

Limited effect of fly-wheel and spinal mobilization exercise countermeasures on lumbar spine deconditioning during 90 d bed-rest in the Toulouse LTBR study

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ABSTRACT

We examined the effect of high-load fly-wheel (targeting the lower-limb musculature and concurrent loading of the spine via shoulder restraints) and spinal movement countermeasures against lumbar spine muscle atrophy, disc and spinal morphology changes and trunk isokinetic torque loss during prolonged bed-rest. Twenty-four male subjects underwent 90 d head-down tilt bed-rest and performed either fly-wheel (FW) exercises every three days, spinal movement exercises in lying five times daily (SpMob), or no exercise (Ctrl). There was no significant impact of countermeasures on losses of isokinetic trunk flexion/extension ($p \geq 0.65$). Muscle volume change by day-89 of bed-rest in the psoas, iliacus, lumbar erector spinae, lumbar multifidus and quadratus lumborum, as measured via magnetic resonance imaging (MRI), was statistically similar in all three groups ($p \geq 0.33$). No significant effect on MRI-measures of lumbar intervertebral disc volume, spinal length and lordosis ($p \geq 0.09$) were seen either, but there was some impact ($p \leq 0.048$) on axial plane disc dimensions (greater reduction than in Ctrl) and disc height (greater increases than in Ctrl). MRI-data from subjects measured 13 and 90-days after bed-rest showed partial recovery of the spinal extensor musculature by day-13 after bed-rest with this process complete by day-90. Some changes in lumbar spine and disc morphology parameters were still persistent 90-days after bed-rest. The present results indicate that the countermeasures tested were not optimal to maintain integrity of the spine and trunk musculature during bed rest.

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Abbreviations: CSA, cross-sectional area; MRI, magnetic resonance imaging; SpMob, spinal mobilization group; Ctrl, inactive control group; FW, Fly-wheel exercise group; LTBR study, 90 d Long Term Bed Rest study conducted in 2001 and 2002 at MEDES Toulouse France; R+, day of post-bed-rest recovery; HDT, day of head down tilt bed-rest; BDC, baseline data collection; ANOVA, analysis of variance; Ps, psoas muscle; QL, quadratus lumborum muscle; ES, erector spinae muscles; MF, multifidus muscle; IL, iliacus muscle

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1. Introduction

In recent years, there has been increased study of the effect of exercise countermeasures against lumbar spine deconditioning during prolonged bed-rest [1–6], a methodology used to simulate the effect of spaceflight on the human body [7]. Low back pain has been shown to occur in astronauts and cosmonauts in the initial adaptation period to spaceflight [8] and recent data has shown significantly increased incidence of intervertebral disc

herniation in astronauts in the period after spaceflight [9]. In bed-rest, low back pain typically occurs in the first few days of bed-rest [10], but recent work has also shown increased incidence in the first few days after the end of bed-rest [4,11]. Works studying the effect of countermeasures in bed-rest [1–6] have shown losses in lumbar extensor muscle size, reductions in force generation capacity, increases in disc volume and disc height, spinal lengthening and altered response of the intervertebral disc shape to loading. There is evidence to suggest that these kinds of changes are linked to low back pain occurring after bed-rest [4,11], and hence possibly to disc herniation in astronauts: the extent of muscular atrophy and intervertebral disc changes *during* prolonged bed-rest is associated with the occurrence of low back pain *after* bed-rest [4,11]. An underlying idea in the prevention of spinal injury is that the muscle system is, under guidance of the neural controlling system, a major factor in determining the character and extent of loads imposed on passive soft tissue structures, such as the intervertebral discs [12]. Thus, studying both the musculature and soft tissue structures of the lumbar spine is relevant for countermeasure research. Whilst there has been increased attention to countermeasures against changes at the lumbar spine in prolonged bed-rest in recent years, none of the countermeasures are, of course, “perfect” and it is appropriate to further expand our knowledge base for the prescription of exercises against lumbar spine changes in bed-rest and spaceflight.

In the “Long Term Bed Rest” (LTBR) study [13–15], sponsored by the European, French and Japanese space agencies, two countermeasures were performed: one involving the “fly-wheel” (FW) exercise device [15,16] and another involving daily low-load spinal movement exercises¹ [17]. The FW-device is currently in use on the International Space Station and there are plans to expand its use for aerobic, rowing, type exercises. Whilst the effectiveness of the FW-exercise protocol performed in the LTBR-study has been evaluated for lower-limb muscle [15,16] and bone [13,14], with a view to developing more efficient countermeasures, it would be relevant to consider the impact of this exercise protocol on the lumbar spine. The low-load spinal movement exercises were targeted at reducing low back pain incidence during bed-rest and it would be relevant to consider their impact on factors associated with lumbar spine muscle and soft-tissue deterioration in prolonged bed-rest. To achieve these aims, we recently examined magnetic resonance imaging (MRI) and isokinetic force testing data collected as part of the LTBR study.

Data from bed-rest studies to date suggest that low-load (~100% body-weight loading or less) exercise countermeasures (such as with lower-body negative pressure during treadmill running [3] or whole-body vibration with accompanying axial loading of the spine [4]) is

capable of reducing expansion of the intervertebral disc during bed-rest. It appears, however, that the duration of loading (per day or week) may play an important role, given that high-load resistive vibration exercise is capable of reducing disc expansion when conducted in 11 sessions a week [2], but not when only three exercise sessions are conducted [1]. Nonetheless, completely preventing changes in the lumbar discs in bed-rest, or spaceflight, is likely difficult as prior work [18] has shown a limited impact of an eight hour walking protocol in reducing overnight disc volume increases. In terms of the musculature, low-load exercise [3,4] appears ineffective in reducing extensor muscle size loss during bed-rest, although this kind of exercise may still have an effect upon retention of force development capacity [5]. High(er)-load resistive exercise, in contrast, appears capable of reducing muscle atrophy at the lumbar spine [1,2], though retention of lumbar (extensor) muscle size was still not complete in these latter studies. In consideration of data from studies of the lower-limb musculature in bed-rest, it can be expected that a high-load resistive exercise programme with exercises specific for muscle groups most affected by bed-rest will be more effective in preventing muscle atrophy (e.g. compare effects of countermeasures on the calf muscles in Refs. [19,20]). At the lumbar spine this would imply that direct loading of the lumbar extensor muscles with specific exercises would be more effective as a countermeasure than indirect loading, such as via shoulder straps.

Based upon these prior data, we hypothesized that the FW-exercise protocol would reduce lumbar extensor muscle atrophy, but the effect size would be limited as loading of the spine occurred only indirectly. We predicted also, that the FW-exercise protocol would have a relatively limited impact upon changes in disc and spine morphology due to its infrequent (every three days) schedule. We also hypothesized that the daily low-load spinal movement exercises would impact neither muscle volume nor changes in disc and spine morphology.

2. Materials and methods

2.1. Bed-rest study protocol

Twenty-five healthy male subjects underwent 90-days of 6° head-down tilt bed-rest (HDT) as part of the “Long Term Bed Rest” (LTBR) study at MEDES in Toulouse, France, in 2001 and 2002 (clinical trial identifier: NCT00311571; www.clinicaltrials.gov). The LTBR study was supported by the European, French and Japanese space agencies (ESA, CNES and NASDA). The LTBR study was approved by the Toulouse I ethics committee (CCPPRB Toulouse I) of the Ranguel University Hospital as well as the ethical committee of the Free University of Berlin. All subjects gave their informed written consent. For logistical reasons, two separate campaigns were conducted with 14 subjects in the first campaign and 11 in the second campaign. Initially it was planned to include 14 subjects in the second campaign as well, but due to difficulties in subject recruitment this quota could not be fulfilled. Further information regarding the LTBR study is

¹ For readers more familiar with the LTBR-study, this group of subjects also constituted the “pamidronate” group which received pamidronate, an anti-resorptive drug, to prevent bone loss. This drug has, however, no known effects on muscle (size or function) or on the soft-tissues such as the intervertebral discs.

available in prior publications [13–15] as well as in the internet (http://www.medes.fr/home_en/clinical_research/experiments/bed_Rest_2001.html). Data on subjects' physical activity levels ('Freiburg Questionnaire' [21]) before and after bed-rest have been published elsewhere [22]. After leaving the facility 14-days after bed-rest, subjects returned to their regular daily activities but 90 days after bed-rest, subjects total physical activity scores were significantly less than before bed-rest [22].

2.2. Countermeasures

Subjects were randomized to one of three groups: an inactive control group (Ctrl; $n=9$), a group that performed high-load resistance exercise on the "fly-wheel" exercise device (FW; $n=9$) or a third group that performed spinal mobilization exercises but also received pamindronate (SpMob group; $n=7$). The primary outcome parameter for the LTBR study was bone mineral content at the distal tibia for the FW-group vs. Ctrl-group comparison [13]. Due to insufficient pre-existing bed-rest data, a sensitivity analysis could not be performed given these subject numbers for the lumbar spine outcome parameters of the current investigation. Although one FW-subject completed the bed-rest study phase, he ceased the training protocol after 7-weeks bed-rest due to a previously unreported knee injury. This subject was therefore excluded from analysis. Subject characteristics are given in Table 1.

The FW-exercise programme was designed to target muscle and bone in the lower limbs. Nonetheless, indirect loading of the spine occurred via a padded shoulder restraint that resisted motion of the subject in a cephalad direction during exercise manoeuvres. The FW-group performed supine squat exercises (4 sets of 7 repetitions, 2 min between sets; targeting the hip and knee extensor

groups) with then 5 min rest and then calf press exercises (4 sets of 14 repetitions, 2 min between sets; targeting plantar flexors). For each set of exercises, 2 sub-maximal manoeuvres were performed and then the prescribed number of repetitions of maximal voluntary eccentric and concentric actions was performed. Exercises were started on the 5th day of bed-rest and then repeated every third day thereafter. Further details on the FW-group programme and the exercise device are available in prior publications [15,16]. Whilst no specific exercise manoeuvres were performed for the lumbar spine, axial loading of the spine during exercises was produced via padded shoulder restraint.

In the second countermeasure group, the SpMob group, subjects received a single 60 mg intravenous infusion of pamindronate 14-days prior to the start of the bed rest. Pamindronate is a bone anti-resorptive drug [14] and has no known effect on muscle (size or force) or on soft-tissue structures such as the intervertebral discs. As part of the spinal mobilization countermeasure (described in detail in Ref. [17], but as it is in the German language has been reproduced here in abbreviated form), subjects performed, in supine lying, large amplitude, low load, slow speed movements of the spine in the frontal (lateral flexion), sagittal (flexion-extension) and longitudinal (trunk rotation) planes five times every day during bed-rest with approximately 3 h pause between each movement session [17]. Movements were performed in the full range of motion available in each plane over the course of approximately 5 s with the end-position held for 5 s. Each movement was performed twice. The total duration of each movement session was approximately 4 min. The exercises were practiced under supervision prior to two days prior to the beginning of bed-rest. During bed-rest, subjects performed the exercises independently but with regular control of the conduct of the exercises. This movement countermeasure was designed and implemented by another research group in the LTBR study [17] with the aim to prevent or reduce the incidence of low back pain during bed-rest.²

Table 1
Subject characteristics and available magnetic resonance data.

