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Exercise and transversus abdominis muscle atrophy after 60d bed-rest

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**Author contributions:**

**Belavý:** Secured funding. Conception and design of the experiments. Data collection. Statistical analysis and interpretation of the data. Drafting the article.

**Gast:** Data collection. Interpretation of the data. Revision of article.

**Felsenberg:** Secured funding. Conception and design of the experiments. Interpretation of the data.
Abstract

Purpose: To investigate atrophy in the deep abdominal muscles, spinal extensors and the impact of high-load resistive exercise with and without whole body vibration following 60d of strict bed-rest.

Methods: 24 subjects underwent 60-days of head-down tilt bed-rest and performed either resistive vibration exercise (RVE), resistive exercise only (RE) or no exercise control (2nd Berlin BedRest Study). The thickness of the transversus abdominis, internal oblique and erector spinae muscles and area of the multifidus muscle were measured bilaterally via real-time ultrasound. "Intent to treat" analysis was implemented and p-values were adjusted by the false discovery rate method.

Results: At end-bed-rest, transversus abdominis thickness was reduced by 18.3% in the inactive group (p=0.00011) with no significant change in the RVE (-4.0%; p=0.014 versus control) or RE (-5.0%; p=0.10 versus control) groups. In the inactive subjects, internal oblique thickness reduced by 10.6% (p=0.0025) and by 7% (p>0.05) in each of the training groups. Lengthening of the lumbar spine was greatest on day 1 (+7.4%, p=0.004) and 2 (+6.3%, p=0.004; day 54: +4.1%, p=0.023). Extensor atrophy and spinal lengthening was not impacted by exercise. No significant difference was seen between RVE and RE.

Conclusion: Bed-rest leads to atrophy of the transversus abdominis and internal oblique muscles. The exercise program, which implemented lower-limb and back extension exercises against shoulder restraints, was able to reduce atrophy seen in transversus abdominis in bed-rest.

Keywords: spine; muscle; astronauts; spaceflight; back pain; herniation
Introduction

Optimising countermeasures against musculoskeletal deterioration is a priority for space agencies around the world. With a long-term view to missions to Mars or a Moon base, it is important to maintain the musculoskeletal system to enable completion of mission tasks in the hypogravitational fields on Mars or Moon, and also to safeguard the return to Earth’s 1-g environment. At the lumbar spine in particular astronauts are at increased risk of injury (28) and muscular deficiency is considered (3) to be one likely risk factor for this. Beyond the importance (29) of the extensor musculature, in particular the lumbar multifidus (40), for controlling the lumbar spine, the deep abdominal musculature, in particular transversus abdominis, has an important role in posture and in stabilising the lumbar spine. Specifically, contraction of the deep abdominal musculature increases the stiffness of the lumbar spine via the thoracolumbar fascia (19) and contraction of the transversus abdominis muscle with the other muscles of the abdominal cavity has also been shown to increase intra-abdominal pressure (21) which has a direct effect in stiffening the lumbar spine (20). Furthermore, contraction of the deep abdominal muscles increases the stiffness of the sacroiliac joint (35). Overall, the deep abdominal muscles play a role in control of upright posture and likely a role in lumbar spine stability.

In disuse, specifically strict bed-rest, it is known that the major postural muscles of the lower-limbs and lumbar spine atrophy. For example, atrophy is greatest in the plantarflexors (medial gastrocnemius, soleus), knee extensors (vasti) and hip extensors (hamstrings) (33). At the lumbar spine, atrophy of the extensors occurs during prolonged bed-rest (31). Based on our knowledge of the postural roles of the deep abdominal musculature, it would therefore be intuitive to expect this musculature to atrophy in strict disuse (prolonged bed-rest). However, there is no data available on these muscles. The primary aim of this work was, therefore, to investigate the impact of disuse (60d...
bed-rest) on the internal oblique and transversus abdominis and the primary hypothesis was that atrophy of these muscles would occur with bed-rest.

In terms of preventing muscle atrophy during prolonged bed-rest, the available data (11) indicates that muscle specific higher-load resistance exercise appears to be more effective in maintaining muscle mass, with aerobic and/or low-load type exercise being less effective. For the spinal extensors some studies have shown significant effects on preventing muscle atrophy, such as with muscle specific resistance exercise (37), resistive exercise with whole-body vibration (6) and supine treadmill running in lower-body negative pressure combined with lower-limb fly-wheel exercise (26), yet other exercise approaches, such as supine treadmill running in lower-body negative pressure alone (14), lower-limb fly-wheel exercise alone (13) and whole-body vibration without additional exercise (25) had limited impact on extensor muscle atrophy. For the deep abdominal musculature there is no data available in the literature. A secondary aim of the current work was to examine the impact of exercise countermeasures, specifically resistive exercise with or without whole-body vibration (5), on ameliorating adaptations at in the deep abdominal and spinal extensor muscles in prolonged bed-rest.

