in 60-day head-down tilt bed rest (2nd Berlin BedRest Study; BBR2–2). We hypothesized that individual muscles would not atrophy uniformly along their length such that different regions of an individual muscle would atrophy to different extents. This hypothesis was confirmed for the adductor magnus, vasti, lateral hamstrings, medial hamstrings, rectus femoris, medial gastrocnemius, lateral gastrocnemius, tibialis posterior, flexor hallucis longus, flexor digitorum longus, peroneals, and tibialis anterior muscles ($P \leq 0.004$). In contrast, the hypothesis was not confirmed in the soleus, adductor brevis, gracilis, pectineus, and extensor digitorum longus muscles ($P \geq 0.20$). The extent of atrophy only weakly correlated ($r = -0.30$, $P < 0.001$) with the location of greatest cross-sectional area. The rate of atrophy during bed rest also differed between muscles ($P < 0.0001$) and between some synergists. Most muscles recovered to their baseline size between 14 and 90 days after bed rest, but flexor hallucis longus, flexor digitorum longus, and lateral gastrocnemius required longer than 90 days before recovery occurred. On the basis of findings of differential atrophy between muscles and evidence in the literature, we interpret our findings of intramuscular atrophy to reflect differential disuse of functionally different muscle regions. The current work represents the first lower-limb wide survey of intramuscular differences in disuse atrophy. We conclude that intramuscular differential atrophy occurs in most, but not all, of the muscles of the lower limb during prolonged bed rest.

musculature; immobilization; spaceflight

RECENT WORKS HAVE DEMONSTRATED that, during physical inactivity, such as during prolonged bed rest, differential atrophy occurs between the muscles of the lower limbs (5, 6, 13, 29, 36, 46). By use of the term differential atrophy, we mean that some muscles atrophy faster and/or to a greater extent than other muscles. Typically, due to prolonged bed rest, greater atrophy is seen in the calf musculature, followed by the knee extensors and hamstrings. Additionally, differential atrophy occurs between synergistic muscles. For example, among the knee extensors, the vasti, a monoarticular extensor of the knee, atrophies to a much greater extent than rectus femoris, a muscle that also acts at the hip (13). Such information is not only interesting for understanding the physiological process of muscle atrophy, but it is also important to guide rehabilitation programs in individuals subject to muscle atrophy.

Within an individual muscle, however, it is often assumed in the literature that muscle atrophy is greatest at the point of greatest cross-sectional area (CSA) (30). In searching the literature, we have identified works in bed rest (2, 3) and spaceflight (1) that provide insight into this issue. In these works, the knee extensors and plantar flexors were considered as a whole, and it was found that muscle atrophy was indeed typically greatest at the point of greatest CSA. It has, however, been reported that quadriceps muscle hypertrophy differs along its length in response to exercise (31), which suggests that, within the same muscle, specific regions are recruited differently. A further example of differential muscle recruitment can be found in a functional magnetic resonance imaging study of knee extension exercises, which demonstrated that the distal part of rectus femoris is recruited greater than its proximal section, with the relationship reversed for the vastus lateralis (4).

This selected "regional" recruitment of muscle during functional tasks has been previously referred to as "muscle partitioning" (19). The muscle-partitioning hypothesis describes muscles as being divided into separate regions or neuromuscular compartments as defined by differences in muscle architecture and innervation patterns. A number of studies in humans have demonstrated that muscles are partitioned into architectural subdivisions (35) and show regional differences in fiber-type distribution (18, 26) and innervation patterns (15, 45). Presumably, these different muscle subregions would have slightly different functions. Potentially, this could mean that atrophy of an individual muscle is not consistent along its length and that the effect may not relate to where a muscle is largest. Thus the questions we wished to investigate in this study were as follows: does differential regional atrophy occur within individual muscles during prolonged bed rest? Also, does this differ from one muscle to the next in the lower limb? Aside from better understanding of muscle atrophy, this information will provide us with more information regarding compartmentalization of function within individual muscles.

Our hypothesis was that intramuscular differential atrophy would occur within muscles of the lower limb but that its pattern would be muscle specific. The 2nd Berlin BedRest Study (BBR2–2) (12) provided the opportunity to assess this hypothesis. Additionally, as part of this bed rest project, the opportunity arose to examine muscle recovery up to 180 days after 60-day bed rest, a process that has not been investigated in detail to date.

MATERIALS AND METHODS

Ethical approval, bed rest protocol, and subject characteristics. This study was approved by the ethical committee of the Charité Universitätsmedizin Berlin and conformed to the latest revision of the Declaration of Helsinki. All subjects gave their written informed

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consent. Nine medically and psychologically healthy males [age 33.1(7.8) yr, height 181.3(6.0) cm, weight 80.6(5.2) kg] were randomized to an inactive control group in the BBR2–2 and received no form of countermeasure. The study protocol and screening process is discussed in detail elsewhere (12). In brief, however, subjects attended the facility for the baseline data collection (BDC) from 9 days before a 60-day six-degree head-down tilt (HDT) bed rest period and remained in the facility for a 7-day post-bed rest recovery period (R+1 to R+7). During the HDT phase, subjects performed all hygiene activities in the HDT position, were required to move in bed only to the extent necessary for hygiene and meals, and were not allowed to stand up for any reason. After bed rest, subjects returned to the facility for follow-up appointments 14, 90, and 180 days post-bed rest (R+14, R+90, R+180). Exclusion criteria specifically relevant to the current study were any muscle or bone disease, any kind of cartilage or joint disease, prior knee surgery, performance of resistive exercise in the last 6 mo, or participation in competitive sport in the last 5 yr. After discharge from the facility 7 days after bed rest, subjects returned to their daily lives. Subjects completed a physical activity questionnaire (7), which showed no significant differences 180 days after bed rest compared with before bed rest (9).

The sample size of the BBR2–2 was based on bone parameters (11) and not the parameters of the current study. On the basis of data from a prior study (13), we estimated a correlation of 0.95 between repeated measures of muscle CSA at the point of greatest soleus muscle CSA and a between-subject standard deviation of 13.0% (relative to the mean CSA). Given these data and assuming a power of 0.8 and an α-level of 0.05, the current study design, with one baseline measure and two following measures during bed rest, should be able to detect a 1.9% difference in muscle atrophy between subsections of the soleus muscle. G*Power version 3.1.2 (http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/) was used for these calculations.

Magnetic resonance imaging protocol. MRI was conducted in the horizontal supine position 9 or 8 days before bed rest (BDC), on *day 27* or *28* (HDT27/28) of bed rest and then on *day 55* or *56* (HDT55/56) using a 1.5T Siemens Avanto scanner with a peripheral angio matrix coil (Erlangen, Germany). Follow-up scanning was done 14, 90, and 180 days after bed rest (R+14, R+90, R+180). Cushions provided by the MRI manufacturer were positioned behind the knee to support the joint in slight flexion, and the MRI coil positioned over the legs ensured the subject did not need to actively contract their lower-limb muscles to maintain position. The MRI coil placed over the legs and feet helped to standardize ankle and foot position. To allow time for shift of body fluids from the extremities (16), subjects remained in horizontal lying for at least 2 h before each scanning session. During the HDT bed rest phase, beds were placed in the horizontal position 2 h before scanning to ensure comparability to pre- and post-bed rest data.

Depending on subject height, up to 180 axial images (slice thickness 6 mm, inter-slice distance 0.6 mm, echo time: 36 ms) were collected to encompass the left and right sides of the body from the iliac crest to the end of the lateral malleolus. Scan regions were divided into three blocks at the hip (repetition time: 8,000 ms, field of view: 420 × 294 mm interpolated to 320 × 224 pixels), thigh (repetition time: 5,820 ms, field of view: 450 × 270 mm interpolated to 320 × 192 pixels), and calf (repetition time: 6,190 ms, field of view: 400 × 200 mm interpolated to 320 × 160 pixels). The images were stored for offline analysis.

Image analysis and further data processing. Each dataset was coded with a random number (www.random.org) to blind the operator to study time point. The same experienced operator (T. Miokovic) used ImageJ (http://rsb.info.nih.gov/ij/) to measure the area of muscles of the lower limb in every image on the left and right sides of the body (Fig. 1). The first few and last few slices for each muscle typically contained a mixture of muscle (gray) and tendon (black) although the two were usually straightforward to distinguish. Intramuscular fat and nerves were carefully traced around during measurements. After measurement of a subject's entire data, the data were plotted and checked visually by the operator and last author, while maintaining blinding, to screen for any errors such as measurements being pasted into the wrong section of the data entry spreadsheet. The volume of each muscle was calculated via linear interpolation, given the slice thickness of 6 mm and inter-slice gap of 0.6 mm. The length of each muscle in centimeters was calculated based on the number of images in which it was present and the slice thickness and gap settings.

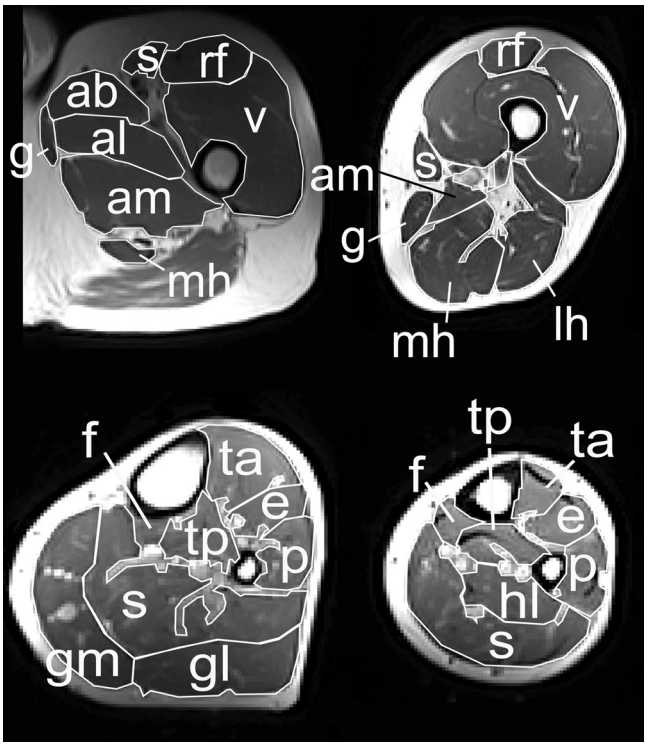


Fig. 1. Muscle measurements on magnetic resonance images. Images are from the proximal thigh (*top*, *left*), distal thigh (*top*, *right*), proximal lower leg (*bottom*, *left*), and distal lower leg (*bottom*, *right*). Thigh: ab, adductor brevis; al, adductor longus; am, adductor magnus; g, gracilis; s, sartorius; rf, rectus femoris; v, vasti; mh, medial hamstrings (semimembranosus and semitendinosus); lh, lateral hamstrings (biceps femoris long and short heads). Pectineus is not shown. Lower leg: f, flexor digitorum longus; s, soleus; gm, medial gastrocnemius; gl, lateral gastrocnemius; ta, tibialis anterior; tp, tibialis posterior; e, extensor digitorum longus; p, peroneals; hl, flexor hallucis longus.

Because the lengths of muscles differed from one individual to the next, to permit examination of intramuscular differences in CSA loss during bed rest, it was necessary to standardize the length of each muscle measured. Each set of CSA measurements from each muscle, measurement day, and subject was linearly interpolated into 21 individual CSA measurements. Each area measurement was interpolated at 5% intervals of total muscle length such that 0% was at the proximal end of the muscle, 50% at the middle of the muscle, and 100% at the distal end of the muscle. This interpolation algorithm was implemented via custom written software in the Labview environment (version 6.1, National Instruments, Dallas, TX). Data from the left and right sides of the body were then averaged before statistical analysis. Left and right side differences in atrophy were 5.5% in pectineus at end-bed rest and otherwise less than 2.5% in the remaining muscles.

Statistical analysis. To test the hypothesis that the extent of atrophy differed across the length of each muscle, percentage change in CSA at each position along the length of each muscle in the bed rest phase was evaluated. The factors of length (0%, 5%, . . . , 100% of muscle length), study date (HDT27/28, HDT55/56), and their interaction were modeled. Allowances for heterogeneity of variance were made for the

Table 1. *Muscle length, volume, and relationship between atrophy and muscle size*

Muscle	Baseline Length, cm	*r*	Volume, cm³		
			Baseline	Mid-bed rest	End-bed rest
Medial thigh muscles					
Adductor brevis	11.7 (1.3)	0.36*	111.3 (16.6)	112.3 (21.0)	111.1 (17.8)
Adductor longus	19.2 (1.9)	−0.52‡	182.5 (18.0)	180.0 (18.2)	172.5 (17.5)*
Adductor magnus	26.6 (2.4)	−0.60‡	599.2 (79.4)	565.8 (82.5)†	539.8 (82.8)‡
Pectineus	10.8 (1.4)	−0.45†	72.0 (9.1)	70.4 (8.8)	71.0 (10.0)
Gracilis	30.7 (2.8)	−0.19	109.1 (17.6)	113.4 (16.1)	106.5 (16.0)
Sartorius	51.3 (1.8)	0.11	181.8 (28.0)	178.5 (27.7)	168.3 (27.4)†
Knee extensors					
Rectus femoris	33.3 (1.4)	−0.38*	307.9 (41.5)	302.1 (43.2)	290.8 (43.7)†
Vasti	42.2 (2.5)	−0.53‡	1949 (147)	1765 (116)‡	1637 (132)‡
Hamstrings					
Medial hamstrings	37.2 (1.8)	−0.19	521.3 (51.6)	483.8 (46.7)‡	454.1 (45.3)‡
Lateral hamstrings	35.2 (2.0)	−0.42†	375.2 (46.1)	351.4 (42.7)†	323.6 (42.5)‡
Anterolateral leg muscles					
Extensor digitorum longus	38.6 (2.0)	−0.13	122.0 (19.6)	117.8 (19.7)†	114.9 (19.8)‡
Tibialis anterior	33.8 (2.4)	−0.79‡	166.8 (20.9)	153.9 (21.3)‡	146.6 (21.3)‡
Peroneals	38.9 (2.0)	−0.44†	155.3 (29.3)	137.6 (29.7)‡	129.6 (30.8)‡
Posteromedial calf muscles					
Flexor digitorum longus	23.1 (1.9)	−0.48†	31.6 (3.3)	28.0 (3.3)‡	26.6 (3.4)‡
Flexor halluces longus	20.1 (1.9)	0.18	86.5 (8.0)	77.3 (7.6)‡	71.5 (9.3)‡
Tibialis posterior	32.3 (2.6)	−0.75‡	118.5 (17.9)	102.9 (17.6)‡	99.0 (18.0)‡
Lateral gastrocnemius	24.6 (2.3)	−0.66‡	188.1 (31.0)	164.7 (29.9)‡	149.7 (29.6)‡
Medial gastrocnemius	27.3 (1.6)	−0.36*	304.8 (53.3)	254.5 (55.6)‡	230.1 (56.5)‡
Soleus	34.6 (2.2)	−0.45†	574.8 (93.2)	475.8 (94.0)‡	441.3 (97.3)‡

Values for baseline muscle length and muscle volume before and during bed rest are mean(SD). *r*: Pearson's correlation coefficient comparing baseline cross-sectional area along the length of the muscle to subsequent atrophy (percentage change in cross-sectional area) at the same point. Negative values imply that larger baseline muscle cross-sectional area correlates with greater subsequent atrophy. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

position of across the length of the muscle. Linear mixed-effects models (32) were implemented. Where the length factor or study-date × length interaction were significant, this indicated that intramuscular differential atrophy occurred.

Correlation analyses were performed to examine whether the region of greatest muscle atrophy, in percentage terms, correlated with the location of greatest muscle CSA. Mean estimates from statistical modeling of baseline muscle CSA along the entire length of the muscle were compared with the mean estimates from percentage change during bed rest vs. baseline. Pearson's correlation coefficient was calculated for each muscle and for all muscles pooled.

To examine whether the rates of atrophy differed between muscles, nonlinear mixed effects modeling was used to calculate exponential decay rates per week of bed rest. To enable this, the volume data from baseline, mid-bed rest, and end-bed rest were converted to fractional change in muscle volume and compared with the pre-bed rest value set as 1 (i.e., a 20% loss in muscle volume was equivalent to $1-0.2 = 0.8$ and a complete loss of muscle volume would have been 0). A factor of muscle was examined. Allowances for heterogeneity of variance between muscles were made. Although similar results were achieved with a linear model, given that muscle is of a finite size and a negative muscle volume is not possible, it would be invalid to assume a linear model of muscle atrophy and a model of exponential decay was more appropriate (14). The nonlinear mixed-effects model calculated an exponential rate for each muscle within each subject. Then the model calculated the mean of all subjects for each muscle and also the variance for each muscle. The R^2 for the exponential decay model including all muscles was 0.87. A series of a priori contrasts was performed between synergistic muscles to examine whether synergistic muscles atrophied at differential rates.

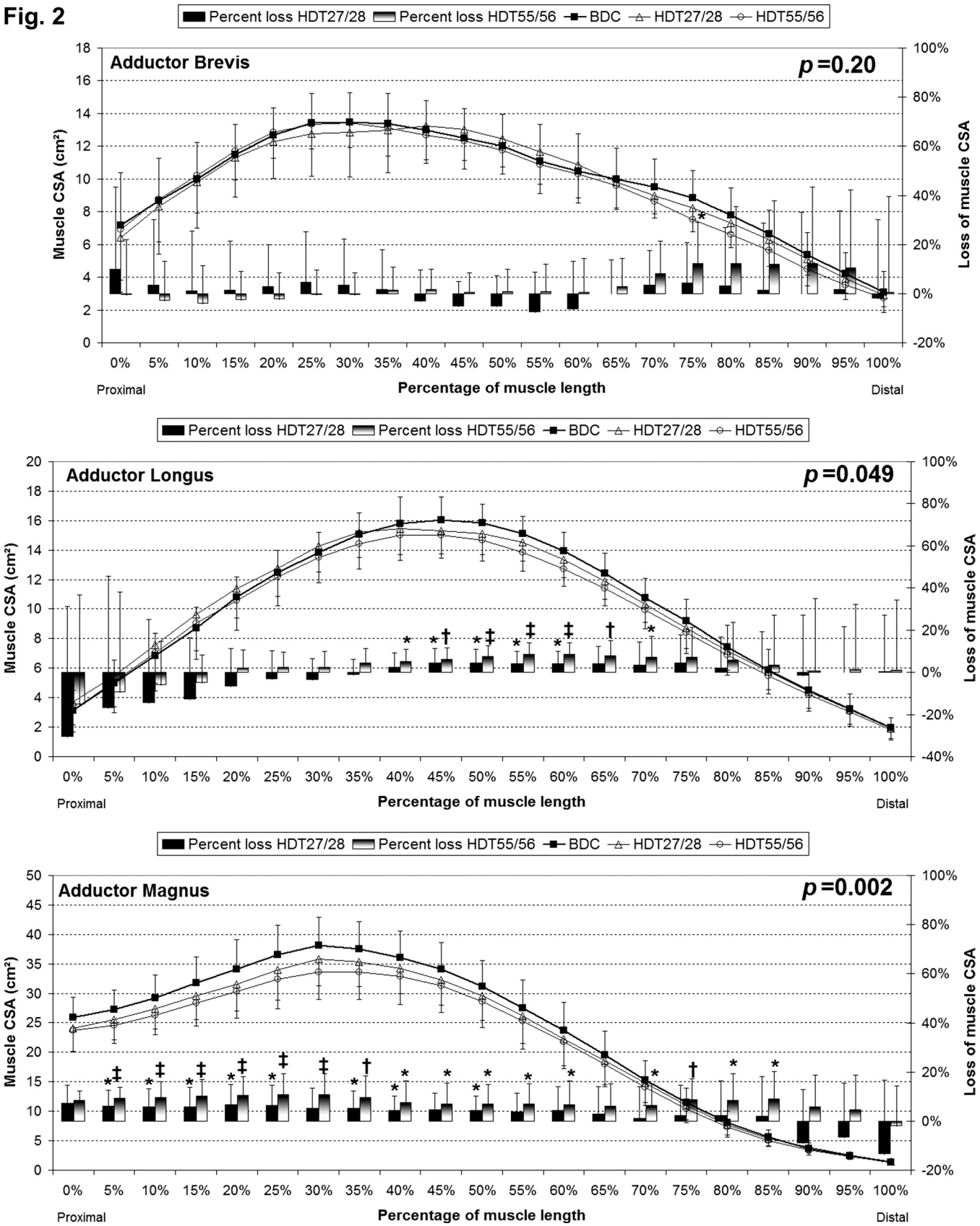
To examine the recovery of the musculature after bed rest, the data on whole muscle volume were used. A study-date effect with a priori contrasts comparing each time point to baseline was evaluated. Linear mixed-effects models were implemented. Similar models were performed to examine changes in muscle length during bed rest. Data on changes in muscle CSA data along muscle length were also evaluated during recovery. Where these findings differed to those provided by volume data, they have been reported.

Adherence to the statistical assumption of normal distribution of residuals was checked visually using quantile-quantile plots. Due to the number of comparisons performed, we considered an α of 0.01 to denote statistical significance, and *P* values equal to 0.01 but less than 0.05 were considered trends. The nlme package was used for linear mixed-effects modeling in the 'R' statistical environment (version 2.10.1, www.r-project.org). Unless otherwise specified, results are presented as means(SD).

RESULTS

All nine subjects completed the examinations up until *day 14* after bed rest. One subject did not return for the 90- and 180-day post-bed rest scanning. Data on muscle length at baseline and muscle volumes before and during bed rest are presented in Table 1. Only in tibialis anterior was a significant ($P = 0.003$) effect for changes in muscle length during bed rest observed. The length of this muscle decreased by 3.1(2.6)% ($P = 0.0002$) at mid-bed rest and 2.3(3.2)% ($P = 0.031$) at end-bed rest.

Fig. 2



Differential atrophy within individual muscles. During the bed rest phase, the results from ANOVA showed that differential atrophy occurred within the vasti, adductor magnus, lateral hamstrings, medial hamstrings, rectus femoris, medial gastrocnemius, lateral gastrocnemius, tibialis posterior, flexor hallucis longus, flexor digitorum longus, peroneals, and tibialis anterior muscles (P all ≤ 0.004). In adductor longus ($P = 0.048$) and sartorius ($P = 0.049$), there was a trend for differential atrophy along the length of the muscles. In adductor brevis ($P = 0.20$), pectineus ($P = 0.51$), gracilis ($P = 0.95$), soleus ($P = 0.59$), and extensor digitorum longus ($P = 0.49$), analysis indicated that, if atrophy occurred, it was even throughout the muscles.

In the thigh, adductor magnus atrophy was most pronounced in the proximal third of the muscle (Fig. 2). In the adductor longus, there was only a trend to suggest differential atrophy occurred within this muscle, with decreases in CSA being concentrated between 40% and 70% of muscle length. In adductor brevis (Fig. 2), gracilis (Fig. 3), and pectineus (Fig. 3), no significant atrophy occurred. For sartorius (Fig. 3), atrophy was greatest between 60 and 95% of muscle length, followed by 15 to 40% of muscle length, but this effect was only a trend on ANOVA. Both the vasti and rectus femoris (Fig. 4) demonstrated more atrophy toward the proximal end of the muscle, with this effect in the vasti being more prominent at mid-bed rest. The lateral hamstrings demonstrated the greatest losses between 15% to 70% of total muscle length (Fig. 5), whereas the medial hamstrings displayed the greatest atrophy in the distal half of the muscle.

In the lower leg, tibialis anterior showed the greatest losses between 10% and 55% of its length and the peroneals demonstrated the greatest CSA losses in the proximal 40% (Fig. 6). In the toe extensors (extensor digitorum longus), some atrophy did occur but did not differ significantly along its length (Fig. 6). Tibialis posterior (Fig. 7) showed consistent atrophy between 5% and 85% of its length. The toe flexors (flexor digitorum longus) displayed the greatest atrophy in the proximal half of the muscle, between 10% to 40% of muscle length, with the flexor of the great toe (flexor hallucis longus), demonstrating atrophy at 25–50% of muscle length and also at the distal end of the muscle. In the calf, atrophy of the medial gastrocnemius (Fig. 8) was greatest between 30% and 100% of its length with a similar pattern of atrophy demonstrated in its lateral counterpart. In the soleus muscle, the extent of atrophy did not differ significantly throughout its volume (Fig. 8).