	Subject-group		
	Ctrl	FW	SpMob
<i>Subject characteristics</i>			
Age (years)	31.9(3.6)	30.8(5.8)	33.3(3.2)
Height (cm)	173(3)	176(5)	175(5)
Weight (kg)	71.7(5.4)	71.0(5.7)	71.0(8.6)
<i>Number of subjects available for analyses</i>			
BDC ^a	9	8	7
HDT89 ^a	9	8	7
R+13	2	4	3
R+90 ^b	5	4	6

Subject anthropometric data are mean(SD) and no significant differences between subject groups were apparent for these data. Ctrl: inactive control group; FW: fly-wheel exercise countermeasure group; SpMob: spinal mobilization. BDC: baseline data collection; HDT89: 89th day of head-down tilt bed-rest; R+13: 13th day after 90-days bed-rest; R+90: 90th day after 90-days bed-rest. Only two subjects (both SpMob group) were scanned on both R+13 and R+90. One subject dropped out of the FW-group during bed-rest and was hence not included in these analyses.

^a Due to missing data, for the iliocostalis muscle at BDC and HDT89, 8 Ctrl, 5 FW, 7 SpMob subjects were available for analysis.

^b At R+90 data on iliocostalis muscle volume were available from 4 Ctrl, 1 FW, 7 SpMob subjects.

2.3. MRI protocol

MR-imaging was conducted in supine in all subjects either 17 or nine days prior to bed-rest (BDC) and on day 89 of bed-rest (HDT89). To avoid potential influence of fluid volume shifts on muscle size with changes in posture [23], subjects were recumbent for one-hour prior

² As these data have only been published in a German thesis, we summarize the low back pain questionnaire methodology and results here: spinal pain questionnaires were completed three time-points before bed-rest, daily in bed-rest phase and four days in recovery phase up to R+10. Spinal pain referred to the entire spine, not just the lumbar region. The author stated that 76.5% of back pain reports were in lumbar region but did not give further information as to how this was divided between the groups or over time. The author found no impact of the spinal movement protocol on spinal pain during bed-rest compared to control but found lower pain intensity on the first two-days after bed-rest in this group. In contrast to the other two groups, the fly-wheel group showed pain throughout the bed-rest phase with significantly higher spinal pain intensity in this group in the first week of bed-rest.

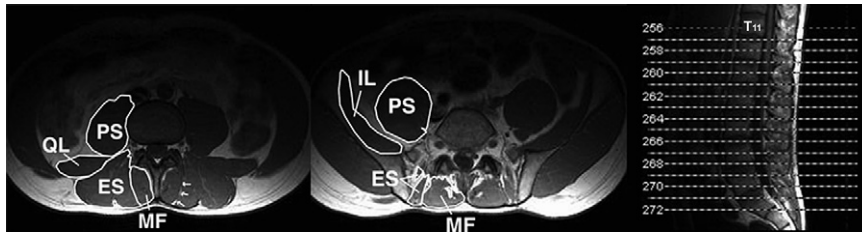


Fig. 1. Measurement of muscle volume. On each true axial image, cross-sectional area measurements were made where the psoas (Ps), erector spinae (ES), multifidus (MF), iliacus (IL) and quadratus lumborum (QL) muscles were present. These measurements were interpolated to estimate muscle volume, given a slice thickness of 5 mm and interslice distance of 10 mm. For the MF and ES muscles, only the measurements from L1 and below were included in the estimation of muscle volume. Arrows indicate the fascial border between MF and ES which aided delineation of these two muscles.

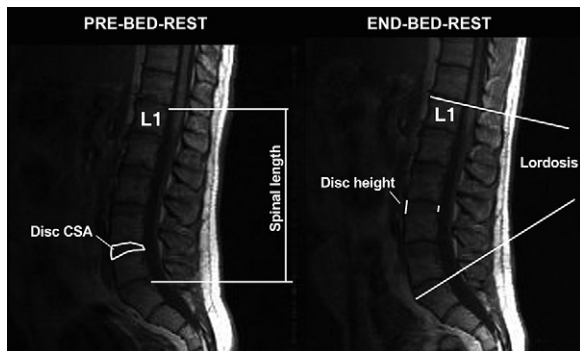


Fig. 2. Measurement of spinal morphology on sagittal images. On each sagittal image, anterior and posterior disc height was measured from $T_{12}L_1$ to L_5S_1 (shown on right image at L_{34}). Spinal length (left image) and lumbar lordosis (right image) were measured between L_1 and S_1 . Images are from the same subject before (left) and on day-89 of bed-rest (right). Note the lengthening of the spine and increases in disc height.

to the beginning of each MR-scanning session. For logistical reasons, the nine subjects measured at 13 days after bed-rest ($R+13$) were those of the 2nd campaign and the 15 subjects measured 90 days after bed-rest ($R+90$) participated in the 1st campaign with a further two subjects (A2, B2). A 1.0 T Siemens Somatom Impact (Erlangen, Germany) scanner was used to conduct the following sequences:

- 1) Seventeen true axial plane scans from the lower thoracic spine (typically T_{11}) to the sacrum (Fig. 1): thickness 5 mm, interslice distance 5 mm, repetition time 782 ms, echo time 12 ms, field of view $280 \times 280 \text{ mm}^2$ interpolated to 256×256 pixels
- 2) Five contiguous sagittal plane scans centred at the spinous processes (Fig. 2): thickness 5 mm, repetition time 800 ms, echo time 12 ms, field of view $400 \times 400 \text{ mm}^2$ interpolated to 256×256 pixels
- 3) Twelve paraxial scans positioned either through the middle of each intervertebral disc or through the centre of each vertebra from T_{12} to L_5S_1 . The images were orientated parallel to the respective vertebral endplates (Fig. 3): thickness 5 mm, repetition time 552 ms, echo time 12 ms, field of view $200 \times 200 \text{ mm}^2$ interpolated to 256×256 pixels.

Data were then stored in an offline database.

2.4. MR-image processing

In 2010, the lumbar spine MR-images were extracted from the database and each data set was assigned a random number (www.random.org) to blind the operator to study time-point. The same operator (DLB) used ImageJ 1.38 × (<http://rsb.info.nih.gov/ij/>) to conduct all image measurements. On the true axial plane images, the cross-sectional area (CSA) of the lumbar multifidus (MF), erector spinae (ES), quadratus lumborum (QL), psoas (Ps) and iliacus (IL) muscles were measured on the left and right sides (Fig. 1). To accurately delineate MF and the more laterally placed longissimus muscle, the fascial border [24] separating these two muscles was used as an anatomical landmark. As Ps and IL muscles fuse distally to form the iliopsoas muscle, these muscles were only measured in images where they could be readily delineated as separate muscles. The volume of each of the muscles was interpolated from the individual CSA measurements (i.e. total CSA × [slice thickness + distance between slices]). The volume of the MF and ES muscles was calculated from images between the 1st lumbar vertebra to the sacrum only (Ps and QL do not extend superior to L_1 and IL is confined to the pelvis).

For the assessment of spinal and disc morphology, the following parameters were measured from the five sagittal MR-images (Fig. 2):

- 1) Anterior and posterior disc height from $T_{12}L_1$ to L_5S_1 .
- 2) Sagittal plane disc CSA from $T_{12}L_1$ to L_5S_1 .
- 3) Spinal length: sagittal distance between the dorsorostral corner of S_1 and L_1
- 4) Lumbar lordosis between the superior endplates of L_1 and S_1 . A positive angle denoted a “lordosis”.

The data from each of the five images for each subject/scanning date were averaged.

On the paraxial MR-images (Fig. 3) the following parameters were measured:

- 1) Intervertebral disc CSA ($T_{12}L_1$ to L_5S_1): measurements were repeated twice and if there was difference of more than 3% between the two measures, then a third measurement was made and the closest two results then averaged. These data were then also used to calculate disc volume (calculated as the average of the anterior and posterior disc height (from sagittal images) multiplied by the axial plane disc CSA)

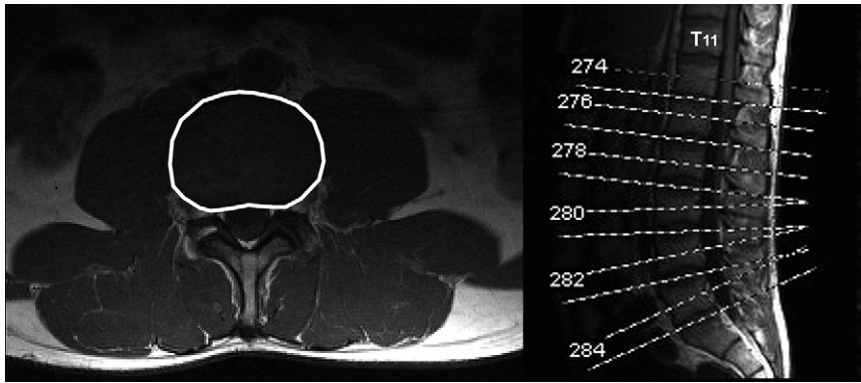


Fig. 3. Measurement of spinal morphology on para-axial images. A series of para-axial images were taken from the lumbar spine through the centre of each vertebra and also the centre of each disc. On each para-axial image passing through the intervertebral discs, the disc cross-sectional area was measured (left image). The lengths of the minor and major axes of an ellipse fitted to the region of interest were taken as, respectively, the anteroposterior and transverse diameter of the disc. These para-axial images were also used for some subjects in the assessment of muscle size (see results section for further details). Right image: positioning of MR-images.

- 2) Anteroposterior plane and transverse plane diameter of the intervertebral discs (T₁₂L₁ to L₅S₁) were quantified as, respectively, the minor and major axes of an ellipse fitted to the paraxial plane CSA measures.

2.5. Isokinetic trunk flexion/extension force

Concentric isokinetic (60°/s) trunk flexion and extension force was measured using an isokinetic dynamometer with the “trunk extension/flexion unit” (Cybex 6000, Lumex Inc. Ronkonkoma, NY, USA) according to standard Cybex testing protocol. Testing was performed twice prior to bed-rest (with the results from both baseline measures averaged), on the 2nd day after bed-rest (R+1) and then again on R+10 and R+90. Following a sub-maximal warm-up on a cycle ergometer, subjects were positioned in standing in the testing apparatus with padded restraints positioned against their upper back and chest as well as hip and thigh/knee restraints. Four repetitions each of trunk flexion and extension from an upright position were performed. Subjects were given verbal encouragement but no visual feedback was provided. The greatest value from each of the four trials was taken for further analysis.

