

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

The authors have no conflict of interest.

*Author contributions: Mehdi Kordi: Image analysis. Drafting the article. Daniel L. Belavý: Secured funding. Conception and design of the experiments. Statistical analysis and interpretation of the data. Drafting the article. Gabriele Armbrecht: Collection and interpretation of data. Revision of the article. Adnan Sheikh: Interpretation of the data. Revision of the article. Dieter Felsenberg: Secured funding. Conception and design of the experiments. Revising the article. Guy Trudel: Secured funding. Conception and design of the experiments. Interpretation of the data. Revision of the article.

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Edited by: F. Rauch
Accepted 30 July 2015

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 size²⁻⁵ and water content⁶⁻⁸. 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 height⁹⁻¹⁴, volume^{9,10,14}

and/or sagittal plane IVD area^{11,15} but no change or reduction in anteroposterior and transverse IVD diameters¹² 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 bedrest⁶⁻⁸, 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 rates¹⁶⁻¹⁸, 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 study¹⁵ showed a continued increase in lumbar IVD T2-time (a measure of IVD water and glycosaminoglycan content¹⁹) at the end of 5 or 17 weeks of bedrest. However, yet another bedrest study from the same research group²⁰ 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^{21,22}) and muscle (typically 2-12 weeks depending on muscle and duration of unloading²³), we can assume the time-course for IVD re-adaptation post-bedrest will be long. For example, the metabolic response of bone after bedrest²⁴ is characterised by an increase in bone formation, reduction of bone resorption and a gradual turnaround²² 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^{6,8} and the response of spine length to loading²⁵, 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 2nd 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 elsewhere²⁶. Exclusion criteria relevant to this investi-

gation 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 elsewhere²⁶⁻²⁸. 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 variables²⁹. 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 spine³⁰. 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

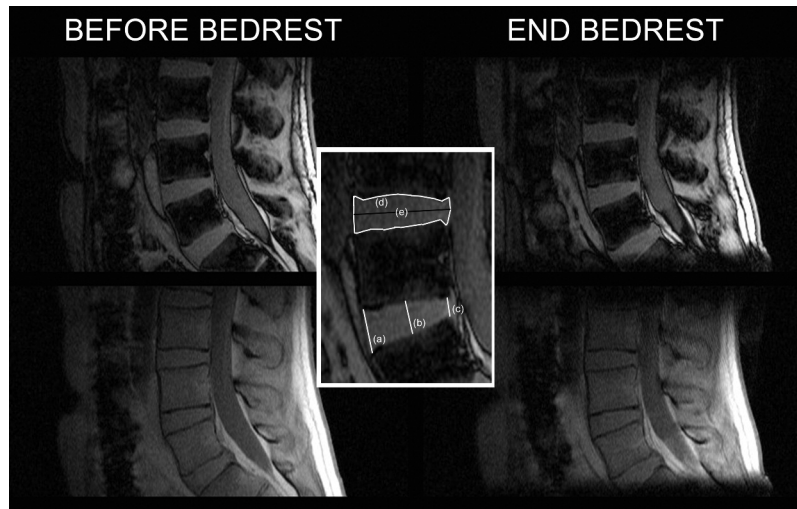


Figure 1. Magnetic resonance imaging and image measurements Top: Out-of-phase images. Bottom: In-phase images. Left: prior to bed-rest; right: at end of bed-rest in same subject. Increases in disc height (in particular at the posterior aspect of the disc) and area can be seen in this subject. Measurements 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.