Finally, whilst it is clear that extensor muscle atrophy occurs in disuse, it is not clear how quickly this sets in and to what extent. It is known that lower-limb muscle atrophy can occur rapidly in disuse (39). A tertiary goal of this work was to examine the rate of lumbar spine extensor musculature atrophy in disuse.
Methods

*Bed-rest study design and subjects*

Twenty-four psychologically and medically healthy males participated in the 2nd Berlin Bed Rest Study and underwent 60d six-degree head-down tilt (HDT) strict bed rest (5). Exclusion criteria included a history of chronic low back pain, a current episode of low back pain, or any history of spinal operation. During bed-rest subjects were permitted to have a pillow underneath their head in lying, but were instructed that their shoulder blades must be in contact with the mattress. The study was approved by the ethical committee of the Charité Universitätsmedizin Berlin. All subjects gave their informed written consent prior to participation in the study. Subjects were randomized to three different groups: one that performed resistive exercises with whole-body vibration during bed-rest (RVE; n=8; mean(SD) age 32.0(10.7)years, height 179.5(5.9)cm, weight 80.5(6.4)kg), one that performed resistive exercise only (RE; n=8; 31.1(5.1) years, 179.3(7.7)cm, 75.0(12.8)kg) and finally one that performed no exercise and served as a control group (CTR; n=8; 34.7(6.8)years, 182.4(5.4)cm, 81.2(5.3)kg). Randomisation occurred after baseline data collection. For the RVE and RE groups, training was performed three days a week during the bed-rest phase. Subjects were positioned in head-down tilt on a moveable platform with shoulder pads and hand grips preventing downward movement and permitting application of force via the platform. After a short warm up, the following exercises were performed on the Galileo Space exercise device (Novotec Medical GmbH, Pforzheim, Germany; Figure 1): bilateral squats (~75-80% of pre bed-rest maximum voluntary contraction: in RVE-group vibration frequency 24Hz, amplitude 3.5-4mm, peak acceleration ~8.7g where g=9.81ms-2), single leg heel raises (~1.3 times body-weight; in RVE-group vibration frequency 26Hz, amplitude 3.5-4mm, peak acceleration ~10.2g), double leg heel raises (~1.8 times body-weight; in RVE-group vibration frequency 26Hz, amplitude 3.5-4mm, peak acceleration ~10.2g), back and heel raise (performing hip and lumbar spine extension against
gravity with ankle dorsiflexion but with ~1.5 times body-weight applied at the shoulders; in RVE-
group vibration frequency 16Hz, amplitude 3.5-4mm, acceleration ~3.9g). The RVE-group
performed the same exercises as the RE-group except that whole-body vibration was applied (5).

**Measurement protocol**

Two testing sessions (four or five days before bed-rest and then two days before bed-rest) were
performed with subjects before bed-rest to obtain baseline data. All measurements were performed
on days 12, 26, 40, and 54 of bed-rest. To assess rate of atrophy in the extensor musculature, the
erector spinae and multifidus were imaged additionally on days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13
and 14 of bed-rest. Lumbar spine length was measured at every testing session. The HDT position
was maintained in all testing sessions.

To ensure blinding of the examiner to study-date for later offline image measurements, each testing
session was assigned a random number (obtained from [www.random.org](http://www.random.org)) and this was encoded in
the image data. A portable GE LOGIQe (GE Healthcare, Berlin, Germany) ultrasound machine was
used with a convex transducer (4C-RS) set at 5 MHz. The same operator performed all
measurements with each subject. Prior to each measurement session, the vertebral levels were
marked on the subject's skin: first the operator palpated the posterior superior iliac spines and
marked this position at the midline (typically S2). The operator then palpated the spinous process of
L4 and then marked the interspinous space at L4/5. The interspinous spaces from L4/5 up to T12/L1
were marked. The location of the marked vertebral levels was then confirmed via real-time
ultrasound, using known anatomical landmarks (i.e. [a] transverse process of L5 is the shortest and
deepest anatomically, [b] the L5 spinous process is smaller than at L4 and, [c] the twelfth rib is
located near the L1 transverse process in parasagittal section).
Lumbar spine length: The same standard tape measure was used to measure the distance along the lumbar lordosis between the marking centrally between the posterior superior iliac spines and the T12/L1 interspinous space. Measurements were performed in prone lying. The measurement was performed three times and the average value was used in further analysis.

