Published in final edited form as:

Spine J. 2013 April ; 13(4): 428–436. doi:10.1016/j.spinee.2013.01.046.

Low-Intensity Vibrations Partially Maintain Intervertebral Disc Mechanics and Spinal Muscle Area During Deconditioning

Nilsson Holguin, PhD¹, John T. Martin, MS², Dawn M. Elliott, PhD³, and Stefan Judex, PhD¹

¹Department of Biomedical Engineering, Stony Brook University Stony Brook, NY, USA

²Department of Mechanical Engineering, University of Pennsylvania, Philadelphia, PA, USA

³Department of Biomedical Engineering, University of Delaware, Newark, DE, USA

INTRODUCTION

The intervertebral disc (IVD) receives a variety of complex loads during daily activities, engendered from gravity and back musculature, that are diverse in magnitude, frequency and duration [1]. These loads are supported by the central nucleus pulposus which transfers axial load to the inferior endplate-vertebrae and distributes radial load to the outer collagenous annulus fibrosus. Prolonged, reduced dynamic loading, as occurs with immobilization, bed rest or exposure to microgravity, alters spine physiology [2–4] and remodels the intervertebral disc [5]. Ultimately, these changes unbalance the load distribution of the disc, potentially stimulating nociceptive nerves [6] and increasing the risk of mechanical IVD failure [7].

Although overnight bed rest reduces the spinal range of motion and swells the IVD [8], these alterations return to normal following brief reloading [9] and do not produce mechanical features associated with degeneration [10]. On the other hand, prolonged bed rest induces discal swelling and also differentially changes spinal musculature [11] such that 5 months of reambulation does not restore the discal morphology induced by 3 wk of bed rest [12]. Two months of bed rest increases the axial compressibility of the spine [3], which may increasingly strain the annulus as shown in human degenerated discs [13]. During bed rest, the posterior region incurs greater morphological changes [11] which may amplify functional alterations in this thinner and weaker region.

Animal models of degeneration offer unique opportunities to test the mechanical outcome of potential countermeasures to disc degeneration via invasive procedures which are not possible to perform in humans. Hindlimb unloading is a widely utilized animal model for simulating musculoskeletal, orthostatic and cardiovascular changes that arise from diminished functional weightbearing. Although hindlimb unloading primarily reduces axial spine loads, rather than removing most components of the complex mechanical environment, many outcome variables show similar IVD effects to those observed during spaceflight and degeneration, including decreased hydrophilic glycosaminoglycan content and discal hydrostatic pressure, altered collagen organization, and up-regulation of catabolic

© 2013 Elsevier Inc. All rights reserved

Correspondence to: Stefan Judex, Ph.D. Professor of Biomedical Engineering Bioengineering Building (Rm 213) Stony Brook University Stony Brook, NY 11794-5281 Voice: 631-632-1549 Fax: 631-632-8577 stefan.judex@stonybrook.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

molecules [4,14,15]. Hindlimb unloaded discs also contain larger collagen fiber diameters in the posterior region [4] which, while priming the structure for tensile loading, may render the IVD less capable of recovering creep [16], and more susceptible to mechanical damage. Exposure to space flight for five days increases the stiffness of lumbar discs [17] while hindlimb unloading diminishes the speed of trunk rotation by reducing the electromyogram activity of the internal oblique, increasing the number of slow type I fibers, and reducing the metabolic activity of fast type II fibers [18]. However, the effects of spaceflight or hindlimb unloading on the mechanics of the rodent IVD, back musculature, and how they relate are largely unknown.

Effective countermeasures following disc deconditioning have not been identified. Mechanical signals applied as whole body vibrations may attenuate changes in IVD morphology, lower the incidence of back pain, and prevent muscle changes during spinal unloading [19,20]. In rats standing on a vertically vibrating plate in erect posture, facilitating the transmission of the oscillatory signal from the feet into the spine, 90 Hz low-level vibrations maintained glycosaminoglycan content and height, but not area, of the IVD compromised by hindlimb unloading [21]. While this study demonstrated the benefits of 90 Hz erect body vibrations to the composition and height in rats during unloading, it was not clear whether the mechanical behavior of the disc also benefited from the vibrational countermeasure during unloading. In an effort to address this question, here, we evaluated the consequences of 90 Hz low-level vibrations on disc mechanics and its relation to disc morphology following immobilization. Further, we assessed spinal muscles to clarify the role of endogenous loading in modulating the potential benefits of low-intensity vibrations.