Group	Baseline	Bedrest day		Days post bedrest		
		27/28	55/56	90	180	720
<i>Signal intensity</i>						
CTR	103.4(13.1)	-3.7(9.6)%	3.3(12.9)%	-5.4(10.5)%	-3.9(10.3)%	-4.5(8.9)%
RE	105.9(9.2)	1.0(11.7)%	-6.2(18.0)%	-2.5(9.7)%	-9.0(14.1)%	-5.6(13.5)%
RVE	113.7(21.9)	-7.9(14.6)%	0.4(17.0)%	-11.6(12.1)%†	-9.5(12.1)%*	-2.4(19.6)%
<i>Average disc height (mm)</i>						
CTR	8.9(1.3)	6.8(4.2)%‡	8.1(3.6)%‡	4.1(5.3)%*	4.7(3.8)%†	4.0(4.3)%*
RE	8.5(1.4)	5.8(5.0)%†	6.2(6.0)%*	3.5(5.2)%	5.4(5.5)%*	0.3(4.3)%
RVE	8.9(0.8)	5.3(4.1)%†	7.0(5.4)%†	2.7(3.4)%	4.1(3.6)%*	-2.8(8.9)%
<i>Disc area (mm²)</i>						
CTR	298.5(64.1)	7.5(4.2)%‡	10.7(4.6)%‡	5.5(5.4)%†	7.0(4.5)%‡	2.7(5.2)%
RE	272.6(91.5)	7.2(5.9)%†	8.8(7.4)%†	5.5(3.5)%‡	5.1(4.4)%†	3.6(4.6)%
RVE	305.6(43.8)	2.6(7.0)%	5.8(3.5)%‡	-0.1(4.5)%	3.0(4.4)%	-1.5(10.1)%
<i>Anteroposterior disc diameter (mm)</i>						
CTR	36.8(2.8)	0.5(2.1)%	0.3(1.8)%	0.8(2.3)%	1.5(1.5)%†	0.9(2.1)%
RE	35.5(4.2)	0.4(1.8)%	0.0(1.7)%	1.1(1.8)%	0.7(2.2)%	0.7(1.7)%
RVE	36.9(2.0)	-0.9(1.2)%*	-1.3(1.6)%*	-1.2(1.2)%*	-1.0(1.4)%	-1.4(2.3)%
<i>Number of subjects</i>						
CTR	9	9	9	8	8	8
RE	8	8	7	7	7	7
RVE	7	7	7	6	6	6

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.

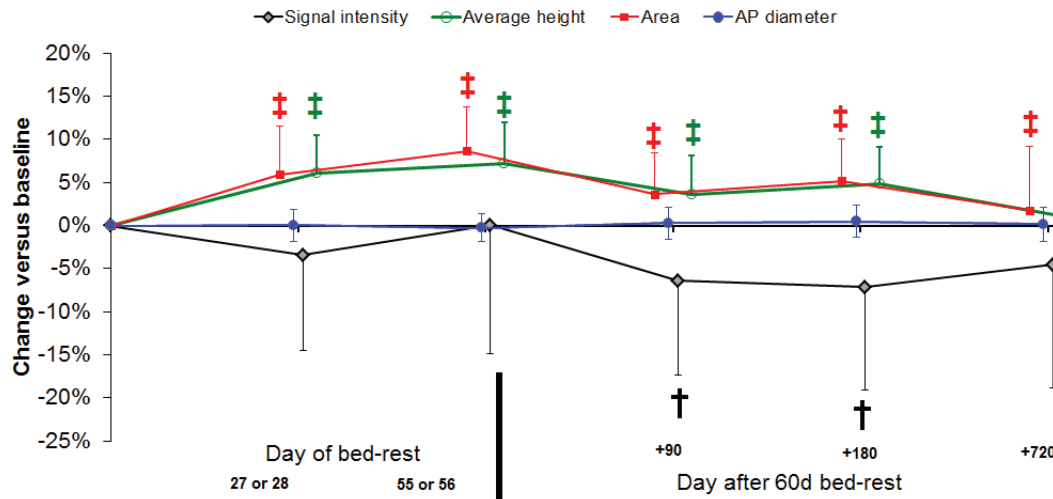


Figure 2. Changes in lumbar disc signal intensity and dimensions during and after bedrest. Mean (SD) percentage (%) changes from baseline of average (of anterior, central and posterior) disc height, disc area in the sagittal plane, disc anteroposterior (AP) diameter and water signal intensity during and after bedrest. *: $p < 0.05$, †: $p < 0.01$, ‡: $p < 0.001$ and denotes difference to baseline. HDT: day of head-down tilt bedrest; R: day of post bedrest recovery. 24 subjects assessed at baseline and day 27/28 of bedrest, 23 on day 55/56 of bedrest and 21 subjects from 90 days after bedrest onwards. Including only those subjects with complete data sets in the analysis did not change the findings of the study (*data not shown*). Data are pooled from the L3/4 and L4/5 intervertebral discs.