Correlation analyses. Correlation analyses were performed between the percentage change in muscle CSA during bed rest and the fraction of total muscle area present at each muscle length. When considered across all muscles on all testing days during bed rest, there was only a weak correlation between the region of greatest CSA at baseline and the subsequent extent of muscle atrophy ($r = -0.30$, $P < 0.0001$). This correlation, however, varied from muscle to muscle (Table 1) indicating that some, but not all, muscles tended to atrophy at the region of greatest CSA.

Differential atrophy between muscles. Analysis of exponential decay rates of muscle volume loss during bed rest showed that the muscles of the lower limb atrophied at different rates ($P < 0.0001$; Fig. 9). The fastest rates of atrophy were seen in the posterior calf muscles (specifically gastrocnemius medialis and soleus) followed by the vasti muscles of the thigh, tibialis anterior, hamstrings, and adductor magnus.

The rates of atrophy differed between synergistic muscles. In the adductor muscle group, adductor magnus demonstrated faster atrophy than adductor brevis ($P = 0.0008$) and pectineus ($P = 0.004$), but this did not differ significantly to the rate observed in adductor longus ($P = 0.055$). In the knee extensors, the vasti atrophied faster than its synergist, rectus femoris ($P < 0.0001$). For the plantar flexor muscles, both the soleus and medial gastrocnemius displayed faster rates of atrophy (P both < 0.028) than the lateral head of gastrocnemius. The other postero-lateral muscles of the ankle and foot arch (tibialis posterior, peroneals, flexor hallucis longus, flexor digitorum longus) all atrophied at similar rates. The peroneals, flexor hallucis longus, and tibialis posterior in turn demonstrated a faster rate of atrophy than the tibialis anterior muscle ($P < 0.041$). The other dorsiflexor, extensor digitorum longus, atrophied slower than the tibialis anterior ($P = 0.002$).

Recovery of the musculature after bed rest. Muscle volume recovery after bed rest is presented in Figs. 10 and 11. The volumes of adductor magnus, adductor longus, sartorius, and extensor digitorum longus, which showed losses during bed rest (Table 1), were no longer significantly different to baseline 14 days after bed rest. Tibialis anterior, peroneals, flexor digitorum longus, flexor hallucis longus, tibialis posterior, lateral gastrocnemius, medial gastrocnemius, soleus, vasti, rectus femoris, medial hamstrings, and lateral hamstrings still showed significant volume losses at 14 days after bed rest. The volumes of these muscles were recovered by 90 days after bed rest, with the exception of flexor hallucis longus, which recovered by the final measurement time point 180 days after bed rest.

Only a few differences were seen in the muscle recovery rates when the data from muscle CSA along the entire length of the muscles were examined. In the lateral gastrocnemius, atrophy persisted at 55% to 70% of muscle length 90 days after bed rest (data not shown). Similarly, flexor digitorum longus was still smaller 90 days after bed rest at 25% to 35% of its length (data not shown). Also, atrophy of the adductor longus persisted at 40% to 60% of muscle length 14 days after bed rest (data not shown).

DISCUSSION

The current study found that, during prolonged bed rest, the majority of the lower-limb muscles (12 of a total of 19 muscles) showed differences in the extent of muscle atrophy

Figs. 2 to 8 (following pages). Heterogeneous atrophy within individual muscles. Line plots show muscle cross-sectional area (CSA; left axis), and bar plots show percentage loss in muscle CSA (positive values indicate atrophy; right axis) from the proximal (0%) to distal (100%) ends of each muscle. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ and indicate significance of change in muscle CSA. P value at top right of each panel, obtained from analysis of variance, indicates whether atrophy was nonuniform (i.e., $P < 0.01$), showed a trend to nonuniformity ($0.01 \leq P < 0.05$), or was uniform ($P \geq 0.05$) across the entire muscle. BDC, baseline data collection; HDT, days of head-down tilt position.

Fig. 3

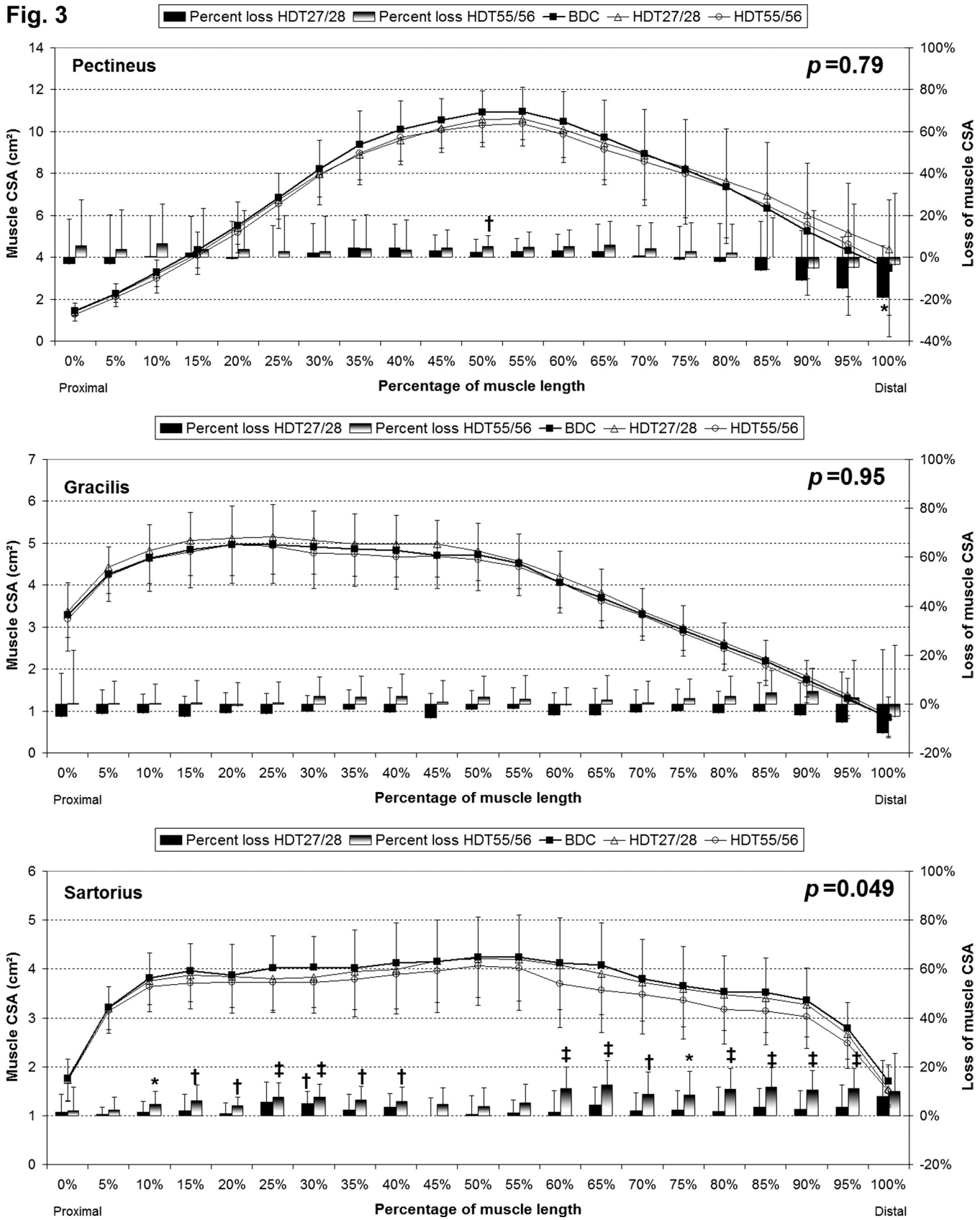
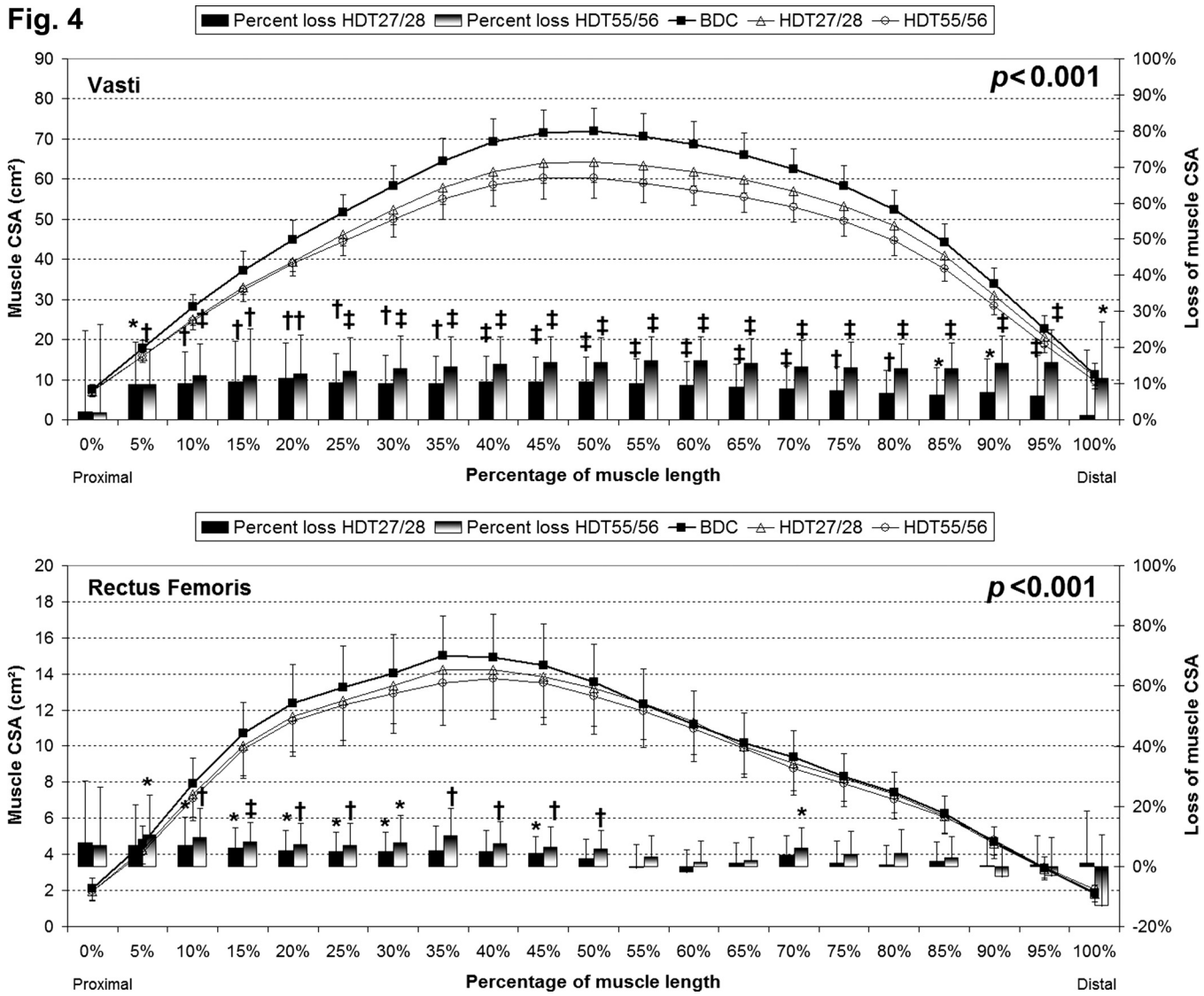


Fig. 4

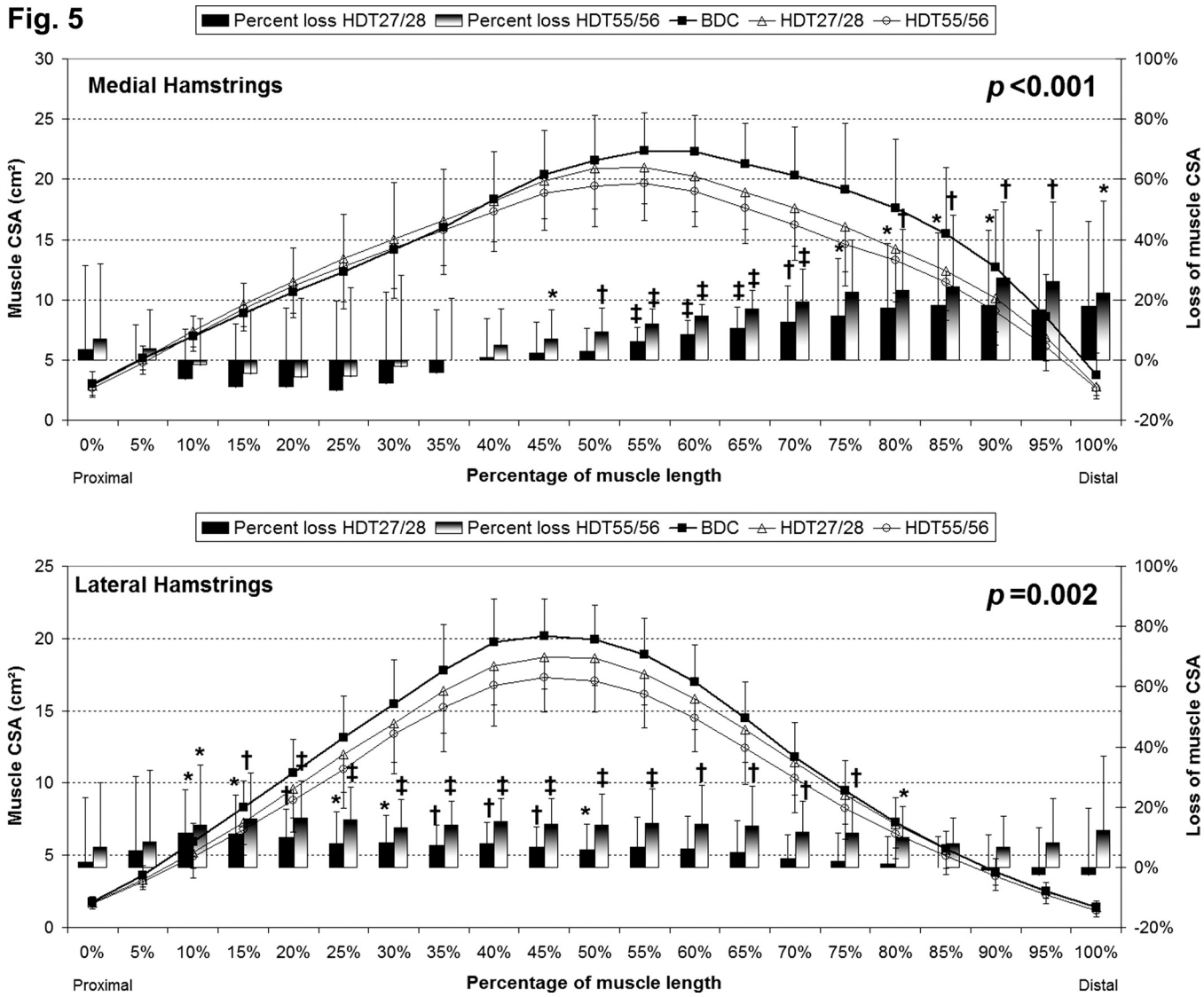


along their length. The pattern of intramuscular atrophy was muscle specific. The correlation analyses showed that muscles do not necessarily atrophy most where muscle bulk is greatest. These data extend the finding that, not only do different muscles atrophy at different rates, but that individual regions of a muscle also respond nonuniformly during prolonged bed rest.

To understand differences in atrophy between different regions of an individual muscle, it is helpful to consider differences between synergistic muscles. In our study, the vasti atrophied more, or faster, than the rectus femoris. In combined knee and hip extension tasks, functional MRI (20, 33, 39) and electromyographic (43) studies have shown greater recruitment of the vasti than rectus femoris. MRI studies of muscle damage after exercise have suggested the vasti are recruited more than the rectus femoris during a sit-to-stand task (38). Based on the existing literature, it is expected that the vasti are much more highly recruited during activities of daily life, such as standing up and walking, than the rectus femoris. Thus, during bed rest, it is probable that the overall daily loading of the vasti is reduced more than the rectus femoris, leading to greater atrophy of this muscle. Differences between muscles in their functions and how their usage changes during bed rest are likely to be responsible for different rates of atrophy in the different muscles we examined (see Ref. 13 for recent review).

Compartmentalization of function within muscles has been described previously (19) and likely plays the most important role for the intramuscular differences in atrophy that we observed within a number of different muscles. For example, the adductor magnus muscle atrophied most in its proximal third. Anatomically, adductor magnus has two parts: an extensor portion, originating at the ischial tuberosity, which is innervated by the sciatic nerve, and an adductor portion, originating from the pubic ramus and innervated by the obturator nerve (8). In lower vertebrates, this extensor portion is actually a separate presemimembranosus muscle grouped with the hamstring muscles (25), whereas, in human embryos, these two sections of the adductor magnus muscle develop separately and later fuse (8).

Fig. 5



In the rectus femoris and vasti, greater atrophy was seen proximally in these muscles. Nonuniform recruitment within the quadriceps during loading tasks has been noted in functional MRI (4) and electromyographic (40) studies and may be an important factor in explaining our findings. A further explanation could be found in data from anatomical studies, which have noted that at the distal half of the rectus femoris there is a separate "muscle within a muscle" (22) and that the blood and nerve supply of this muscle are typically split into distal and proximal portions (44).

In the triceps surae, the soleus atrophied consistently along its entire length; however, both heads of gastrocnemius atrophied more so in their distal parts. Functional MRI (27, 34) and electromyographic (27) studies on the triceps surae have shown intramuscular differences in recruitment of the gastrocnemius medialis but not in the soleus. Another study (42) has also demonstrated regional intramuscular differences in activation of the gastrocnemius lateralis muscle during a knee flexion-extension task in standing. Furthermore, three anatomical compartments of this muscle have been described (35). These anatomically distinct sections of a muscle and regionalization of function within muscles may well lie behind the intramuscular differences in atrophy that we observed in triceps surae.

Similarly, the tibialis anterior has been described as being divided anatomically into two proximal heads, one superficial and one deep, which have longitudinally orientated fibers and a distal head with obliquely oriented fibers (41). This anatomical compartmentalization may play a role in the differences in atrophy that we observed in the tibialis anterior muscle where greatest losses of CSA occurred between 10% and 55% of its length.

Although it should be stressed for the sartorius muscle that the differences between muscle sections in terms of atrophy were not significant, it is nonetheless interesting that atrophy was most prominent in its proximal and distal thirds. That subsections of the sartorius play different functional roles that have been well described in cats (24). Although there are no electromyographic or functional MRI investigations in humans on functional compartmentalization in this muscle, anatomical studies have often sub-