MATERIALS AND METHODS

Experimental Design

This study was reviewed and approved by the Institutional Use and Care Committee of Stony Brook University. Similar to a previous study [21], female Sprague-Dawley rats (18 wk of age) were assigned to the following groups upon two weeks of acclimatization in our animal facility. One group of rats was hindlimb unloaded for 4 wk without interruption (**HU**, n=8). A sham control group of rats (**HU+SC**, n=8) was also subjected to HU but unloading was interrupted by brief periods (15 min/day) of upright posture, facilitated by standing in a vertical plastic cylinder (height: 30.5 cm, inner diameter: 10.2 cm). A third group of experimental rats (**HU+90**, n=8) was also hindlimb unloaded and in these animals, unloading was interrupted by standing in the upright cylinder on a plate that was vertically oscillating (15 min/day, peak acceleration = 0.2 g, frequency = 90 Hz). With a surface area of ~1000 cm², we were able to fit six rats in their cylinders on the vibrating plate. The plate has been used and characterized previously [20–22]. Normally ambulatory age-matched (**AC**) and baseline (**BC**) rats were used as controls (n=8, each). Animals had access to standard rat chow and water ad libitum.

Micro Computed Tomography of IVD and Muscle

An *in vivo* two-dimensional x-ray image at 0-and 4-wk was taken of the spine (“scout view” of VivaCT 75; Scanco, Brüttisellen, Switzerland) to determine the change in anterior, center, posterior and average IVD height. Average IVD height was calculated as the mean of anterior, center and posterior height. Following each scout view image, the inferior L5 vertebra to the superior L4 vertebra region was imaged at a resolution of 78 µm (45 kV, 177 µA, 300 ms integration time). An automated algorithm [23] segmented muscle from bone to determine muscle volume including the paraspinal (e.g., erector spinae, multifidus, quadratus lumborum, which could not be individually distinguished due to low in-plane contrast) and psoas. Since both unloading and vibrations coupled with resistive exercise

differentially alter the spinal musculature [19], the psoas and paraspinal muscles were contoured in 3 consecutive images at inferior L4 and L5. After sacrifice, the lumbar spine was stored *en bloc* at -20°C .

Mechanical Testing

Prior to mechanical testing, the L4-L5 bone-disc-bone segment was excised, facets and extraneous tissue removed, and hydrated in $1\times$ PBS for 18 h at 4°C . The L4 vertebra was gripped by custom-made microvises and the L5 vertebra was fixed with bone cement into a cylinder. Once secured, the sample was immersed in $1\times$ PBS. Controlled mechanical tests were performed on the L4-L5 segment to describe physiological loading modalities (i.e., axial compression-tension, creep and torsion) [24–27]. An Instron 5542 testing system (Instron, Canton, MA) applied a load range of -6 N to 3 N at a frequency of 0.1 Hz . These loads were selected to be physiological and to be in the linear-region of the force and displacement response. After twenty compression-tension cycles, a 1 sec ramp from 0 to 6 N compression was applied and maintained for 1 h. Immediately following creep testing and while maintaining the 6 N compression load, a stepper-motor (AM15E0045; Faulhaber, Clearwater, FL) superimposed 10 cycles of torsion to $\pm 8^{\circ}$ rotation at $1.6^{\circ}/\text{s}$ [27].