2.6. Statistical analyses

As not all subjects (at R+13 nine subjects and at R+90 fifteen subjects; Table 1) were scanned on both post-bed-rest scanning sessions, analysis of variance (ANOVA) first considered the bed-rest phase (BDC vs. HDT89) in isolation. Factors of ‘study-date’, ‘subject-group’ (Ctrl, FW and SpMob) and their interaction were considered. ‘Vertebral-level’ and interactions were also considered for the appropriate spinal morphology variables. If a significant group × study-date interaction was seen, subsequent separate two-group (i.e., Ctrl vs. FW, Ctrl vs. SpMob and FW vs. SpMob) ANOVAs evaluated differences between groups in their response over the course of the study. As an additional goal of the study was to understand

the effects of bed-rest on the lumbar spine, additional models were conducted with data pooled across subject groups.

For analyses of recovery-phase data, only if an effect of the countermeasure(s) were seen in the bed-rest phase, was ‘subject-group’ considered in subsequent separate ANOVAs of BDC vs. R+13 and BDC vs. R+90. Note that only two subjects attended both R+13 and R+90, hence these analyses were also conducted separately. Otherwise only a factor of ‘study-date’ was considered in recovery-phase analyses. Similar analyses were performed for the isokinetic trunk flexion/extension data except that all study-dates were evaluated in the same ANOVA. Linear mixed-effects models [25] were used for statistical modelling. Allowances for heterogeneity of variance according to study-date, subject-group and/or vertebral level were applied when necessary. Due to the number of individual analyses performed, an alpha-level of 0.01 was chosen for significance of results on ANOVA (although raising the alpha-level to 0.05 does little to alter the outcomes of the current study with respect to the countermeasures), with *p*-values for the study-date factor or group × study-date interaction less than 0.05 but greater than or equal to 0.01 described as “marginal”. All analyses were performed in the “R” statistical environment (version 2.4.1, www.r-project.org). Unless otherwise stated, values are reported as mean(SD).

3. Results

For three out of eight fly-wheel (subjects A1, B1, C1) and one out of nine control subjects (subject E1), baseline (BDC) true axial sequences, used in calculating muscle volume, were not conducted due to technical difficulties, although the other (paraxial and sagittal) sequences were performed. To ensure that these subjects’ muscle size data could still be used in analyses, we examined whether muscle CSA measures from the paraxial sequences could be used to estimate muscle volume. Using one time-point chosen at random from all 25 (i.e. including the

one drop-out) subjects, linear regression was used to compare the average CSA measure from the paraxial images to the muscle volume data from the true axial images ($\text{volume}_{\text{axial}} = a * [\text{average CSA}_{\text{paraxial}}] + b$; where a represents the gradient of linear regression and b the intercept). For the psoas ($a=0.116$, $b=9.92$, $R^2=0.98$), quadratus lumborum ($a=0.059$, $b=16.51$, $R^2=0.90$), multifidus (from L₁ to sacrum; $a=0.116$, $b=10.71$, $R^2=0.95$) and erector spinae (from L₁ to sacrum; $a=0.102$, $b=14.79$, $R^2=0.995$) muscles the average paraxial muscle CSA explained more than 90% of the variation in muscle volume. The iliacus muscle, however, could typically not be completely visualized on the paraxial images and hence estimation of this muscle's volume could not be performed from the paraxial images. To ensure comparability of volume data between study-dates, for subjects A1, B1, C1 and E1, muscle volume was estimated from the paraxial images for *all* study-dates, rather than just at baseline.

3.1. Muscle size

For the MF, ES, QL and IL muscles, ANOVA provided evidence (F all ≥ 8.7 , p all ≤ 0.0057) of a significant change in muscle volume between the start (BDC) and end (HDT89) of bed-rest, but this was not the case for the Ps muscle ($F=2.4$, $p=0.13$). The percentage change in volume of each muscle at the end of bed-rest is displayed in Fig. 4. There was no evidence of effect of the countermeasures on changes in muscle volume (F all ≤ 1.15 , p all ≥ 0.33). Excluding data from the subjects whose muscle volume was estimated from paraxial images did not alter this finding for the MF, ES and QL muscles, but for the psoas muscle a marginal effect was observed ($F=3.3$, $p=0.0484$) with a greater increase in psoas muscle volume seen in the FW-group (+4.7[2.2]%, $p < 0.0001$) than in the

Ctrl (+1.4[3.7]%, $p=0.27$) or SpMob (+1.4[4.1]%, $p=0.37$) groups.

The extent of muscle volume change at the end of bed-rest (HDT89) did not differ (all muscles $p \geq 0.57$) between those subjects who were subsequently scanned 13 days after bed-rest ($R+13$) and those scanned 90-days after bed-rest ($R+90$). In those subjects who were measured on $R+13$, there was evidence that volume loss persisted in the ES ($F=10.9$, $p=0.011$) and QL ($F=5.5$, $p=0.047$) muscles, but that the Ps muscle was increased in size compared to baseline in these subjects ($F=8.8$, $p=0.018$; for MF and IL $p \geq 0.39$; Table 2). The subjects who were measured on $R+90$ exhibited a similar volume in the QL and IL muscles as prior to bed-rest (F all ≤ 0.8 , p all ≥ 0.40), but that MF ($F=8.1$, $p=0.013$) and ES ($F=6.0$, $p=0.028$) muscle volume was greater than at baseline and Ps muscle volume was less than at baseline ($F=8.7$, $p=0.010$; Table 2). Evaluation of muscle CSA change at each intervertebral level, as conducted in prior work [1,11], did not alter the findings for the impact of the countermeasures and time-course of recovery in the current study and also confirmed prior findings [11] of greater atrophy of the MF muscle at the lower lumbar spine and ES at the upper lumbar spine (data not shown).

3.2. Spinal morphology

The spinal morphology parameters of disc volume, paraxial plane disc CSA, anteroposterior disc diameter, transverse disc diameter, anterior disc height, posterior disc height, lumbar lordosis and lumbar spine length all changed as a consequence of bed-rest (F all ≥ 5.9 , p all ≤ 0.0167) and only sagittal plane disc CSA showed no significant change (F all ≤ 2.5 , p all ≥ 0.09). Whilst reductions in paraxial plane disc CSA, and correspondingly in anteroposterior and transverse plane disc

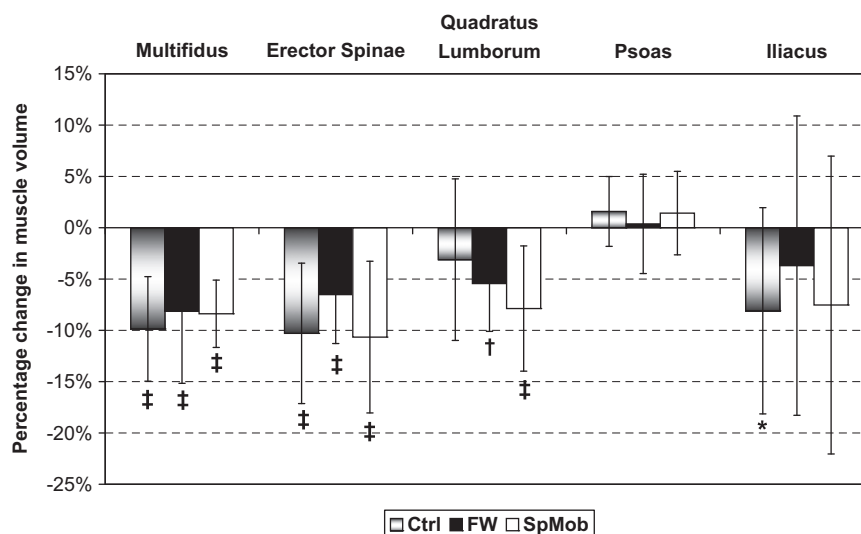


Fig. 4. Percentage change in muscle volume after 89-days bed-rest in each subject group. Values are mean(SD) percentage change in muscle volume compared to baseline (BDC) values. Ctrl: inactive control group; FW: fly-wheel exercise group; SpMob: spinal mobilization group. *: $p < .05$; †: $p < .01$; ‡: $p < .001$ indicate significance of difference of the mean to baseline. There was no evidence on ANOVA of a different response in the three groups (p all ≥ 0.33). See Table 2 for data on baseline (BDC) muscle volume and changes in muscle volume when all subjects are pooled together.

Table 2

Muscle volume at baseline in each group and after bed-rest.

Subject-group	Study-date	Muscle				
		Multifidus	Erector Spinae	Quadratus Lumborum	Psoas	Iliacus
<i>Baseline (BDC) volume in each group</i>						
Ctrl	BDC (cm ³)	75.4(12.3)	189.5(43.2)	41.7(10.7)	153.0(20.8)	29.6(14.3)
FW	BDC (cm ³)	74.4(13.2)	200.9(40.1)	48.8(10.2)	159.7(22.3)	36.4(17.0)
SpMob	BDC (cm ³)	73.8(11.6)	192.4(44.2)	46.7(10.5)	151.4(19.8)	39.0(14.0)
Pooled	BDC (cm ³)	74.5(11.6)	192.6(40.8)	45.7(10.8)	154.4(20.2)	35.0(12.0)
	HDT89(%)	-8.8(4.5) [‡]	-9.0(6.1) [‡]	-5.8(6.0) [‡]	+1.4(3.8)	-6.8(9.4) [†]
<i>Subjects measured 13-days post bed-rest (R+13; n=9; data pooled for all groups)</i>						
Pooled	BDC (cm ³)	76.7(12.6)	202.0(40.7)	45.8(8.8)	157.2(25.1)	37.9(13.3)
	HDT89(%)	-11.4(10.7) [†]	-9.6(4.7) [‡]	-8.1(5.9) [‡]	+5.0(2.9) [‡]	-3.1(13.7)
	R+13(%)	-2.4(8.5)	-4.9(4.8) [†]	-3.5(4.7) [*]	+4.9(4.8) [†]	+6.3(24.8)
<i>Subjects measured 90-days post bed-rest (R+90; n=15; data pooled for all groups)</i>						
Pooled	BDC (cm ³)	73.6(12.2)	184.8(41.6)	45.1(12.7)	153.8(20.0)	35.1(18.1)
	HDT89(%)	-7.6(5.7) [‡]	-7.6(9.6) [†]	-4.1(7.3) [*]	-1.0(5.5)	-9.6(19.4)
	R+90(%)	+4.5(7.0) [*]	+4.7(10.9)	+0.4(7.6)	-3.2(4.5) [†]	-6.3(27.0)

Values at baseline (BDC) are mean(SD) in cm³. Values at end-bed-rest (HDT89), 13-days post bed-rest (R+13) and 90-days post-bed-rest (R+90) are mean(SD) percentage change compared to BDC value. See Fig. 4 for changes in muscle volume within each group during bed-rest. The extent of muscle volume change at the end of bed-rest (HDT89) did not differ (all muscles $p \geq 0.57$) between those subjects who were scanned on R+13 and those scanned on R+90. Ctrl: inactive control group; FW: fly-wheel exercise countermeasure group; SpMob: spinal mobilization.