Fig. 6

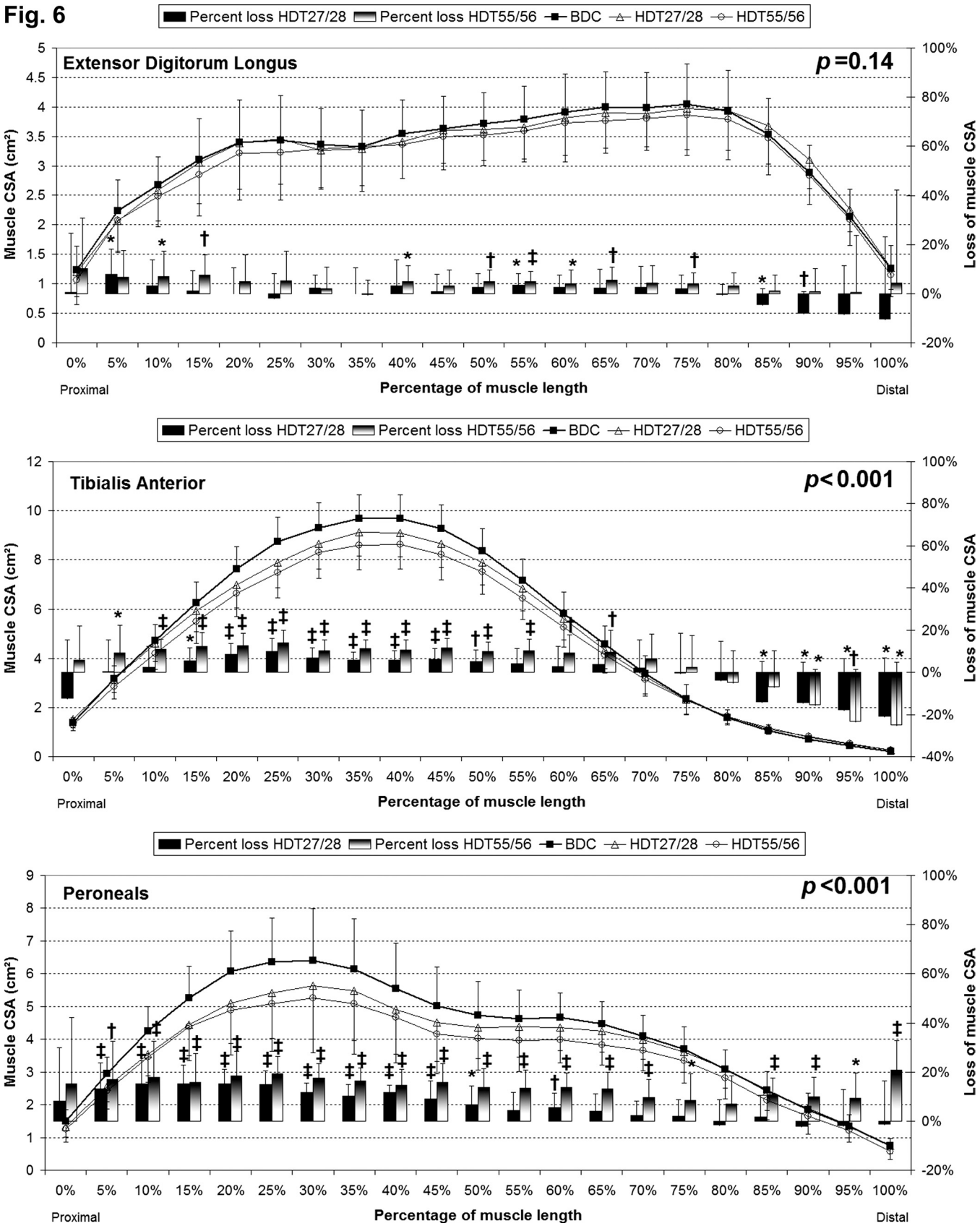


Fig. 7

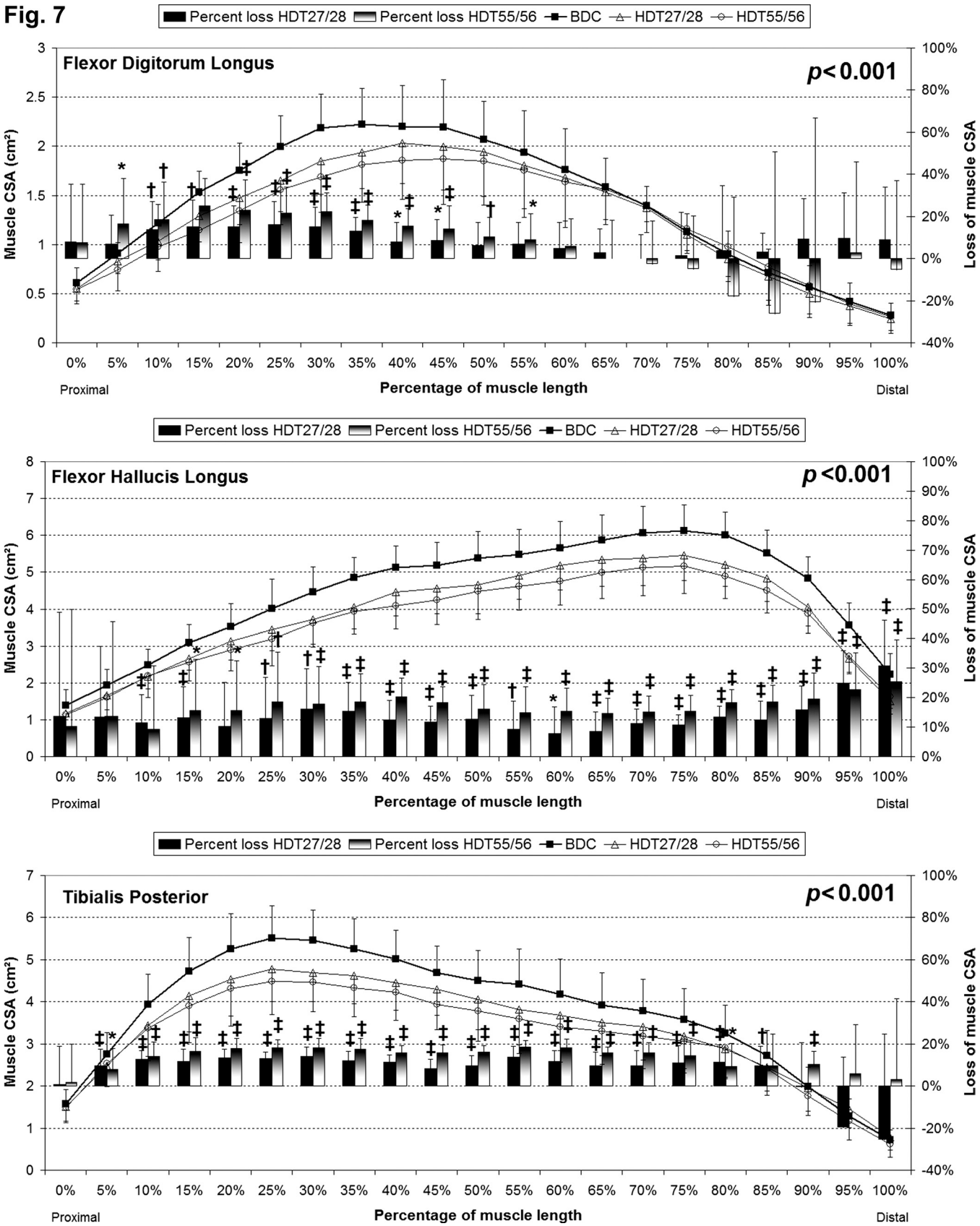
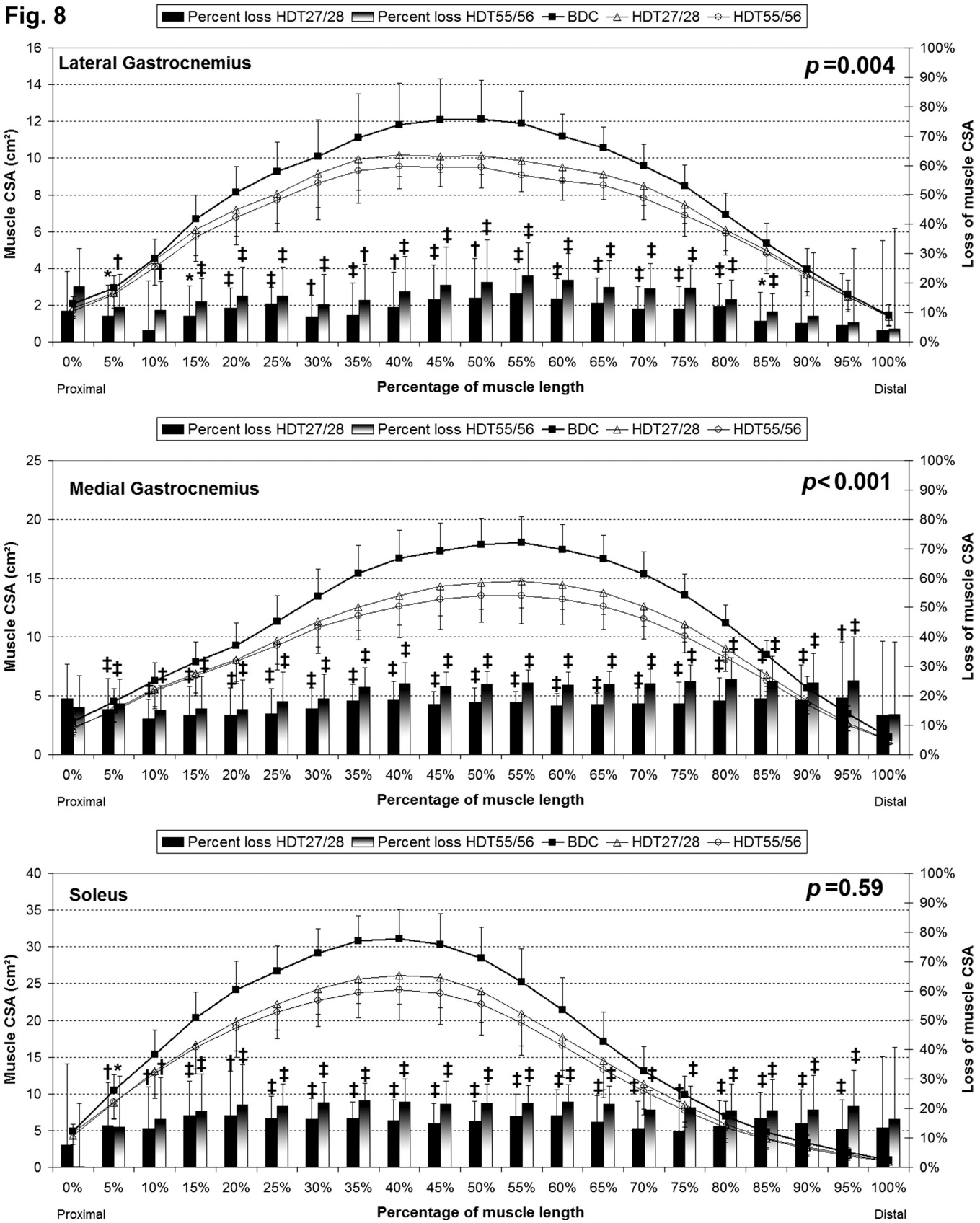


Fig. 8



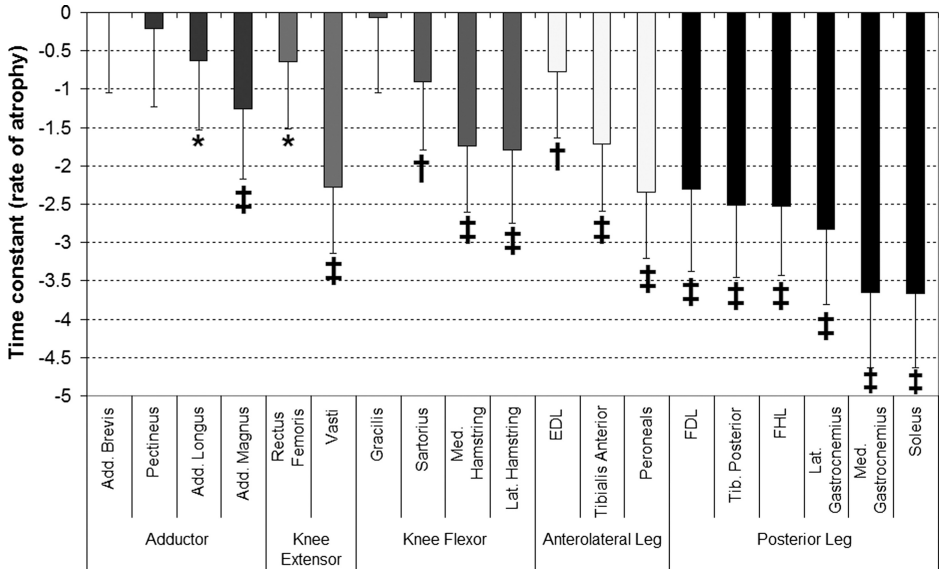


Fig. 9. Differential atrophy between muscles. Values are mean ± SD time constants of exponential decay of muscle volume per week of bed rest. Values have been multiplied by 100 to enable ease of comparison between muscles. A more negative time constant indicates a faster rate of atrophy. $*P < 0.05$; $\dagger P < 0.01$; $\ddagger P < 0.001$ and indicate significance of difference of time constant to 0 (i.e., whether atrophy occurred). Note: for differences in the rates of atrophy between muscles, $P < 0.05$ where a difference of 0.63 or more exists between time constants. EDL, extensor digitorum longus; FDL, flexor digitorum longus; FHL, flexor halluces longus.

divided the blood and nerve supply to this muscle into proximal, middle, and distal portions (15, 45). The majority of its fibers do not extend from origin to insertion as in a number of other muscles, but rather are restricted to one- or two-thirds of its length and are hence placed in series (21).

Hence, our argument in interpreting and explaining our findings is that the function of a muscle, or a subregion of it, in daily life and the consequent change in activity due to bed rest is the most important factor in determining what extent of

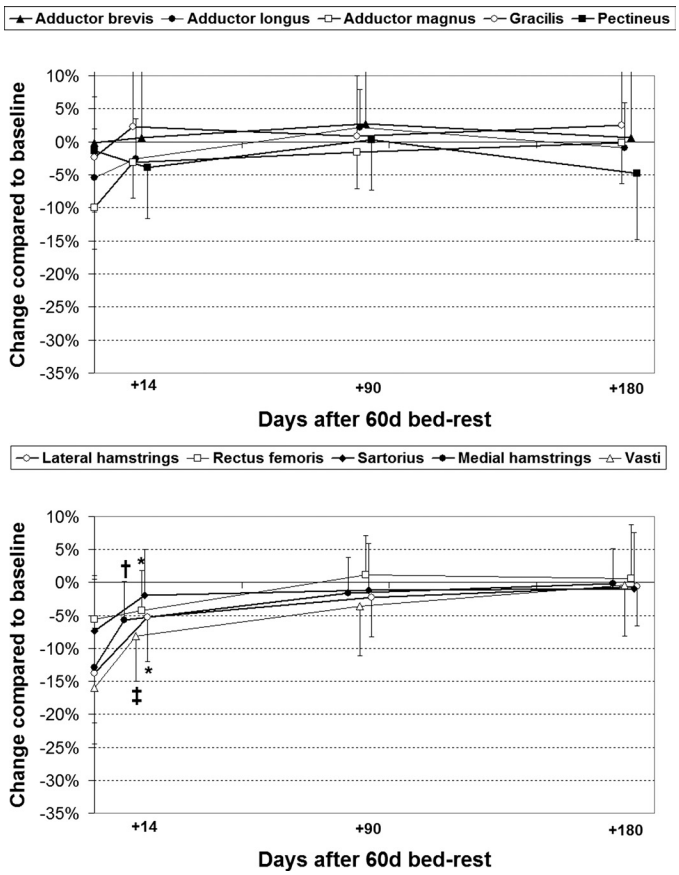


Fig. 10. Recovery of the thigh musculature after bed rest. Values are means ± SD percentage volume difference compared with baseline. $*P < 0.05$; $\dagger P < 0.01$; $\ddagger P < 0.001$ and indicate significance of difference to baseline. The value at end-bed rest is given at 0 on the *x*-axis.

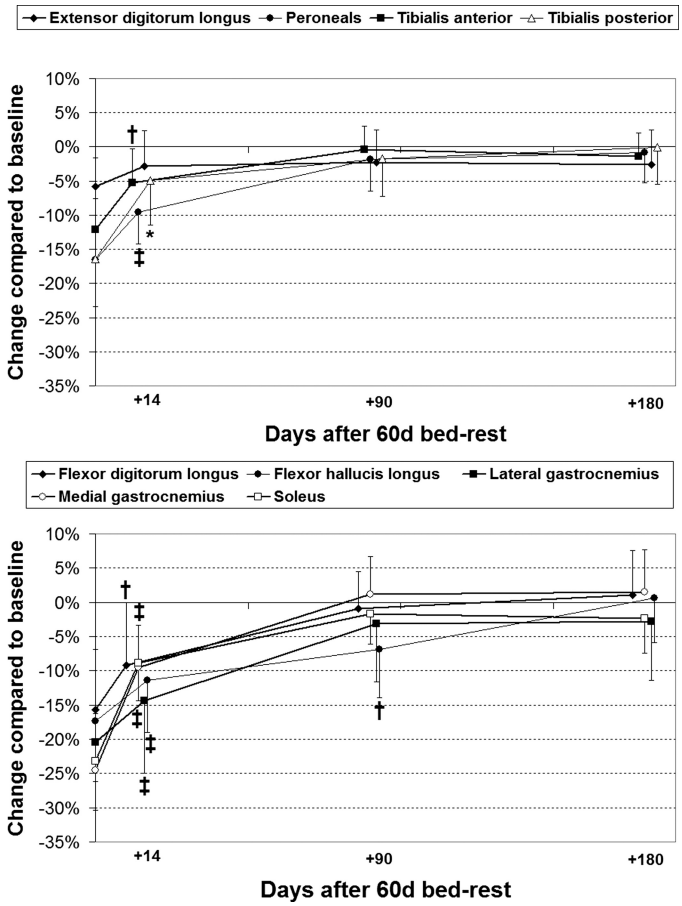


Fig. 11. Recovery of the lower-leg musculature after bed rest. Values are means ± SD percentage volume difference compared with baseline. $*P < 0.05$; $\dagger P < 0.01$; $\ddagger P < 0.001$ and indicate significance of difference to baseline. The value at end-bed rest is given at 0 on the *x*-axis.

muscle atrophy occurs. Quantifying this function during daily life and then correlating the change of function in bed rest with the atrophy that occurs is, of course, a very difficult task. For a number of muscles, we could not find information in the literature on intramuscular compartmentalization, be it functional or anatomical. For those muscles, the current findings on intramuscular atrophy could help spur further work on understanding the function of these muscles.

One might be tempted to try to explain the differences in muscle atrophy based on muscle size, fiber type percentages, pennation angles, muscle spindle innervation patterns, and other variables. We have attempted such correlations in prior work (13) by comparing our results to prior published data, but with limited success. In future work, one could investigate and measure all these variables in the same set of subjects as an attempt to explain the findings of intramuscular differential atrophy. In any case, we caution the reader against examining these other factors to find a "cause". In our opinion, these characteristics of muscles reflect the function of a muscle; the adaptation of muscle architecture in response to hypertrophic overload (37) and unloading (17) underscore this idea.

The current study also gives insight into the recovery of the musculature after prolonged inactivity. The duration of recovery differed between muscles. The process of recovery was largely complete by 3 mo after bed rest and finalized 6 mo after bed rest. Prior data (see discussion section of Ref. 10 for review) had suggested that late in recovery some muscles may end up being larger than before bed rest. These prior data were typically based on individual CSA measurements. In our data, the tibialis anterior muscle became shorter during bed rest, and this was associated with increases in muscle CSA at its distal end. Because this muscle atrophied overall, we speculate that a change in muscle "shape" drove the increases in muscle size at its distal end. In recovery also, a number of muscles shortened, and this was also associated with increases in CSA, typically at the distal end (data not shown). This may help to explain prior findings of greater muscle CSA after in late recovery after bed rest. Overall, the current data show that lower-limb muscle volume returned to pre-bed rest levels within 6 mo after bed rest and did not show any "hypertrophy" after bed rest.

The results of the current study have some clinical relevance. It is likely that individuals who are subject to bed rest as part of medical care or undergo prolonged periods of limb disuse (e.g., extended use of crutches) will show similar patterns of muscle atrophy. The current data help to define which muscles would be most affected in such patients, which will then influence exercise selection during rehabilitation. For example, implementing a leg press exercise, involving hip and knee extension, rather than isolated knee extension or hip adduction will activate muscles such as the vasti and extensor portion of adductor magnus (20) without strongly activating their synergists, such as rectus femoris and adductor longus, which in turn, are not strongly affected by immobilization. Although it should be kept in mind that joint position during immobilization or fixation of individual joints impacts on individual muscle changes (23), patients subject to joint immobilization, such as after bone fracture or joint surgery, could show similar patterns of muscle atrophy.

The current study also has some methodological implications. First, measuring the muscle volume as a whole will miss some information on intramuscular differences in atrophy in most muscles. Conversely, where only a few images are taken at a specific section of a muscle, this will often not perfectly reflect changes in the muscle as a whole or in other sections of the same muscle.

It is important to mention some of the limitations of the current study. As is typical of bed rest studies, the number of subjects was limited due to logistical and financial restraints. Due to the limited number of subjects, some nonsignificant results for intramuscular atrophy may represent false negatives. Conversely, although we reduced our threshold for statistical significance to 0.01, rather than the more typical 0.05, there is still the possibility that some findings may represent false positives. Differences in muscle size correlate to differences in maximum voluntary force (28). It is therefore reasonable to assume that the relative differences between synergists in muscle atrophy observed in the current study may alter the normal balance between individual muscles during force production. However, the presence of such functional differences would need to be evaluated in future work. Also, the large, and typically highly variable, changes at the ends of the muscles should be viewed with some skepticism; due to the small size of the muscle at the distal ends, measurement variability will be comparatively higher. It should be stressed that increases in muscle size at the distal and proximal ends of some muscles may not be of functional relevance and more likely relate to the shortening of the muscle with a subsequent change in muscle shape. Variations from one day to the next in joint positioning in the MRI scanner, with subsequent variability in muscle length and shape, could also have played a role in the large variability of the measurements at the distal ends of the muscle. Also, the measurement of muscle length was based on the number of slices in which the muscle was present. In the future, more precise measures of muscle length could be obtained via MRI images along the length of each muscle. The exponential decay modeling of muscle volume loss in bed rest would have been better performed with three or more measurement time points during bed rest. Due to logistical reasons, we were limited to two scanning sessions per subject during bed rest. The MRI measurements implemented in the current study quantified overall muscle size and could not differentiate between muscle fiber, connective tissue, fat, and fluid within muscles. No doubt, there is not a 1:1 relationship between changes in muscle size and changes in muscle tissue subcomponents. A next research step would be to attempt to separate out these different components in the bed rest model of muscle atrophy.

In conclusion, to the best of our knowledge, the current study is the first to examine the topic of differential atrophy within muscles during bed rest or similar models (spaceflight, lower limb suspension) in such a wide range of lower-limb muscles. We found that intramuscular differences in the extent of atrophy during bed rest occurred in the adductor magnus, vasti, lateral hamstrings, medial hamstrings, rectus femoris, medial gastrocnemius, lateral gastrocnemius, tibialis posterior, flexor hallucis longus, flexor digitorum longus, peroneals, and tibialis anterior muscles. In the adductor longus and sartorius muscles, there was a statistical trend suggesting that different muscle subcompartments atrophied nonuniformly. In contrast, for the soleus, adductor brevis, gracilis, pectineus, and extensor digitorum longus muscles, when atrophy occurred, it was uniform throughout the muscle.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: T.M., G.A., D.F., and D.L.B. performed experiments; T.M. and D.L.B. analyzed data; T.M., G.A., D.F., and D.L.B. interpreted results of experiments; T.M. and D.L.B. prepared figures; T.M. and D.L.B. drafted manuscript; T.M., G.A., D.F., and D.L.B. approved final version of manuscript; G.A. and D.F. edited and revised manuscript; D.F. and D.L.B. conception and design of research.

REFERENCES

1. **Akima H, Kawakami Y, Kubo K, Sekiguchi C, Ohshima H, Miyamoto A, Fukunaga T.** Effect of short-duration spaceflight on thigh and leg muscle volume. *Med Sci Sport Exer* 32: 1743–1747, 2000.
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* 172: 269–278, 2001.
3. **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* 82: 30–38, 2000.
4. **Akima H, Takahashi H, Kuno SY, Katsuta S.** Coactivation pattern in human quadriceps during isokinetic knee-extension by muscle functional MRI. *Eur J Appl Physiol* 91: 7–14, 2004.
5. **Akima H, Ushiyama J, Kubo J, Fukuoka H, Kanehisa H, Fukunaga T.** Effect of unloading on muscle volume with and without resistance training. *Acta Astronaut* 60: 728–736, 2007.
6. **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* 181: 345–357, 2004.
7. **Baecke JA, Burema J, Frijters JE.** A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr* 36: 936–942, 1982.
8. **Bardeen CR.** Development and variation of the nerves and the musculature of the inferior extremity and of the neighboring regions of the trunk in man. *Am J Anat* 6: 259–390, 1906.
9. **Belavý DL, Armbrecht G, Gast U, Richardson CA, Hides JA, Felsenberg D.** Countermeasures against lumbar spine deconditioning in prolonged bed rest: resistive exercise with and without whole-body vibration. *J Appl Physiol* 109: 1801–1811, 2010.
10. **Belavý DL, Bansmann PM, Bohme G, Frings-Meuthen P, Heer M, Rittweger J, Zange J, Felsenberg D.** Changes in intervertebral disc morphology persist 5 months after 21-days bed rest. *J Appl Physiol* 111: 1304–1314, 2011.
11. **Belavý DL, Beller G, Armbrecht G, Perschel FH, Fitzner R, Bock O, Börst H, Degner C, Gast G, Felsenberg D.** Evidence for an additional effect of whole-body vibration above resistive exercise alone in preventing bone loss during prolonged bed rest. *Osteoporos Int* 22: 1581–1591, 2011.
12. **Belavý DL, Bock O, Börst H, Armbrecht G, Gast U, Degner C, Beller G, Heer M, de Haan A, Stegeman DF, Ceretteli P, Blottner D, Rittweger J, Gelfi C, Kornak U, Felsenberg D.** The 2nd Berlin Bedrest Study: protocol and implementation. *J Musculoskelet Neuronal Interact* 10: 207–219, 2010.
13. **Belavý DL, Miokovic T, Armbrecht G, Richardson CA, Rittweger J, Felsenberg D.** Differential atrophy of the lower-limb musculature during prolonged bed rest. *Eur J Appl Physiol* 107: 489–499, 2009.
14. **Booth FW.** Time course of muscular atrophy during immobilization of hindlimbs in rats. *J Appl Physiol* 43: 656–661, 1977.
15. **Buckland A, Pan WR, Dhar S, Edwards G, Rozen WM, Ashton MW, Taylor GI.** Neurovascular anatomy of sartorius muscle flaps: implications for local transposition and facial reanimation. *Plast Reconstr Surg* 123: 44–54, 2009.
16. **Conley MS, Foley JM, Ploutz-Snyder LL, Meyer RA, Dudley GA.** Effect of acute head-down tilt on skeletal muscle cross-sectional area and proton transverse relaxation time. *J Appl Physiol* 81: 1572–1577, 1996.
17. **de Boer MD, Maganaris CN, Seynnes OR, Rennie MJ, Narici MV.** Time course of muscular, neural and tendinous adaptations to 23 day unilateral lower-limb suspension in young men. *J Physiol* 583: 1079–1091, 2007.
18. **Elder GC, Bradbury K, Roberts R.** Variability of fiber type distributions within human muscles. *J Appl Physiol* 53: 1473–1480, 1982.
19. **English AW, Wolf SL, Segal RL.** Compartmentalization of muscles and their motor nuclei: the partitioning hypothesis. *Phys Ther* 73: 857–867, 1993.
20. **Enocson AG, Berg HE, Vargas R, Jenner G, Tesch PA.** Signal intensity of MR-images of thigh muscles following acute open- and closed chain kinetic knee extensor exercise - index of muscle use. *Eur J Appl Physiol* 94: 357–363, 2005.
21. **Harris AJ, Duxson MJ, Butler JE, Hodges PW, Taylor JL, Gandevia SC.** Muscle fiber and motor unit behavior in the longest human skeletal muscle. *J Neurosci* 25: 8528–8533, 2005.
22. **Hasselman CT, Best TM, Hughes Ct Martinez S, Garrett WE Jr.** An explanation for various rectus femoris strain injuries using previously undescribed muscle architecture. *Am J Sports Med* 23: 493–499, 1995.
23. **Hnik P, Vejsada R, Goldspink DF, Kasicki S, Krekule I.** Quantitative evaluation of electromyogram activity in rat extensor and flexor muscles immobilized at different lengths. *Exp Neurol* 88: 515–528, 1985.
24. **Hoffer JA, Loeb GE, Sugano N, Marks WB, O'Donovan MJ, Pratt CA.** Cat hindlimb motoneurons during locomotion. III. Functional segregation in sartorius. *J Neurophysiol* 57: 554–562, 1987.
25. **Howell AB.** Morphogenesis of the architecture of hip and thigh. *J Morphol* 62: 177–218, 1938.
26. **Johnson MA, Polgar J, Weightman D, Appleton D.** Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. *J Neurol Sci* 18: 111–129, 1973.
27. **Kinugasa R, Kawakami Y, Fukunaga T.** Muscle activation and its distribution within human triceps surae muscles. *J Appl Physiol* 99: 1149–1156, 2005.
28. **Maughan RJ, Watson JS, Weir J.** Strength and cross-sectional area of human skeletal muscle. *J Physiol* 338: 37–49, 1983.
29. **Miokovic T, Armbrecht G, Felsenberg D, Belavý DL.** Differential atrophy of the postero-lateral hip musculature during prolonged bed rest and the influence of exercise countermeasures. *J Appl Physiol* 110: 926–934, 2011.
30. **Mulder ER, Horstman AM, Stegeman DF, de Haan A, Belavy DL, Miokovic T, Armbrecht G, Felsenberg D, Gerrits KH.** Influence of vibration resistance training on knee extensor and plantar flexor size, strength, and contractile speed characteristics after 60 days of bed rest. *J Appl Physiol* 107: 1789–1798, 2009.
31. **Narici MV, Hoppeler H, Kayser B, Landoni L, Claassen H, Gavardi C, Conti M, Cerretelli P.** Human quadriceps cross-sectional area, torque and neural activation during 6 months strength training. *Acta Physiol Scand* 157: 175–186, 1996.
32. **Pinheiro JC, Bates DM.** *Mixed-Effects Models in S and S-PLUS*. Berlin, Germany: Springer, 2000.
33. **Richardson RS, Frank LR, Haseler LJ.** Dynamic knee-extensor and cycle exercise: functional MRI of muscular activity. *Int J Sports Med* 19: 182–187, 1998.
34. **Segal RL, Song AW.** Nonuniform activity of human calf muscles during an exercise task. *Arch Phys Med Rehabil* 86: 2013–2017, 2005.
35. **Segal RL, Wolf SL, DeCamp MJ, Chopp MT, English AW.** Anatomical partitioning of three multiarticular human muscles. *Acta Anat (Basel)* 142: 261–266, 1991.
36. **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* 97: 119–129, 2004.
37. **Suetta C, Andersen JL, Dalgas U, Berget J, Koskinen S, Aagaard P, Magnusson SP, Kjaer M.** Resistance training induces qualitative changes in muscle morphology, muscle architecture, and muscle function in elderly postoperative patients. *J Appl Physiol* 105: 180–186, 2008.
38. **Takahashi H, Kuno S, Miyamoto T, Yoshioka H, Inaki M, Akima H, Katsuta S, Anno I, Itai Y.** Changes in magnetic resonance images in