Axial IVD Geometry

Following mechanical testing, the L4-L5 IVD was bisected and imaged using a stereomicroscope (Leica MZ6, Bannockburn, IL). The horizontal image was analyzed using a custom Matlab algorithm [28] to determine the antero-posterior width for the entire disc (W_{AP}) and nucleus pulposus (N_{AP}), the lateral width for the entire disc (W_L) and nucleus pulposus (N_L), as well as the area for the annulus fibrosus and nucleus pulposus. Assuming the nucleus pulposus supports negligible torsion, polar moment of inertia (J) of the IVD was calculated as $J = (\pi/64) \left[W_L^3 W_{AP} + W_{AP}^3 W_L - (N_L^3 N_{AP} + N_{AP}^3 N_L) \right]$ [25, 27]

Mechanical Data Analysis

Mechanical data were analyzed by custom Matlab routines. The trilinear fit model determined the compressive, tensile and neutral zone stiffness of the L4-L5 motion segment during the 20th compression-tension cycle [26]. Briefly, the compressive and tensile loading curves were isolated and a 5th order polynomial was fit. The lowest derivative of the curve represented the neutral zone stiffness and the maximum derivative measured between 80% and 100% of the maximum load in the compressive and tensile direction constituted the compressive and tensile stiffness, respectively. Compressive range of motion (ROM) was normalized to IVD height. Similarly, the preconditioned 10th cycle of torsion was fit with a trilinear model to obtain neutral zone (K_{NZ}) and linear region (K) stiffnesses in both the clockwise and counterclockwise. The apparent torsional neutral zone modulus (G_{NZ}) and linear region modulus (G) were calculated as $G_i = K_i h / J$ ($\text{MPa}/^{\circ}$) where h was the IVD height and J was the polar moment of inertia. K and G represented the mean from the linear regions of the clockwise and counterclockwise rotations. After the 1 sec step load of 6 N of creep force (F_C), 1 h of creep displacement was fit to a rheological model [29]: $d(t)/F_C = 1/S_1(1 - e^{-S_1 t/\eta_1}) + 1/S_2(1 - e^{-S_2 t/\eta_2}) + 1/S_3$ where $S_{1,2}$ are the elastic damping coefficients, S_3 is the instantaneous elastic stiffness, $\eta_{1,2}$ are the viscous damping coefficients, and $\tau_{1,2}$ are the time constants. The apparent elastic and viscoelastic moduli (MPa) were determined by normalizing the stiffness and damping coefficient by IVD geometry (height/area).

Statistics

Paired t-tests compared temporal changes in disc height and muscle morphology within each group. Unpaired t-tests compared age-related changes between BC and AC rats. The rationale of including a sham control group (HU+SC) was to separate the effect of weight

bearing from the effect of vibrations during weight bearing. As expected, t-tests confirmed demonstrated that no outcome variable (except for torsional stiffness and modulus) was significantly different between HU and HU+SC groups (i.e., no effect of weight bearing). In an effort to preserve statistical power and to avoid raising the probability of committing a type II error (by including a group in the comparisons that was not critical for addressing the hypotheses), HU and HU+SC were pooled and referred to as HU±SC. The pooling was not performed for analyses involving torsional stiffness and modulus. One-way ANOVAs with post-hoc Tukey tests compared the temporal change in IVD height and muscle morphology, and endpoint mechanical properties of the IVD between groups.

For each group of rats, linear regressions were performed between the neutral zone modulus and the change in posterior IVD height. Morphology of the posterior region was selected because it is more susceptible to mechanical deterioration than the anterior region and its altered mechanics relate well to degeneration [13]. Linear regressions also tested for associations between muscle area and mechanical variables, using each group mean as a data point. Due to high variability in the mechanical outcomes, one-sample t-tests with a significance threshold of 0.01 were used to remove outliers from each group [30]. This test was applied to all groups without bias. If there were two outliers in a group that satisfied the above rule, only the farthest outlier was removed. Data were expressed as mean ± standard deviation (mean ± SD). Relative differences between sample means were denoted as percent difference ± SD of the sampling distribution of the relative difference (relative standard error of the difference). Statistical significance was considered at $p < 0.05$ (SPSS 18; SPSS Inc., Chicago, IL), unless otherwise stated. One author (SJ) owns (provisional) patents regarding the application of vibrations to the musculoskeletal system.