* $p < .05$ indicate significance of difference of the mean to baseline.

† $p < .01$ indicate significance of difference of the mean to baseline.

‡ $p < .001$ indicate significance of difference of the mean to baseline.

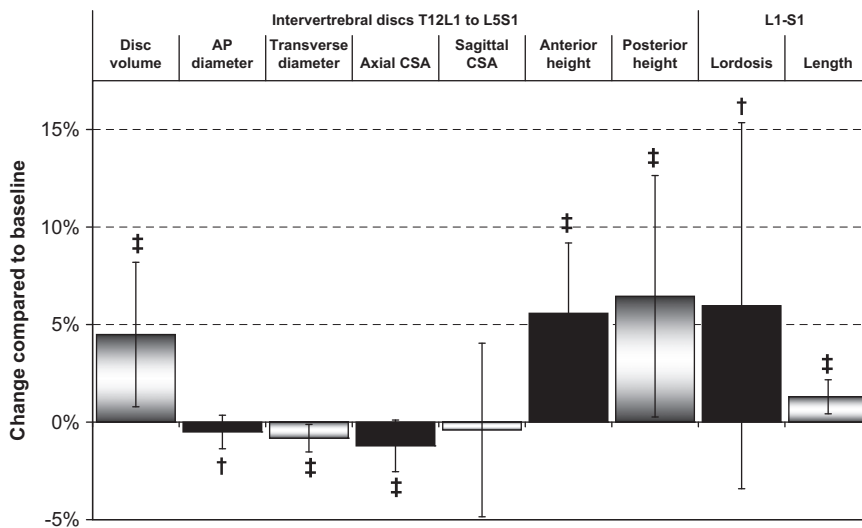


Fig. 5. Percentage change in spinal morphology parameters after 89-days bed-rest. Values are mean(SD) percentage change in each spinal morphology parameter compared to baseline (BDC) values. Data have been pooled for each of the subject groups. Only for disc diameters and axial plane CSA was there evidence for a significant difference between groups ($p \leq 0.017$, otherwise $p \geq 0.048$; see text and Table 3 for further details). *: $p < .05$; † $p < .01$; ‡ $p < .001$ indicate significance of difference of the mean to baseline. CSA: cross-sectional area; AP: anteroposterior.

diameters, were seen, the relatively greater increase in disc height resulted in an overall significant increase in disc volume (Fig. 5). Lumbar spine length and the lumbar lordosis were increased at the end of bed-rest. There was little evidence of a differential response during bed-rest across vertebral levels on measurements made from each disc (p all ≥ 0.048).

There was evidence for an effect of the countermeasures on paraxial plane disc CSA as well as the anteroposterior and

transverse plane disc diameters (F all ≥ 4.2 , p all ≤ 0.017 ; Table 3). The effect was largely consistent across the countermeasures subjects with only one or two subjects in each group showing an increase in these parameters at the end of bed-rest with the remaining showing a decrease. There was marginal evidence for a different response in posterior disc height between subject-groups ($F=3.1$, $p=0.048$). The FW- and SpMob-groups showed greater decreases in paraxial plane disc CSA/diameters than the

Table 3
Changes in spinal morphology during and after bed-rest.

Study-date	All intervertebral discs T12L1 to L5S1							L1-S1	
	Disc volume (cm ³)	AP diameter (mm)	Transverse diameter (mm)	Axial CSA (cm ²)	Sagittal CSA (cm ²)	Anterior height (mm)	Posterior height (mm)	Lordosis (deg.)	Length (cm)
<i>Inactive control group (Ctrl; n=9)</i>									
BDC	12.0(2.3)	38.5(1.7)	51.2(2.4)	15.4(131.4)	2.5(0.5)	9.7(1.2)	5.6(0.9)	42.4(6.0)	16.5(0.7)
HDT89(%)	+5.0(3.8) [‡]	+0.2(1.0)	-0.3(0.7)	-0.1(1.3)	+2.4(5.4)	+6.1(4.6) [‡]	+3.0(6.6)	+5.2(10.7)	+1.2(1.1) ⁻
<i>Fly-wheel group (FW; n=8)</i>									
BDC	14.1(2.3)	40.4(1.7)	54.5(2.4)	17.5(125.1)	3.1(0.5)	10.3(1.2)	5.6(0.8)	43.4(6.6)	17.3(0.6)
HDT89(%)	+2.8(4.9)	-1.1(0.8) [‡]	-1.8(0.9) [‡]	-2.9(1.5) [‡]	-2.8(5.0)	+5.3(4.6) [†]	+7.8(7.0) [†]	+3.2(10.5)	+1.3(0.7) [‡]
<i>Spinal mobilization exercise (SpMob; n=7)</i>									
BDC	13.0(2.2)	38.9(1.7)	52.4(2.4)	16.1(124.0)	2.8(0.4)	10.2(1.1)	5.6(0.8)	44.8(6.1)	17.0(0.7)
HDT89(%)	+4.6(3.2) [‡]	-0.4(0.8)	-0.9(0.6) [‡]	-1.4(1.1) [†]	-0.8(3.4)	+5.2(2.6) [‡]	+8.5(5.1) [‡]	+8.9(7.9) [*]	+1.4(1.5) [*]
<i>Subjects measured 13-days post bed-rest (R+13; n=9; data pooled for all groups)</i>									
BDC	12.9(1.9)	39.5(1.7)	52.0(2.3)	1617.7(151.0)	281.0(41.2)	10.3(1.2)	5.5(0.8)	46.2(6.7)	16.9(7.9)
HDT89(%)	+4.0(4.3) [†]	-0.4(0.9)	-1.2(0.8) [‡]	-1.8(1.5) [‡]	-4.3(5.6) [*]	+3.5(4.2) [*]	+10.2(6.3) [‡]	+0.4(8.9)	+1.9(0.7) [‡]
R+13(%)	+1.8(4.5)	+0.5(1.0)	-0.4(0.8)	-0.1(1.4)	-3.4(6.4)	+1.3(4.8)	+3.9(5.4) [*]	-0.5(6.7)	-0.1(1.3)
<i>Subjects measured 90-days post bed-rest (R+90; n=15; data pooled for all groups)</i>									
BDC	12.9(2.7)	39.0(1.9)	52.6(3.2)	16.3(1.7)	2.8(0.5)	9.9(1.2)	5.7(0.7)	42.3(5.7)	17.0(7.0)
HDT89(%)	+4.8(4.1) [‡]	-0.3(0.8)	-0.8(0.8) [‡]	-1.2(1.4) [‡]	+1.0(4.4)	+6.6(3.9) [‡]	+6.5(6.4) [‡]	+9.8(9.1) [‡]	+1.0(1.1) [†]
R+90(%)	+2.3(3.5) [*]	+0.2(1.1)	-0.2(0.8)	+0.0(1.5)	-1.3(4.0)	+4.1(3.5) [‡]	0.0(6.4)	+11.0(7.3) [‡]	-1.0(1.2) [†]

Values at baseline (BDC) are mean(SD) in units given at top of column. Values at end-bed-rest (HDT89), 13-days post bed-rest (R+13) and 90-days post-bed-rest (R+90) are mean(SD) percentage change compared to BDC value. See text for further details of significance of differences between groups. Only for disc diameters and axial plane CSA was there evidence for a significant difference between groups for the BDC vs. HDT89 comparison ($p \leq 0.017$, otherwise $p \geq 0.048$; see text for further details). Further two-group comparisons on these parameters showed evidence for differences in the response of the FW and SpMob groups for axial disc CSA only ($p=0.009$, otherwise $p \geq 0.018$). Ctrl and FW-groups differed ($p \leq 0.002$) for axial disc CSA and transverse and anteroposterior disc diameters. Ctrl and SpMob-groups differed ($p \leq 0.009$) for axial disc CSA and transverse disc diameter only. For data in each subject group in the post-bed-rest recovery phase, see supplementary data.

* $p < .05$ indicate significance of difference of the mean to baseline.

† $p < .01$ indicate significance of difference of the mean to baseline.

‡ $p < .001$ indicate significance of difference of the mean to baseline.

Ctrl-group, but correspondingly greater increases in disc height. Consequently, there were no significant differences between groups (F both ≤ 1.0 , p both ≥ 0.44) for changes in disc volume (Table 3). For all other spinal morphology parameters, there was no evidence for an influence of the countermeasures (F all ≤ 2.5 , p all ≥ 0.09).

In the subjects scanned 13 days after bed-rest (R+13), spinal morphology parameters had largely returned to pre-bed-rest levels, though posterior disc height was still marginally higher than at baseline ($F=4.1$, $p=0.048$; Table 3). In the subjects scanned 90 days after bed-rest (R+90), only two of whom were scanned at R+13, disc volume, anterior disc height and lordosis angle were greater (F all ≥ 6.0 , p all ≤ 0.016) and spinal length was less ($F=9.1$, $p=0.0094$) than at baseline. The remaining parameters were not significantly different to pre-bed-rest ($p \geq 0.20$). Of the spinal morphology parameters for which there was evidence of an effect of subject-group during bed-rest, only posterior disc height at R+13 showed a strong effect for a different response in the three groups ($F=8.5$, $p=0.0007$; Supplementary Table 5) with greater posterior disc height seen at R+13 in the SpMob-group.

3.3. Isokinetic trunk flexion/extension

Due to subject absence or technical difficulties, one subject (SpMob group) could not be measured on R+1 and

another (Ctrl group) could not be measured on R+90. Significant changes in isokinetic force measures were seen over the course of the study ($F \geq 6.3$, $p \leq 0.0009$) with mean(SD) decreases on average in all subjects in trunk isokinetic extension force of $-28.7(30.6)\%$ ($p < 0.001$) at R+1, $-15.4(26.8)\%$ ($p=0.0015$) at R+10 and $-5.8(23.4)\%$ ($p=0.21$) at R+90. Isokinetic trunk flexion force was decreased on average in all subjects by $-9.4(20.5)\%$ ($p=0.018$) at R+1, $-10.0(16.9)\%$ ($p=0.0019$) at R+10 and $+1.5(17.5)\%$ ($p=0.69$) at R+90. Reductions in extension force were significantly greater than flexion at R+1 only ($p=0.00008$, otherwise $p \geq 0.53$).