Imaging of transversus abdominis and internal oblique: Subjects were positioned in supine lying. The ultrasound head was positioned over on the abdominal wall on a line midway between the inferior angle of the rib cage and the iliac crest (18). The ultrasound head was oriented to be perpendicular to the skin and rotated along its axis such that the anterior insertion of the transversus abdominis muscle into the fascia could be visualised (Figure 2). Prior work on transversus abdominis muscle thickness (30) has shown that performing and averaging three measurements of muscle thickness provided the bulk of improvement in repeatability possible with multiple measurements. Therefore, three repetitions of measurements were performed each on the left and right sides of the abdomen and the results averaged. No trials were omitted from the averaging procedures. For each repetition, the subject was instructed to breathe in and out slowly, pause their breathing. A video of ultrasound image was recorded during this time. At the end of the out-breath a still image was generated, labelled with the side of body and repetition number.

Imaging of the erector spinae in parasaggital section: Erector spinae thickness was measured at L1, L2, L3, L4 and L5 on the left and right sides of the body in prone lying. The ultrasound probe was positioned in sagittal section over the spinous processes and then moved laterally until the transverse processes could be seen (Figure 2). To ensure a consistent anatomical position was used, the thickness of the erector spinae was measured at the most lateral tip of the transverse process. At this anatomical position, the image was saved for offline processing. To aid offline processing, the vertebral level of the transverse process and side of body was marked in the image.
Imaging of the multifidus muscle in paraxial section: The multifidus muscle was imaged at L1, L2, L3, L4 and L5 on the left and right sides of the body in prone lying. Images were taken of the multifidus muscle at each vertebral level where the vertebral lamina could be identified (Figure 3). The lateral border of the multifidus muscle (next to the longissimus muscle) was identified. At this anatomical position, the image was saved for offline processing. To aid offline processing, the vertebral level, side of body and positioning of the lateral border of the multifidus muscle was then marked on the image.

Further image data processing and image analysis

Blinding was retained for image measurements. After exporting images from the ultrasound device, date and time of measurement information, which was automatically saved in the exported images, was removed from all images prior to analysis. Image analysis was conducted by the same operator and performed using publicly available ImageJ (version 1.39u; http://rsb.info.nih.gov/ij/) software. No trials were omitted from any averaging procedures.

Transversus abdominis and internal oblique muscle thicknesses: Lateral to the insertion of transversus abdominis into the anterior fascia, the connective tissues deep and superficial to the transversus abdominis muscle can be seen on the ultrasound image to run parallel for a short distance (Figure 2). The thickness of the transversus abdominis muscle was measured at a point half-way along this length. The thickness of the internal oblique muscle was measured at the same point. The results of the three repetitions on each side were averaged prior to further analysis. Based on the duplicate baseline measurements performed on two separate days, the between day coefficients of variation (CVs) for this measurement approach are 9.2% and 6.7% for the transversus abdominis and internal oblique muscles respectively.
_Erector spinae thickness:_ Erector spinae thickness was measured on each image at each vertebral level as the linear distance from the highest point of the transverse process to the superior border of muscle. To reduce measurement error, the data from all vertebral levels on each side were also averaged. The primary analysis considered the average thickness. Based on the duplicate baseline measurements, the between day CV for average erector spinae thickness area was 4.4%.

_Multifidus muscle area:_ The multifidus muscle was traced around on each image and care was taken to ensure the more laterally place longissimus muscle was not included in the measurement. To reduce measurement error, the data from all vertebral levels on each side were also averaged. The primary analysis considered the average area. Based on the duplicate baseline measurements, the between day CV for average multifidus area was 6.2%.

Results from the two baseline measurements averaged prior to further analysis.

**Statistical analyses**

An “intent to treat” (ITT) approach was used in primary analysis. In assessing the impact of exercise in bed-rest on abdominal muscle thickness, linear mixed-effects models with subsequent repeated measures analysis of variance (ANOVA) were used to assess the interaction of ‘date’ (i.e. baseline and bed-rest days 12, 26, 40 and 54) and ‘group’ (i.e. CTR, RE and RVE). If the group×study-time interaction was significant on ANOVA, then secondary 2-group (i.e. CTR vs. RE, CTR vs. RVE and RE vs. RVE) models were built using the same approach to assess which groups had a different response. A priori t-tests were then performed comparing each study-date to baseline within each group. A similar approach was used for the erector spinae thickness and multifidus area, however for the analysis of the impact of ‘group’, to avoid spurious effects, the data collected from days 1 to 7 were averaged and the data from days 8 to 14 were averaged. To assess whether
lengthening of the spine contributed to paraspinal muscle size changes, Pearson’s correlation co-efficient between the change in lumbar spine length and the change in average multifidus area and average erector spinae area on each day of bed-rest was calculated.