RESULTS

IVD Geometry

Discs of rats that were hindlimb unloaded with and without brief upright weight bearing were hypotrophied. Compared to AC, change in IVD height of the HU±SC rats was smaller by $96 \pm 24\%$ ($p=0.005$) (Figure 1). Compared to AC, area of the nucleus pulposus of HU±SC discs was less by $39 \pm 8\%$ ($p<0.001$) (Figure 2). High-frequency vibrations prevented changes to IVD morphology. Change in mean height of HU+90 IVD was greater than of HU±SC by $2052 \pm 893\%$ ($p=0.016$) (Figure 1) with no difference in baseline height of the IVD of HU±SC (0.83 ± 0.07 mm) and HU+90 (0.82 ± 0.07 mm). Compared to HU±SC, nucleus pulposus area of HU+90 discs was greater by $35 \pm 12\%$ ($p=0.016$) (Figure 2). There were no differences between the morphology of IVD from AC and HU+90 rats ($p = 0.108$). Annulus fibrosus area was not different between any groups ($p=0.949$).

Compression-Tension

Hindlimb unloading altered the axial compression properties of the L4-L5 motion segment. Compared to AC, compressive modulus of HU±SC IVD was greater by $26 \pm 8\%$ ($p=0.020$) and axial NZ modulus of HU±SC discs was smaller by $60 \pm 23\%$ ($p=0.043$) (Figure 3). A similar increase was noted with the axial NZ stiffness of HU±SC discs but not with the compressive stiffness (Table 1). Tensile modulus and stiffness was not different between the 4 wk groups ($p = 0.432$).

The introduction of 90 Hz vibrations quelled the increase in L4-L5 IVD stiffness seen in unloaded rats. Compared to HU±SC, the compressive modulus of HU+90 IVD was smaller by $18 \pm 9\%$ ($p=0.047$) and the NZ modulus of HU+90 IVD was greater by $185 \pm 74\%$ ($p=0.012$) (Figure 3). The intervention provided a similar protection for the respective

structural mechanical properties (Table 1) but no differences in elastic properties existed between the IVD of AC and HU+90 rats ($p = 0.482$).

Creep

Compared to AC, the long time constant τ_2 of HU $\pm$ SC IVD was 101 ± 32 s longer ($p = 0.009$) and the instantaneous elastic modulus S_3 was $25 \pm 8\%$ greater ($p = 0.008$) (Table 2). The structural equivalent of the instantaneous elastic modulus, stiffness S_3 , of HU $\pm$ SC discs was similarly greater than of AC (Table 1). Superposition of 90 Hz low-intensity vibrations provided few benefits to creep property changes induced by hindlimb unloading. Vibrations did not mitigate the greater τ_2 of HU $\pm$ SC discs ($p = 0.858$) which was not different from the τ_2 of AC ($p = 0.067$) (Table 2). Contrarily, stiffness S_3 of HU+90 discs was smaller by $16 \pm 5\%$ ($p = 0.004$) (Table 1) but the modulus was not different to that of HU $\pm$ SC ($p = 0.278$) or AC ($p = 0.305$) (Table 2). Compressive range of motion, short time constant τ_1 , S_1 , and S_2 were not different between the 4 wk groups ($p = 0.303$).

Torsion

Torsional neutral zone stiffness K_{NZ} of AC discs was smaller than of HU $\pm$ SC by $25 \pm 6\%$ ($p < 0.001$) but torsional stiffness K ($p = 0.067$) (Table 1), torsional NZ modulus G_{NZ} ($p = 0.059$) (Table 2), and modulus G ($p = 0.105$) of the L4-L5 IVD were not different between groups. Vibrations did not maintain K_{NZ} during unloading. There was no difference in K_{NZ} between HU+90 and HU $\pm$ SC ($p = 0.161$) but was $37 \pm 8\%$ smaller ($p < 0.001$) in HU+90 than AC (Table 1).