human skeletal muscle after eccentric exercise. *Eur J Appl Physiol Occup Physiol* 69: 408–413, 1994.
39. **Tesch PA.** *Target Bodybuilding*. Champaign, IL: Human Kinetics Publishers, 1999.
40. **Watanabe K, Kouzaki M, Moritani T.** Task-dependent spatial distribution of neural activation pattern in human rectus femoris muscle. *J Electromyogr Kinesiol* 22: 251–258, 2012.
41. **Wolf SL, Kim JH.** Morphological analysis of the human tibialis anterior and medial gastrocnemius muscles. *Acta Anat (Basel)* 158: 287–295, 1997.
42. **Wolf SL, Segal RL, English AW.** Task-oriented EMG activity recorded from partitions in human lateral gastrocnemius muscle. *J Electromyogr Kinesiol* 3: 87–94, 1993.
43. **Yamashita N.** EMG activities in mono- and bi-articular thigh muscles in combined hip and knee extension. *Eur J Appl Physiol* 58: 274–277, 1988.
44. **Yang D, Morris SF.** Neurovascular anatomy of the rectus femoris muscle related to functioning muscle transfer. *Plast Reconstr Surg* 104: 102–106, 1999.
45. **Yang D, Morris SF, Sigurdson L.** The sartorius muscle: anatomic considerations for reconstructive surgeons. *Surg Radiol Anat* 20: 307–310, 1998.
46. **Zange J, Mester J, Heer M, Kluge G, Liphardt AM.** 20-Hz whole body vibration training fails to counteract the decrease in leg muscle volume caused by 14 days of 6 degrees head down tilt bed rest. *Eur J Appl Physiol* 105: 271–277, 2008.

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Differential atrophy of the postero-lateral hip musculature during prolonged bedrest and the influence of exercise countermeasures

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Miokovic T, Armbrecht G, Felsenberg D, Belavý DL. Differential atrophy of the postero-lateral hip musculature during prolonged bedrest and the influence of exercise countermeasures. *J Appl Physiol* 110: 926–934, 2011. First published January 13, 2011; doi:10.1152/japplphysiol.01105.2010.—As part of the 2nd Berlin BedRest Study (BBR2-2), we investigated the pattern of muscle atrophy of the postero-lateral hip and hamstring musculature during prolonged inactivity and the effectiveness of two exercise countermeasures. Twenty-four male subjects underwent 60 days of head-down tilt bedrest and were assigned to an inactive control (CTR), resistive vibration exercise (RVE), or resistive exercise alone (RE) group. Magnetic resonance imaging (MRI) of the hip and thigh was taken before, during, and at end of bedrest. Volume of posterolateral hip and hamstring musculature was calculated, and the rate of muscle atrophy and the effect of countermeasure exercises were examined. After 60 days of bedrest, the CTR group showed differential rates of muscle volume loss ($F = 21.44$; $P \leq 0.0001$) with fastest losses seen in the semi-membranosus, quadratus femoris and biceps femoris long head followed by the gluteal and remaining hamstring musculature. Whole body vibration did not appear to have an additional effect above resistive exercise in preserving muscle volume. RE and RVE prevented and/or reduced muscle atrophy of the gluteal, semimembranosus, and biceps femoris long head muscles. Some muscle volumes in the countermeasure groups displayed faster recovery times than the CTR group. Differential atrophy occurred in the postero-lateral hip musculature following a prolonged period of unloading. Short-duration high-load resistive exercise during bedrest reduced muscle atrophy in the mono-articular hip extensors and selected hamstring muscles. Future countermeasure design should consider including isolated resistive hamstring curls to target this muscle group and reduce the potential for development of muscle imbalances.

muscle atrophy; magnetic resonance imaging; microgravity; whole body vibration

THE DEVELOPMENT OF APPROPRIATE countermeasure exercises to combat the effects of human spaceflight on the musculoskeletal system (21, 31, 33) continues to be a prime consideration for space agencies worldwide. Bedrest is a common model used to simulate the effects of space travel on the human body (40); however, it is also a good model to understand changes that occur in the muscles during prolonged levels of reduced physical activity (16).

Although a number of studies have examined the effects of bedrest on the lower limb and lumbar spine musculature (1–3, 9, 12, 30, 31, 44, 50, 57), limited information is known regarding the effects of prolonged inactivity on the muscles of the hip. The hip musculature is considered to play an integral role in control and stabilization of the hip joint during locomotion and in facilitation of upright stance (34–36). Furthermore, few works have examined the time course of recovery in the musculature after bedrest. An understanding of the recovery process of the muscles is also essential to help guide rehabilitation protocols. Thus the first aim of this work is to investigate the effect of bedrest and subsequent recovery of the muscles of the hip region.

Previous studies examining the effects of spaceflight and bedrest on the trunk and lower limb musculature have demonstrated that not all muscles within a region are affected to the same extent, with the degree of muscle atrophy found to differ between muscle synergists. During unloading, greater atrophy has been reported in the vastii muscles compared with their synergist, rectus femoris (1, 3, 58), with varying degrees of atrophy also documented in the three heads of triceps surae, soleus, and gastrocnemius medials and lateralis (12). Ground-based unloading studies have found decreases in muscle force and power to exceed reductions in muscle size in the hip (14), knee (14, 39, 52, 58), and calf extensors (30). If similar changes were to occur in the postero-lateral hip musculature, this may create muscle force imbalances in this region. Muscle force imbalances have been reported to be associated with increased risk of pain and/or injury in the hip (41), back (47), and lower limb (15, 22). Thus another aim of this study was to examine differential atrophy among synergists of the postero-lateral hip-hamstring muscle complex.

A further consideration is the development of appropriate countermeasure exercises against such changes at the hip. Thus far, development for countermeasures against unloading has focused largely on the lower limb muscles with little information available on the hip musculature. High load resistive countermeasure exercises have been shown to be effective at reducing/preventing muscle atrophy in the lower limb during bedrest (1–4, 13, 49, 52, 53, 58); however, effects have been inconsistent and often limited to a select number of muscles. Therefore, the second aim of this work is to evaluate the effectiveness of two different countermeasure exercises, resistive exercise and resistive exercise with the addition of whole body vibration, in protecting the hip musculature against atrophy during prolonged inactivity.

Resistive whole body vibration (WBV) is thought to provide additional stimulus to muscles during training with reported effects including increased muscle strength, power, and flexibility (17, 19, 48, 50). It has been proposed that vibration applied directly to muscle or tendon facilitates muscle contraction via the excitation of primary and secondary muscle spindle endings (18) and leads to a greater recruitment of motoneuron pools and subsequent increased muscle contraction via the tonic vibration reflex (48). WBV applied at the feet is transmitted to the lower limbs during loading (51); hence, it is possible that resistive WBV could be more effective in reduc-

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ing muscle atrophy than resistive exercise alone. Since no studies to date have investigated this theory on the muscles of the hip, we wish to examine this as part of our countermeasure exercise protocol.

This study aimed to investigate: *1*) the pattern of muscle atrophy that occurs in the postero-lateral muscles of the hip during 60 days of prolonged bedrest, in addition to the rate of muscle recovery up to 6 mo post-bedrest and *2*) the effectiveness of two exercise countermeasures, resistive exercise and resistive vibration exercise, in protecting against muscle atrophy at the hip.

METHODS

Subjects and bedrest protocol. Twenty-four medically and psychologically healthy males were recruited for the 2nd Berlin Bedrest Study (BBR2-2), conducted at the Charité Campus Benjamin Franklin in Berlin, Germany, by the Centre for Muscle and Bone Research. The protocol of the BBR2-2 has been described in detail elsewhere (11). In brief, however, subjects underwent 60 days of 6° head-down tilt bedrest (HDT), were required to perform all activities, including hygiene, in the head-down tilt position, and were discouraged from moving excessively or unnecessarily. Twenty-four hour nursing care and video surveillance permitted monitoring of subjects' activities. The Charité Universitätsmedizin Berlin ethics committee approved this study. Subjects gave their informed, written consent before participating in the study and were aware that they were permitted to withdraw from the study at any time.

Subjects were randomized to three different groups: one that performed resistive exercises with whole body vibration during bedrest (RVE; $n = 7$), one that performed resistive exercise only (RE; $n = 8$), and one that performed no exercise and served as a control group (CTR; $n = 9$). For medical reasons unrelated to the current study, one RE subject dropped out of the study after HDT30 (*day 30* of bedrest). At R+90 (*day 90* of recovery) and R+180 (*day 180* of recovery), one RE and one CTR subject did not attend scanning.

The sample size estimates of the BBR2-2 were based on bone parameters [specifically distal tibia bone mineral content (10)] and were not powered to the muscle volume measurements considered in the present investigations. Since there is limited data on the hip musculature with countermeasures in bedrest in similar detail as in the present study, it was difficult to conduct a sensitivity analysis for this study. Thus we consider the present work to be an exploratory study for the comparison between RE and RVE. For all comparisons, particularly between the RVE and RE groups, where no significant differences are found, the findings should be considered in light of the fact that a meaningful effect size of vibration may not be able to be detected for the hip musculature given the present study design.

Exercise countermeasures. Details of the countermeasure exercise programme have been published in detail elsewhere (11). In brief, however, exercise maneuvers were chosen to target those load-bearing regions of the body that are most affected by bedrest (i.e., lower-quadrant and lumbar region). Both countermeasure groups performed their exercise on the Galileo Space exercise device (Novotec Medical, Pforzheim, Germany; Fig. 1), with the device switched off for the resistive exercise only group. Training was performed 3 days/wk during the HDT phase. After a short warm-up, the following exercises were performed: bilateral squats (~75–80% of pre-bedrest maximum voluntary contraction; in RVE group vibration frequency 24 Hz, amplitude 3.5–4 mm, peak acceleration ~8.7 g where $g = 9.81$ ms^{-2}), single leg heel raises (~1.3 times body weight; in RVE group vibration frequency 26 Hz, amplitude 3.5–4 mm, peak acceleration ~10.2 g), double leg heel raises (~1.8 times body weight; in RVE group vibration frequency 26 Hz, amplitude 3.5–4 mm, peak acceleration ~10.2 g), back and heel raises (performing hip and lumbar spine extension against gravity with ankle dorsiflexion, with ~1.5 times body weight applied at the shoulders; in RVE group vibration frequency 16 Hz, amplitude 3.5–4 mm, acceleration ~3.9 g). The RVE group performed the same exercises as the RE group except that whole body vibration was applied. Note that the acceleration parameters stated refer to the acceleration of the platform itself; effective accelerations on the subject are much lower. The maximum resulting ground reaction forces transmitted to the feet of the subjects result in effective acceleration at the feet in the order of 0.7 g.

Fig. 1. Countermeasure exercise. This figure depicts a training subject performing a bilateral squat. Bilateral squats were included in the countermeasure exercise program to target the hip musculature, in particular the gluteal and hamstring muscles, which contract concentrically during the ascension phase of the squat. Other exercises included single and bilateral heel raises and back extension maneuvers. Both the resistance exercise only (RE) and resistance exercise with whole body vibration (RVE) groups performed their exercises on the Galileo Space exercise device (Novotec Medical, Pforzheim, Germany), with the RE group performing their exercises without vibration. Subjects were positioned in head-down tilt on a moveable board with their feet placed on either side of the Galileo platform. Shoulder pads and hand grips ensured subjects remained in the desired position and enabled application of force via the vibrating platform. A pneumatic system generated the force, applied through the moveable board, against which the subject was required to resist and move (via the shoulder pads and hand grips). A sport scientist supervised all exercises to ensure correct performance, and a monitor positioned in the subjects field of view provided visual feedback regarding exercise motion and speed and the subjects actual and target exercise position.

Magnetic resonance imaging protocol. Baseline magnetic resonance (MR) scanning was conducted 8 or 9 days before bed rest (BDC −8/9), at mid-bedrest (BR27/28), and at end bedrest (HDT55/56). Post-bedrest scans were conducted on *days 14*, *90*, and *180* of recovery (R+14, R+90, R+180). Before baseline and post-bedrest MRI scanning, subjects remained horizontal, lying for at least 2 h to allow time for shift of body fluids from the extremities (20). During the head-down tilt bedrest phase, beds were placed in the horizontal position 2 h before MR scanning to ensure comparability to pre- and post-head down tilt data.

Subjects were positioned on the scanning bed supine with their knees and hips supported in slight flexion by a pillow under the knee. Sandbags were used to support their legs in neutral rotation (kneecaps oriented to the ceiling and first metatarsal of the foot oriented vertically). Using a 1.5-T Avanto MR system (Siemens, Erlangen, Germany), transverse MR images were acquired from the iliac crest to the inferior-most portion of the gluteus maximus with a second sequence overlapping the gluteus maximus and extending to the knee joint line (slice thickness = 6 mm; interslice distance = 0.6 mm, TR = 8,000 ms, TE = 36 ms, FA = 150°, field of view: 400 × 294 mm interpolated to 320 × 224 pixels). Typically, 39–43 images from

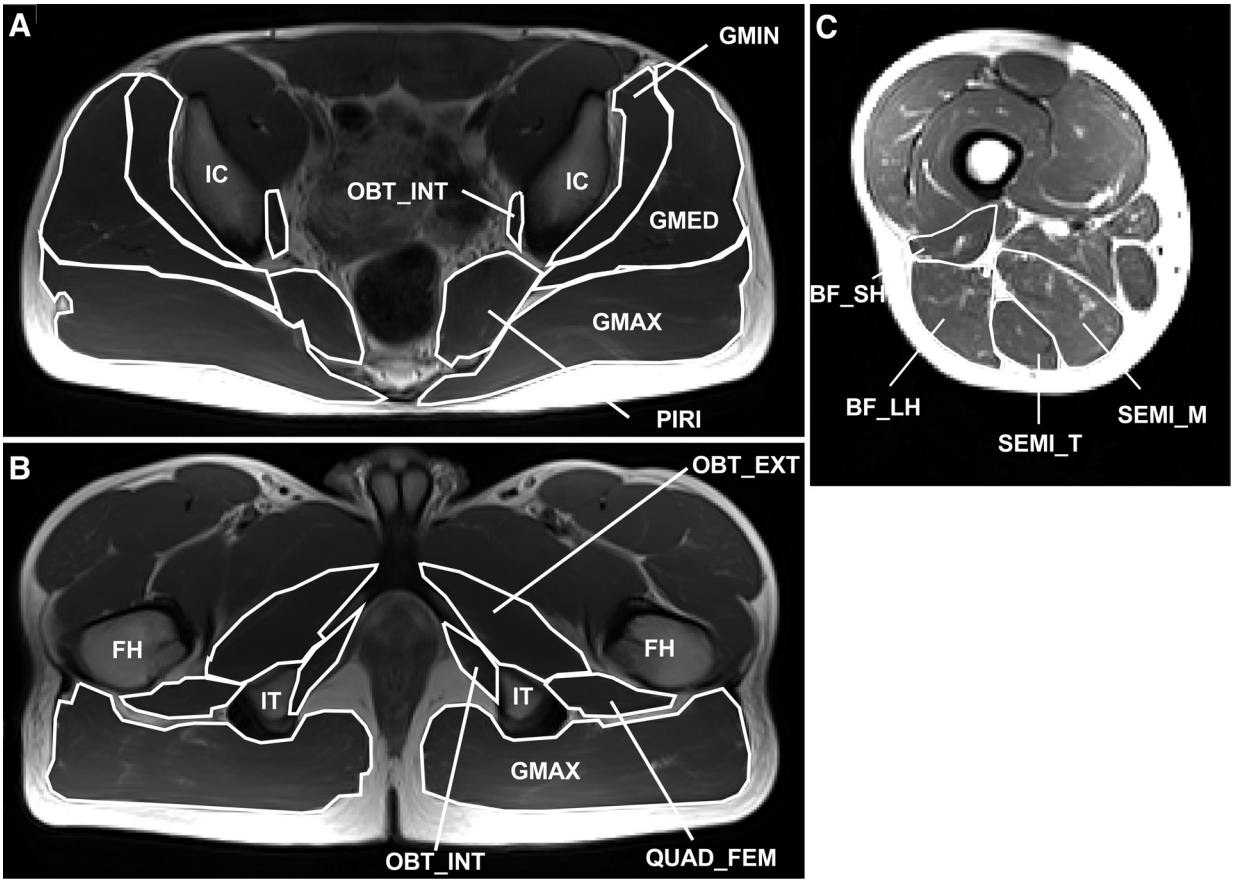

Fig. 2. Hip and thigh muscle image measurements. Example images from the proximal hip (*A*), distal hip (*B*) and mid thigh (*C*). Thirty-four to 39 images were acquired from the iliac crest to the inferior-most portion of the gluteus maximus for the hip musculature. A second sequence of 63–69 images were acquired overlapping the inferior-most portion of the gluteus maximus and extending to the knee joint line to capture the hamstring musculature. The cross-sectional area (when present) of the muscles: upper gluteus maximus (UGM), lower gluteus maximus (LGM), gluteus medius (GMED), gluteus minimus (GMIN), obturator internus (OBT_INT), obturator externus (OBT_EXT), quadratus femoris (QUAF_FEM), piriformis (PIRI), biceps femoris short head (BF_SH), biceps femoris long head (BF_LH), semi-membranosus (SEMI_M), and semi-tendinosus (SEMI_ T) were measured in each image. Note the bony landmarks: iliac crest (IC), femoral head (FH), and ischial tuberosity (IT).

the hip and 63–69 images from the thigh were obtained during scanning, although for taller subjects additional images were added to ensure the region of interest was captured. Images were stored for offline analysis.

Image measurements. One operator (TM) performed all image measurements. To ensure operator blinding to study time point and subject group, each data set was assigned a random numeric code (http://www.random.org). ImageJ (version 1.38x, http://rsb.info.nih.gov/ij/) was used for MR image analysis. Cross-sectional areas (CSA) of the following muscles in the hip (Fig. 2) were measured in each image for both the left and right sides: upper gluteus maximus (UGM), lower gluteus maximus (LGM), gluteus medius (GMED), gluteus minimus (GMIN), obturator internus (OBT_INT), obturator externus (OBT_EXT), quadratus femoris (QUAD_FEM), piriformis (PIRI), semi-membranosus (SEMI_M), semi-tendinosus (SEMI_T), biceps femoris long head (BF_LH), and biceps femoris short head (BF_SH). The gluteus maximus was divided into upper and lower portions at the point of greatest CSA of the femoral head (28), since these have been shown to have functional differences (8, 28, 36, 59). Due to difficulties in defining muscle borders, the superior and inferior gemelli muscles were grouped together with the obturator internus. These muscles have often been proposed to have a common embryological origin (8), function, and anatomy (55).

Further data processing and statistical analyses. To efficiently check for any errors in image measurement, another operator (D. L. Belavý), also blinded to study time point and subject group, plotted data from each muscle for every scanning date for a particular subject. Changes in muscle volume compared with muscle volume at baseline and relative amount (percentage) of muscle atrophy were calculated. Muscle volume was calculated by interpolating muscle cross-sectional areas.

Statistical analysis focused on two aspects: *1*) the effect of bedrest in the inactive control group only and *2*) the impact of countermeasures on muscle changes in the bedrest and recovery phases. To examine the effect of inactivity alone, the following process was used.

1) Using raw muscle volume, linear-mixed effects models (44), with subsequent ANOVA, were constructed with a main effect of "study date" to evaluate changes within each muscle in the CTR group subjects over all measurement dates.

2) Subsequently, to facilitate comparisons between muscles and to determine which muscles atrophied the most/fastest in the CTR group, the fractional changes in muscle volume at HDT27/28 and HDT55/56 compared with baseline were calculated. Nonlinear mixed effects models (44) were then used to compute an exponential decay model $e^{[k\cdot(\text{HDT week})]}$, where k is the time constant for muscle volume loss and HDT week is the week of head-down tilt bedrest. In this model, a more negative k value indicates a faster rate of muscle volume loss. (Similar results were achieved when linear models were used, but since it is to be expected that muscle atrophy occurs in a nonlinear fashion, the use of an exponential decay model was more appropriate.)

To examine the effect of countermeasures, the following process was used.

3) Using raw muscle volume data, the bedrest (HDT27/28, HDT55/56 vs. BDC) and recovery (R+14, R+90, R+180 vs. HDT55/56) phases were examined separately. Linear-mixed effects models, with subsequent ANOVA, were built with main effects of study date and group with their two-way interaction.

4) If the group × studydate interaction from this first model showed a P value of <0.05, a further model, considering only the RVE and RE groups, was constructed in a similar fashion to determine whether there was a difference in response between the two countermeasure groups.

For all statistical models, random effects for each subject and side-of-body within subject and, where necessary, allowances for heterogeneity of variance (such as due to group or study date) were modeled. Due to the number of muscles examined and number of comparisons performed, an alpha level of 0.01 was used for statistical significance for the "study date" main effect, for "group × study date" interaction, and for between-muscle comparisons on ANOVA; P values for these model terms being <0.05 but >0.01 were considered trends. For analysis of changes during and after bedrest, since multiple measurement sessions were undertaken on the same subjects, a Bonferroni adjustment was not performed; rather we looked for consistent "significant" differences across time points. Subject age, height, and weight had little influence on the findings if they were incorporated in the models as linear covariates and were hence excluded from the analyses presented here. Where raw muscle volume data was used in statistical analyses, using percentage change in muscle volume data yielded similar results; however, these additional analyses are not shown. The R statistical environment (version 2.10.1, http://www.r-project.org) was used for all analyses.

RESULTS

In addition to the RE subject that dropped out after the HDT27/28 measurement, two further subjects, one from the RE and one from the CTR group, did not return for the R+90 and R+180 data collections. No differences existed in baseline muscle volume for any muscles between groups ($F \leq 2.2$ for all; $P \geq 0.14$ for all).