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

influx³¹, 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^{15,20} were equivocal findings as to whether IVD T2-time (which positively correlates with IVD water and glycosaminoglycan content¹⁹) 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 increase⁶⁻⁸ 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 aggrecan³² one might assume that a change in IVD metabolism and glycosaminoclycan 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 metabolic²⁴ (increases in bone resorption and marginal reductions in bone formation) and subsequently losses of bone mineral content^{22,29} 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 evidence³³ 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 needed¹⁶⁻¹⁸ 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^{34,35}, hindlimb-suspension³⁴⁻³⁹ and tail vertebra immobilization^{40,41} have typically^{34-39,41} found losses in glycosaminoglycan content. Some authors⁴² caution against assuming that findings from animal models of the IVD translate to what occurs in humans. Whilst 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 dissimilarities⁴³, muscle atrophy in both humans⁴⁴ and rodents⁴⁵ in disuse in particular affects the postural and locomotor musculature of both species. Similarly, bone losses in animal models⁴⁵ of disuse do differ in their location and extent to what is observed in humans⁴⁶, 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 (*data not shown*). This gradual return to baseline may represent a long-duration, and slow, re-adaptation of the IVD after prolonged bedrest. Data from an animal model of unloading³⁹ 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 bedrest²² and spaceflight²¹ 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-bedrest²³. 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 occurs⁴⁷. 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 content³¹. 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.

Acknowledgements

We thank the people who volunteered for the bedrest study as well as the nurses, staff, and entire research team at the Charité for the support in the implementation of the study. The authors would also wish to thank Dr Ian Cameron and Elizabeth Coletta for their support. The 2nd Berlin Bed Rest Study was supported by grant 14431/02/NL/SH2 from the European Space Agency and grant 50WB0720 from the German Aerospace Center (DLR). The 2nd Berlin Bed rest Study was also sponsored by Novotec Medical, Charité Universitätsmedizin Berlin, Siemens, Osteomedical Group, Wyeth Pharma, Servier Deutschland, P&G, Kubivent, Seca, Astra-Zeneca and General Electric. Guy Trudel was supported by Canadian Institutes of Health Research Grant BRS 67811.

References

1. Johnston SL, Campbell MR, Scheuring R, Feiveson AH. Risk of herniated nucleus pulposus among U.S. astronauts. *Aviat Space Env Med* 2010;81(6):566-574.
2. Botsford DJ, Esses SI, Ogilvie-Harris DJ. *In vivo* diurnal variation in intervertebral disc volume and morphology. *Spine* 1994;19(8):935-940.
3. Malko JA, Hutton WC, Fajman WA. An *in vivo* magnetic resonance imaging study of changes in the volume (and fluid content) of the lumbar intervertebral discs during a simulated diurnal load cycle. *Spine* 1999;24(10):1015-1022.
4. Malko JA, Hutton WC, Fajman WA. 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* 2002;15(2):157-163.
5. Hutton WC, Malko JA, Fajman WA. Lumbar disc volume measured by MRI: effects of bed rest, horizontal exercise, and vertical loading. *Aviat Space Env Med* 2003;74(1):73-78.
6. Boos N, Wallin Å, Gbedegbegnon T, Aebi M, Boesch C.

