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Loss and re-adaptation of lumbar intervertebral disc water signal intensity after prolonged bedrest

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Abstract

The adaptation and re-adaptation process of the intervertebral disc (IVD) to prolonged bedrest is important for understanding IVD physiology and IVD herniations in astronauts. Little information is available on changes in IVD composition. In this study, 24 male subjects underwent 60-day bedrest and In/Out Phase magnetic resonance imaging sequences were performed to evaluate IVD shape and water signal intensity. Scanning was performed before bedrest (baseline), twice during bedrest, and three, six, and twenty-four months after bedrest. Area, signal intensity, average height, and anteroposterior diameter of the lumbar L3/4 and L4/5 IVDs were measured. At the end of bedrest, disc height and area were significantly increased with no change in water signal intensity. After bedrest, we observed reduced IVD signal intensity three months (p=0.004 versus baseline), six months (p=0.003 versus baseline), but not twenty-four months (p=0.25 versus baseline) post-bedrest. At these same time points post-bedrest, IVD height and area remained increased. The reduced lumbar IVD water signal intensity in the first months after bedrest implies a reduction of glycosaminoglycans and/or free water in the IVD. Subsequently, at two years after bedrest, IVD hydration status returned towards pre-bedrest levels, suggesting a gradual, but slow, re-adaptation process of the IVD after prolonged bedrest.

Keywords: Herniation, Disuse, Diurnal, Low Back Pain, Spaceflight

Introduction

In recent years the adaptation of the intervertebral disc (IVD) to and its recovery after bedrest has received greater attention. This is in large part due to the findings that IVD herniation risk is increased in astronauts1. However, investigating the adaptation of the IVD to reduced load is important for the basic understanding of the interaction between loading on the spine, the subsequent response of the IVD, and hence IVD physiology. Bedrest is considered a model of reduced loading on the lumbar spine.

Magnetic resonance imaging (MRI) is used to study the impact of bedrest on the IVD. Acute (i.e. for a few hours) and overnight bedrest has been shown to result in increases in lumbar IVD size2,3 and water content4,5. In prolonged (i.e. over a series of weeks or months) bedrest, our knowledge of the response of the shape of the IVD (height, width, volume, area) has now been well established. Increases in IVD height6,8, volume6,10,14
and/or sagittal plane IVD area\textsuperscript{11,13} but no change or reduction in anteroposterior and transverse IVD diameters\textsuperscript{12} have been observed in a series of prolonged bedrest studies. Whilst it is useful to understand adaptations in IVD shape with loading, it is important to understand changes in IVD composition. An equivalent analogy would be to investigate muscle or bone geometry without consideration of, respectively, muscle fibre type or bone mineral density.

Based on data from overnight bedrest\textsuperscript{4,8}, we can safely assume that water content is increased in the IVD at least in the first few days of bedrest. Given that IVD hyperhydration results in reductions in glycosaminoglycan synthesis rates\textsuperscript{16-18}, what impact might this have on the composition of the IVD during prolonged bedrest and also in the readaptation after bedrest? In prolonged bedrest, one study\textsuperscript{15} showed a continued increase in lumbar IVD T2-time (a measure of IVD water and glycosaminoglycan content\textsuperscript{19}) at the end of 5 or 17 weeks of bedrest. However, yet another bedrest study from the same research group\textsuperscript{20} showed a decrease in lumbar IVD T2-time after 5 weeks of bedrest. As such, it is unclear whether initial increases in IVD hydration in acute bedrest persist in prolonged bedrest.

Furthermore the time-course of recovery of the IVD after bedrest is unclear. Based upon knowledge for other tissues such as bone (recovery time course of typically 1-2 years depending on bone examined and duration of unloading\textsuperscript{21,22}) and muscle (typically 2-12 weeks depending on muscle and duration of unloading\textsuperscript{23}), we can assume the time-course for IVD re-adaptation post-bedrest will be long. For example, the metabolic response of bone after bedrest\textsuperscript{24} is characterised by an increase in bone formation, reduction of bone resorption and a gradual turnaround\textsuperscript{22} in bone mineral density. However, the recovery of the IVD post-bedrest still needs to be investigated.

Our aims were to study the changes in IVD composition (water signal intensity on an In/Out MR-sequence) and morphology during and after prolonged bedrest. Specifically, we focussed on the recovery of the IVD in a two-year follow-up period after prolonged bedrest. Data from overnight bedrest\textsuperscript{4,8} and the response of spine length to loading\textsuperscript{25}, suggest that the recovery of IVD hydration after unloading occurs rapidly (within minutes). Therefore, our primary hypothesis was that increases in IVD water signal during bedrest would return to pre-bedrest levels rapidly post-bedrest.