Effect of bedrest on muscle volume (CTR group only). Tables 1–3 represent the percentage changes in muscle volume across all groups during the course of the study. With the exception of obturator externus (study date: $F = 2.0$, $P = 0.086$), piriformis (study date: $F = 2.3$, $P = 0.054$) and obturator internus (study date: $F = 3.1$, $P = 0.014$), analysis of CTR group only data showed strong effects for changes in volume across the muscles examined over the course of the study (study date: $F \geq 3.8$ for all; $P \leq 0.004$ for all).

At the end of bedrest, the greatest losses in muscle volume were observed in the quadratus femoris (−18.1% at HDT 55/56: $t \geq -2.4$; $P \leq 0.02$), semi-membranosus (−18.1% at HDT 55/56: $t \geq -5.7$; $P \leq 0.0001$), and biceps femoris long head (−17.7% at HDT 55/56: $t \geq -4.9$; $P \leq 0.0001$ for both). The following muscles also demonstrated significant losses in muscle volume ($P \leq 0.0090$ for all): gluteus minimus (−11.2%), upper gluteus maximus (−9.9%), lower gluteus maximus (−5.7%), semi-tendinosus (−5.2%), and gluteus medius (−3.7%). Nonsignificant changes in muscle volume were observed in the biceps femoris short head, obturator internus, obturator externus, and piriformis during bedrest ($P \geq 0.13$ for all).

To facilitate easier comparison between muscles, the rates of muscle volume change during bedrest were calculated (see Fig. 3). ANOVA showed that rates of muscle atrophy differed between muscles ($F = 20.1$; $P < 0.0001$). Table 4 displays data comparing the rates of muscle atrophy between muscles. Interestingly, this data reveals that a number of synergistic muscles atrophies at significantly different rates. From the hamstring muscle group, semi-membranosus atrophied at a significantly faster rate than its synergists, biceps femoris short head, and semi-tendinosus ($P < 0.0001$ for both), with the latter demonstrating a faster rate of atrophy than biceps femoris short head ($P < 0.0001$). Within the gluteal muscle group, gluteus minimus atrophied at a faster rate than both gluteus medius and lower gluteus maximus ($P < 0.007$), whereas

Table 1. *Hamstring musculature: percentage change in muscle volume during bedrest and recovery in CTR, RE, and RVE groups*

Group	Day of Bedrest/Recovery					
	BDC	HDT27/28	HDT55/56	R + 14	R + 90	R + 180
Biceps femoris long head						
CTR	235.1 (26.2)	−9.6 (5.9)%‡	−17.7 (5.8)%‡	−9.1 (6.8)%‡	−3.1 (5.7)%	−1.1 (5.6)%
RE	245.6 (54.3)	−9.3 (4.0)%‡	−13.1 (3.9)%‡	−5.3 (4.9)%†	4.1 (5.3)%	1.8 (4.8)%
RVE	209.9 (24.0)	−10.2 (5.9)%‡	−13.6 (5.4)%‡	−3.2 (5.5)%	4.4 (5.7)%*	2.4 (5.8)%
Biceps femoris short head						
CTR	140.1 (29.7)	0.4 (10.1)%	−6.5 (11.2)%	3.2 (10.2)%	4.4 (5.7)%*	1.2 (9.8)%
RE	134.8 (29.9)	0.6 (6.5)%	−2.7 (6.8)%	3.2 (6.2)%	3.9 (6.0)%	3.0 (5.7)%
RVE	128.8 (35.4)	0.8 (9.1)%	−2.7 (9.0)%	3.1 (8.7)%	3.4 (9.2)%	2.5 (9.2)%
Semi-membranosus						
CTR	300.4 (42.8)	−10.6 (5.5)%‡	−18.1 (4.9)%‡	−7.7 (5.5)%‡	−2.1 (5.3)%	0.0 (5.2)%
RE	292.1 (56.0)	−10.0 (4.1)%‡	−12.8 (4.1)%‡	−4.6 (4.0)%†	2.9 (3.6)%	1.8 (4.1)%
RVE	256.1 (43.9)	−11.2 (6.3)%‡	−14.1 (6.1)%‡	−2.2 (5.8)%	3.9 (6.0)%	3.5 (5.5)%
Semi-tendinosus						
CTR	220.9 (30.0)	−2.0 (5.8)%	−5.2 (5.8)%†	−1.8 (6.1)%	0.5 (6.9)%	1.0 (6.8)%
RE	236.1 (55.9)	−2.0 (4.6)%	−5.3 (5.0)%†	1.0 (6.8)%	5.0 (5.6)%*	1.1 (6.3)%
RVE	207.7 (59.5)	−0.4 (9.7)%	−2.4 (9.4)%	5.6 (8.8)%	7.6 (8.6)%*	3.9 (8.8)%

BDC, baseline data; HDT27/28, head-down-tilt bedrest *day 27/28*; HDT 55/56, head-down-tilt bedrest *day 55/56*; R + 14, *day 14* of recovery; R + 90, *day 90* of recovery; R + 180, *day 180* of recovery; CTR, control; RE, resistive exercise; RVE, resistive vibration exercise. At BDC, values are means (SD) muscle volume (in ml). Subsequent to BDC, values are means (SD) percentage change compared with BDC. Significant difference of the percentage change in muscle volume compared with baseline (BDC): *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

upper gluteus maximus demonstrated a trend toward faster atrophy than both lower gluteus maximus and gluteus medius ($P < 0.0383$ for both). Concerning the muscles involved in hip rotation, quadratus femoris atrophied significantly faster than all other rotator muscles ($P < 0.0001$ for all).

Not only did rate of muscle atrophy differ within the mono-articular gluteal muscles and bi-articular hamstring muscles but also between these two groups, with the biceps femoris long head and semi-membranosus displaying a tendency toward a faster rate of atrophy than the gluteal muscles ($P < 0.0234$). Similar results were achieved with a linear percentage change per week model (data not shown).

Effect of countermeasures: gluteal musculature. ANOVA results provided strong evidence for an effect of the countermeasure exercise on the volume of all of the gluteal muscles ($F \geq 4.6$; $P \leq 0.02$; Table 2). There were no significant differences between the two training groups ($F \leq 3.96$; $P \geq 0.068$). For the lower gluteus maximus, both countermeasure groups demonstrated significant increases in muscle volume at the end of bedrest (HDT 55/56) compared with a decrease in the CTR group. Muscle loss was attenuated in the upper gluteus maximus in both training groups up to mid-bedrest (HDT27/28); however, by end of bedrest there appeared to be a non-significant increase in muscle volume in the RE group and a significant decrease in muscle volume in RVE group, although the difference between the two training groups was not statistically significant. Countermeasure exercises reduced muscle loss in the gluteus minimus in both training groups compared with the CTR group. For the gluteus medius, although muscle volume loss was quite minor in the CTR group, countermeasure exercises appeared to prevent this in the RE group only.

Effect of countermeasures: hamstring musculature. ANOVA demonstrated a strong effect for the countermeasure on muscle volume in semi-membranosus and biceps femoris long head ($F \geq 9.04$; $P \leq 0.0005$; Table 1), but not for the semi-tendinosus and biceps femoris short head ($F \leq 1.86$; $P \geq 0.18$). There was no difference between the two countermeasure groups for any of the hamstring muscles ($F \leq 0.67$; $P \geq 0.43$). In the semi-membranosus and biceps femoris long head, atrophy was reduced in both training groups, although this effect was not complete.

Effect of countermeasures: hip rotators. The quadratus femoris was the only hip rotator to demonstrate a significant loss of muscle volume throughout bedrest; however, according to ANOVA, there was very weak statistical evidence for an effect of the countermeasure ($F \leq 3.4$; $P \geq 0.053$; Table 3). Significant muscle hypertrophy was seen in the RE group for piriformis and obturator internus ($F \geq 4.9$; $P \leq 0.01$), but there was some statistical evidence of difference in the RVE group for piriformis only ($F \geq 6.9$; $P \leq 0.02$).

Recovery of the musculature after bedrest. In the CTR group (Tables 1–3), at R+14, muscles still significantly decreased in volume compared with baseline included: quadratus femoris (−10%), biceps femoris long head (−9.2%), semi-membranosus (−7.7%), upper gluteus maximus (−7.4%), and gluteus minimus (−5.5%) ($P \leq 0.04$). By R+90, across all groups, the volume of these muscles was no longer significantly reduced compared with baseline.

Generally, the countermeasure groups displayed faster recovery of the muscles. According to ANOVA, there was some statistical evidence of this for the upper gluteus maximus, gluteus minimus, biceps femoris long head, semi-tendinosus ($F \geq 2.6$ for all; $P \leq 0.04$ for all), and lower gluteus maximus ($F = 9.6$; $P = 0.001$), which continued to display a degree of muscle hypertrophy throughout the recovery period. Of these, the upper gluteus maximus was the only muscle to demonstrate a significantly different effect for the two countermeasure groups ($F = 5.8$; $P = 0.006$) with the RVE group displaying a significant degree of hypertrophy at R+90 compared with the RE group and a reversal of this effect at R+180.

The post-bedrest rehabilitation program implemented evaluating two different exercise programs for the recovery of the lumbar spine (27) did not impact the recovery of the hip or thigh muscles examined in this paper (all $P \geq 0.04$). Although ANOVA suggested a different response between the "specific motor control" and "trunk flexor strengthening" rehabilitation groups for the quadratus femoris muscle ($P = 0.0069$), this effect was largely due to a greater loss of quadratus femoris muscle volume at the end of bedrest in the "specific motor control" group before the beginning of the rehabilitation intervention (data not shown).

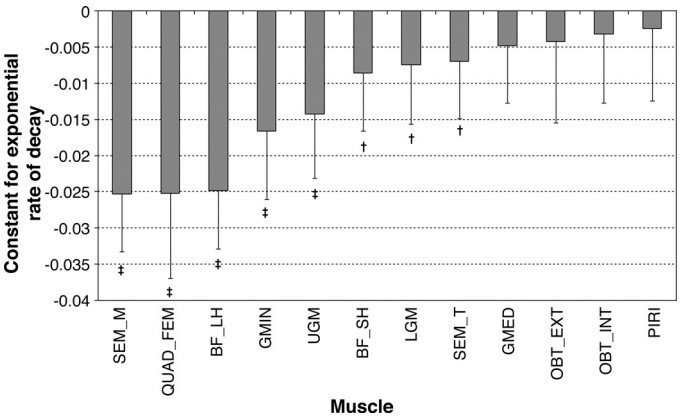

Fig. 3. Estimates of rates of volume loss (exponential rates of decay) in the postero-lateral hip muscles during bedrest. Values are means and SD estimates of the time constant k in the fitted exponential decay model $e^{[k\cdot(\mathrm{BR}x-1)]}$ (where BRx is the xth day of bedrest). More negative time constants indicate faster loss of muscle volume during bedrest. BF_LH, biceps femoris long head; BF_SH, biceps femoris short head; GMED, gluteus medius; GMIN, gluteus minimus; LGM, lower gluteus maximus; OBT_EXT, obturator externus; OBT_INT, obturator internus; PIRI, piriformis; QUAD_FEM, quadratus femoris; SEMI_M, semi-mebranosus; SEMI_T, semi-tendinosus; UGM, upper gluteus maximus. Significant difference of the percentage change in muscle volume compared with zero: *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ indicate. Otherwise $P > 0.05$.

DISCUSSION

Although prior works have investigated the effects of unloading on the anterior hip (23), gluteus maximus (14, 57) and selected hamstring muscles (1, 3, 32, 33, 53), this study is, to our knowledge, the first work to investigate in detail the effect of bedrest on the muscles of the postero-lateral hip. Our main finding was that muscles of the postero-lateral hip atrophy at different rates with fastest rates of atrophy observed in the quadratus femoris, semi-membranosus and biceps femoris long head, followed by the gluteal and remaining hamstring musculature. The remaining hip rotators were not significantly affected by prolonged unloading. The second main finding was that high-load resistive exercise

Table 2. *Gluteal musculature: percentage change in muscle volume during bedrest and recovery in CTR, RE, and RVE groups*

Group	Day of BedRest/Recovery					
	BDC	HDT27/28	HDT55/56	R + 14	R + 90	R + 180
Gluteus minimus						
CTR	124.8 (16.0)	−11.3 (4.4)%‡	−11.2 (4.2)%‡	−5.5 (3.8)%‡	−1.6 (4.2)%	−2.7 (4.5)%
RE	112.5 (21.6)	−3.7 (4.6)%	−5.4 (4.0)%‡	−2.5 (4.6)%	2.2 (3.6)%	7.1 (7.6)%*
RVE	112.6 (19.5)	−9.4 (6.8)%‡	−5.6 (6.3)%*	0.8 (6.2)%	2.5 (5.8)%	1.6 (5.7)%
Gluteus medius						
CTR	373.1 (41.9)	−2.2 (2.8)%*	−3.7 (2.8)%‡	−1.0 (2.7)%	−0.1 (2.5)%	−0.2 (3.1)%
RE	363.0 (75.4)	−1.9 (2.7)%	0.0 (3.1)%	1.9 (2.9)%	2.7 (2.8)%*	2.1 (3.4)%
RVE	373.6 (43.3)	−4.8 (5.1)%*	−3.6 (6.2)%	−1.2 (5.3)%	0.6 (5.6)%	1.0 (5.7)%
Lower gluteus maximus						
CTR	573.3 (128.6)	−1.9 (5.3)%	−5.7 (4.9)%‡	−2.7 (5.3)%	−0.9 (3.9)%	−2.4 (3.8)%
RE	609.5 (113.3)	6.0 (4.8)%†	8.2 (5.6)%‡	7.4 (5.6)%‡	7.4 (5.7)%†	3.2 (6.1)%
RVE	510.5 (58.2)	1.2 (5.7)%	7.0 (6.0)%†	5.8 (6.2)%*	4.6 (5.5)%*	6.8 (8.5)%*
Upper gluteus maximus						
CTR	397.4 (37.9)	−8.5 (6.9)%‡	−9.9 (7.2)%‡	−7.4 (7.3)%†	−2.7 (5.3)%	−1.0 (6.2)%
RE	398.2 (87.8)	−0.9 (5.5)%	2.5 (7.6)%	2.6 (7.9)%	2.5 (4.9)%	5.3 (5.2)%*
RVE	397.4 (52.2)	−1.6 (5.6)%	−4.3 (5.3)%*	−2.0 (5.7)%	6.7 (6.6)%†	0.5 (6.6)%

At BDC, values are means (SD) muscle volume (in ml). Subsequent to BDC, values are means (SD) percentage change compared with BDC. Significant difference of the percentage change in muscle volume compared with baseline (BDC): *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

countermeasures (with and without vibration) incorporating squat exercises, reduced and/or prevented atrophy of the gluteal muscles and demonstrated a "significant" effect on semi-membranosus and biceps femoris long head; however, the effect size was relatively minor for these two muscles. Third, the addition of whole body vibration did not appear to have a supplementary effect in protecting against atrophy of the postero-lateral hip and hamstring musculature. Finally, muscle volume remained reduced 2 wk after bedrest but appeared completely recovered after 90 days of bedrest.

When we consider the activity of the postero-lateral hip musculature during loading and functional tasks, it is not surprising that, first, these muscles are affected by unloading and that, second, they respond differently to unloading and exercise stimuli. Regarding the hamstring muscles, a similar pattern of differential atrophy to that in the present study has been described in previous unloading studies (2, 3, 12), with semi-tendinosus and biceps femoris short head comparatively less affected than semi-membranosus and biceps femoris long head. The biarticular semi-membranosus and biceps femoris long head are active during the loading and swing phases of walking and running (36, 37, 46) and are thought to play an important role in providing hip extension force for propulsion. In contrast, the biceps femoris short head does not cross the hip joint and is thought to be more involved in knee flexion and control (37).

Although the gluteal muscles can act synergistically with the hamstring muscles, they do of course have different functions and, hence, demonstrate a different response to unloading/bedrest. Interestingly, our findings showed significantly greater atrophy in the upper gluteus maximus compared with the lower gluteus maximus. These two muscle portions are not only embryologically (59) and functionally different, with lower gluteus maximus thought to act primarily as a hip extensor and

Table 3. *Thigh external rotators: percentage change in muscle volume during bedrest and recovery in CTR, RE, and RVE groups*

Group	Day of BedRest/Recovery					
	BDC	HDT27/28	HDT55/56	R + 14	R + 90	R + 180
Obturator externus						
CTR	73.3 (13.9)	0.6 (12.1)%	−4.3 (8.9)%	−1.7 (9.6)%	0.4 (6.7)%	0.6 (7.2)%
RE	77.4 (21.2)	−2.8 (10.1)%	−1.2 (11.0)%	4.1 (12.8)%	5.1 (12.3)%	1.4 (12.4)%
RVE	68.5 (11.8)	−6.3 (7.1)%*	−3.7 (6.3)%	−2.6 (5.2)%	0.2 (7.0)%	0.7 (5.1)%
Obturator internus						
CTR	80.3 (12.4)	−0.6 (5.1)%	−3.0 (5.9)%	3.3 (5.3)%	−2.5 (5.4)%	1.4 (5.0)%
RE	82.1 (10.4)	4.3 (4.5)%*	8.3 (5.9)%‡	6.4 (5.0)%†	2.8 (5.2)%	5.5 (5.8)%*
RVE	75.9 (5.5)	−0.9 (8.1)%	−0.2 (7.4)%	3.8 (7.8)%	1.3 (7.8)%	5.4 (8.3)%
Quadratus femoris						
CTR	46.3 (13.0)	−9.8 (12.2)%*	−18.1 (13.3)%‡	−10.0 (11.4)%*	−4.5 (9.8)%	−3.6 (9.8)%
RE	41.4 (9.4)	−5.5 (8.1)%	−8.2 (9.4)%*	−4.0 (9.5)%	1.0 (7.5)%	−2.8 (8.1)%
RVE	43.7 (13.6)	−9.5 (7.2)%†	−14.4 (9.8)%‡	−2.9 (6.8)%	7.3 (8.5)%*	3.9 (10.0)%
Piriformis						
CTR	44.3 (10.4)	−0.6 (8.2)%	−2.0 (6.7)%	3.1 (6.4)%	−2.4 (5.2)%	−0.6 (5.1)%
RE	43.2 (4.4)	12.1 (9.1)%†	12.9 (9.2)%‡	7.5 (11.8)%	10.2 (10.0)%*	5.4 (8.5)%
RVE	43.9 (6.8)	0.2 (7.1)%	1.9 (7.2)%	6.8 (8.4)%*	5.0 (8.2)%	4.0 (7.7)%

At BDC, values are means (SD) muscle volume (in ml). Subsequent to BDC, values are means (SD) percentage change compared with BDC. Significant difference of the percentage change in muscle volume compared with baseline (BDC): *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

upper gluteus maximus as a hip abductor (28), but they also have been found to respond differently in hip pathology (25). We find two alternate explanations for the differential atrophy demonstrated in this study to be plausible. First, we cannot exclude the possibility that, for the purpose of muscle measurements, our division of the upper and lower gluteus maximus was imperfect. Second, although subjects were instructed to limit load bearing through their feet during bedrest, during changes in position, daily transfers, and use of bed pans, it is possible that one or both feet were partially loaded. Although relatively infrequent, these hip-extension movements may have activated the lower gluteus maximus sufficiently enough to maintain its muscle volume. Unfortunately, previous works in spaceflight and bedrest, which investigated the effect of unloading on the gluteal musculature, grouped the muscles together, making comparisons to our findings difficult. Interestingly, inconsistent responses of the gluteal muscles to unloading were reported in these studies, with 2.3% muscle volume loss documented after 35 days of bed rest (14) compared with 8% muscle volume after 17 days of spaceflight (57). These findings demonstrate that, although bedrest may generally represent physical inactivity, this does not mean that all muscles are inactive, and, moreover, it may be possible for increased usage of some muscles to occur.

An interesting finding from our study was that of quadratus femoris, which appears to have a unique response among the hip external rotators to unloading. Originating on the lateral border of the ischial tuberosity and inserting on the quadrate tubercle of the femur, this deep muscle of the hip (38) demonstrated the fastest rate of atrophy and greatest loss of muscle volume of all the muscles we examined. The current understanding of quadratus femoris and its function is limited, with research on this muscle in humans largely comprising of anatomical and embryological (7, 8, 61) studies. Case studies on injury of the quadratus femoris (6, 29, 42, 43, 60) may help us to understand that it is active in load bearing, thigh adduction, and external rotation and may also be involved in hamstring tears (6); however, further investigation is necessary to elucidate the role of this muscle in control of the hip joint and function.

Our findings of differential atrophy between different muscle groups and among muscle synergists, have important implications for exercise countermeasure development and rehabilitation of the muscles after loading. Data from modeling studies show that decreased force from the gluteal musculature during hip extension results in increased superior (35) and anterior (34) forces on the hip joint, with subsequent substitution and increased muscle activity in the semi-membranosus (34, 35), gluteus medius, vastii, and tensor fascia latae (35). Furthermore, research suggests that hip muscle force imbalances contribute to increased risk of hamstring tears (22) and hip and lower limb overuse injuries (41, 47). Hence, it is possible that the differential atrophy occurring in the muscles at the hip during bedrest could lead to altered joint loading and subsequent increased risk of injury in this area.

The countermeasure exercise program reduced/prevented muscle volume loss in the mono-articular gluteal musculature but had less effect on the bi-articular hamstring musculature. The strongest effect of the countermeasure was observed in lower gluteus maximus, which is strongly (55–60% MVIC) activated during squats (5) and hence resulted in the hypertrophic response observed in both training groups by end bedrest. The hamstring musculature, however, is only moderately activated during squats (3–30% MVIC) (5, 26, 56), and, as a result, this muscle group underwent significant atrophy in the training groups in our study. Isolated hamstring curls are reported to produce twice as much hamstring muscle activity (between 67 and 70% MVIC) as squats or leg press (5, 62) and, furthermore, facilitate more equal recruitment of semi-membranosus and biceps femoris (5, 24), with increased muscle activation with greater resistance (54). Future countermeasure exercise programs should consider including resisted isolated hamstring curls to help reduce muscle atrophy and potential development of muscle force imbalances in this area.

Whole body resistive vibration did not provide a significant additional effect above resistive exercise alone in ameliorating loss of muscle volume during bed rest. Due to limited existing data on the effects of bedrest on the hip musculature, it was not possible to perform a meaningful sensitivity analysis for the RE-RVE comparison; hence, low subject numbers may have prevented us from accurately assessing any potential additional effect(s) of vibration. Another possibility is that, although resistive vibration training has been reported to have positive effects on functional muscle parameters such as muscle strength, power, and flexibility (17, 19, 48, 50), muscle volume is a morphological parameter and, as such, may not be ideal to reflect additional effects of vibration.

Table 4. *Comparisons of rates of atrophy in the hip muscles*

Muscle	BF_LH	QF	SEMI_M	GMIN	UGM	BF_SH	LGM	SEMI_T	OBT_EXT	PIRI	OBT_INT
QF	0.91										
SEMI_M	0.87	0.99									
GMIN	0.02	0.05	0.01								
UGM	**0.001**	0.01	**0.0007**	0.51							
BF_SH	**<0.0001**	**<0.0001**	**<0.0001**	0.012	0.08						
LGM	**<0.0001**	**<0.0001**	**<0.0001**	**0.008**	0.04	0.7					
SEMI_T	**<0.0001**	**<0.0001**	**<0.0001**	**0.004**	0.03	0.59	0.89				
OBT_EXT	**<0.0001**	**<0.0001**	**<0.0001**	**0.004**	0.017	0.27	0.43	0.48			
PIRI	**<0.0001**	**<0.0001**	**<0.0001**	**0.0003**	**0.002**	0.09	0.17	0.2	0.68		
OBT_INT	**<0.0001**	**<0.0001**	**<0.0001**	**0.0005**	**0.003**	0.12	0.23	0.26	0.81	0.85	
GMED	**<0.0001**	**<0.0001**	**<0.0001**	**0.0005**	**0.004**	0.19	0.39	0.44	0.89	0.51	0.64

Values are P values of direct comparisons between muscles of estimates of rates of volume loss. Differences of $P < 0.01$ are in **bold**. BF_LH, biceps femoris long head; BF_SH, biceps femoris short head; GMED, gluteus medius; GMIN, gluteus minimus; LGM, lower gluteus maximus; OBT_EXT, obturator externus; OBT_INT, obturator internus; PIRI, piriformis; QF, quadratus femoris; SEMI_M, semi-mebranosus; SEMI_T, semi-tendinosus; UGM, upper gluteus maximus.