An alpha-level of 0.05 was taken for statistical significance. To avoid type I errors, all p-values were adjusted by the false discovery rate method. The “R” statistical environment (version 2.10.1, www.r-project.org) was used for all analyses. Unless otherwise specified all values are reported as mean(SD).
Results

One RE subject dropped out of the study on day 30 of bed-rest and data for him on day 40 and 54 of bed-rest were not available. One RVE subject experienced exercise induced headaches during training in the first days of bed-rest which prevented him from further performing the exercises. The independent medical advisor determined that the subject could remain in the study, but only as an inactive CTR subject (5). This subject was classified as RVE-group on the ITT-analysis and as CTR-group on the “per protocol” (PP) analysis. The main findings of the study did not differ on PP-analysis.

No differences existed between groups at baseline. Initial analyses showed no significant difference between the left and right sides of the body in the response during bed-rest (p>0.13). Hence, left and right side measurements were pooled in the analyses presented here.

Atrophy of the anterolateral abdominal muscles and impact of exercise

Losses of the thickness of the transversus abdominis and internal oblique muscles occurred in bed-rest with these effects being greatest in magnitude in the inactive control group (Table 1, Figure 4). The thickness of the transversus abdominis reduced by 18.3% (p= 0.00011) in the inactive group by the end of bed-rest, with no significant change in the RE (-5.0%, p=0.36) and RVE (-4.0%, p=0.33) groups at this time point. Internal oblique muscle thickness reduced by 10.6% (p=0.0025) in the inactive group by the end of bed-rest (-7.0%, p=0.055 and -6.9%, p=0.12 in the RE and RVE groups respectively). ANOVA showed the differences between the groups to be significant for transversus abdominis only (p=0.030 [p=0.0031 on PP-analysis]; internal oblique p=0.34) with subsequent 2-group ANOVAs showing the CTR and RVE groups to differ significantly in their response for transversus abdominis thickness changes (p=0.014, PP-analysis: p=0.0008). No significant
difference was observed between CTR and RE (p=0.10, PP-analysis: p=0.086) or RE and RVE (p=0.59, PP-analysis: p=0.30) for transversus abdominis thickness changes.

Atrophy of the spinal extensors and impact of exercise

At the end of bed-rest a 5.2% loss (p=0.035) of average erector spinae thickness was observed in the inactive control group, with 1.4% (p=0.55) and 5.2% (p=0.077) loss in the RE and RVE groups respectively (Table 1 and Figure 4). At the same time point, average multifidus area was reduced by 4.1% in the inactive group (p=0.039), 4.4% in the RE group (p=0.010) and 4.6% in the RVE group (p=0.030). The changes within each group at each vertebral level are presented in Supplemental Tables 1 and 2. For average multifidus area, ANOVA showed a difference between the groups (p=0.026; PP-analysis: p=0.036) and subsequent ANOVAs comparing group pairs reached significance for RE vs RVE (p=0.0023; PP-analysis: p=0.0025) but not for CTR vs RE (p=0.54) or CTR vs RVE (p=0.16). However, inspection of the data (Supplemental Table 2) did not indicate a consistent pattern of greater loss in either the RE or RVE group. For average erector spinae thickness, differences between the groups were not observed (p=0.78).

Rate of atrophy of the spinal extensors early in bed-rest and relationship to spinal length changes

Since minimal impact of the countermeasures was observed on the spinal extensors, in evaluating the changes in the first two weeks of bed-rest, data from all subjects were pooled. Significant reductions in spinal extensor size were seen within the first two days of bed-rest (Figure 5). Average multifidus area was reduced by 4.7% (p=0.0049) on day 1 of bed-rest. Average erector spinae area was reduced by 4.2% (p=0.0011) on day 2 of bed-rest.
Lumbar spine length increases peaked on days 1 and 2 of bed-rest with a subsequent partial return towards baseline. On day 1 of bed-rest, the length of the lumbar spine was increased by 7.4% (p=0.0036) compared to baseline and by 6.3% on day 2 (p=0.0036). After this lumbar spine length returned towards baseline levels and remained stable at between +3.5% and +5% versus baseline for the remainder of the bed-rest phase and was 4.1% greater (p=0.023) at the end of bed-rest. Lumbar spine length changes during bed-rest did not differ significantly between groups. Secondary correlation analysis indicated a correlation on day 2 of bed-rest only between average multifidus area change (r=0.43, unadjusted p=0.035 adjusted p=ns), but not average erector spinae thickness change, and lumbar spine length change.
Discussion

This was the first study to have examined atrophy of the deep abdominal muscles in disuse, the impact of exercise on this atrophy and also the rate of atrophy in the spinal extensors. The main finding of the current study was that atrophy of the transversus abdominis and internal oblique muscles occur during strict disuse. In relative terms, the thickness reduction of the transversus abdominis was greater than seen in the internal oblique. Another important finding was that the exercise protocol reduced these changes and was this effect was statistically significant for the transversus abdominis muscle.