- Quantitative MR imaging of lumbar intervertebral disks and vertebral bodies: influence of diurnal water content variations. *Radiology* 1993;188:351-354.
7. Matsumura Y, Kasai Y, Obata H, Matsushima S, Inaba T, Uchida A. Changes in water content of intervertebral discs and paravertebral muscles before and after bed rest. *J Orthop Sci* 2009;14(1):45-50. doi:10.1007/s00776-008-1288-5.
 8. Roberts N, Hogg D, Whitehouse GH, Dangerfield P. Quantitative analysis of diurnal variation in volume and water content of lumbar intervertebral discs. *Clin Anat* 1998;11:1-8.
 9. Belavý DL, Armbrecht G, Richardson CA, Felsenberg D, Hides JA. Muscle atrophy and changes in spinal morphology: is the lumbar spine vulnerable after prolonged bed-rest? *Spine* 2011;36(2):137-145.
 10. Belavý DL, Bansmann PM, Bohme G, et al. Changes in intervertebral disc morphology persist 5 months after 21-days bed-rest. *J Appl Physiol* 2011;111(5):1304-1314.
 11. Belavý DL, Hides JA, Wilson SJ, et al. Resistive simulated weightbearing exercise with whole body vibration reduces lumbar spine deconditioning in bed-rest. *Spine* 2008;33(5):E121-E131.
 12. Belavý DL, Ohshima H, Bareille M-P, Rittweger J, Felsenberg D. Limited effect of fly-wheel and spinal mobilization exercise countermeasures on lumbar spine deconditioning during 90d bed-rest in the Toulouse LTBR study. *Acta Astronaut* 2011;69(7-8):406-419.
 13. Cao P, Kimura S, Macias BR, Ueno T, Watenpaugh DE, Hargens AR. Exercise within lower body negative pressure partially counteracts lumbar spine deconditioning associated with 28-day bed rest. *J Appl Physiol* 2005;99(1):39-44.
 14. Holguin N, Muir J, Rubin C, Judex S. Short applications of very low-magnitude vibrations attenuate expansion of the intervertebral disc during extended bed rest. *Spine J* 2009;9(6):470-477.
 15. Le Blanc AD, Evans HJ, Schneider VS, Wendt RE, Hedrick TD. Changes in intervertebral disc cross-sectional area with bed rest and space flight. *Spine* 1994;19(7):812-817.
 16. Bayliss MT, Urban JP, Johnstone B, Holm S. *In vitro* method for measuring synthesis rates in the intervertebral disc. *J Orthop Res* 1986;4(1):10-17. doi:10.1002/jor.1100040102.
 17. Ohshima H, Tsuji H, Hirano N, Ishihara H, Katoh Y, Yamada H. Water diffusion pathway, swelling pressure, and biomechanical properties of the intervertebral disc during compression load. *Spine* 1989;14(11):1234-1244.
 18. Ohshima H, Urban JP, Bergel DH. Effect of static load on matrix synthesis rates in the intervertebral disc measured *in vitro* by a new perfusion technique. *J Orthop Res* 1995;13(1):22-29. doi:10.1002/jor.1100130106.
 19. Marinelli NL, Houghton VM, Munoz A, Anderson PA. T2 relaxation times of intervertebral disc tissue correlated with water content and proteoglycan content. *Spine* 2009;34(5):520-524.
 20. Le Blanc AD, Schonfeld E, Schneider VS, Evans HJ, Taber KH. The spine: changes in T2 relaxation times from disuse. *Radiology* 1988;169(1):105-107. doi:10.1148/radiology.169.1.3420243.
 21. Sibonga JD, Evans HJ, Sung HG, et al. Recovery of spaceflight-induced bone loss: bone mineral density after long-duration missions as fitted with an exponential function. *Bone*. 2007;41(6):973-978.
 22. Rittweger J, Felsenberg D. Recovery of muscle atrophy and bone loss from 90 days bed rest: results from a one-year follow-up. *Bone* 2009;44(2):214-224.
 23. Miokovic T, Armbrecht G, Felsenberg D, Belavý DL. Heterogeneous atrophy occurs within individual lower limb muscles during 60d bed-rest. *J Appl Physiol* 2012;113(10):1545-1559. doi:10.1152/jappphysiol.00611.2012.
 24. Armbrecht G, Belavý DL, Gast G, et al. Resistive vibration exercise attenuates bone and muscle atrophy in 56 days of bed rest: biochemical markers of bone metabolism. *Osteoporos Int* 2010;21(4):597-607.
 25. Tyrrell AR, Reilly T, Troup JD. Circadian variation in stature and the effects of spinal loading. *Spine* 1985;10(2):161-164.
 26. Belavý DL, Bock O, Börst H, et al. The 2nd Berlin BedRest Study: protocol and implementation. *J Musculoskelet Neuronal Interact* 2010;10(3):207-219.
 27. Belavý DL, Armbrecht G, Gast U, Richardson CA, Hides JA, Felsenberg D. Countermeasures against lumbar spine deconditioning in prolonged bed-rest: resistive exercise with and without whole-body vibration. *J Appl Physiol* 2010;109(6):1801-1811.
 28. Gast U, John S, Runge M, Rawer R, Felsenberg D, Belavý DL. Short-Duration Resistive Exercise Sustains Neuromuscular Function after Bed Rest. *Med Sci Sports Exerc* 2012;44(9):1764-1772.
 29. Belavý DL, Beller G, Armbrecht G, et al. Evidence for an additional effect of whole-body vibration above resistive exercise alone in preventing bone loss during prolonged bed-rest. *Osteoporos Int* 2011;22(5):1581-1591.
 30. Belavý DL, Miokovic T, Armbrecht G, Felsenberg D. Hypertrophy in the cervical muscles and thoracic discs in bed-rest? *J Appl Physiol* 2013;115(5):586-596. doi:10.1152/jappphysiol.00376.2013.
 31. McMillan DW, Garbutt G, Adams MA. Effect of sustained loading on the water content of intervertebral discs: implications for disc metabolism. *Ann Rheum Dis* 1996;55(12):880-887.
 32. Sivan SS, Wachtel E, Tsitron E, et al. Collagen turnover in normal and degenerate human intervertebral discs as determined by the racemization of aspartic acid. *J Biol Chem* 2008;283(14):8796-8801.
 33. Cui Y, Lee S, Yu J, Urban JP. MMP expression by intervertebral disc cells is responsive to changes in extracellular osmolarity. *FASEB J* 2012;26:206.4.
 34. Pedrini-Mille A, Maynard JA, Durnova GN, et al. Effects of microgravity on the composition of the intervertebral disk. *J Appl Physiol Bethesda Md* 1985 1992;73(2 Suppl):26S-32S.