Regarding recovery of the musculature after bedrest, the countermeasure groups appeared to recover more quickly than the control group. Not unexpectedly, the muscles most affected by bedrest took longer to recover, with significant reductions in volume evident in a number of muscles at 14 days post-bedrest. By R+90, recovery appeared complete for all muscles across all groups. Muscle injury and inflammation have been documented post-immobilization and have been found to be associated with increases in muscle volume (45). Hence, we cannot be certain that inflammation-induced fluid changes masked a lack of recovery of muscle volume in the initial recovery scans. Future unloading studies could reduce this uncertainty by monitoring recovery of musculature at more frequent intervals and/or using muscle biopsies for comparison of muscle fiber cross-sectional area and muscle volume measurements.

In conclusion, the present study found that differential rates of atrophy occur between both functional and synergistic muscle groups of the postero-lateral hip. The fastest rates of muscle volume loss were seen in the quadratus femoris, semimembranosus and biceps femoris long head, followed by the gluteal and remaining hamstring musculature. Short-duration, high-load, resistive exercises, performed 3 days/wk were sufficient to preserve muscle volume of the mono-articular gluteal muscles during bedrest but were less effective in preserving muscle volume of the bi-articular hamstring muscles involved in hip extension and knee flexion. The addition of whole body vibration to resistive exercise did not appear to have a supplementary effect in reducing muscle volume loss during bedrest on the hip muscles we studied, although limited subject numbers may have played a role in this finding. Recovery of muscle volume changes after bedrest took longer than 2 wk but appeared complete by 90 days post-bedrest. Future countermeasure exercise programs should consider including hamstring-specific exercises, such as isolated, resisted hamstring curls, to target a wider range of muscles in the hip and minimize the risk of the development of muscle force imbalances in this region.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

1. **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* 172: 269–278, 2001.
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* 82: 30–38, 2000.
3. **Akima H, Ushiyama J, Kubo J, Fukuoka H, Kanehisa H, Fukunaga T.** Effect of unloading on muscle volume with and without resistance training. *Acta Astronaut* 60: 728–736, 2007.
4. **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* 181: 345–357, 2004.
5. **Andersen LL, Magnusson SP, Nielsen M, Haleem J, Poulsen K, Aagaard P.** Neuromuscular activation in conventional therapeutic exercises and heavy resistance exercises: implications for rehabilitation. *Phys Ther* 86: 683–697, 2006.
6. **Askling CM, Tengvar M, Saartok T, Thorstensson A.** Acute first-time hamstring strains during slow-speed stretching: clinical, magnetic resonance imaging, and recovery characteristics. *Am J Sports Med* 35: 1716–1724, 2007.
7. **Aung HH, Sakamoto H, Akita K, Sato T.** Anatomical study of the obturator internus, gemelli and quadratus femoris muscles with special reference to their innervation. *Anat Rec* 263: 41–52, 2001.
8. **Bardeen RC.** Development and variation of the nerves and musculature of the inferior extremity and of the neighboring regions of the trunk in man. *Am J Anat* 6: 263–390, 1907.
9. **Belavý DL, Armbrecht G, Richardson CA, Felsenberg D, Hides JA.** Muscle Atrophy and Changes in Spinal Morphology: Is the Lumbar Spine Vulnerable After Prolonged Bedrest? *Spine* 36: 137–145, 2011.
10. **Belavý DL, Beller G, Armbrecht G, Perschel FH, Fitzner R, Bock O, Börst H, Degner C, Gast U, Felsenberg D.** Evidence for an additional effect of whole-body vibration above resistive exercise alone in preventing bone loss during prolonged bedrest. *Osteoporosis International*; DOI:10.1007/s00198-010-1371-6 http://dx.doi.org/10.1007/s00198-101-1371-6.
11. **Belavý DL, Bock O, Börst H, Armbrecht G, Gast U, Degner C, Beller G, Heer M, de Haan A, Stegeman DF, Ceretteli P, Blottner D, Rittweger J, Gelfi C, Kornak U, Felsenberg D.** The 2nd Berlin Bed-Rest Study: protocol and implementation. *J Musculoskelet Neuronal Interact* 10: 207–219, 2010.
12. **Belavý DL, Miokovic T, Armbrecht G, Richardson CA, Rittweger J, Felsenberg D.** Differential atrophy of the lower-limb musculature during prolonged bedrest. *Eur J Appl Physiol* 107: 489–499, 2009.
13. **Belavý DL, Miokovic T, Armbrecht G, Rittweger J, Felsenberg D.** Resistive vibration exercise reduces lower limb muscle atrophy during 56-day bedrest. *J Musculoskelet Neuronal Interact* 9: 225–235, 2009.
14. **Berg HE, Eiken O, Miklavcic L, Mekjavic IB.** Hip, thigh and calf muscle atrophy and bone loss after 5-week bedrest inactivity. *Eur J Appl Physiol* 99: 283–289, 2007.
15. **Beukeboom C, Birmingham TB, Forwell L, Ohrling D.** Asymmetrical strength changes and injuries in athletes training on a small radius curve indoor track. *Clin J Sport Med* 10: 245–250, 2000.
16. **Booth FW, Gollnick PD.** Effects of disuse on the structure and function of skeletal-muscle. *Med Sci Sports Exerc* 15: 415–420, 1983.
17. **Bosco C, Cardinale M, Tsarpela O.** Influence of vibration on mechanical power and electromyogram activity in human arm flexor muscles. *Eur J Appl Physiol Occup Physiol* 79: 306–311, 1999.
18. **Burke D, Hagbarth KE, Lofstedt L, Wallin BG.** The responses of human muscle spindle endings to vibration during isometric contraction. *J Physiol* 261: 695–711, 1976.
19. **Cardinale M, Bosco C.** The use of vibration as an exercise intervention. *Exerc Sport Sci Rev* 31: 3–7, 2003.
20. **Conley MS, Foley JM, Ploutz-Snyder LL, Meyer RA, Dudley GA.** Effect of acute head-down tilt on skeletal muscle cross-sectional area and proton transverse relaxation time. *J Appl Physiol* 81: 1572–1577, 1996.
21. **Convertino VA.** Neuromuscular aspects in development of exercise countermeasures. *Physiologist* 34: 125–128, 1991.
22. **Croisier JL, Ganteaume S, Binet J, Genty M, Ferret JM.** Strength imbalances and prevention of hamstring injury in professional soccer players: a prospective study. *Am J Sports Med* 36: 1469–1475, 2008.
23. **Dilani Mendis M, Hides JA, Wilson SJ, Grimaldi A, Belavý DL, Stanton W, Felsenberg D, Rittweger J, Richardson C.** Effect of prolonged bed rest on the anterior hip muscles. *Gait Posture* 30: 533–537, 2009.
24. **Escamilla RF, Fleisig GS, Zheng N, Barrentine SW, Wilk KE, Andrews JR.** Biomechanics of the knee during closed kinetic chain and open kinetic chain exercises. *Med Sci Sports Exerc* 30: 556–569, 1998.
25. **Grimaldi A, Richardson C, Durbridge G, Donnelly W, Darnell R, Hides J.** The association between degenerative hip joint pathology and size of the gluteus maximus and tensor fascia lata muscles. *Man Ther* 14: 611–617, 2009.

26. **Gryzlo SM, Patek RM, Pink M, Perry J.** Electromyographic analysis of knee rehabilitation exercises. *J Orthop Sports Phys Ther* 20: 36–43, 1994.
27. **Hides JA, Lambrecht G, Richardson CA, Stanton WR, Armbrecht G, Pruett C, Damann V, Felsenberg D, Belavý DL.** The effects of rehabilitation on the muscles of the trunk following prolonged bed rest. *Eur Spine J*. In press.
28. **Jaegers S, Dantuma R, de Jongh HJ.** Three-dimensional reconstruction of the hip muscles on the basis of magnetic resonance images. *Surg Radiol Anat* 14: 241–249, 1992.
29. **Klinkert P Jr, Porte RJ, de Rooij TP, de Vries AC.** Quadratus femoris tendinitis as a cause of groin pain. *Br J Sports Med* 31: 348–349, 1997.
30. **Le Blanc A, Gogia P, Schneider V, Krebs J, Schonfeld E, Evans H.** Calf muscle area and strength changes after five weeks of horizontal bed rest. *Am J Sports Med* 16: 624–629, 1998.
31. **Le Blanc A, Lin C, Shackelford L, Sinitsyn V, Evans H, Belichenko O, Schenkman B, Kozlovskaya I, Oganov V, Bakulin A, Hedrick T, Feeback D.** Muscle volume, MRI relaxation times (T2), and body composition after spaceflight. *J Appl Physiol* 89: 2158–2164, 2000.
32. **Le Blanc A, Rowe R, Schneider V, Evans H, Hedrick T.** Regional muscle loss after short duration spaceflight. *Aviat Space Environ Med* 66: 1151–1154, 1995.
33. **Le Blanc A, Schneider V, Shackelford L, West S, Oganov V, Bakulin A, Voronin L.** Bone mineral and lean tissue loss after long duration space flight. *J Musculoskelet Neuronal Interact* 1: 157–160, 2000.
34. **Lewis CL, Sahrmann SA, Moran DW.** Anterior hip joint force increases with hip extension, decreased gluteal force, or decreased iliopsoas force. *J Biomech* 40: 3725–3731, 2007.
35. **Lewis CL, Sahrmann SA, Moran DW.** Effect of position and alteration in synergist muscle force contribution on hip forces when performing hip strengthening exercises. *Clin Biomech (Bristol, Avon)* 24: 35–42, 2009.
36. **Lyons K, Perry J, Gronley JK, Barnes L, Antonelli D.** Timing and relative intensity of hip extensor and abductor muscle action during level and stair ambulation. An EMG study. *Phys Ther* 63: 1597–1605, 1983.
37. **Montgomery WH 3rd, Pink M, Perry J.** Electromyographic analysis of hip and knee musculature during running. *Am J Sports Med* 22: 272–278, 1994.
38. **Moore KL, Dalley AF.** *Clinically Orientated Anatomy*. Baltimore, MD: Lippincott Williams & Wilkins, 2006.
39. **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* 97: 706–715, 2006.
40. **Nicogossian AE, Dietlein LF.** Microgravity simulation and analogues. In: *Space Physiology and Medicine*, edited by Nicogossian AE. Philadelphia, PA: Lea and Febiger, 1982, p. 240–248.
41. **Niemuth PE, Johnson RJ, Myers MJ, Thieman TJ.** Hip muscle weakness and overuse injuries in recreational runners. *Clin J Sport Med* 15: 14–21, 2005.
42. **O'Brien SD, Bui-Mansfield LT.** MRI of quadratus femoris muscle tear: another cause of hip pain. *Am J Roentgenol* 189: 1185–1189, 2007.
43. **Peltola K, Heinonen OJ, Orava S, Mattila K.** Quadratus femoris muscle tear: an uncommon cause for radiating gluteal pain. *Clin J Sport Med* 9: 228–230, 1999.
44. **Pinheiro JC, Bates DM.** *Mixed-Effects Models in S and S-PLUS*. Berlin: Springer, 2000.
45. **Ploutz-Snyder LL, Tesch PA, Hather BM, Dudley GA.** Vulnerability to dysfunction and muscle injury after unloading. *Arch Phys Med Rehabil* 77: 773–777, 1996.
46. **Prilutsky BI, Gregor RJ.** Swing- and support-related muscle actions differentially trigger human walk-run and run-walk transitions. *J Exp Biol* 204: 2277–2287, 2001.
47. **Renkawitz T, Boluki D, Grifka J.** The association of low back pain, neuromuscular imbalance, and trunk extension strength in athletes. *Spine J* 6: 673–683, 2006.
48. **Rittweger J.** Vibration as an exercise modality: how it may work, and what its potential might be. *Eur J Appl Physiol* 108: 877–904, 2010.
49. **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* 36: 1019–1029, 2005.
50. **Rittweger J, Just K, Kautzsch K, Reeg P, Felsenberg D.** Treatment of chronic lower back pain with lumbar extension and whole-body vibration exercise: a randomized controlled trial. *Spine* 27: 1829–1834, 2002.
51. **Rubin C, Pope M, Fritton JC, Magnusson M, Hansson T, McLeod K.** Transmissibility of 15-hertz to 35-hertz vibrations to the human hip and lumbar spine: determining the physiologic feasibility of delivering low-level anabolic mechanical stimuli to skeletal regions at greatest risk of fracture because of osteoporosis. *Spine* 28: 2621–2627, 2003.
52. **Schulze K, Gallagher P, Trappe S.** Resistance training preserves skeletal muscle function during unloading in humans. *Med Sci Sports Exerc* 34: 303–313, 2002.
53. **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* 97: 119–129, 2004.
54. **Shields RK, Madhavan S, Gregg E, Leitch J, Petersen B, Salata S, Wallerich S.** Neuromuscular control of the knee during a resisted single-limb squat exercise. *Am J Sports Med* 33: 1520–1526, 2005.
55. **Shinohara H.** Gemelli and obturator internus muscles: different heads of one muscle? *Anat Rec* 243: 145–150, 1995.
56. **Stuart MJ, Meglan DA, Lutz GE, Growney ES, An KN.** Comparison of intersegmental tibiofemoral joint forces and muscle activity during various closed kinetic chain exercises. *Am J Sports Med* 24: 792–799, 1996.
57. **Tesch PA, Berg HE, Bring D, Evans HJ, LeBlanc AD.** Effects of 17-day spaceflight on knee extensor muscle function and size. *Eur J Appl Physiol* 93: 463–468, 2005.
58. **Tesch PA, Trieschmann JT, Ekberg A.** Hypertrophy of chronically unloaded muscle subjected to resistance exercise. *J Appl Physiol* 96: 1451–1458, 2004.
59. **Tichy M, Grim M.** Morphogenesis of the human gluteus maximus muscle arising from two muscle primordia. *Anat Embryol (Berl)* 173: 275–277, 1985.
60. **Willick SE, Lazarus M, Press JM.** Quadratus femoris strain. *Clin J Sport Med* 12: 130–131, 2002.
61. **Wilson JT.** Abnormal distribution of the nerve to the quadratus femoris in man, with remarks on its significance. *J Anat Physiol* 23: 354–357, 1889.
62. **Wright GA, Delong TH, Gehlsen G.** Electromyographic activity of the hamstrings during performance of the leg curl, stiff-leg deadlift, and back squat movements. *J Str Cond Res* 13: 168–174, 1999.

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Hylonome

Original Article

Resistive vibration exercise reduces lower limb muscle atrophy during 56-day bed-rest

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Abstract

Objectives: The current study aimed to examine the effectiveness of a resistive vibration exercise countermeasure during prolonged bed-rest in preventing lower-limb muscle atrophy. **Methods:** 20 male subjects underwent 56-days of bed-rest and were assigned to either an inactive control, or a countermeasure group which performed high-load resistive exercises (including squats, heel raises and toe raises) with whole-body vibration. Magnetic resonance imaging of the lower-limbs was performed at two-weekly intervals. Volume of individual muscles was calculated. **Results:** Countermeasure exercise reduced atrophy in the triceps surae and the vastii muscles ($F>3.0$, $p<.025$). Atrophy of the peroneals, tibialis posterior and toe flexors was less in the countermeasure-subjects, though statistical evidence for this was weak ($F\leq2.3$, $p\geq.071$). Atrophy in the hamstring muscles was similar in both groups ($F<1.1$, $p>.38$). The adductor longus, sartiorius and rectus femoris muscles showed little loss of muscle volume during bed-rest ($F<1.7$, $p>.15$). **Conclusions:** The countermeasure exercise programme was effective in reducing atrophy in the extensors of the knee and ankle but not the hamstrings.

Keywords: Spaceflight, Berlin Bed Rest Study, Magnetic Resonance Imaging, Microgravity, Countermeasures

Introduction

With space agencies and governments striving towards manned missions to Mars, an important research question is the development of exercise countermeasures to maintain various body systems for optimal function upon landing. As part of this, it is important to assess the effectiveness of countermeasures in maintaining the musculature of the lower-limbs. The role of the lower-limb musculature, particularly with regard to the plantarflexors and knee and hip extensors[1-3], in upright posture and locomotion imply that the maintenance of these muscle systems during long-duration spaceflight, an environment where such functions are not required, would be critical. Prolonged bed-rest is a frequently used ground-based methodology to simulate the effects of spaceflight on the human musculoskeletal system[4]. Basic principles of exercise physiology suggest that low load endurance exercise is inappropriate to maintain muscle mass during prolonged bed-rest and that higher load resistance exercise is required[5]. Indeed, bed-rest studies implementing aerobic countermeasures[6,7] or low-load exercise[8] have found that they were ineffective for the maintenance of the lower-limb musculature, whereas bed-rest studies implementing resistive exercise were much more successful, though the effect on particular muscle groups depended upon the types of exercise performed[9-14].

More recently, whole body vibration during resistive exercise has received attention in sport science as a method to provide an additional stimulus during training[15]. It is thought that the vibratory inputs stimulate additional muscle activity during contraction[16] *via* the muscle spindle system[17-19], to produce a greater force of muscle contraction[20], and hence stimulus for muscle maintenance and/or hypertrophy. Vibratory stimuli, applied at the feet are transmitted up to the hip and lumbar spine[21] and hence can potentially modulate muscle activity throughout the entire lower quadrant. We hypothesized that high-load resistive exercise with whole body vibration could be an effective countermeasure against muscle atrophy in the lower-limbs during prolonged bed-rest.

Dieter Felsenberg and Jörn Rittweger are acting as consultants to the European Space Agency and Novotec Medical for the exploitation of this study's results. All other authors have no conflicts of interest.

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Figure 1. Resistive vibration exercise during bed-rest. Subjects were required to perform lower-limb exercises against a resistive force transmitted via belts at the waist and shoulders and via hand-grips. The feet were placed on a suspended platform. Vibratory stimuli in the lower-limbs were generated by rotation of the platform around a vertically oriented axis.

Magnetic resonance imaging (MRI) is a commonly used methodology to assess muscle size. In studies where subject numbers are limited, such as bed-rest, measures of muscle atrophy during bed-rest and response to countermeasures are best performed using measures of muscle volume, thus avoiding greater imprecision associated with taking single cross-sectional area measures[22,23]. In the current work, we wished to examine the effectiveness of resistive exercise with whole body vibration during prolonged bed-rest on changes in muscle volume in the lower-limbs as measured with MRI.

Materials and methods

Bed-rest protocol

The "Berlin Bed-Rest Study" was undertaken at the Charité Campus Benjamin Franklin Hospital in Berlin, Germany, from February 2003 to June 2005. Twenty medically and psychologically healthy male subjects underwent 8-weeks of strict bed-rest. The sample size of the Berlin Bed-Rest Study was based on power analyses of expected distal tibia bone density changes, rather than muscle measures. It is reasonable to expect, however, that muscle is more susceptible to inactivity/training, and other works have shown strongly significant findings, including the effect of countermeasure exercise, in sample sizes half than that in the current study[12]. The bed-rest protocol, as well as inclusion and exclusion criteria, is discussed in detail elsewhere[24]. In brief, however, subjects were randomly allocated to either an inactive control group (CTRL) or a group that underwent a whole body resistive vibration exercise countermeasure programme (RVE group) using the Galileo Space exercise device (Novotec Medical, Pforzheim, Germany). The mean (SD) baseline age, height and weight of the RVE group were: 32.6(4.8) years, 183(9) cm and 81.7(14.4) kg respectively and in the CTRL group: 33.4(6.6) years, 185(7) cm and 79.4(9.7) kg.

Horizontal bed-rest was employed, though subjects were permitted to be positioned in up to thirty degrees head-up tilt for recreational activities during daylight hours (such as watching television). Subjects performed all hygiene in the supine position and were discouraged from moving excessively or unnecessarily. Force sensors placed in the bed supports, 24-hour nursing care and video surveillance permitted monitoring of subjects' activities. The institutional ethics committee approved this study and subjects gave their informed written consent. Subjects were aware that their participation in the study was voluntary and that they were permitted to withdraw from the study at any time.

Countermeasure exercise

Resistive vibration exercise (RVE) was performed using a dedicated prototype (Galileo Space) of a commercially available vibration platform (Novotec Medical, Pforzheim, Germany). The exercise device permitted exercise in the supine position throughout bed rest. The exercise regime targeted the lower leg muscle groups with resistive loading and neuromuscular stimuli via whole-body vibration applied at the feet[25,26]. The countermeasure exercise protocol is described in detail elsewhere[24]. In brief, the subjects were placed in a supine position (Figure 1), with their feet resting on the vibration platform and an applied vibration amplitude between 3.5-4 mm. An axial force between 1.0 and 1.8 times body weight was placed through the subjects' trunk and spine via elastic shoulder straps (targeted to be approximately 75-85% of the subject's 1-repetition maximum). With the exception of Sundays and Wednesday afternoons, two exercise sessions per day, of 30 min duration (between 4-6 min pure exercise time) were performed. A total of 89 exercise sessions were scheduled for each subject.

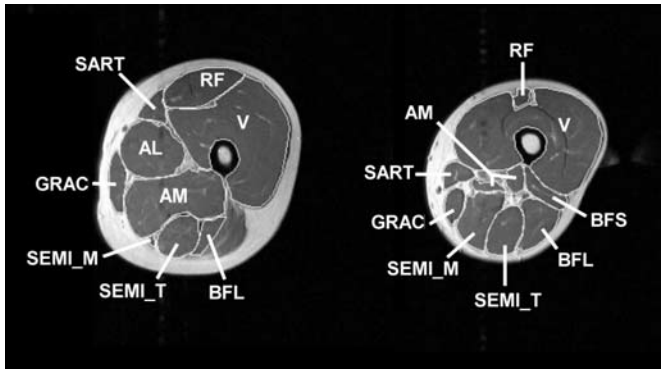

Figure 2. Thigh muscle image measurements. Example images from the upper (left) and lower (right) thigh. Thirty-five images were acquired from the superior aspect of the femoral head to the knee joint line. The cross-sectional area (when present) of the muscles rectus femoris (RF), vastii (V), sartorius (SART), gracilis (GRAC), adductor magnus (AM), adductor longus (AL), biceps femoris long head (BFL), biceps femoris short head (BFS), semitendinosus (SEMI_T) and semimembranosus (SEMI_M) were measured in each image.

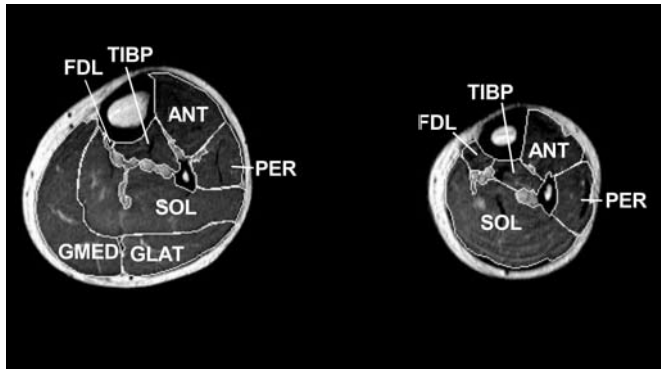

Figure 3. Lower leg muscle image measurements. Example images from the upper (left) and lower (right) calf. Thirty images were acquired from the knee joint line to the lateral malleolus. The cross-sectional area (when present) of the muscles gastrocnemius lateralis (GLAT), gastrocnemius medialis (GMED), soleus with flexor hallucis longus (SOL), tibialis posterior (TIBP), flexor digitorum longus (FDL), peroneals (PER; peroneus longus, brevis and tertius), anterior tibial (ANT; tibialis anterior, extensor digitorum longus, extensor hallucis longus) were measured in each image.