The findings of atrophy in transversus abdominis and internal oblique contrast to what has been observed in the other abdominal muscles during bed-rest. The psoas (37) and rectus abdominis (17) muscles hypertrophy in prolonged bed-rest. In contrast to spaceflight, where atrophy of psoas is seen, bed-rest offers a stimulus for hypertrophy of some trunk and neck (7) flexor muscles. Other works have used superficial electromyography to investigate the trunk muscles after bed-rest. These works have found increased activation of the superficial trunk muscles during isometric trunk holding tasks (11) and earlier activation of the superficial abdominal muscles in postural control tasks (36) after bed-rest, indicating that the stabilisation of the trunk is impacted after bed-rest. Less information is available about the deep abdominal muscles, but one other work examined the trunk musculature via ultrasound in bedridden elderly people compared ambulant elderly people (27). These authors found the bedridden elderly to have thinner transversus abdominis (45% thinner), erector spinae (39%), lumbar multifidus (2%), external oblique (42%), but not internal oblique, muscles compared to their ambulant group. Overall, the deep abdominal muscles are ascribed postural and lumbar spine stabilisation roles (2). In other body regions (8, 33), the postural and anti-gravity muscles are those that most strongly atrophy in disuse strict bed-rest. The results of the current study provide evidence that this pattern is consistent at the trunk: the need for the postural
and spine stabilisation roles of the deep abdominal muscles is reduced and atrophy of the transversus abdominis and internal oblique muscles occurs.

The current work is also the first examination of the effect of exercise countermeasures in preventing atrophy of the deep abdominal muscles in strict disuse. The magnitude of transversus abdominis and internal oblique thickness loss was less in the training groups and this effect was statistically significant for the resistive vibration exercise countermeasure group for preventing atrophy of the transversus abdominis. It is important to note that the exercise protocols did not explicitly target the abdominal musculature. Rather, loading of the spine and trunk occurred via shoulder restraints during training and exercise was performed with high intensity. Prior work has shown that transversus abdominis and internal oblique are activated when the knee is loaded in isolated isometric knee extension and flexion (15) or in an isometric leg press task with shoulder restraints (16). This means that the deep abdominal muscles will have been activated by the exercises performed in the current study. Typically, to prevent lower-limb muscle atrophy in prolonged bed-rest, it is more effective to have specific exercise manoeuvres targeting select muscle groups (reviewed in (9)). In clinical treatment of low back pain, some authors (13) argue for the selective training of transversus abdominis. The current study, however, provides evidence that in disuse, exercises specific for the transversus abdominis muscle are not necessary to prevent its atrophy. Rather, high-intensity lower limb and spine extension exercise manoeuvres with restraints at the shoulders activate the transversus abdominis muscle sufficiently to reduce its atrophy.

The tertiary aim of the current work was to investigate the rate of atrophy of the spinal extensors. We observed reductions in multifidus muscle area from day 1 of bed-rest. Erector spinae thickness reductions were observed as early as day 2 of bed-rest. Losses of muscle mass have been observed within the first few days of immobilisation in animal models (12). Also, prior work on the lower-limb musculature (39) found significant thigh muscle atrophy at the earliest follow-up day after five
days of immobilization. Water content reduction in the musculature occurs in early stages of atrophy: an animal injury model at the spine (22) found losses of water content of the multifidus muscle of between four to six percentage points within three to six days of injury. This loss of muscle water content contributed to the loss of muscle size (22). It is well known (1) that as atrophy progresses, reductions in muscle fibre cross-sectional area, and changes in fibre type contribute to overall loss of muscle size. In the current study we also examined changes in spinal length as they may potentially confound the assessment of the atrophic response in the paraspinal muscles. We found some evidence, albeit weak and not significant after adjustment of p-values for type I errors, of a relationship between lengthening of the spine on day 2 of bed-rest and reductions in multifidus muscle area. This weak relationship was seen only on day 2 of bed-rest and was not seen for the erector spinae. This may indicate that some extent of the measured area reduction of multifidus early in bed-rest might be due to “stretching” of the muscle volume with lengthening of the spine. Overall, however, the current study found atrophy of the multifidus and erector spinae muscles occurs rapidly in bed-rest and is measurable within two days of bed-rest.

The findings of the current study may have some clinical relevance. There is evidence (15, 16, 23, 24) that the function deep abdominal musculature is impaired in low back pain. With reduced muscle size, a muscle cannot produce as high forces with contraction (32). Presumably, the capacity of the transversus abdominis and internal oblique muscles to generate intra-abdominal pressure (20) and tense the thoracolumbar fascia (19) to stiffen the spine will be impaired. Astronauts have been shown to have a higher incidence of intervertebral disc herniation (28), and although it is likely (3) that this effect is mostly due hyperhydration of the intervertebral disc, impairment of musculature that stabilise the spine could contribute to this risk.