**Methods**

**Bedrest study, ethical approval and sample size**

In the 2\textsuperscript{nd} Berlin Bedrest (BBR2-2) study, 24 male subjects underwent 60d head-down tilt (HDT) bedrest and 2 yr follow-up and was performed by the Center of Muscle and Bone Research at the Charité in Berlin, Germany. Ethical approval for BBR2-2 was provided by the ethics committee of the Charité Universitätsmedizin Berlin. Each subject gave their informed written consent and was aware of their right to withdraw from the study without prejudice.

A more detailed account of the BBR2-2 protocol can be found elsewhere\textsuperscript{25}. Exclusion criteria relevant to this investigation included any history of chronic low back pain, spinal injury and surgery. Subjects were randomized to one of three groups: 1) resistive exercises with whole body vibration during bedrest, 2) resistive exercise only, 3) control subjects without countermeasure. Details of the exercise protocols can be found elsewhere\textsuperscript{26-28}. The main aim of the BBR2-2 was to compare the effects of resistive exercise and whole-body vibration countermeasures for their impact on bone variables\textsuperscript{29}. Preventing changes in the IVD was not the primary goal of these interventions. Hence, the present investigation should be considered an “exploratory study” of the effects the countermeasures on IVD water signal.

**Magnetic resonance imaging protocols**

All subjects underwent MRI 8 or 9 d before the start of bedrest (baseline), after 27 or 28 d of bedrest (mid-HDT), and after 55 or 56 d of bedrest (end-HDT). During the re-adaptation period, they were imaged 90, 180 and 720 days after bedrest. MR images were acquired using a 1.5-T Siemens Magnetom Symphony scanner with a body coil. To allow time for equalization of body fluid, subjects rested in bed in the horizontal position for 2 hours before scanning. The lower lumbar vertebrae were imaged in the sagittal plane using a T1 In/Out (TR=160 milliseconds, TE=2.4/4.6 (in/out) milliseconds, flip angle=25°, field of view=300 millimeter, slice thickness=6 millimeter, number of slices=3; Figure 1) sequence.

**Image analysis**

Each data set was assigned (by D. Belavý) a random number (obtained from www.random.org) to blind the operator (M. Kordi) who used ImageJ 1.38x (http://rsb.info.nih.gov/ij/) to perform the blinded image measurements. The L3/4 and L4/5 IVDs were measured. IVD area in the sagittal plane was measured as well as signal intensity in this area. IVD height in the sagittal plane was measured as the average of the anterior, central and posterior IVD heights. Anteroposterior diameter was also measured. IVD variables were averaged from those measured on each of the images at three anatomical slices through the disc.

**Statistical analyses**

Linear mixed-effects models were used to examine whether the ‘study-date’, ‘group’ and/or ‘vertebral level’ impacted upon IVD variables over the course of the study. Changes of IVD variables versus baseline were examined using a priori T-tests. An alpha-level of 0.05 was taken for statistical significance on ANOVA. The “R” statistical environment (version 2.10.1, www.r-project.org) was used.