There was no evidence from ANOVA of an effect of the countermeasures on isokinetic trunk flexion/extension ($F \leq 0.69$, $p \geq 0.65$). The values for isokinetic trunk flexion and extension in each group on each study-date are presented in Table 4.

4. Discussion

We found no statistically significant effects of fly-wheel exercise (every third day, with exercises targeting the lower-limb musculature and concurrent loading of the spine via shoulder restraints) and low-load spinal movement (five times daily) countermeasures on lumbar muscle volume changes or maximal trunk isometric force capacity after 90 d of head-down tilt bed-rest. In addition,

these countermeasures had little impact upon changes in spinal morphology with similar changes in disc volume, lumbar lordosis and spinal length as the inactive control

Table 4

Isokinetic trunk flexion and extension torque at 60 degrees/second over the course of the study.

Group	Study-date			
	BDC (nm)	R+1 (%)	R+10 (%)	R+90 (%)
<i>Isokinetic (60°/s) trunk extension</i>				
Ctrl	227.6(79.6)	-34.9(42.5) [‡]	-19.2(35.3)*	-7.7(31.2)
FW	269.3(24.2)	-27.8(27.3) [‡]	-15.4(9.9)	-10.5(21.6)
SpMob	273.8(71.3)	-21.0(29.2)*	-9.9(22.8)	5.6(21.3)
<i>Isokinetic (60°/s) trunk flexion</i>				
Ctrl	190.3(58.7)	-9.6(7.9)	-9.8(6.3)	6.0(8.7)
FW	215.8(19.1)	-17.7(8.1)*	-13.6(5.6) [†]	-4.8(5.7)
SpMob	207.4(53.8)	-1.3(6.5)	-3.4(8.3)	5.9(5.6)

Values at baseline (BDC) are mean(SD) in Newton-metres (nm) and at subsequent dates, values are mean(SD) percentage change compared to baseline. Ctrl: inactive control group; FW: fly-wheel exercise countermeasure group; SpMob: spinal mobilization group. R+1, R+10 and R+90: indicate respectively 1, 10 and 90 day(s) after bed-rest. ANOVA showed no significant difference between groups on changes over time in these parameters ($p \geq .65$) and greater percentage losses were seen in extension than flexion (see text for further details).

* $p < .05$ indicate significance of difference of the mean to baseline.

† $p < .01$ indicate significance of difference of the mean to baseline.

‡ $p < .001$ indicate significance of difference of the mean to baseline.

subjects. There was some statistical evidence, however, of a greater decrease in axial plane intervertebral disc dimensions in the two countermeasure groups than in the control-group with marginally greater increases in sagittal plane disc height.

Whilst the effect of the countermeasures on para-axial plane disc dimensions and disc height was largely consistent across all countermeasure subjects, and thus do appear to be a general effect of the countermeasures, it is difficult to explain the mechanism by which the countermeasures may have had these effects. Data from biomechanical studies [27] suggest that an increased likelihood of disc failure occurs when axial plane disc CSA decreases with concurrent increase in disc height. Also, an increase in disc height is commonly seen as an effect of bed-rest [2,3,11] which countermeasures typically aim to prevent. Furthermore, greater increases in disc height during bed-rest have been associated with low back pain incidence after bed-rest [11]. Based upon these data, the effects of the countermeasures seen of greater decreases in axial plane disc dimensions and marginally greater increases in posterior disc height may well be detrimental, though further work would be needed to better assess this. Paradoxically, the countermeasures had limited positive effect upon the trunk musculature, but a possibly negative impact upon disc morphology.

In addressing the other, non-significant, findings on the countermeasures, it is important to try to understand whether there was actually “no effect” or whether (a) there were too few subjects, (b) measurement reproducibility was too low, (c) the effect size was too small or (d)

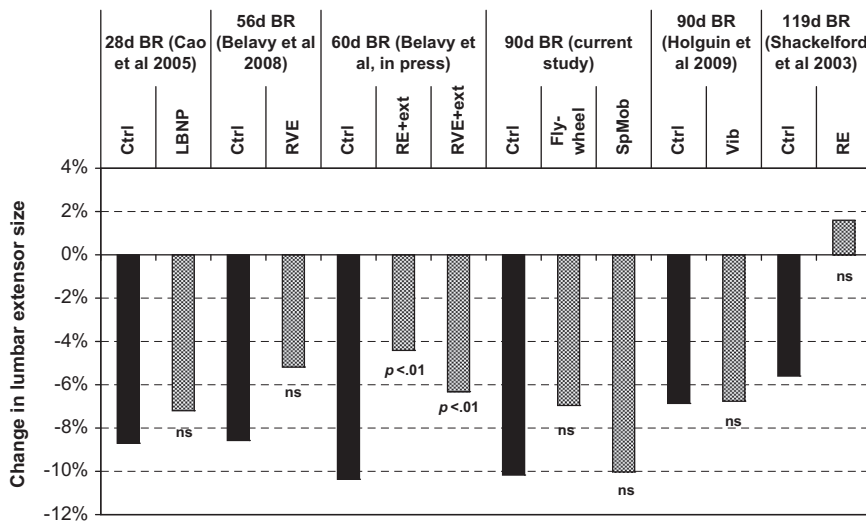


Fig. 6. Effects of various countermeasures on paraspinal muscle size. Values are mean percentage change in extensor (all paraspinal muscles – erector spinae and multifidus – grouped together) in the control (Ctrl; black) and countermeasure (hashed) groups of each study. “ns”: no significant difference in comparison to control group (p -values otherwise indicate significance of difference to control); “BR”: days of bed-rest. Cao et al. [3]: Lower body negative pressure (LBNP) and treadmill (~100% body weight), no specific exercises for the spine; 40 min/d, 5 d/week; muscle size measured as CSA at the L34 interspace. Belavý et al. [2]: High-load resistive exercises with whole-body vibration (RVE) targeted at lower-limb musculature, loading of spine via shoulder straps, no specific exercises for the spine; 11 sessions/week; muscle size measured as CSA at the L4 vertebral body. Belavý et al. [1]: High-load resistive exercises targeted at lower-limb musculature (loading of spine via shoulder restraints) plus back extension exercise, with (RE+ext) and without (RVE+ext) vibration; 3 sessions/week; muscle size measured as average CSA from images taken at each vertebral body L1 to L5. Holguin et al. [4]: Low load axial loading of spine via straps (60% body weight) with vibration (Vib); no exercises; 10 min/d, 7 d/week; muscle size measured as volume between T12 and L3. Shackelford et al. [6]: High-load exercises included manoeuvres for the spinal extensor and hip musculature conducted 3 d/week. Overall programme consisted of 3 d/week of lower-body work and 3 d/week of upper-body work with the upper and lower body were exercised on alternate days. Heel raises done 6 d/week.

some combination of these three factors resulted in insufficient statistical power. Limited subject numbers are a common limitation of bed-rest studies due to the costs and logistics of implementing such projects. Despite this, other studies have been able to detect significant effects of countermeasures on muscle size [1] or disc morphology [2,3] despite similarly low sample sizes whilst using similar MR-methodologies as in the current study. Precise data on measurement reproducibility is not available to us for the sequences performed using the 1 Tesla MR-scanner of the current study. As suggested by the data presented in Fig. 6, the fly-wheel exercise in the current study may well have had an effect on lumbar extensor muscle atrophy in bed-rest, but that the effect was too small to be detected given the experimental design. In all fairness, also, the primary end-points for the fly-wheel and spinal movement countermeasures laid elsewhere and the fly-wheel countermeasure was capable of reducing bone [13,14] and lower-limb muscle loss [15,16]. The spinal movement countermeasure did not achieve its primary aim of reducing back pain incidence during bed-rest, but these subjects did report fewer incidents of back pain after bed-rest [17]. Nonetheless, the findings of the current study, in conjunction with data from other works, can help to suggest which exercise approaches may be more effective in preventing changes at the lumbar spine in bed-rest.

In terms of the maintenance of muscle size, studies in the lower-limbs have shown low-load exercise [28–30] is typically less effective than high-load (resistive) exercise [6,15,16,20,31–33], and this is exemplified in findings to date on the lumbar extensor musculature (see Fig. 6). High-load exercise can reduce muscle atrophy at the lumbar spine even when performed on a relatively infrequent (every 2–3 days) schedule ([1]; see also findings from the LTBR study on the lower-limb musculature [15,16]). However, relying upon loading of the spine as a secondary by-product of performing exercises for the lower-limb ([2] and FW-group of current study) can be of limited value (Fig. 6): muscle maintenance in bed-rest is much more effective when high-load resistive exercises target specific muscle groups (compare [19,20] in the effect of resistance exercise on the plantar flexors; see also Fig. 6). Thus, the effect of countermeasures on the lumbar spine is more efficient when specific exercises are included for the lumbar extensors ([1] and Fig. 6). The use of shoulder restraints during countermeasure exercise will result in a compressive force on the spine and subjects typically alter their spinal posture to resist this force. As the maintenance of S-curve of the spine is important for optimal activation of the musculature [34,35], specific exercises for the spinal extensors could be done starting at low loads and progressing to high-load as subjects maintain spinal posture and their motor skill in the exercise improves. Nonetheless, specific resisted spinal extension exercises would be appropriate for better maintenance of the lumbar multifidus and lumbar erector spinae. Such exercises, of course, need to be done with care, given findings of increased low back pain incidence with high-load exercise during bed-rest [1,17]. Overall, for the maintenance of the musculature during bed-rest, there

is a relatively broad evidence base, when data from the lower-limbs are taken into account. Nonetheless, there are still a number of aspects of countermeasure exercise prescription for the musculature which need to be better delineated, such as exercise duration and frequency. In bed-rest studies the countermeasure or countermeasures investigated can typically act via a mixed bag of physiological pathways. This makes assessing specific exercise principles, such as low-load vs. high-load or daily vs. less frequent exercise, quite difficult. We argue that it would be beneficial in future work to assess specific exercise prescription principles, rather than simply combining different countermeasure devices (such as is the plan for future bed-rest studies in Europe as part of the 2009 announcement of opportunity) and leaving the actual exercise programme on such devices as an afterthought.