We also noted a peak in lumbar spine lengthening on days 1 and 2 in bed-rest with a return back to a still lengthened new steady state. Another research group (38) has made a similar observation,
specifically that increases in lumbar disc height were greatest in the first days of bed-rest. The lengthening of the spine in bed-rest is most strongly related (4) to increases in disc size in bed-rest. Future work should consider what occurs in the spine tissues to potentiate the reduction from peak disc size and spine length in the early days of bed-rest and whether this is a critical period for the adaptation of the spine and disc to unloading.

It is important to consider some limitations of the current work. As is typical of bed-rest studies in Europe, only one gender was included in the study. Although we are of the opinion that the results would be readily applicable to female bed-rest participants, formally we cannot be sure of this as we included only male subjects. The number of subjects was limited due to logistical and financial restraints. Due to the limited number of subjects, some non-significant results for the effect of the exercise protocol may represent false-negatives. Furthermore, as this was an observational study, we cannot comment on the underlying mechanisms (e.g. ref (34)) of the trunk muscle atrophy observed. However, the results of the current work justify future investigation of mechanisms of trunk muscle atrophy in disuse, which would typically require invasive procedures, such as biopsy.

In conclusion, we found that with strict bed-rest, atrophy of the transversus abdominis muscle and internal oblique muscle occurs. The atrophy of transversus abdominis (19% reduction of muscle thickness) was greater than internal oblique (10% reduction of thickness). Critically, exercise reduced atrophy of the transversus abdominis muscle despite the exercise regime not specifically targeting this muscle group. This indicates loading of the spine during high-intensity lower-limb and back extension exercises with shoulder restraints activate the deep abdominals muscle sufficiently during exercise to reduce their atrophy in bed-rest. The current study was also the first to examine the rate of spinal muscle atrophy in disuse. We found significant atrophy of the spinal extensor musculature occurred within two days of bed-rest. Overall, the findings show that the
musculature which contributes to the control of spinal posture and stability atrophies during bed-
rest.
References


Supplemental Digital Content

Supplemental Table 1 (SDC Table 1.doc)
Supplemental Table 2 (SDC Table 2.doc)
Here the subject is performing bilateral squat exercises. Subjects were positioned in head-down tilt on a moveable platform. Shoulder pads and hand grips permitted application of force via the platform. The feet were positioned on either side of a platform which was set to vibrate in the group which received additional whole-body vibration.
Figure 2: Ultrasound measurement of transversus abdominis, internal oblique and erector spinae thickness

To measure transversus abdominis and internal oblique (left), the ultrasound head was positioned over on the abdominal wall on line midway between the inferior angle of the rib cage and the iliac crest. The ultrasound head was positioned such that the anterior insertion of the transversus abdominis muscle ('TrA') into the fascia (arrow at 'a') and the portion where connective tissues deep and superficial to the transversus abdominis muscle run parallel (between 'b' and 'c') were positioned at the centre of the field of view. In offline image analysis thickness of the transversus abdominis muscle was measured at a point half-way along this length. The thickness of the internal oblique ('IO') muscle was measured at the same point. 'EO': external oblique muscle. The coding to ensure blinding of the operator during image analysis (in this case 108640, S2R) can be seen at the top of the image.

To measure erector spinae thickness, using parasagittal images, the most lateral point of the transverse processes was visualized and the vertebral level of each transverse process marked on the image. Images were taken on both the left and right sides of the body. The transverse process can be seen as a short bright line at the lower part of the image. Typically, a "shadow" could be seen below the transverse process. This is most evident in this particular set of images at L4 and L2. The distance between the transverse process and the superficial border of the muscle was measured. Where a vertebral level was captured in more than one image (typically L3), it was measured twice and the result averaged prior to further analysis.
Figure 3: Ultrasound measurement of multifidus muscle area at each lumbar vertebral level
Transverse plane images of the multifidus muscle were taken at each vertebral level. To guide offline processing, the vertebral level of each measurement was marked in the image and markers ("<-") were placed in the image at the lateral border of the muscle.
**Figure 4:** Atrophy of the deep abdominal muscles (top) and spinal extensors (bottom)

Values are mean(SD) percentage change compared to pre-bed-rest. Data have been pooled from measurements in the first half of bed-rest (days 12 and 26) and the second half of 60d bed-rest (days 40 and 54). See also Table 1. CTR: control group, RE: resistance exercise group, RVE: resistance exercise + vibration group *: p <0.05; †: p <0.01; ‡: p <0.001 and indicate significance of within-group difference compared to baseline. To guard against false positives, p-values have been adjusted via the false discovery rate method. Intent-to-treat analysis presented.
**Figure 5**: Extensor muscle size and lumbar spine length in the first two weeks of bed-rest