Trained staff supervised all training sessions, and subjects were given feedback and encouragement. For each morning session, four resistive exercises were performed once in the following order:

- squats: knees were straightened from 90° to full extension in cycles of 6 s for 60 s whilst the vibration frequency was set to 18 Hz in the first training sessions and progressed in subsequent sessions up to a maximum of 24 Hz.
- heel raises: in almost full knee extension, the heels were raised into plantar flexion as long as the subjects could sustain this (up to 40 seconds). The vibration frequency was retained at 26 Hz.
- toe raises: with knees in full extension, the forefoot was raised into dorsiflexion up to 40 seconds. The vibration frequency was retained at 26 Hz.
- explosive kicks: 10 explosive pushes from a flexed knee and hip position against the vibrating platform were performed. These "kicks" were targeted at generating peak forces to stimulate bone formation[27] rather than muscle *per se*.

In the afternoon session, subjects were asked to exercise with a lower resting platform reaction force (60-80% of the value achieved in the morning). Only one exercise was performed and subjects kept their feet on the platform with their knees in a nearly extended position and performed no movement. Vibration frequency was retained at 19 Hz and the exercise performed between 4 and 6 minutes (depending on the physical ability of subject).

MRI protocol

Baseline MR scanning was conducted on the first day of bed-rest (BR1) and then at two week intervals (BR14, BR28, BR42 and BR56) through to the end of the bed-rest period. Subjects were positioned on the scanning bed in supine with their knees and hips supported in slight flexion by a pillow under the knees. Transverse MR images were acquired from the lower-limbs using a 1.5 Tesla Magnetom Vision system (Siemens, Erlangen, Germany). Typically, 35 images of the thigh (from the superior aspect of the head of femur to the knee joint line; thickness=10 mm, inter-slice distance=5 mm, TR=6000 ms, TE=15 ms, FA=180 degrees, field of view: 480 x 480 mm interpolated to 512 x 512 pixels) and 30 images of the lower leg (knee joint line to the distal most portion of the lateral malleolus; thickness=10 mm; inter-slice distance=5 mm, TR=4800 ms, TE=15 ms, FA=180 degrees, field of view: 340 x 340 mm interpolated to 512 x 512 pixels) were acquired, though for taller subjects, additional images were added to ensure the region of interest was captured. Images were stored for offline analysis.

Image measurements

One operator (TM) performed all image measurements. To ensure operator blinding to study time-point and subject group, each image was assigned a random number (www.random.org). ImageJ (Ver. 1.38x, http://rsb.info.nih.gov/ij/) was used for MR image analysis. The cross sectional area (CSA) of the following muscles in the thigh (Figure 2) was measured in each image: rectus femoris (RF), vastii (V; vastus medialis, lateralis and intermedius), sartorius (SART), gracilis (GRAC), adductor magnus (AM), adductor longus (AL), biceps femoris long head (BFL), biceps femoris short head (BFS), semitendinosus (SEMI_T) and semimembranosus (SEMI_M). Whilst AM and AL could be readily differentiated from adductor brevis, this adductor muscle could not easily be differentiated from pectineus and hence was not measured. In the lower leg (Figure 3) the following muscles were measured: gastrocnemius lateralis

Study-date	Lower leg musculature		Thigh musculature	
	CTRL	RVE	CTRL	RVE
BR1	8[a]	9[b]	6[a,b]	9[c]
BR14	10	8[b]	10	9[b]
BR28	10	8[b]	9[b]	10
BR42	10	8[b]	10	9[b]
BR56	10	9[b]	10	9[b]

[a]Two data sets missing due to MRI scanner failure
[b]Scanning performed but data not appropriate for analysis (e.g. movement artefacts)
[c]Data set missing due to MRI scanner failure
CTRL: control group; RVE: resistive vibration exercise group. BR=day of bed-rest.

Table 1. Number of data sets available for analysis on each study-date.

(GLAT), gastrocnemius medialis (GMED), soleus with flexor hallucis longus (SOL; due to a lack of consistent anatomical landmarks (e.g. fascia) on MRI, soleus was difficult to separate from flexor hallucis longus in a number of subjects. The results of the current study do not change when soleus is considered separately from flexor hallucis longus, thus the data presented is pooled from both muscles), tibialis posterior (TIBP), flexor digitorum longus (FDL), peroneal group (PER; peroneus longus, brevis and tertius), anterior tibial muscles (ANT; tibialis anterior, extensor digitorum longus, extensor hallucis longus). Due to experiments conducted during bed-rest on the right lower-limb[28,29], all image measurements were performed on the left lower-limb only. 33,073 individual manual CSA measurements comprised the final data set, requiring approximately 830 person-hours of image analysis. To enable assessment of changes in entire muscle volume, individual CSA measurements for each muscle were interpolated given an image thickness of 1.0 cm and inter-image distance 0.5 cm. The resulting muscle volume data was used in further analyses.

Data processing and statistical analyses

Linear mixed-effects models[30] were used for each muscle, to fit fixed-effects for study-date, training-group and a study-date×training-group interaction. Baseline subject age, height and weight were included in the model as linear covariates. Random effects for each subject were permitted. Where necessary, allowances were made for heterogeneity of variance (such as due to training-group and/or study-date). Subsequent analysis of variance (ANOVA) examined the significance of each of the factors and the interaction term. An α of 0.05 was taken for statistical significance. Where significant effects were seen, subsequent post-hoc analyses determined which study-days differed from baseline (BDC). As multiple imaging sessions were undertaken on the same subjects, we looked for consistent significant differences across time points. All analyses were performed in the "R" statistical environment (version 2.4.1, www.r-project.org).

Results

Due to issues such as movement artefacts or scanner failure, data sets were not available for analysis for all subjects from every scanning session. Table 1 lists the number of data sets available for analysis in the current work. No differences existed in baseline muscle volume between the groups at baseline scanning (BR1) for any of the muscles (F all<2.73, p all >0.12). Tables 2, 3 and 4 show, respectively, the baseline (BR1) volume of each muscle group in the lower leg and thigh.

Effect of resistive vibration exercise countermeasure – lower leg musculature

For ease of interpretation, changes in muscle volume during bed-rest in the lower leg muscles are expressed as percentage change compared to baseline in Table 2 from BR14 and beyond. ANOVA showed very strong effects for changes in volume in all muscles of the lower leg over the course of the study (study-date: $F_{4,63}$ all ≥3.8, p all ≤.0075). Very strong statistical evidence for an effect of the countermeasure exercise on muscle volume were seen in the gastrocnemius medialis (group: $F_{1,14}$=5.9, p=.029, group×study-date: $F_{4,63}$=6.3, p=.0003) and soleus muscles (group: $F_{1,14}$=1.5, p=.24, group×study-date: $F_{4,63}$=5.9, p=.0004), with the RVE group showing preservation of soleus muscle volume up to the BR42 scanning session with some losses thereafter. Losses in gastrocnemius medialis muscle volume in the RVE group also occurred later (BR28 and beyond) than in the CTRL group but to a lesser extent. A moderate statistical effect (group: $F_{1,14}$=8.3, p=.012, group×study-date: $F_{4,63}$=3.0, p=.0247) was also seen in the gastrocnemius lateralis for an influence of the countermeasure on muscle volume, with no significant changes in muscle volume in the RVE group over the course of the study, but with significant losses in the CTRL group.

In the peroneal (group: $F_{1,14}$=0.9, p=.35, group×study-date: $F_{4,63}$=1.1, p=.36) and tibialis posterior (group: $F_{1,14}$=0.5, p=.49, group×study-date: $F_{4,63}$=2.3, p=.071) muscle groups, little or no evidence existed in ANOVA for an effect of the countermeasure exercise. Inspection of the data in Table 2 shows, however, significant losses of volume in both muscles in the CTRL group, but no significant losses of muscle volume in tibialis posterior in the RVE group. The peroneal muscles showed a much later (BR56 in the RVE group as compared to BR28 in the CTRL group) appearance of statistically significant muscle volume loss. Evidently, a greater number of subjects would be needed to more precisely examine the extent of effectiveness of the countermeasure in these two muscle groups.

In the anterior tibial muscles, no evidence (group: $F_{1,14}$=0.3, p=.60, group×study-date: $F_{4,63}$=0.6, p=.66) existed for an influence of the countermeasure, despite a decrease in muscle volume, with loss of muscle marginally greater in the RVE group. The toe flexor, flexor digitorum longus also showed little evidence of a different response in the RVE group (group: $F_{1,14}$=2.0, p=.18, group×study-date: $F_{4,63}$=1.8, p=.13), though some loss in muscle volume occurred in the CTRL group by the end of bed-rest, with no significant change in the RVE group. The RVE group showed a marginal increase in muscle volume early in bed-rest (BR14) and subsequently no losses in muscle volume with respect to baseline thereafter.

Subject-group	Study-date				
	BR1 (cm³)	BR14	BR28	BR42	BR56
			Anterior Tibial Muscles		
CTRL	256.1(5.1)	-0.7 (1.5)%	-0.8 (1.4)%	-1.2 (1.5)%	-5.1 (1.7)%†
RVE	263.5(4.7)	-1.3 (0.8)%	-3.6 (0.6)%‡	-4.3 (1.4)%†	-6.5 (1.7)%‡
			Flexor Digitorum Longus		
CTRL	30.7(2.5)	+2.9 (1.3)%*	-4.1 (2.1)%	-2.3 (1.6)%	-8.7 (1.8)%‡
RVE	34.8(2.8)	+7.8 (4.2)%	+4.2 (3.1)%	+3.6 (3.4)%	+0.7 (3.4)%
			Gastrocnemius Lateralis		
CTRL	150.5(9.5)	-7.7 (3.8)%*	-11.2 (2.9)%‡	-10.5 (1.8)%‡	-14.4 (2.8)%‡
RVE	177.7(10.3)	+2.7 (2.3)%	-3.3 (2.7)%	-0.1 (2.4)%	-0.2 (3.4)%
			Gastrocnemius Medialis		
CTRL	229.7(16.8)	-9.4 (1.5)%‡	-13.8 (1.6)%‡	-18.1 (1.1)%‡	-22.3 (1.5)%‡
RVE	276.8(18.1)	-1.4 (1.8)%	-6.1 (2.6)%*	-4.6 (1.5)%†	-8.7 (2.1)%‡
			Peroneals		
CTRL	143.5(9.4)	-1.4 (1.6)%	-4.3 (2.0)%*	-7.5 (1.9)%‡	-10.8 (2.2)%‡
RVE	153.4(9.9)	+0.6 (1.3)%	-2.7 (1.5)%	-3.8 (1.8)%*	-5.0 (2.1)%*
			Soleus with Flexor Hallucis Longus		
CTRL	589.9(22.2)	-6.2 (1.8)%†	-9.1 (1.8)%‡	-12.3 (1.8)%‡	-16.5 (1.8)%‡
RVE	588.6(22.9)	+0.9 (1.7)%	-1.5 (1.7)%	-1.1 (1.7)%	-7.2 (1.6)%‡
			Tibialis Posterior		
CTRL	112.9(7.4)	-4.2 (1.5)%†	-6.1 (1.7)%‡	-6.2 (1.5)%‡	-10.2 (1.7)%‡
RVE	115.9(7.7)	+0.8 (1.2)%	-1.0 (1.1)%	-1.5 (1.8)%	-3.2 (1.8)%

At 1st day of bed-rest (BR1) values are mean(SEM) volume in cm³, beyond BR1 values are mean and standard error of the mean percentage change compared to BR1. *: $p<.05$; †: $p<.01$; ‡: $p<.001$ and indicate significance of difference to baseline value. BR= day of bed-rest. Anterior tibial muscles comprise the tibialis anterior, extensor digitorum longus and extensor hallucis longus muscles. No differences between groups existed at baseline (BR1; $F_{1,12}$ all<2.73, p all >0.12)

Table 2. Changes in lower leg muscle volume during bed-rest and effect of countermeasure.

Subject-group	Study-date				
	BR1 (cm³)	BR14	BR28	BR42	BR56
			Anterolateral Tibial Muscles		
CTRL	400.1(12.1)	-1.0 (1.5)%	-2.0 (1.5)%	-3.4 (1.5)%*	-7.2 (1.8)%‡
RVE	416.3(12.0)	-0.6 (0.4)%	-3.4 (0.7)%‡	-4.2 (1.0)%‡	-5.9 (1.8)%†
			Triceps Surae		
CTRL	908.6(38.2)	-7.8 (1.9)%‡	-11.2 (1.8)%‡	-14.4 (1.8)%‡	-18.3 (2.0)%‡
RVE	971.9(37.6)	+0.7 (1.0)%	-3.0 (1.0)%†	-1.9 (0.7)%†	-6.6 (1.8)%‡
			Quadriceps Femoris		
CTRL	2235.1(92.4)	-6.4 (3.5)%	-9.1 (3.4)%*	-12.0 (3.4)%‡	-14.4 (3.5)%‡
RVE	2133.3(63.0)	-1.0 (1.6)%	-1.4 (1.4)%	-1.7 (1.5)%	-3.3 (1.6)%*
			Hamstrings		
CTRL	880.6(42.6)	-6.0 (3.3)%	-6.4 (3.2)%*	-9.3 (3.2)%†	-11.3 (3.1)%‡
RVE	868.1(38.6)	-6.5 (2.4)%†	-8.3 (2.0)%‡	-10.9 (2.0)%‡	-10.9 (2.2)%‡
			Knee Flexors		
CTRL	1176.1(55.7)	-5.3 (3.0)%	-5.0 (3.0)%	-7.6 (3.0)%*	-9.6 (3.0)%†
RVE	1146.1(52.7)	-6.0 (2.5)%*	-7.3 (2.2)%†	-9.5 (2.1)%‡	-9.7 (2.3)%‡
			Adductors		
CTRL	769.6(27.1)	-3.2 (2.4)%	-3.8 (3.0)%	-4.5 (1.8)%*	-5.1 (2.1)%*
RVE	780.6(30.6)	-2.4 (2.7)%	-5.8 (2.4)%*	-5.4 (2.7)%*	-6.9 (2.5)%†

At 1st day of bed-rest (BR1) values are mean(SEM) volume in cm³, beyond BR1 values are mean and standard error of the mean percentage change compared to BR1. *: $p<.05$; †: $p<.01$; ‡: $p<.001$ and indicate significance of difference to baseline value. BR=day of bed-rest. Anterolateral tibial muscles = tibialis anterior, extensor hallucis longus, extensor digitorum longus, peroneus longus, brevis and tertius; triceps surae= soleus, gastrocnemius medials and lateralis; quadriceps= vastii, rectus femoris, hamstrings= biceps femoris (short and long heads), semitendinosus, semimembranosus; knee flexors= hamstrings with gracilis and sartorious; Adductors= adductor magnus and longus.

Table 3. Changes in muscle volume in different muscle groups.

Subject-group	Study-date				
	BR1 (cm³)	BR14	BR28	BR42	BR56
			Adductor Longus		
CTRL	181.7(11.8)	+2.7 (3.7)%	+0.4 (3.6)%	+0.5 (3.2)%	+0.8 (3.1)%
RVE	192.9(11.5)	-1.2 (2.2)%	-3.4 (1.6)%*	-1.2 (2.0)%	-3.1 (2.6)%
			Adductor Magnus		
CTRL	588.7(22.5)	-5.1 (2.8)%	-5.0 (3.6)%	-6.2 (2.3)%*	-7.0 (2.6)%*
RVE	586.6(25.0)	-2.5 (3.2)%	-6.3 (2.9)%*	-6.5 (3.2)%*	-7.8 (2.9)%†
			Gracilis		
CTRL	118.4(7.0)	-2.9 (2.2)%	-2.7 (2.3)%	-4.0 (2.2)%	-4.4 (2.2)%*
RVE	114.2(7.2)	-3.9 (2.4)%	-3.8 (1.9)%	-4.9 (1.6)%†	-5.0 (2.0)%*
			Sartorius		
CTRL	177.7(10.1)	-3.8 (3.0)%	-0.7 (3.0)%	-2.1 (2.7)%	-4.9 (3.3)%
RVE	164.3(10.9)	-5.1 (3.5)%	-4.8 (3.4)%	-5.4 (3.4)%	-6.3 (3.5)%
			Biceps Femoris Long Head		
CTRL	232.8(16.1)	-5.2 (5.6)%	-6.7 (5.6)%	-10.2 (5.5)%	-12.5 (5.5)%*
RVE	229.0(12.0)	-6.0 (3.0)%*	-9.4 (2.5)%‡	-12.3 (2.6)%‡	-13.3 (2.7)%‡
			Biceps Femoris Short Head		
CTRL	123.7(8.4)	-3.8 (3.1)%	-2.1 (3.0)%	-3.3 (2.7)%	-7.3 (3.0)%*
RVE	119.2(8.3)	-3.6 (3.1)%	-1.8 (1.4)%	-4.8 (0.9)%‡	-3.7 (1.8)%*
			Semimembranosus		
CTRL	273.6(11.5)	-6.5 (2.4)%†	-6.5 (2.4)%†	-11.1 (1.4)%‡	-12.3 (1.3)%‡
RVE	274.2(15.8)	-7.5 (3.8)%	-10.1 (3.7)%†	-14.3 (3.7)%‡	-13.8 (4.0)%†
			Semitendinosus		
CTRL	250.1(16.8)	-6.5 (4.9)%	-7.6 (4.9)%	-8.7 (4.9)%	-10.4 (4.9)%*
RVE	240.2(12.9)	-5.5 (2.3)%*	-6.9 (2.1)%†	-7.7 (1.6)%‡	-7.8 (1.8)%‡
			Rectus Femoris		
CTRL	318.2(19.8)	-4.1 (3.5)%	-2.7 (3.5)%	-2.9 (3.4)%	-5.1 (3.5)%
RVE	311.5(18.7)	+1.8 (2.2)%	+3.7 (1.7)%*	+4.6 (2.0)%*	+4.0 (2.1)%
			Vastii		
CTRL	1914.5(83.1)	-6.7 (3.7)%	-9.9 (3.6)%†	-13.3 (3.5)%‡	-15.9 (3.7)%‡
RVE	1822.4(57.7)	-1.5 (1.6)%	-2.4 (1.5)%	-2.8 (1.5)%	-4.6 (1.6)%†

At 1st day of bed-rest (BR1) values are mean (SEM) volume in cm³, beyond BR1 values are mean and standard error of the mean percentage change compared to BR1. *: $p<.05$; †: $p<.01$; ‡: $p<.001$ and indicate significance of difference to baseline value. BR= day of bed-rest. No differences between groups existed at baseline (BR1; $F_{1,9}$ all<1.76, p all >0.22).

Table 4. Changes in thigh muscle volume during bed-rest and effect of countermeasure.

To facilitate comparison to other studies, the muscles measured were grouped together into larger muscle groups. Both the triceps surae and anterolateral muscle groups showed significant losses of volume over time (study-date: $F_{4,63}$ both >15.8, p both <.0001), though this reached statistical significance in triceps surae from BR14 and in the anterolateral tibial muscles from BR42 (Table 3). The triceps surae (group: $F_{1,14}$=7.9, p=.013, group×study-date: $F_{4,63}$=25.9, p<.0001) but not the anterolateral tibial muscles (group: $F_{1,14}$=1.49, p=.24, group×study-date: $F^{4,63}$=.53, p=.71) showed statistical evidence for an effect of the countermeasure exercise.

Effect of resistive vibration exercise countermeasure – thigh musculature

Of the thigh musculature, the vastii, adductor magnus, semimembranosus, semitendinosus, biceps femoris (long and short heads) and gracilis all showed strong evidence for changes in muscle volume during bed-rest (study-date: $F_{4,62}$ all >5.08, p all ≤.0013) with reductions in muscle volume seen in the CTRL group during bed-rest (Table 4). Of these muscles, only the vastii demonstrated strong statistical evidence for an different response in the RVE group (group: $F_{1,15}$=2.63, p=.13, group×study-date: $F_{4,62}$=30.7, p<.0001), where losses in muscle volume in the RVE only reached statistical significance at the end of bed-rest (BR56), but were evident in the CTRL group from bed-rest (BR28). Of the other muscles, the RVE group appeared to show losses in muscle volume slightly earlier in the biceps femoris (long and short heads), semitendinosus, semimembranosus, adductor magnus and gracilis, though these differences were not statistically significant (group: $F_{1,15}$ all <2.28, p all >.15, group×study-date: $F_{4,62}$ all <1.1, p all >.38).

In both the adductor longus and sartorius muscles, little statistical evidence existed for changes in muscle volume during bed-

	Day of bed-rest (BR), head-down tilt (HDT) or lower-limb suspension (ULLS)											
Muscle	BR14	14d HDT Zange 2008	20d HDT Akima 2001	20d HDT Akima 2007	BR28	29d HDT Alkner 2004	30d HDT Berry 1993	35d ULLS Tesch 2004	BR42	BR56	89d HDT Alkner 2004	119d BR Shackelford 2004
ANT	-1.3%		-9.4%	-2.6%	-3.6%‡		-7.6%*		-4.3%†	-6.5%‡		
PER	+0.6%				-2.7%				-3.8%*	-5.0%*		
FDL	+7.8%				+4.2%				+3.6%	+0.7%		
GLAT	+2.7%	-5.5%	-16.8%†	+1.3%	-3.3%		-6.1%*		-0.1%	-0.2%		-9.2%
GMED	-1.4%	-7.3%*	-12.3%†	-1.1%	-6.1%*		-8.6%*		-4.6%†	-8.7%‡		
SOL	+0.9%	-5.8%	-9.8%†	-5.4%	-1.5%		-12.6%*		-1.1%	-7.2%‡		-6.8%
TS	+0.7%	-6.4%*	-12.2%‡	-3.1%	-3.0%†	-8.0%*	-10.2%*	-11.1%*	-1.9%†	-6.6%‡	-15.0%*	
TIBP	+0.8%				-1.0%				-1.5%	-3.2%		
AL	-1.2%			-2.4%	-3.4%*				-1.2%	-3.1%		
AM	-2.5%			-0.2%	-6.3%*				-6.5%*	-7.8%†		
ADD	-2.4%			-0.7%	-5.8%*				-5.4%*	-6.9%†		-3.4%
GRAC	-3.9%		+3.4%	-1.3%	-3.8%				-4.9%†	-5.0%*		
SART	-5.1%		-3.4%	-4.5%	-4.8%				-5.4%	-6.3%		+4.8%
BFL	-6.0%*		+1.4%	-3.1%	-9.4%‡				-12.3%‡	-13.3%‡		
BFS	-3.6%		-4.6%	+0.3%	-1.8%				-4.8%‡	-3.7%*		
SEMI_M	-7.5%		-5.9%	-7.1%	-10.1%†				-14.3%‡	-13.8%†		
SEMI_T	-5.5%*		-5.9%	-0.9%	-6.9%†				-7.7%‡	-7.8%‡		
HAMS	-6.5%†	-6.1%*			-8.3%‡				-10.9%‡	-10.9%‡		-8.1%
KF	-6.0%*		-3.0%*	-3.4%	-7.3%†				-9.5%‡	-9.7%‡		
RF	+1.8%	-3.6%*	-2.2%	+3.2%	+3.7%*	NS	-12.0%*	+16.7%*	+4.6%*	+4.0%	-9.0%*	
V	-1.5%	-7.0%*	7.4%	0.6%	-2.4%	NS			-2.8%	-4.6%†	NS	
QUADS	-1.0%	-6.6%*	+6.0%‡	+1.0%	-1.4%	NS	-11.0%*	+7.7%*	-1.7%	-3.3%*	NS	+5.2%

ANT: anterior tibial muscles (tibialis anterior, extensor digitorum longus, extensor hallucis longus), PER: peroneals (peroneus longus, brevis and tertius), FDL: flexor digitorum longus, GLAT: gastrocnemius lateralis, GMED: gastrocnemius medialis, SOL: soleus (with flexor hallucis longus in current work), TS: triceps surae, TIBP: tibialis posterior, AL: adductor longus, AM: adductor magnus, ADD: adductors combined, GRAC: gracilis, SART: sartorius, BFL: biceps femoris long head, BFS: biceps femoris short head, SEMI_M: semimembranosus, SEMI_T: semitendinosus, RF: rectus femoris, V: vastii, QUADS: quadriceps femoris. *: $p<.05$; †: $p<.01$; ‡: $p<.001$ indicate significance of difference to baseline value; NS indicates non-significant difference to baseline value (percentage change not reported or volume values reported in graphical form). Vastii data from Akima 2001 and 2007 summarized from reported pre- and post volumes of individual heads of the vastii. *P*-values not reported in male training subjects by Shackelford et al (2004). Data for men and women not reported separately by Tesch et al (2004). BR: bed-rest; HDT: head-down tilt bed-rest; ULLS: unilateral lower-limb suspension.