**Results**

One subject withdrew after day 30 of bedrest due to an injury to the thoracic spine\textsuperscript{30}. Two additional subjects did not return for testing 90-days and beyond after bedrest. One subject was tested 360 days after bedrest instead of 180 after bedrest
**Figure 1.** Magnetic resonance imaging and image measurements Top: Out-of-phase images. Bottom: In-phase images. Left: prior to bed-rest; right: at end of bed-rest in same subject. Increases in disc height (in particular at the posterior aspect of the disc) and area can be seen in this subject. Measurements were made of the L3/4 and L4/5 intervertebral discs in the sagittal plane. The inset shows the measurements performed on each disc in each image. Disc heights were measured at left side of disc (a), centre of disc (b) and right side (c). These values were then averaged to generate average disc height. Disc area (white region of interest traced at (d)) was measured and the signal intensity was also measured in this region of interest. Transverse disc diameter (black line at (e)) was also measured.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Bed rest day</th>
<th>Days post bedrest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>27/28</td>
<td>55/56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Signal intensity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>103.4(13.1)</td>
<td>-3.7(9.6)%‡</td>
<td>3.3(12.9)%⁺</td>
</tr>
<tr>
<td>RE</td>
<td>105.9(9.2)</td>
<td>1.0(11.7)%‡</td>
<td>-6.2(18.0)%⁺</td>
</tr>
<tr>
<td>RVE</td>
<td>113.7(21.9)</td>
<td>-7.9(14.6)%‡</td>
<td>0.4(17.0)%⁺</td>
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<tr>
<td><strong>Average disc height (mm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>8.9(1.3)</td>
<td>6.8(4.2)%‡</td>
<td>8.1(3.6)%‡</td>
</tr>
<tr>
<td>RE</td>
<td>8.5(1.4)</td>
<td>5.8(5.0)%⁺</td>
<td>6.2(6.0)%⁺</td>
</tr>
<tr>
<td>RVE</td>
<td>8.9(0.8)</td>
<td>5.3(4.1)%⁺</td>
<td>7.0(5.4)%⁺</td>
</tr>
<tr>
<td><strong>Disc area (mm²)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>298.5(64.1)</td>
<td>7.5(4.2)%‡</td>
<td>10.7(4.6)%‡</td>
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<tr>
<td>RE</td>
<td>272.6(91.5)</td>
<td>7.2(5.9)%⁺</td>
<td>8.8(7.4)%⁺</td>
</tr>
<tr>
<td>RVE</td>
<td>305.6(43.8)</td>
<td>2.6(7.0)%</td>
<td>5.8(3.5)%‡</td>
</tr>
<tr>
<td><strong>Anteroposterior disc diameter (mm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>36.5(2.8)</td>
<td>0.5(2.1)%</td>
<td>0.3(1.8)%</td>
</tr>
<tr>
<td>RE</td>
<td>35.5(4.2)</td>
<td>0.4(1.8)%</td>
<td>0.0(1.7)%</td>
</tr>
<tr>
<td>RVE</td>
<td>36.9(2.0)</td>
<td>-0.9(1.2)%*</td>
<td>-1.3(1.6)%*</td>
</tr>
<tr>
<td><strong>Number of subjects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>9</td>
<td>9</td>
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</tr>
<tr>
<td>RE</td>
<td>8</td>
<td>8</td>
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</tr>
<tr>
<td>RVE</td>
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<td>7</td>
<td>6</td>
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</table>

Values are mean(SD). Baseline values are in absolute units and changes during and after bedrest are in percentage change to baseline. *: p<0.05, †: p<0.01, ⁂: p<0.001 and denotes difference to baseline. CTR: inactive control group, RE: resistive exercise group, RVE: resistive exercise plus vibration group. Including only those subjects with complete data sets in the analysis did not change the findings of the study (data not shown). ANOVA showed no significant differences between groups at baseline (p>0.25) nor in their response during or after bed-rest (p>0.08).

Table 1. Disc water signal and morphology in each group.
as planned as he could not attend the earlier appointment. Excluding these subjects from the analyses did not change the findings of the study (data not shown). In all ANOVAs ‘vertebral level’ was not significant (p all ≥0.28), therefore data for L3/4 and L4/5 were pooled. There was no significant difference between the sub-groups, therefore we focus here on the aggregate data here but also present the data from each group in Table 1.

ANOVA showed changes over the course of the study (‘date’ main-effect) in IVD height (p<0.001), IVD cross-sectional area (p<0.001) and MR water signal intensity (p<0.05) but not anteroposterior IVD diameter (p=0.67). Changes from baseline measurements for water signal intensities and IVD height are shown in Figure 2.

IVD height and area increased during bedrest (p<0.001) and this remained so 90, 180 and 720 days after bedrest (p all<0.001). Despite increases in IVD height and area, water signal intensity did not increase during bedrest. After bedrest, however, reductions in IVD water signal intensity were observed. The reduction in water signal intensity was statistically significant 90 and 180 days after bedrest (p=0.004 and p=0.003, respectively; Figure 2) with signal intensity 720 days after bedrest returning towards pre-bedrest levels (p=0.25).

Discussion

The MR-sequences implemented in the current study were specifically targeted at assessing water MR signal intensity in the lumbar intervertebral discs. Although disc size increase due to unloading must, in the acute phase, be accompanied by fluid influx, we found no increase in IVD water signal intensity during bedrest. It is possible that the overall fluid per unit volume need not necessarily increase to an extent that it can be detected as a signal intensity increase on MRI. Contrary to our expectations, we saw significant reductions in disc water signal intensity, despite continually increased disc height and area, 3 and 6 months after bedrest.