Spinal Muscle Morphology

Contrary to the lack of longitudinal change in AC (Figure 4, Table 1), HU $\pm$ SC rats gained $17 \pm 15\%$ ($p < 0.001$) and $12 \pm 20\%$ ($p = 0.038$) psoas muscle area at L4 and L5, and lost $14 \pm 9\%$ ($p < 0.001$) paraspinal area at L4. Compared to AC, HU $\pm$ SC rats lost three times ($p < 0.001$) more paraspinal muscle area at L4 and no difference in change at L5 ($p = 0.220$). Change in area of the psoas muscle at L4 and L5 was not different among the 4 week groups. Vibrations at 90 Hz superimposed on brief upright posture provided partial benefits to the altered spinal muscle size during hindlimb unloading. HU+90 animals did not gain psoas muscle area at either vertebral level ($p = 0.138$) (Figure 4). However, HU+90 rats lost $17 \pm 2\%$ and $16 \pm 2\%$ paraspinal muscle area at L4 and L5 ($p < 0.001$) and, compared to HU $\pm$ SC, HU+90 rats lost $74 \pm 38\%$ more paraspinal muscle area at L5 ($p = 0.029$). Compared to AC, the change in paraspinal muscle area of HU+90 rats was smaller by $425 \pm 71\%$ and $591 \pm 87\%$ at L4 and L5 ($p < 0.002$). Over 4 wk, unloading groups gained lumbar muscle volume ($p = 0.002$) (Table 3).

Relationship between Disc Mechanics and Geometry or Musculature

There was a moderate negative relationship between axial neutral zone modulus and change in posterior disc height in the AC group ($R^2 = 0.60$, $p = 0.040$) (Figure 5). Hindlimb unloading with and without brief weightbearing uncoupled the relationship between disc mechanics and changing geometry ($R^2 = 0.00$, $p = 0.905$). The application of 90 Hz vibrations normalized the relationship between axial neutral zone modulus and change in posterior disc height ($R^2 = 0.65$, $p = 0.028$). Across all groups, mean paraspinal muscle area was positively and highly related ($R^2 = 0.99$, $p < 0.001$) to torsional neutral zone stiffness of the L4-L5 IVD. No other significant relationships were detected between mean muscle area and material properties of the L4-L5 IVD.

Age-Related Changes

Baseline IVD height of AC rats was 0.79 ± 0.05 mm and did not change over the 4 wk, but nucleus pulposus area of AC rats was $25 \pm 10\%$ greater ($p=0.023$) than that of BC (Figures 1 and 2). For mechanical structural properties, torsional stiffness K of AC discs was greater than of BC by $46 \pm 17\%$ ($p=0.016$) (Table 1). During creep, the short time constant τ_1 of AC discs was longer by 3.6 ± 1.3 s ($p=0.017$) (Table 2). No age-related differences were measured in creep properties ($p=0.262$), torsional properties ($p=0.085$), axial stiffness ($p=0.143$), and modulus properties ($p=0.115$) of the L4-L5 motion segment (Tables 1 and 2, Figure 3). Total lumbar muscle volume of the AC rats increased over 4 wk of aging ($p=0.003$) but individual psoas or paraspinal muscle area did not change significantly ($p=0.086$) (Figure 4, Tables 3–4).

DISCUSSION

The aim of this study was to assess the degradation of lumbar IVD biomechanics/morphology and the surrounding spinal muscle morphology of hindlimb unloaded rats and to evaluate the ability of low intensity vibrations to mitigate these changes. Four weeks of hindlimb unloading hypotrophied the disc, deconditioned the spinal segment and altered the axial and torsional mechanical behavior of the disc with almost no impact from brief interruptions of bipedal posture in sham controls (SC). Superimposing 90 Hz, low-magnitude mechanical oscillations on brief periods of upright weight bearing maintained the morphology of the IVD and psoas muscle but not paraspinal muscle, and partially countered the disruption of the axial, but not torsional, mechanics of the L4-L5 segment during hindlimb unloading. Thus, the incorporation of brief periods of low-intensity, high-frequency mechanical signals to weight bearing activities may not only provide morphological and biochemical benefits to the IVD as previously shown [21], but can also positively impact biomechanical function during long periods of spinal deconditioning.