Values are mean(SD) percentage change compared to pre-bed-rest. Data from all groups have been pooled. Muscle size was averaged across all vertebral levels. *: $p < 0.05$; †: $p < 0.01$; ‡: $p < 0.001$ and indicate significance of difference compared to baseline. To guard against false positives, p-values have been adjusted via the false discovery rate method.
Table 1: Abdominal and lumbar extensor muscle atrophy

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Day 26</th>
<th>Day 40</th>
<th>Day 54</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transversus abdominis thickness (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>4.4(0.6)</td>
<td>-</td>
<td>-21.9(13.5)%‡</td>
<td>-19.3(11.7)%‡</td>
<td>-17.9(12.4)%‡</td>
<td>-18.3(10.6)%‡</td>
</tr>
<tr>
<td>RE</td>
<td>4.2(0.9)</td>
<td>-</td>
<td>-9.0(11.7)%*</td>
<td>-6.2(13.4)%</td>
<td>-4.0(12.3)%</td>
<td>-5.0(12.2)%</td>
</tr>
<tr>
<td>RVE</td>
<td>3.5(0.5)</td>
<td>-</td>
<td>-6.6(11.8)%</td>
<td>-8.7(11.9)%</td>
<td>-13.0(13.8)%*</td>
<td>-4.0(11.1)%</td>
</tr>
<tr>
<td></td>
<td>Internal oblique thickness (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>9.8(1.4)</td>
<td>-</td>
<td>-12.2(10.0)%†</td>
<td>-12.1(9.1)%‡</td>
<td>-11.6(9.4)%†</td>
<td>-10.6(9.1)%†</td>
</tr>
<tr>
<td>RE</td>
<td>11.3(2.0)</td>
<td>-</td>
<td>-13.3(8.5)%‡</td>
<td>-4.5(9.5)%</td>
<td>-6.6(8.5)%</td>
<td>-7.0(8.7)%</td>
</tr>
<tr>
<td>RVE</td>
<td>9.1(1.8)</td>
<td>-</td>
<td>-10.0(12.7)%</td>
<td>0.1(13.9)%</td>
<td>-7.8(10.8)%</td>
<td>-6.9(11.5)%</td>
</tr>
<tr>
<td></td>
<td>Average erector spinae thickness (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>3.9(0.5)</td>
<td>-4.3(5.8)%</td>
<td>-4.0(5.5)%</td>
<td>-4.3(6.0)%</td>
<td>-3.9(6.0)%</td>
<td>-5.2(6.2)%*</td>
</tr>
<tr>
<td>RE</td>
<td>3.8(0.4)</td>
<td>-2.6(5.8)%</td>
<td>-2.3(5.0)%</td>
<td>-2.9(5.7)%</td>
<td>-3.1(5.1)%</td>
<td>-1.4(5.4)%</td>
</tr>
<tr>
<td>RVE</td>
<td>3.8(0.4)</td>
<td>-1.7(3.2)%</td>
<td>-3.0(3.6)%*</td>
<td>-4.1(3.9)%*</td>
<td>-4.5(5.6)%*</td>
<td>-5.2(7.5)%</td>
</tr>
<tr>
<td></td>
<td>Average multifidus area (cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>6.4(0.8)</td>
<td>-5.4(4.1)%†</td>
<td>-4.6(3.3)%‡</td>
<td>-6.5(5.1)%†</td>
<td>-3.7(4.3)%*</td>
<td>-4.1(4.9)%*</td>
</tr>
<tr>
<td>RE</td>
<td>5.4(0.7)</td>
<td>-5.3(5.2)%*</td>
<td>-5.3(3.5)%‡</td>
<td>-4.3(4.1)%*</td>
<td>-3.7(4.1)%*</td>
<td>-4.4(3.9)%*</td>
</tr>
<tr>
<td>RVE</td>
<td>5.9(1.0)</td>
<td>-2.9(4.4)%</td>
<td>-1.0(3.8)%</td>
<td>-6.1(7.1)%*</td>
<td>-1.1(5.2)%</td>
<td>-4.6(5.2)%*</td>
</tr>
</tbody>
</table>