35. Sinha RK, Shah SA, Hume EL, Tuan RS. The effect of a 5-day space flight on the immature rat spine. *Spine J* 2002;2(4):239-243.
36. Holguin N, Judex S. Rat intervertebral disc health during hindlimb unloading: brief ambulation with or without vibration. *Aviat Space Env Med* 2010;81(12):1078-1084.
37. Holguin N, Uzer G, Chiang F-P, Rubin C, Judex S. Brief daily exposure to low-intensity vibration mitigates the degradation of the intervertebral disc in a frequency-specific manner. *J Appl Physiol* 2011;111(6):1846-1853. doi:10.1152/jappphysiol.00846.2011.
38. Hutton WC, Yoon ST, Elmer WA, et al. Effect of tail suspension (or simulated weightlessness) on the lumbar intervertebral disc: study of proteoglycans and collagen. *Spine Phila Pa 1976* 2002;27(12):1286-1290.
39. Yasuoka H, Asazuma T, Nakanishi K, et al. Effects of reloading after simulated microgravity on proteoglycan metabolism in the nucleus pulposus and anulus fibrosus of the lumbar intervertebral disc: an experimental study using a rat tail suspension model. *Spine Phila Pa 1976* 2007;32(25):E734-40.
40. Iatridis JC, Mente PL, Stokes IA, Aronsson DD, Alini M. Compression-induced changes in intervertebral disc properties in a rat tail model. *Spine* 1999;24(10):996-1002.
41. MacLean JJ, Lee CR, Grad S, Ito K, Alini M, Iatridis JC. Effects of immobilization and dynamic compression on intervertebral disc cell gene expression *in vivo*. *Spine Phila Pa 1976* 2003;28(10):973-981.
42. Alini M, Eisenstein SM, Ito K, et al. Are animal models useful for studying human disc disorders/degeneration? *Eur Spine J* 2008;17(1):2-19. doi:10.1007/s00586-007-0414-y.
43. Baldwin KM, Haddad F. Plasticity in Skeletal, Cardiac, and Smooth Muscle. Invited Review: Effects of different activity and inactivity paradigms on myosin heavy chain gene expression in striated muscle. *J Appl Physiol* 2001;90:345-357.
44. Le Blanc AD, Schneider VS, Evans HJ, Pientok C, Rowe R, Spector E. Regional Changes in Muscle Mass Following 17 Weeks of Bed Rest. *J Appl Physiol* 1992;73(5):2172-2178.
45. Le Blanc A, Marsh C, Evans H, Johnson P, Schneider V, Jhingran S. Bone and muscle atrophy with suspension of the rat. *J Appl Physiol* 1985;58(5):1669-1675.
46. Le Blanc A, Schneider V, Shackelford L, et al. Bone mineral and lean tissue loss after long duration space flight. *J Musculoskelet Neuronal Interact* 2000;1(2):157-160.
47. Wu N, Liu H, Chen J, et al. Comparison of apparent diffusion coefficient and T2 relaxation time variation patterns in assessment of age and disc level related intervertebral disc changes. *PloS One* 2013;8(7):e69052. doi:10.1371/journal.pone.0069052.