The evidence base for the maintenance of spinal morphology and the intervertebral discs during bed-rest is, compared to muscle, less well developed. The intervertebral discs are complex structures with a similarly complex response to mechanical loading, with a range of as yet ill-defined loading levels and frequencies considered to result in positive effects on disc metabolism (for review see Ref. [36]). Even less well understood is the response of the “chronically unloaded” disc to mechanical loading, such as may be afforded by exercise during bed-rest. Perhaps not unexpectedly, high levels of loading on a previously immobilized disc can prove deleterious [37]. Based upon the data available from long-term human bed-rest studies ([1–5]; with data common to these studies summarized in Fig. 7) it is possible to say that infrequent loading cycles (irrespective of the magnitude of the load), such as on a schedule of every 2–3 days will not impact upon changes in the intervertebral disc. It appears that the duration and frequency of loading are more important with lower body negative pressure with treadmill running for approximately 40 min/d (5 d/week; [3,5]), 10-min daily whole-body vibration with static spinal loading [4], as well as resistive vibration exercise 11-times a week [2] being more successful in reducing morphological changes in the discs during bed-rest. Nonetheless, it does appear the magnitude of loading is still important, given that the SpMob group of the current study showed little benefit in terms of discal changes, despite performing regular spinal movements every day. In ambulant individuals, the spine is loaded for many hours in upright posture, and as prior work in overnight bed-rest [18] has pointed out, it would be difficult to perfectly maintain the intervertebral discs at their pre-bed-rest (or pre-spaceflight) levels. Regardless, longer duration exercise with at least “moderate” dynamic loading levels of the spine would be more effective as a countermeasure against changes in discal and spinal morphology. There is, however, much work still to be done in developing more efficient countermeasures for the lumbar spine in spaceflight/bed-rest.

The findings of the current study also contribute to a deeper understanding of the effects of bed-rest, and hence potentially spaceflight, on the human lumbar spine. To the best of our knowledge, in no other long-term [1–5,11,38] or overnight [18,39–41] bed-rest study which

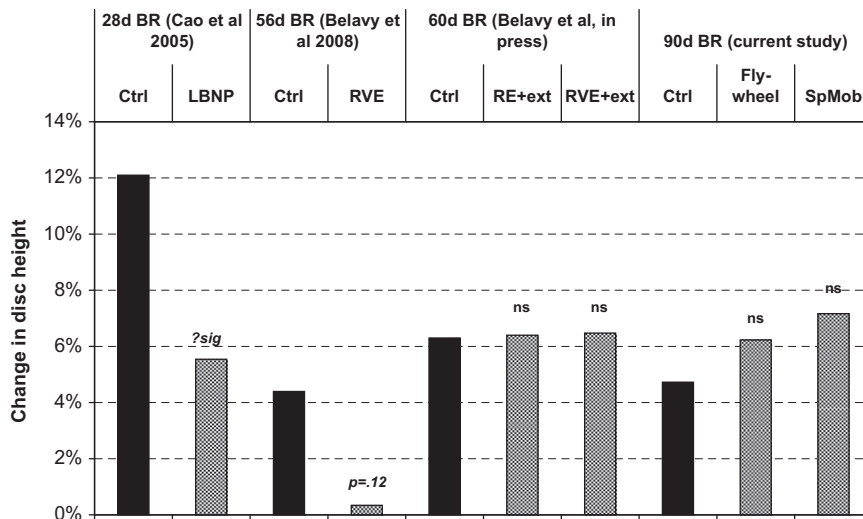


Fig. 7. Effects of various countermeasures on disc height. Values are mean percentage change in disc height from L12 to L5S in the control (Ctrl; black) and countermeasure (hashed) groups of each study. “ns”: no significant difference in comparison to control group (p -values otherwise indicate significance of difference to control); “?sig”: in Cao et al., significances were given for each vertebral level and not for average measures, but based upon the data presented in their paper, the difference here is assumed to be significant. “BR”: days of bed-rest. Cao et al. [3]: Lower body negative pressure (LBNP) and treadmill (~100% body weight), no specific exercises for the spine; 40 min/d, 5 d/week; disc height measured as average of anterior and posterior disc height—estimated from their Fig. 4. Belavý et al. [2]: high-load resistive exercises with whole-body vibration (RVE) targeted at lower-limb musculature, loading of spine via shoulder straps, no specific exercises for the spine; 11 sessions/week; disc height measured as the height of a rectangle fitted to sagittal plane CSA measures of the disc. Belavý et al. [1]: high-load resistive exercises targeted at lower-limb musculature (loading of spine via shoulder restraints) plus back extension exercise, with (RE+ext) and without (RVE+ext) vibration; 3 sessions/week; disc height measured as the average of anterior and posterior disc height from L12 to L5S. Data on disc height changes not available from Holguin et al. [4].

examined the intervertebral disc, were changes in the transverse (paraxial) plane of the lumbar intervertebral disc investigated. Given that the current and other bed-rest studies invariably show an *increase* in disc volume and height, it was initially somewhat surprising to see a *reduction* of disc transverse and anteroposterior diameters and correspondingly CSA. Re-analysis of MR-data published by our group previously [1] showed a significant 0.4% reduction of anteroposterior disc diameter at both four and eight-weeks of bed-rest, which is comparable to that seen in the current work despite a somewhat different measurement approach. It thus seems that the increase in disc volume seen in bed-rest is accompanied by disproportionate changes in its dimensions. This effect seems to be clinically relevant: previous biomechanical studies [27] have shown that a relative reduction of disc CSA in the axial plane with increases in disc height in the sagittal plane increase the likelihood of disc tissue failure. Hence, these alterations of disc shape along with increase in disc volume, could be one of the contributing factors, aside from other factors such as muscle atrophy, to the spike of low back pain incidence seen in the days following prolonged bed-rest (see Ref. [17] for data from the subjects of the current study as well as [1,4] for low back pain data from other bed-rest studies) and perhaps also to increased incidence of disc protrusion seen in astronauts after spaceflight [9].

The current study found significant reduction in iliacus muscle volume after 90 d bed-rest with a non-significant increase in the volume its synergist, the psoas muscle. These results are interesting as they help to stress that

bed-rest, whilst being a model of “physical inactivity” may not be a perfect model of spaceflight for all muscles. Reports from spaceflight [42] have shown *decreases* in psoas muscle size, whereas studies in bed-rest have shown either significant [6,11,43] or non-significant *increases* in the size of the psoas muscle ([3,44] and current study; for Le Blanc et al. mean values were not reported and were calculated directly from their Fig. 4). The iliacus muscle has not been studied after spaceflight. During bed-rest, the hip flexor muscles, primarily psoas and iliacus [45], will act in lifting of the leg as part of position changes in bed and the psoas muscle will also assist in movements of the spine [46]. The use of the hip flexor muscles as part of daily activities during bed-rest could in part explain the increase in psoas size seen during bed-rest, as well why these findings are not seen in spaceflight [42], where such loading patterns do not occur. Whilst psoas and iliacus are synergists, there is of course some divergence in their activation patterns during functional tasks [46], and any effect of daily activity on stimulating psoas muscle activity during bed-rest evidently did not prevent significant reductions in size of the iliacus muscle after 90 d bed-rest. One prior study in 56 d bed-rest [47] showed no change in either of these muscles, although a different measurement approach was used in this prior study (CSA measures at the level of the ilium). The contrasting data on psoas between bed-rest and spaceflight suggest that bed-rest may not be the best model for simulating the effects of spaceflight on muscles involved in hip and spine flexion. Direct comparison of data from bed-rest to data from astronauts would be

appropriate to better understand the extent to which bed-rest may model spaceflight for the remaining muscles of the spine and hip, such as the iliacus and other hip flexors.

The current study also affords some insight into the recovery of the lumbar musculature and discs after prolonged bed-rest. Whilst much work has concentrated upon the deterioration of the musculoskeletal system with bed-rest, comparatively few works have considered its recovery afterwards. In the current work, the subjects did not undergo any specific rehabilitation programme, and their recovery after bed-rest can be seen to reflect the “natural” progression. In subjects that attended 13 days after bed-rest, significant reductions in muscle volume persisted in the erector spinae and quadratus lumborum muscles, with increased psoas size was seen and with multifidus and iliacus not significantly different to their pre-bed-rest levels. Ninety days after bed-rest, the multifidus and erector spinae muscles were larger than before bed-rest with the psoas muscle smaller than at baseline in these subjects and iliacus and quadratus lumborum at pre-bed-rest levels. Prior work [2] showed a return to pre-bed-rest levels of (raw) multifidus CSA at the L₄ vertebral level after approximately two-weeks of recovery after bed-rest. In the current work, muscle CSA at each vertebral level was measured as part of volume estimation and the results of the current study do not differ if the L₄ vertebral level is considered alone as in prior work [2]. Overall, it appears that the recovery of muscle size is “automatic” after bed-rest, in contrast to findings from low back pain/injury investigations which suggest persistence of selective atrophy of this muscle [48,49]. One factor that limits the comparison of the two post-bed-rest MRI measurement time points in the current study is that only two subjects attended both sessions. Also, in the immediate post-bed-rest period, increased muscular water content and hence increased muscle size associated with muscular soreness [50] can confound muscle size measurements. Data from our prior work suggest that muscular soreness also occurs at the lumbar spine after bed-rest [1,51]. Hence it is unclear to what extent the increased muscle volumes at R+13 actually reflect true muscle recovery. Another consideration is that prior electromyography work after bed-rest [52,53] has shown persistence of motor control in the short lumbar extensors change long into the recovery period. In the current study, such measurements were not performed and whilst muscle size may recover, this does not mean that other aspects of muscle function, such as motor control, also automatically recover.

In terms of lumbar spine and disc morphology, some of these parameters were also still significantly different to baseline 90 d after bed-rest. Another work [4] showed persistence of disc changes 7d after bed-rest. Whilst one of our prior works [2] suggested recovery of spinal morphology parameters soon after bed-rest, more recent work with more precise measurements [54] showed persistence of increased spinal length, disc volume and disc height up to 90 d after bed-rest. In this recent work [54], however, rehabilitation programmes were evaluated without a no-rehabilitation control and the persistence of these spinal morphology changes may have been due to

this “rehabilitation-intervention” after bed-rest rather than due to a “lack of recovery” *per se*. Overall, however, whilst recovery of the musculature may occur of its own accord after prolonged bed-rest, this of course takes time and potentially leaves a time-window where a higher risk of low back injury may be possible. Also, it may well be that changes in the discs do indeed persist long-term, and it would be warranted to investigate this in more detail. Such investigations will help to understand why intervertebral disc protrusion incidence is increased in astronauts after spaceflight [9] and potentially also help to understand findings [55] suggesting that acute low back pain in the general population is more likely to have its onset in the hours after awakening in the morning and beginning daily activities, a time of the day when the discs are still expanded after an overnight rest.