Values are mean(SD). CTR: control group, RE: resistance exercise group, RVE: resistance exercise + vibration group. Values in bed-rest are percentage change versus baseline. Week 1: data from extensor muscles from first 7 days of bed-rest averaged. Week 2: data from extensor muscles from days 8 to 14 of bed-rest averaged and abdominal muscles measured on day 12 of bed-rest. *: p <0.05; †:p <0.01; ‡: p<0.001 and indicate significance of within-
group difference compared to baseline. To guard against false positives, p-values have been adjusted via the false discovery rate method.
**Supplemental Table 1**: Erector spinae muscle atrophy at the first (L1) through to fifth (L5) vertebral levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline (cm)</th>
<th>Day of bed-rest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1-7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>L1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>3.3(0.4)</td>
<td>-3.5(8.0)%</td>
</tr>
<tr>
<td>RE</td>
<td>3.3(0.3)</td>
<td>-1.4(6.4)%</td>
</tr>
<tr>
<td>RVE</td>
<td>3.3(0.3)</td>
<td>-1.5(6.5)%</td>
</tr>
<tr>
<td><strong>L2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>3.2(0.4)</td>
<td>-4.2(6.6)%</td>
</tr>
<tr>
<td>RE</td>
<td>3.3(0.4)</td>
<td>-3.2(6.9)%</td>
</tr>
<tr>
<td>RVE</td>
<td>3.1(0.3)</td>
<td>-2.2(6.4)%</td>
</tr>
<tr>
<td><strong>L3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>3.8(0.5)</td>
<td>-2.6(6.4)%</td>
</tr>
<tr>
<td>RE</td>
<td>3.8(0.4)</td>
<td>-1.6(6.4)%</td>
</tr>
<tr>
<td>RVE</td>
<td>3.6(0.4)</td>
<td>0.6(5.1)%</td>
</tr>
<tr>
<td><strong>L4</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>4.3(0.7)</td>
<td>-6.6(7.0)%</td>
</tr>
<tr>
<td>RE</td>
<td>4.0(0.6)</td>
<td>-3.9(5.9)%</td>
</tr>
<tr>
<td>RVE</td>
<td>4.1(0.7)</td>
<td>-3.5(5.3)%</td>
</tr>
<tr>
<td><strong>L5</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>5.1(1.0)</td>
<td>-4.3(5.1)%</td>
</tr>
<tr>
<td>RE</td>
<td>4.4(0.5)</td>
<td>-2.8(8.8)%</td>
</tr>
<tr>
<td>RVE</td>
<td>5.0(0.8)</td>
<td>-1.6(3.6)%</td>
</tr>
</tbody>
</table>

Values are mean(SD). CTR: control group, RE: resistance exercise group, RVE: resistance exercise + vibration group. Values in bed-rest are percentage change versus baseline. Data
collected in the first week (days 1-7) and second week (days 8-14) of bed-rest have been pooled. *: p <0.05; †: p <0.01; ‡: p <0.001 and indicate significance of within-group difference compared to baseline. To guard against false positives, p-values have been adjusted via the false discovery rate method. Intent-to-treat analysis presented.
### Supplemental Table 2: Multifidus muscle atrophy at the first (L1) through to fifth (L5) vertebral levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline (cm²)</th>
<th>Day of bed-rest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1-7</td>
</tr>
<tr>
<td>CTR</td>
<td>2.6(0.5)</td>
<td>2.6(0.5)%</td>
</tr>
<tr>
<td>RE</td>
<td>2.2(0.5)</td>
<td>-1.6(7.8)%</td>
</tr>
<tr>
<td>RVE</td>
<td>2.3(0.6)</td>
<td>4.6(11.0)%</td>
</tr>
<tr>
<td>CTR</td>
<td>3.8(1.0)</td>
<td>-2.5(9.0)%</td>
</tr>
<tr>
<td>RE</td>
<td>3.4(0.7)</td>
<td>-5.7(9.5)%</td>
</tr>
<tr>
<td>RVE</td>
<td>3.4(0.8)</td>
<td>1.8(4.3)%</td>
</tr>
<tr>
<td>CTR</td>
<td>6.0(1.3)</td>
<td>-5.4(5.7)%*</td>
</tr>
<tr>
<td>RE</td>
<td>5.1(1.2)</td>
<td>-6.3(6.9)%*</td>
</tr>
<tr>
<td>RVE</td>
<td>5.5(1.5)</td>
<td>-2.9(7.8)%</td>
</tr>
<tr>
<td>CTR</td>
<td>8.8(1.2)</td>
<td>-5.6(6.2)%*</td>
</tr>
<tr>
<td>RE</td>
<td>7.4(1.4)</td>
<td>-3.8(7.3)%</td>
</tr>
<tr>
<td>RVE</td>
<td>8.3(1.8)</td>
<td>-6.1(8.9)%</td>
</tr>
<tr>
<td>CTR</td>
<td>10.2(0.8)</td>
<td>-2.4(4.2)%</td>
</tr>
<tr>
<td>RE</td>
<td>8.8(0.7)</td>
<td>-6.0(6.5)%*</td>
</tr>
<tr>
<td>RVE</td>
<td>9.8(1.9)</td>
<td>-1.8(5.4)%</td>
</tr>
</tbody>
</table>

Values are mean(SD). CTR: control group, RE: resistance exercise group, RVE: resistance exercise + vibration group. Values in bed-rest are percentage change versus baseline. *: p
<0.05; †: p <0.01; ‡: p <0.001 and indicate significance of within-group difference compared to baseline. To guard against false positives, p-values have been adjusted via the false discovery rate method. Intent-to-treat analysis presented.