Table 5. Percentage changes in muscle size in the countermeasure group in the current and other studies.

rest or an effect of the countermeasure (study-date: $F_{4,62}$ both <1.7, p both >.15; group: $F_{1,15}$ both <1.8, p both >.20; group×study-date: $F_{4,62}$ both <.66, p both >.62; Table 4), but when data were pooled between groups, analysis suggested that some atrophy of sartorius did occur, though this was marginal and did not reach statistical significance until BR56 (-8.4[3.9]cm^3; p=.033). Similarly, in the rectus femoris muscle, little evidence existed for changes during bed-rest or an effect of the countermeasure (study-date: $F_{4,62}$=1.5, p=.22; group: $F_{1,15}$=.35, p=.56; group×study-date: $F_{4,62}$=1.4, p=.25), but inspection of the data in Table 4 shows a subtle (but non-significant) decrease in volume of rectus femoris in the CTRL group. The RVE group, in contrast, demonstrated increased volume throughout the bed-rest phase, though this was statistically significant only at BR28 and BR42.

To facilitate comparison to other studies, we combined muscles into differing groups (Table 3). All muscle groups (adductors, hamstrings, knee flexors, quadriceps femoris) showed significant changes over the course of the study (study-date: $F_{4,62}$ all >4.9, p all ≤.0017), with losses in muscle volume in all muscle groups. Statistical evidence existed for a different response of the RVE group for the quadriceps muscle group only (group: $F_{1,15}$=.83, p=.37; group×study-date: $F_{4,62}$=8.0, p<.0001), with the increases in rectus femoris volume tending to mask vastii volume loss. To further facilitate comparison to other studies, the changes in muscle volume in the current and other studies are presented in Table 5.

Discussion

The main findings of the current study were that a high-load resistive vibration countermeasure with exercises comprising heel raises, squats and toe raises was most effective in reducing atrophy of the soleus, medial gastrocnemius and vastii muscles during prolonged bed-rest, with losses in volume of these muscles occurring later and to a much lesser extent than in the control group. The exercise countermeasure also prevented

significant changes in muscle volume of the lateral gastrocnemius muscle. Additionally, muscle volume of the tibialis posterior and flexor digitorum longus was stable in the RVE group, with losses seen in the CTRL group. Reductions in peroneal muscle volume occurred later in the RVE group and the rectus femoris muscle actually increased in volume in the RVE group, with no change in the CTRL group. The effects in the tibialis posterior, flexor digitorum longus, peroneal and rectus femoris muscles were, however, statistically weak. Little evidence existed for an effect of the countermeasure on the four members of the hamstrings group, adductor magnus and gracilis, with significant losses in muscle volume occurring in both subject groups. Bed-rest itself had little or no effect on the adductor longus, sartorius and anterior tibial muscles.

The strongest effect of the countermeasure on the extensors of the ankle and knee attests to the use of squats and heel raise exercises - i.e. exercises to target these specific muscle groups. In contrast, the hip extensors (hamstrings and adductor magnus) were unaffected by the countermeasure, and no exercises were specifically performed for these muscles. In other bed-rest and related studies where countermeasure exercise comprised only squat or leg-press exercise (such as on a Cybex[23] or Flywheel device[9,10,31]; see Table 5) and the calf muscles were not targeted directly, the retention of calf muscle volume was much less effective than in works where exercises also targeted this muscle group (such as the current study and in other works[11,13]). In contrast to the current work, Akima and colleagues[13] showed an effect of their leg-press and plantarflexor exercises on reducing hamstrings atrophy in bed-rest. In walking, the semimembranosus and biceps femoris typically activate to decelerate the lower-limb towards the end of the swing phase and are also active in initial load-bearing[1] (i.e. they contribute to a hip extension moment in a position of hip flexion). A squat-type or combined hip-knee extension exercise from a more flexed hip position could activate the different members of the hamstring muscles to a greater degree[32,33], and this may be appropriate modification to countermeasure exercise in bed-rest. It should, however, be remembered that the gluteal muscles are also important extensors of the hip (particularly the inferior portion of gluteus maximus such as at heel-strike and mid-stance in walking or in stair ascension)[1]. The gluteal muscles do atrophy in bed-rest[34], but as yet, no work has considered the differential effect of bed-rest on the hip extensors (i.e. gluteal muscles and hamstrings) and it should also be noted that different rates of atrophy are apparent amongst the four members of the hamstrings muscle group during bed-rest[22]. Thus, gaining a deeper understanding the effect of bed-rest/spaceflight on the hip extensors as a whole (and not just hamstring muscles) is important for further countermeasure development.

It is worthy to note that although the countermeasure group showed a similar volume loss in the anterior tibial muscles, the exercises in the current study targeted at this muscle group (toe raises) were conducted against low resistance. Whilst the addition of more resistance to the dorsiflexion exercises may result in a greater effect of exercise, this muscle group was also little affected by bed-rest (in the inactive group)[22] and given this lesser susceptibility, the priorities of countermeasure exercise may be better set at exercises for other, more susceptible muscles (e.g. hip extensors[22] or more proximal systems such as the lumbar extensors[35,36]).

Zange and co-workers[8] also implemented whole-body vibration as part of countermeasure exercise for the lower-limb musculature during bed-rest. Compared to the current work, after 14-days of bed-rest their countermeasure subjects exhibited a relatively greater extent of atrophy, in all of the lower-limb muscle groups except the hamstrings (see Table 5). It is noteworthy that the countermeasure by Zange and co-workers was performed in standing with only an addition of 15% of body-weight and no muscle-specific exercises being performed (i.e. the subjects purely retained their position). It is likely therefore, that this kind of training stimulus was insufficient for the lower-limb muscles. Interestingly, another work, examining vibration exercise at the lumbar spine using static loading at approximately 60% of body-weight found no effect of the countermeasure on losses of lumbar extensor muscle cross-sectional area during bed-rest[37]. These findings[8,37] are in line with the argument that higher-load muscle specific exercises are needed if individual muscle size is to be retained. In our opinion, whole body vibration during resistive exercise may help to provide an additional stimulus, above that of resistive exercise alone, for muscle activity and force development and retention of muscle mass during bed-rest. It is important to note that the current study was not intended to examine the extent of any additional benefit of whole-body vibration on muscle loss during bed-rest *per se*, but rather to examine the efficacy of the countermeasure as a whole. Further work is necessary to examine any additive effect of whole-body vibration during resistance exercise.

Deconditioning during spaceflight is not localised to the musculoskeletal system and a variety of body systems (e.g. cardiovascular, vestibular) require countermeasures of their own. Aerobic exercise (such as cycling or lower-body negative pressure) is considered appropriate for the maintenance of the cardiovascular system, but such exercise is ineffective in maintaining muscle[6,7,38] or bone[39-41]. A combination of approaches is therefore needed. Astronauts, however, do not have an indefinite amount of time available for exercises and priorities of countermeasure exercise need to be set such that the available time is used most effectively.

In our opinion, there are four key aspects for countermeasure design to ensure the most effective use of exercise time for preventing muscle atrophy in the lower-limbs: 1) which muscles are indeed affected by bed-rest and/or spaceflight? 2) What exercise manoeuvres need to be implemented to target these muscles in the most time effective fashion? 3) What loading levels should be used? 4) What "dose" of exercise (number of times per day or week, duration of exercises) is necessary? In the current study, the muscles that were most affected (>10% loss of volume at the end of bed-rest in the inactive group) were all members of triceps surae, the vastii, long head biceps femoris, semitendinsosus, semimembranosus, tibialis

posterior and the peroneals (see also Belavý and colleagues[22]). An isolated knee extension exercise would be effective for the vastii muscles, but this would also stimulate the rectus femoris[32,33,42], which is much less affected in bed-rest[22], and potentially lead to the development of muscle imbalances. Modified leg-press exercises (combined hip and knee extension), on the other hand, target the vastii, with less activation of the rectus femoris[32,33,42], but also require contribution from the hamstrings and adductor magnus[32,33,42], thus comprising a more time effective exercise. Calf raises, when appropriately performed, can also be effective in stimulating the peroneal and tibialis posterior muscles groups[43], though care needs to be taken in exercise selection for calf raises as knee position can markedly modify the contribution of the gastrocnemius and soleus muscles[44]. Controlling the posture of the arch of the foot appears to be an important factor for optimal activation of the tibialis posterior muscle[44,45], and this should also be incorporated into exercise. Exercises to target the muscles of the hip and lumbar spine should also be considered[34,35,37,46,47].

Furthermore, based upon the results of the current and prior work, it appears that high (between 75-85% of a person's 1-repetition maximum) loading levels[9-14] during exercise are better at maintaining muscular mass and function than low-load or endurance exercise[6-8]. This does not imply that low-load exercise has no role to play in countermeasure exercise programmes for maintenance of muscle in bed-rest/spaceflight. In the current work, rectus femoris muscle volume increased whilst vastii volume decreased in the countermeasure exercise group. This could imply the development of muscle imbalance due to exercise. Some subjects did report the development of knee pain during training[24], which could imply innapropraite loading and/or muscle activation. One particular benefit of including low-load exercises could be to ensure that the individual can perform the exercises using the correct technique and optimal/appropriate body posture prior to progressing the exercises to a higher-load. This may help to avoid the development of muscle imbalances due to inappropriate movement/loading patterns. However, future work will have to consider this in more detail.

Finally, the frequency (per day or per week) with which exercise countermeasures should be performed during bed-rest/spaceflight is a topic which still requires further study. Works by Alkner and Tesch[9,10] have shown that resistive exercise on a relatively infrequent schedule (every three days) is capable of reducing lower-limb muscle atrophy during bed-rest (Table 5). Further research on exercise dose during bed-rest could provide valuable information to optimise the efficiency of countermeasure exercises.

It should be noted that in the current work we considered only muscle size when evaluating the effectiveness of the countermeasure exercises. Other aspects of muscle function, such as muscle activation, proprioception and postural control were not evaluated. Whilst it may be possible to argue that changes in muscle size do correlate with changes in muscle force production capacity[28,48], other aspects of muscle function, particularly postural control, are dependent upon integration of information from a number of systems (e.g. vestibular, proprioceptive, visual)[49] which may need to be addressed using additional countermeasures.

In conclusion, we examined the effect of a resistive vibration exercise countermeasure comprised of squats, heel raises and toe raises during 56-days of bed-rest on muscle volume loss in the lower-limbs. We found the countermeasure to be most effective in reducing or preventing atrophy in the three heads of triceps surae and the vastii muscles. Conversely, the countermeasure was comparatively ineffective for the hamstring and thigh adductor muscles. Countermeasure design needs to consider which muscular systems are most affected by bed-rest and also ensure that other body systems (e.g. cardiovascular) are trained. Further work is necessary to evaluate the optimal exercise "dose" (per day / per week) for a time- and cost-effective intervention.

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References

1. Lyons K, Perry J, Gronley JK, Barnes L, Antonelli D. Timing and relative intensity of hip extensor and abductor muscle action during level and stair ambulation. An EMG study. Phys Ther 1983;63:1597-605.
2. Montgomery WH III, Pink M, Perry J. Electromyographic analysis of hip and knee musculature during running. Am J Sports Med 1994;22:272-8.
3. Winter DA, Yack HJ. EMG profiles during normal human walking: stride-to-stride and inter-subject variability. Electroencephalogr Clin Neurophysiol 1987;67:402-11.
4. Pavy-Le Traon A, Heer M, Narici MV, Rittweger J, Vernikos J. From space to Earth: advances in human physiology from 20 years of bed rest studies (1986-2006). Eur J Appl Physiol 2007;101:143-94.
5. Komi PV. Strength and Power in Sport. Oxford: Blackwell Scientific; 1992.
6. Suzuki Y, Kashihara H, Takenaka K, Kawakubo K, Makita Y, Goto S, et al. Effects of daily mild supine exercise on physical performance after 20 days bed rest in young persons. Acta Astronaut 1994;33:101-11.
7. Berry P, Berry I, Manelfe C. Magnetic-resonance-imaging evaluation of lower-limb muscles during bed rest - a microgravity simulation-model. Aviat Space Environ Med 1993;64:212-8.

8. Zange J, Mester J, Heer M, Kluge G, Liphardt AM. 20-Hz whole body vibration training fails to counteract the decrease in leg muscle volume caused by 14 days of 6 degrees head down tilt bed rest. Eur J Appl Physiol 2008;105:271-7.
9. 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;181:345-57.
10. 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(3):294-305.
11. Shackelford LC, LeBlanc AD, Driscoll TB, Evans HJ, Rianon NJ, Smith SM, et al. Resistance exercise as a countermeasure to disuse-induced bone loss. J Appl Physiol 2004;97:119-29.
12. 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;82:30-8.
13. Akima H, Ushiyama J, Kubo J, Fukuoka H, Kanehisa H, Fukunaga T. Effect of unloading on muscle volume with and without resistance training. Acta Astronaut 2007;60:728-36.
14. Kouzaki M, Masani K, Akima H, Shirasawa H, Fukuoka H, Kanehisa H, et al. Effects of 20-day bed rest with and without strength training on postural sway during quiet standing. Acta Physiol (Oxf) 2007;189(3):279-92.
15. Cardinale M, Bosco C. The use of vibration as an exercise intervention. Exerc Sport Sci Rev 2003;31:3-7.
16. Roelants M, Verschueren SM, Delecluse C, Levin O, Stijnen V. Whole-body-vibration-induced increase in leg muscle activity during different squat exercises. J Strength Cond Res 2006;20:124-9.
17. Ribot-Ciscar E, Butler JE, Thomas CK. Facilitation of triceps brachii muscle contraction by tendon vibration after chronic cervical spinal cord injury. J Appl Physiol 2003;94:2358-67.
18. Roll JP, Vedel JP, Ribot E. Alteration of proprioceptive messages induced by tendon vibration in man: a microneurographic study. Exp Brain Res 1989;76:213-22.
19. 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;23:428-32.
20. Mischi M, Kaashoek I. Electromyographic hyperactivation of skeletal muscles by time-modulated mechanical stimulation. In: Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society; 2007; p. 5373-6.
21. Rubin C, Pope M, Fritton JC, Magnusson M, Hansson T, McLeod K. Transmissibility of 15-hertz to 35-hertz vibrations to the human hip and lumbar spine: determining the physiologic feasibility of delivering low-level anabolic mechanical stimuli to skeletal regions at greatest risk of fracture because of osteoporosis. Spine 2003; 28:2621-7.
22. Belavý DL, Miokovic T, Armbrecht G, Richardson CA, Rittweger J, Felsenberg D. Differential atrophy of the lower-limb musculature during prolonged bed-rest. Eur J Appl Physiol 2009;107:489-99.
23. Akima H, Kubo K, Imai M, Kanehisa H, Suzuki Y, Gunji A, et al. Inactivity and muscle: effect of resistance training during bed rest on muscle size in the lower limb. Acta Physiol Scand 2001;172:269-78.
24. Rittweger J, Belavy DL, Hunek P, Gast U, Boerst H, Feilcke B, et al. Highly demanding resistive vibration exercise program is tolerated during 56 days of strict bed-rest. Int J Sport Med 2006;27:553-9.
25. Blottner D, Salanova M, Puttmann B, Schiffl G, Felsenberg D, Buehring B, et al. Human skeletal muscle structure and function preserved by vibration muscle exercise following 55 days of bed rest. Eur J Appl Physiol 2006;97:261-71.
26. Gabriel DA, Kamen G, Frost G. Neural adaptations to resistive exercise: mechanisms and recommendations for training practices. Sports Med 2006;36:133-49.
27. Frost HM. From Wolff's law to the Utah paradigm: insights about bone physiology and its clinical applications. Anat Rec 2001;262:398-419.
28. Mulder ER, Stegeman DF, Gerrits KH, Paalman MI, Rittweger J, Felsenberg D, et al. 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;97:706-15.
29. Belavý DL, Richardson CA, Wilson SJ, Rittweger J, Felsenberg D. Superficial lumbo-pelvic muscle overactivity and decreased co-contraction after 8-weeks of bed-rest. Spine 2007;32:E23-29.
30. Pinheiro JC, Bates DM. Mixed-effects models in S and S-PLUS. 1st ed. Berlin: Springer; 2000.
31. Tesch PA, Trieschmann JT, Ekberg A. Hypertrophy of chronically unloaded muscle subjected to resistance exercise. J Appl Physiol 2004;96:1451-8.
32. Tesch PA. Target Bodybuilding. Champaign, IL: Human Kinetics Publishers; 1999.
33. Richardson RS, Frank LR, Haseler LJ. Dynamic knee-extensor and cycle exercise: functional MRI of muscular activity. Int J Sports Med 1998;19:182-7.
34. Berg HE, Eiken O, Miklavcic L, Mekjavic IB. Hip, thigh and calf muscle atrophy and bone loss after 5-week bedrest inactivity. Eur J Appl Physiol 2007;99:283-9.
35. Belavý DL, Hides JA, Wilson SJ, Stanton W, Dimeo FC, Rittweger J, et al. Resistive simulated weightbearing exercise with whole body vibration reduces lumbar spine deconditioning in bed-rest. Spine 2008;33:E121-E131.
36. Hides JA, Belavý DL, Stanton W, Wilson SJ, Rittweger J, Felsenberg D, et al. MRI assessment of trunk muscles during prolonged bed rest. Spine 2007;32:1687-1692.
37. Holguin N, Muir J, Rubin C, Judex S. Short applications

of very low-magnitude vibrations attenuate expansion of the intervertebral disc during extended bed rest. Spine J 2009;9:470-7.
38. Berry P, Berry I, Manelfe C. Magnetic-Resonance-Imaging Evaluation of Lower-Limb Muscles During Bed Rest - a Microgravity Simulation-Model. Aviat Space Environ Med 1993;64:212-8.
39. Oganov VS, Grigoriev AI, Voronin LI, Rakmanov AS, Bakulin AV, Schneider V, et al. Bone mineral density in cosmonauts after 4.5-6 month long flights aboard orbital station MIR. Aerospace Environ Med 1992;26:20-4.
40. Le Blanc A, Schneider V, Shackelford L, West S, Oganov V, Bakulin A, et al. Bone mineral and lean tissue loss after long duration space flight. J Musculoskelet Neuronal Interact 2000;1:157-60.
41. Le Blanc A, Lin C, Shackelford L, Sinitsyn V, Evans H, Belichenko O, et al. Muscle volume, MRI relaxation times (T2), and body composition after spaceflight. J Appl Physiol 2000;89:2158-64.
42. Enocson AG, Berg HE, Vargas R, Jenner G, Tesch PA. Signal intensity of MR-images of thigh muscles following acute open- and closed chain kinetic knee extensor exercise - index of muscle use. Eur J Appl Physiol 2005;94:357-63.
43. Kulig K, Burnfield JM, Requejo SM, Sperry M, Terk M. Selective activation of tibialis posterior: evaluation by magnetic resonance imaging. Med Sci Sports Exerc 2004;36:862-7.
44. Price TB, Kamen G, Damon BM, Knight CA, Applegate B, Gore JC, et al. Comparison of MRI with EMG to study muscle activity associated with dynamic plantar flexion. Magn Reson Imaging 2003;21:853-61.
45. Kaye RA, Jahss MH. Tibialis posterior: a review of anatomy and biomechanics in relation to support of the medial longitudinal arch. Foot Ankle 1991;11:244-7.
46. Cao P, Kimura S, Macias BR, Ueno T, Watenpaugh DE, Hargens AR. Exercise within lower body negative pressure partially counteracts lumbar spine deconditioning associated with 28-day bed rest. J Appl Physiol 2005; 99:39-44.
47. Macias BR, Cao P, Watenpaugh DE, Hargens AR. LBNP treadmill exercise maintains spine function and muscle strength in identical twins during 28-days simulated microgravity. J Appl Physiol 2007;102:2274-8.
48. 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.
49. Massion J. Postural control system. Curr Opin Neurobiol 1994;4:877-87.

Paper 5:

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Miokovic T, Armbrecht G, Felsenberg D, Belavý DL (2011): Differential atrophy of the postero-lateral hip musculature during prolonged bed-rest and the influence of exercise countermeasures. *Journal of Applied Physiology* 110(4): 926-934.

Belavý DL, **Miokovic T**, Rittweger J, Felsenberg D (2010): Estimation of changes in volume of individual lower-limb muscles using magnetic resonance imaging (during bed-rest). *Physiological Measurement*, 32(1): 35-50.

Mulder ER, Horstman AM, Stegeman DF, de Haan A, Belavý DL, **Miokovic T**, Armbrecht G, Felsenberg D, Gerrits KH (2009): Influence of vibration resistance training on knee extensor and plantar flexor size strength, and contractile speed characteristics after 60 days of bed rest. *Journal of Applied Physiology* 107: 1789-1798.

Belavý DL, **Miokovic T**, Armbrecht G, Rittweger J, Felsenberg D (2009): Resistive vibration exercise reduces lower limb muscle atrophy during 56-day bed-rest. *Journal of Musculoskeletal and Neuronal Interactions* 9(4):225-235.

Belavý DL, **Miokovic T**, Armbrecht G, Richardson CA, Rittweger J, Felsenberg D (2009): Differential atrophy of the lower-limb musculature during prolonged bed-rest. *European Journal of Applied Physiology* 107(4):489-499.

Hides JA, **Miokovic T**, Belavý DL, Stanton WR, Richardson CA (2007): Ultrasound imaging assessment of abdominal muscle function during drawing-in of the abdominal wall: an intrarater reliability study. *Journal of Orthopaedic and Sports Physical Therapy*; 37(8): 480-6

Conference Presentations:

Miokovic T, Belavý DL, Ambrecht G, Felsenberg D. Atrophy of the calf muscles during 60 days bed-rest and the effect of two different countermeasure exercises. *International Society for Gravitational Physiology: Life in Space for Life on Earth Symposium. Aberdeen, Scotland, UK, 18-22 June, 2012*

Miokovic T, Belavý DL, Ambrecht G, Felsenberg D. Inter- and Intramuscular atrophy of the calf musculature during 60 days of bed rest. *International Society of Musculoskeletal and Neuronal Interactions. Ispwich, Suffolk, UK, 4-7 May, 2012*

Miokovic T, Belavý DL, Ambrecht G, Felsenberg D. The second Berlin Bed Rest Study-differential atrophy of the hip and thigh musculature during 60 days of bed rest and influence of countermeasures. *International Society for Gravitational Physiology: Life in Space for Life on Earth Symposium, Trieste, Italy, 13-18 June, 2010*

Miokovic T, Belavý DL, Ambrecht G, Felsenberg D. Differential atrophy of the posterolateral hip musculature during 60 days of Bed Rest. *Knochen und Muskeln. Charite, Bejnamin Franklin Campus, Berlin, Germany. March, 2009*

Felsenberg D, **Miokovic T**. Muscle/Bone Adaptation in Weightlessness. *International Scoiety of Musculoskeletal and Neuronal Interactions. Cologne, 20-23 March, 2008*

Hides JA, **Miokovic T**, Belavý DL, Stanton WR, Richardson CA. Ultrasound imaging assessment of abdominal muscle function during drawing-in of the abdominal wall: an intrarater reliability study. *Australian Physiotherapy Association Conference Week. Cairns, 4-8 October, 2007*

Poster Presentations

Miokovic T, Belavy DL, Armbrecht G, Rittweger J, Felsenberg D. Resistive vibration exercise reduces lower limb muscle atrophy during 56-day bed-rest. *2nd Joint Meeting of the International Bone & Mineral Society and the Australian & New Zealand Bone & Mineral Society. Sydney, 21-25 March, 2009*

Miokovic T, Belavy DL, Armbrecht G, Richardson CA, Rittweger J, Felsenberg D. Differential atrophy of the lower-limb musculature during prolonged bed-rest: implications for the management of the immobilised patient. *2nd Joint Meeting of the International Bone & Mineral Society and the Australian & New Zealand Bone & Mineral Society. Sydney, 21-25 March, 2009*

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