5. Conclusions

In conclusion, the current study found limited statistical evidence for a positive impact of fly-wheel (every third day, with exercises targeting the lower-limb musculature and concurrent loading of the spine via shoulder restrains) and spinal movement (five times daily) countermeasures on preventing lumbar muscle atrophy, reduction in trunk flexion and extension isokinetic force production and on changes in the morphology of the lumbar spine and intervertebral discs after 90 d bed-rest. Based on available data from other studies, we do not consider the statistically significant effects of the countermeasures on intervertebral disc morphology to represent beneficial effects: the countermeasures exacerbated the effects seen in the control group rather than reducing them. Whilst there appeared to be few beneficial effects of the countermeasures on the parameters evaluated, the small sample size may have played a role in the non-significance of some findings. The current study also showed that recovery of volume of the spinal extensor musculature after prolonged bed-rest occurred of its own accord, with the majority of the effect seen in the first two-weeks after bed-rest and that the changes in the discs may not fully recover ninety days after bed-rest.

Conflict of interest

Dieter Felsenberg acts as a consultant to the European Space Agency for the exploitation of the results of this study. All other authors have no conflict of interest.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.actaastro.2011.05.015.

References

- [1] D.L. Belavý, G. Armbrrecht, U. Gast, C.A. Richardson, J.A. Hides, D. Felsenberg, Countermeasures against lumbar spine deconditioning in prolonged bed-rest: resistive exercise with and without whole-body vibration, *J. Appl. Physiol.* 109 (2010) 1801–1811.
- [2] D.L. Belavý, J.A. Hides, S.J. Wilson, W. Stanton, F.C. Dimeo, J. Rittweger, D. Felsenberg, C.A. Richardson, Resistive simulated weightbearing exercise with whole body vibration reduces lumbar spine deconditioning in bed-rest, *Spine* 33 (2008) E121–E131.
- [3] P. Cao, S. Kimura, B.R. Macias, T. Ueno, D.E. Watenpaugh, A.R. Hargens, Exercise within lower body negative pressure partially counteracts lumbar spine deconditioning associated with 28-day bed rest, *J. Appl. Physiol.* 99 (2005) 39–44.
- [4] N. Holguin, J. Muir, C. Rubin, S. Judex, Short applications of very low-magnitude vibrations attenuate expansion of the intervertebral disc during extended bed rest, *Spine J.* 9 (2009) 470–477.
- [5] B.R. Macias, P. Cao, D.E. Watenpaugh, A.R. Hargens, LBNP treadmill exercise maintains spine function and muscle strength in identical twins during 28-days simulated microgravity, *J. Appl. Physiol.* 102 (2007) 2274–2278.
- [6] L.C. Shackelford, A.D. LeBlanc, T.B. Driscoll, H.J. Evans, N.J. Rianon, S.M. Smith, E. Spector, D.L. Feeback, D. Lai, Resistance exercise as a countermeasure to disuse-induced bone loss, *J. Appl. Physiol.* 97 (2004) 119–129.
- [7] A.E. Nicogossian, L.F. Dietlein, *Microgravity Simulation and Analogues*, in: A.E. Nicogossian (Ed.), *Space physiology and Medicine*, Lea & Febiger, Philadelphia, 1982, pp. 240–248.
- [8] P.C. Wing, I.K.Y. Tsang, L. Susak, F. Gagnon, R. Gagnon, J.E. Potts, Back pain, and spinal changes in microgravity, *Orthop. Clin. North Am.* 22 (1991) 255–262.
- [9] S.L. Johnston, M.R. Campbell, R. Scheuring, A.H. Feiveson, Risk of herniated nucleus pulposus among U.S. astronauts, *Aviat. Space Environ. Med.* 81 (2010) 566–574.
- [10] K.J. Hutchinson, D.E. Watenpaugh, G. Murthy, V.A. Convertino, A.R. Hargens, Back pain during 6 degrees head-down tilt approximates that during actual microgravity, *Aviat. Space Environ. Med.* 66 (1995) 256–259.
- [11] D.L. Belavý, G. Armbrrecht, C.A. Richardson, D. Felsenberg, J.A. Hides, Muscle atrophy and changes in spinal morphology: is the lumbar spine vulnerable after prolonged bed-rest? *Spine* 36 (2011) 137–145.
- [12] M.M. Panjabi, The stabilizing system of the spine. Part I. Function, dysfunction, adaptation, and enhancement, *J. Spinal Disord.* 5 (1992) 383–389.
- [13] J. Rittweger, H.M. Frost, H. Schiessl, H. Ohshima, B. Alkner, P. Tesch, D. Felsenberg, 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 (2005) 1019–1029.
- [14] Y. Watanabe, H. Ohshima, K. Mizuno, C. Sekiguchi, M. Fukunaga, K. Kohri, J. Rittweger, D. Felsenberg, T. Matsumoto, T. Nakamura, Intravenous pamidronate prevents femoral bone loss and renal stone formation during 90-day bed rest, *J. Bone Miner. Res.* 19 (2004) 1771–1778.
- [15] B.A. Alkner, P.A. Tesch, Efficacy of a gravity-independent resistance exercise device as a countermeasure to muscle atrophy during 29-day bed rest, *Acta Physiol. Scand.* 181 (2004) 345–357.
- [16] B.A. Alkner, P.A. Tesch, Knee extensor and plantar flexor muscle size and function following 90 days of bed rest with or without resistance exercise, *Eur. J. Appl. Physiol.* 93 (2004) 294–305.
- [17] S. Röhrich, Die Auswirkungen einer 90tägigen Liegephase auf Rückenschmerzen und die Bewegungsaktivität der Rückenmuskulatur [The impact of a 90-day bed rest phase on back pain and motion activity of the back musculature], 2007 <http://zb-sport.dshs-koeln.de/Dissertationen/2007/Sven-Roehrich.html> last accessed 28.03.2010;116.
- [18] W.C. Hutton, J.A. Malko, W.A. Fajman, Lumbar disc volume measured by MRI: effects of bed rest, horizontal exercise, and vertical loading, *Aviat. Space Environ. Med.* 74 (2003) 73–78.
- [19] H. Akima, K. Kubo, M. Imai, H. Kanehisa, Y. Suzuki, A. Gunji, T. Fukunaga, Inactivity and muscle: effect of resistance training during bed rest on muscle size in the lower limb, *Acta Physiol. Scand.* 172 (2001) 269–278.
- [20] H. Akima, J. Ushiyama, J. Kubo, H. Fukuoka, H. Kanehisa, T. Fukunaga, Effect of unloading on muscle volume with and without resistance training, *Acta Astronaut.* 60 (2007) 728–736.
- [21] I. Frey, A. Berg, D. Grathwohl, J. Keul, Freiburger Fragebogen zur körperlichen Aktivität—Entwicklung, Prüfung und Anwendung, *Sozial Präventivmedizin* 44 (1999) 55–64.
- [22] J. Rittweger, D. Felsenberg, Recovery of muscle atrophy and bone loss from 90 days bed rest: results from a one-year follow-up, *Bone* 44 (2009) 214–224.
- [23] M.S. Conley, J.M. Foley, L.L. Ploutz-Snyder, R.A. Meyer, G.A. Dudley, Effect of acute head-down tilt on skeletal muscle cross-sectional area and proton transverse relaxation time, *J. Appl. Physiol.* 81 (1996) 1572–1577.
- [24] N. Bogduk, *Clinical anatomy of the lumbar spine and sacrum*, 3rd ed., Churchill Livingstone, London, 1997.
- [25] J.C. Pinheiro, D.M. Bates, *Mixed-effects models in S and S-PLUS*, 1st ed., Springer, Berlin, 2000.
- [26] R.N. Natarajan, G.B. Andersson, The influence of lumbar disc height and cross-sectional area on the mechanical response of the disc to physiologic loading, *Spine* 24 (1999) 1873–1881.
- [27] Y. Suzuki, H. Kashihara, K. Takenaka, K. Kawakubo, Y. Makita, S. Goto, S. Ikawa, A. Gunji, Effects of daily mild supine exercise on physical performance after 20 days bed rest in young persons, *Acta Astronaut.* 33 (1994) 101–111.
- [28] P. Berry, I. Berry, C. Manelfe, Magnetic-resonance-imaging evaluation of lower-limb muscles during bed rest—a microgravity simulation-model, *Aviat. Space Environ. Med.* 64 (1993) 212–218.
- [29] J. Zange, J. Mester, M. Heer, G. Kluge, A.M. Liphardt, 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 (2008) 271–277.
- [30] H. Akima, K. Kubo, H. Kanehisa, Y. Suzuki, A. Gunji, T. Fukunaga, Leg-press resistance training during 20 days of 6 degrees head-down-tilt bed rest prevents muscle deconditioning, *Eur. J. Appl. Physiol.* 82 (2000) 30–38.
- [31] M. Kouzaki, K. Masani, H. Akima, H. Shirasawa, H. Fukuoka, H. Kanehisa, T. Fukunaga, Effects of 20-day bed rest with and without strength training on postural sway during quiet standing, *Acta. Physiol. (Oxf.)* 189 (2007) 279–292.
- [32] D.L. Belavý, T. Miokovic, G. Armbrrecht, J. Rittweger, D. Felsenberg, Resistive vibration exercise reduces lower limb muscle atrophy during 56-day bed-rest, *J. Musculoskelet. Neuronal Interact.* 9 (2009) 225–235.
- [33] A. Kiefer, A. Shirazi-Adl, M. Parnianpour, Stability of the human spine in neutral postures, *Eur. Spine J.* 6 (1997) 45–53.
- [34] A.P. Claus, J.A. Hides, G.L. Moseley, P.W. Hodges, Different ways to balance the spine: subtle changes in sagittal spinal curves affect regional muscle activity, *Spine* 34 (2009) E208–E214.
- [35] L.A. Setton, J. Chen, Cell mechanics and mechanobiology in the intervertebral disc, *Spine* 29 (2004) 2710–2723.
- [36] J.J. MacLean, C.R. Lee, S. Grad, K. Ito, M. Alini, J.C. Iatridis, Effects of immobilization and dynamic compression on intervertebral disc cell gene expression in vivo, *Spine* 28 (2003) 973–981.
- [37] A.D. Le Blanc, H.J. Evans, V.S. Schneider, R.E. Wendt 3rd, T.D. Hedrick, Changes in intervertebral disc cross-sectional area with bed rest and space flight, *Spine* 19 (1994) 812–817.
- [38] N. Roberts, D. Hogg, G.H. Whitehouse, P. Dangerfield, Quantitative analysis of diurnal variation in volume and water content of lumbar intervertebral discs, *Clin. Anat.* 11 (1998) 1–8.
- [39] J.A. Malko, W.C. Hutton, W.A. Fajman, An in vivo study of the changes in volume (and fluid content) of the lumbar intervertebral disc after overnight bed rest and during an 8-hour walking protocol, *J. Spinal Disord. Tech.* 15 (2002) 157–163.
- [40] D.J. Botsford, S.I. Esses, D.J. Ogilvie-Harris, In vivo diurnal variation in intervertebral disc volume and morphology, *Spine* 19 (1994) 935–940.
- [41] A. Le Blanc, C. Lin, L. Shackelford, V. Sinitysyn, H. Evans, O. Belichenko, B. Schenkman, I. Kozlovskaya, V. Oganov, A. Bakulin, T. Hedrick,