What do the current findings add to the existing literature? Prior studies were equivocal findings as to whether IVD T2-time (which positively correlates with IVD water and glycosaminoglycan content) remains increased at the end of prolonged bedrest. The current study showed no changes in our IVD water signal intensity during prolonged bedrest. Overall the existing data in the literature suggest that an acute increase in disc hydration occurs at the initiation of bedrest. Due to persistent unloading and increases in hydration, given our knowledge from basic science studies of the IVD, it is likely that adaptations in IVD metabolism then occur. It is possible that, due to persistent hyperhydration, the IVD begins to lose glycosaminoglycan. Due to the slow turnover of IVD aggregan one might assume that a change in IVD metabolism and glycosaminoglycan content should not occur in the short time-frame of bedrest. However, other tissues, such as bone, which is also “slow” tissue in its adaptation to load changes, subsequently show metabolic increases in bone resorption and marginal reductions in bone formation) and subsequently losses of bone mineral content due to extreme disuse in bedrest. As such, it is possible that the acute hyperhydration of the IVD in bedrest results in a (negative) adaptation of IVD metabolism. The findings from after bedrest help to understand this.
The findings of reduced water signal intensity three and six months after bedrest may be due to reductions of free water in the IVD and/or losses of glycosaminoglycan. There is evidence from animal models that catabolic pathways are activated when spinal IVD water content increases, and we know that an optimal level of IVD hydration is needed for IVD glycosaminoglycan synthesis and nutrient incorporation. Potentially, IVD hyper-hydration during prolonged bedrest activated IVD catabolic pathways in humans resulting in reduced glycosaminoglycan content during the recovery period. Animal investigations of intervertebral IVD in spaceflight and hindlimb-suspension and tail vertebra immobilization have typically found losses in glycosaminoglycan content. Some authors caution against assuming that findings from animal models of the IVD translate to what occurs in humans. While we agree with this caveat, there is ample evidence from other organ systems that responses in animal and human models show some stark similarities, even if specific findings may differ.

For example, whilst there are some dissimilarities, muscle atrophy in both humans and rodents in disuse in particular affects the postural and locomotor musculature of both species. Similarly, bone losses in animal models of disuse do differ in their location and extent to what is observed in humans, but the major load bearing regions of the body are still affected in both species. Consequently, we consider it reasonable that, whilst there will be differences in the extent and character of adaptations in animal and human IVDs due to unloading, there will be striking similarities and parallels between the two as seen for muscle and bone. We therefore interpret our findings of reductions in IVD MR water signal intensity 3 and 6 months after bedrest to represent losses of glycosaminoglycan and/or free water from the IVD.

Two years after bedrest, whilst IVD water signal intensity was marginally below baseline, this was not statistically significant. Disc morphology parameters were also closer to baseline values. This pattern persisted even when subjects who did not complete the entire recovery phase were excluded from the analysis. This gradual return to baseline may represent a long-duration, and slow, re-adaptation of the IVD after prolonged bedrest. Data from a rabbit model of unloading showed that three weeks of normal ambulation after tail-suspension restored glycosaminoglycan levels. Considering other tissues, the re-adaptation process of bone is also slow after prolonged bedrest and spaceflight with a two year period required for most bone density parameters to return to pre-bedrest levels. Similarly, for the musculature, recovery takes a number of weeks post-bedrest. Hence, the approach of IVD parameters to pre-bedrest levels two years after bedrest may represent the tail-end of the readaptation phase for the IVD.

It is appropriate to discuss some of the limitations of the current study. In the current study we did not include female subjects. To reduce variability between subjects, bedrest studies commonly investigate only one gender. Further work is needed to understand whether females respond similarly. For logistical and financial reasons, we had no parallel ambulatory non-bedrest control group which would have enabled us to control for effects such as normal aging. With normal aging in adult individuals under 45 years of age, approximately 0.2-0.3% per year reduction in MR measures disc water signal occurs. We observed losses of signal intensity on the order of 6-7% in the time frame 3-6 months after bedrest. Therefore, we argue “normal aging” is unlikely to be the main reason to explain our findings.

In conclusion, the current study found that whilst disc size increased during bedrest, we could observe no concurrent increase in disc water signal. This does not mean that overall disc water content did not change, we know that increases in disc height is inevitably accompanied by increases in disc water content. However, 3 and 6 months after bedrest we found evidence of reduced disc signal intensity. This finding was no longer present 2 years after bedrest. This implies that whilst negative changes in IVD composition likely occurred in the months after bedrest, that this slowly recovers in the years after bedrest.

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