- D. Feedback, Muscle volume, MRI relaxation times (T2), and body composition after spaceflight, *J. Appl. Physiol.* 89 (2000) 2158–2164.
- [43] J.A. Hides, D.L. Belavý, W. Stanton, S.J. Wilson, J. Rittweger, D. Felsenberg, C.A. Richardson, MRI assessment of trunk muscles during prolonged bed rest, *Spine* 32 (2007) 1687–1692.
- [44] A.D. Le Blanc, V.S. Schneider, H.J. Evans, C. Pientok, R. Rowe, E. Spector, Regional changes in muscle mass following 17 weeks of bed rest, *J. Appl. Physiol.* 73 (1992) 2172–2178.
- [45] C.L. Lewis, S.A. Sahrman, D.W. Moran, Effect of position and alteration in synergist muscle force contribution on hip forces when performing hip strengthening exercises, *Clin. Biomech.* 24 (2009) 35–42.
- [46] E. Andersson, L. Oddsson, H. Grundstrom, A. Thorstensson, The role of the psoas and iliacus muscles for stability and movement of the lumbar spine, pelvis and hip, *Scand. J. Med. Sci. Sports* 5 (1995) 10–16.
- [47] D.M. Mendis, J.A. Hides, S.J. Wilson, A. Grimaldi, D.L. Belavý, W. Stanton, D. Felsenberg, J. Rittweger, C. Richardson, Effect of prolonged bed rest on the anterior hip muscles, *Gait Posture* 30 (2009) 533–537.
- [48] P.W. Hodges, A. Kaigle, T. Hansson, S. Holm, Rapid atrophy of the lumbar multifidus follows experimental disc or nerve root injury, *Spine* 31 (2006) 2926–2933.
- [49] J.A. Hides, C.A. Richardson, G.A. Jull, Multifidus muscle recovery is not automatic after resolution of acute, first-episode low back pain, *Spine* 21 (1996) 2763–2769.
- [50] L.L. Ploutz-Snyder, P.A. Tesch, B.M. Hather, G.A. Dudley, Vulnerability to dysfunction and muscle injury after unloading, *Arch. Phys. Med. Rehabil.* 77 (1996) 773–777.
- [51] J. Rittweger, D.L. Belavý, P. Hunek, U. Gast, H. Boerst, B. Feilcke, G. Armbrrecht, T. Blenk, E. Mulder, H. Schubert, C. Richardson, A. de Haan, D.F. Stegeman, H. Schiessl, D. Felsenberg, Highly demanding resistive vibration exercise program is tolerated during 56 days of strict bed-rest, *Int. J. Sport. Med.* 27 (2006) 553–559.
- [52] D.L. Belavý, J.K. Ng, S.J. Wilson, G. Armbrrecht, D.F. Stegeman, J. Rittweger, D. Felsenberg, C.A. Richardson, Influence of prolonged bed-rest on spectral and temporal electromyographic motor control characteristics of the superficial lumbo-pelvic musculature, *J. Electromyogr. Kinesiol.* 20 (2010) 170–179.
- [53] D.L. Belavý, C.A. Richardson, S.J. Wilson, D. Felsenberg, J. Rittweger, Tonic to phasic shift of lumbo-pelvic muscle activity during 8-weeks of bed-rest and 6-months follow-up, *J. Appl. Physiol.* 103 (2007) 48–54.
- [54] J.A. Hides, G. Lambrecht, C.A. Richardson, W.R. Stanton, G. Armbrrecht, C. Pruett, V. Damann, D. Felsenberg, D.L. Belavý, The effects of rehabilitation on the muscles of the trunk following prolonged bed rest, *Eur. Spine J.* 20 (2010) 808–818.
- [55] K.M. Varma, R.W. Porter, Sudden onset of back pain, *Eur. Spine J.* 4 (1995) 145–147.



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Supplementary Table 5: Spinal morphology changes in each group from subjects measured 13-days after bed-rest

Study-date	All intervertebral discs T12L1 to L5S1							L1-S1	
	Disc volume (cm ³)	AP diameter (mm)	Transverse diameter (mm)	Axial CSA (cm ²)	Sagittal CSA (cm ²)	Anterior height (mm)	Posterior height (mm)	Lordosis (°)	Length (cm)
<i>Subjects measured 13-days post bed-rest (R+13; n=9)</i>									
Inactive control group (Ctrl; n=2)									
BDC	14.6(1.9)	39.8(1.1)	51.5(1.8)	16.0(126.4)	3.1(38.5)	11.2(1.5)	7.0(0.8)	52.9(9.5)	16.6(9.4)
HDT89(%)	+1.6(4.8)	+1.0(1.3)	-0.8(0.6)	+0.5(2.0)	-4.2(9.5)	-0.7(4.7)	+3.3(5.9)	-9.5(6.1)*	+2.4(0.5)‡
R+13(%)	-1.5(2.5)	+1.0(1.2)	-0.2(0.8)	+0.6(1.7)	-0.6(2.9)	-6.5(5.7)	+4.3(3.6)	-12.5(6.3)†	+2.3(0.5)‡
Fly-wheel group (FW; n=4)									
BDC	14.4(1.4)	40.8(1.0)	53.8(1.8)	17.3(88.4)	3.1(31.3)	10.8(1.1)	5.7(0.5)	45.3(6.7)	17.4(6.7)
HDT89(%)	+1.7(4.4)	-1.1(0.7)†	-1.8(0.9)‡	-2.9(1.3)‡	-4.3(5.7)	+3.4(4.4)	+6.6(6.3)	-1.6(6.1)	+1.4(0.6)‡
R+13(%)	-1.7(4.4)	+0.3(0.9)	-0.3(0.7)	+0.0(1.0)	-9.6(6.5)†	-1.3(4.6)	-2.6(5.1)	+0.3(2.8)	-1.1(0.8)*
Spinal mobilization exercise (SpMob; n=3)									
BDC	10.4(1.4)	37.6(1.0)	49.9(1.8)	14.8(88.8)	2.4(27.4)	9.2(1.1)	4.8(0.5)	45.1(6.7)	16.4(6.6)
HDT89(%)	+9.4(2.7)‡	-0.3(0.8)	-0.5(0.6)	-0.8(1.1)	-4.3(3.4)*	+5.3(2.9)†	+19.3(5.2)‡	+7.0(6.9)	+2.5(0.5)‡
R+13(%)	+9.8(5.3)‡	+0.4(1.8)	-0.8(1.3)	-0.5(2.6)	+6.0(5.4)†	+8.6(5.2)‡	+14.0(6.5)‡	+3.2(4.8)	+0.4(0.4)*

Values at baseline (BDC) are mean(SD) in units given at top of column. Values at end-bed-rest (HDT89) and 13-days post bed-rest (R+13) are mean(SD) percentage change compared to BDC value. *: $p < .05$; †: $p < .01$; ‡: $p < .001$ and indicate significance of difference of the mean to baseline. Of the spinal morphology parameters for which there was evidence of an effect of subject-group during bed-rest, only posterior disc height at R+13 showed a strong effect for a different response in the three groups ($F=8.5$, $p=0.0007$).

Supplementary Table 6: Spinal morphology changes in each group from subjects measured 90-days after bed-rest

Study-date	All intervertebral discs T12L1 to L5S1							L1-S1	
	Disc volume (cm ³)	AP diameter (mm)	Transverse diameter (mm)	Axial CSA (cm ²)	Sagittal CSA (cm ²)	Anterior height (mm)	Posterior height (mm)	Lordosis (°)	Length (cm)
<i>Subjects measured 90-days post bed-rest (R+90; n=15)</i>									
Inactive control group (Ctrl; n=5)									
BDC	11.6(2.7)	38.4(2.1)	50.9(3.1)	15.4(161.5)	2.4(53.2)	9.2(1.2)	5.5(0.7)	40.7(6.2)	16.5(6.7)
HDT89(%)	+6.6(4.0)‡	+0.3(0.8)	+0.1(0.7)	+0.4(1.1)	+5.4(4.9)*	+8.6(4.7)‡	+3.1(7.7)	+9.7(10.3)	+0.7(0.5)*
R+90(%)	+0.3(4.1)	+0.6(1.4)	+0.1(1.2)	+0.8(2.2)	-2.4(5.0)	+2.7(4.8)	-4.7(6.3)	+15.0(8.1)†	-1.5(1.1)†
Fly-wheel group (FW; n=4)									
BDC	13.9(2.7)	39.9(2.0)	55.2(3.1)	17.7(160.9)	3.1(53.0)	9.8(1.2)	5.6(0.7)	41.5(5.9)	17.2(6.6)
HDT89(%)	+3.9(5.3)	-1.1(0.8)†	-1.8(0.9)‡	-2.9(1.6)‡	-1.2(4.5)	+7.5(4.6)†	+8.9(7.6)*	+8.4(9.1)	+1.1(0.8)†
R+90(%)	+3.6(3.9)	-0.6(0.7)	-0.7(0.6)†	-1.3(0.9)†	-2.4(3.1)	+6.3(3.1)‡	+4.3(8.2)	+14.2(6.0)‡	-1.1(1.1)
Spinal mobilization exercise (SpMob; n=6)									
BDC	13.4(2.7)	38.9(2.0)	52.2(3.1)	16.0(160.7)	2.8(53.2)	10.4(1.2)	5.8(0.7)	44.3(5.9)	17.2(7.0)
HDT89(%)	+4.0(3.2)†	-0.3(0.8)	-0.9(0.6)‡	-1.3(1.2)†	-0.5(3.5)	+4.6(2.7)‡	+7.7(5.0)‡	+10.7(6.9)†	+1.3(1.6)
R+90(%)	+2.8(3.1)*	+0.5(1.0)	0.0(0.7)	+0.4(1.2)	+0.3(4.0)	+3.7(2.7)‡	+0.9(5.8)	+6.0(6.8)*	-0.4(1.4)

Values at baseline (BDC) are mean(SD) in units given at top of column. Values at end-bed-rest (HDT89) and 90-days post-bed-rest (R+90) are mean(SD) percentage change compared to BDC value. *: $p < .05$; †: $p < .01$; ‡: $p < .001$ and indicate significance of difference of the mean to baseline. Of the spinal morphology parameters for which there was evidence of an effect of subject-group during bed-rest, there was no evidence for a different response between groups at R+90.