Previously, we demonstrated that unloading is detrimental to the glycosaminoglycan content of rat IVD [21]. Using a similar experimental design, here, we assessed changes to the geometry and axial mechanics of the disc. Unloaded discs had a smaller nucleus pulposus area and displayed a weaker axial NZ modulus, a measure of segmental motion from the neutral position with particular sensitivity to degeneration [31] induced by altered glycosaminoglycan content [26]. Discs of hindlimb unloaded rats also had a greater compressive range of motion, modulus and instantaneous stiffness than of age-matched animals. These unloading-induced changes to the compressive properties of the disc were similar to the greater spinal compressibility of bed rest subjects (49% vs 59%) [3] and stiffening of rodent discs following five days of microgravity (25% vs 26%) [17].

The ability of 90 Hz vibrations to fully or partially maintain compression-tension and creep properties of the disc during unloading may be associated with differences in biochemical and morphologic properties between vibrated and control rats. Under normal loading conditions, the incompressible fluid of the nucleus facilitates the transmission of axial forces into circumferential tension and axial deformation [38]. However, discs with less nuclear glycosaminoglycan do not properly engage the inner annular fibers during axial compression and overstress the outer annular fibers in the radial and axial direction [32], leading to increased stiffness. In support of this association between mechanical, structural, and biochemical properties in the IVD subjected to unloading and vibrations, we recently showed that 90 Hz oscillatory motions superimposed on intermittent periods of squat-like posture retain the area and glycosaminoglycan content in the nucleus pulposus of hindlimb unloaded rats [21]. Thus, application of the 90 Hz mechanical signal during altered loading conditions may preserve the effective load transfer through the disc.

Daily application of 90 Hz vibrations during brief periods of erect weight bearing also prevented the uncoupling of disc mechanics and morphology during hindlimb unloading. The negative relationship between NZ modulus and change in posterior disc height of normally ambulating rats may represent the mechanical relaxation with increasing posterior height. The posterior region of the IVD is of note for its sensitivity to damage [7] and degeneration [13], and its greater morphological change during bed rest [11]. Hindlimb unloading eliminated the mechanical-morphological relationship, indicating a disruption of disc constituents and their ability to maintain normal function. While the application of 90 Hz vibrations maintained the mechanical-morphological relationship of HU+90 discs, it did not maintain the posterior height of hindlimb unloaded IVD [21].

Spinal muscular loading is a principal source of axial loading of the rodent spine. Therefore, changes (and differences) in spinal muscle area may provide insight into the mechanical loading environment of discs following the absence of dynamic loading. After four weeks, spinal muscle volume increased similarly in all groups. When stratified into specific muscle groups, hindlimb unloaded rats had increased psoas muscle area and decreased paraspinal muscle area at L4, similar to bed rest studies [2,11,34]. The lost paraspinal area and gained psoas area perhaps reflects the need to recruit alternate muscle fiber types. For example, bed rest may reduce the area of postural muscles, like the paraspinal muscles, by replacing large area type II fibers with small type I fibers [35]. By contrast, the increase in psoas muscle area with hindlimb unloading may derive from a shift of fiber type as occurs with the disc degenerative procedure of limb amputation [36].

Although the consequences of reduced dynamic loading on the musculature remain unclear, physical countermeasures may target paraspinal muscles and translate into mechanical benefits to the IVD. Here, the application of high-frequency (90 Hz), low-intensity (0.2 g) vibrations in an upright posture did not protect the paraspinal muscle but prevented changes to the psoas muscle area. In a previous bed rest study [20,34], the application of high-frequency (30 Hz), low-intensity (0.3, 0.5 g) vibrations superimposed on an axial force (0.6 body weight) did not prevent paraspinal atrophy or affect psoas muscle area. By contrast, resistive exercise with and without high-frequency (16–26 Hz), low-intensity (0.7 g) vibrations superimposed on a larger axial force (1.2–1.8 body weight) countered paraspinal muscle atrophy during prolonged bed rest but was largely ineffective at mitigating increased psoas muscle [19,37]. While both psoas and paraspinal muscles are involved with spinal compression, paraspinal muscles are additionally involved in torso rotation [38]. Because paraspinal muscle area was highly related ($R^2=0.99$) to a torsional property of the disc, muscle contractions from the extremely brief bouts of postural stabilization (15 min/day), rather than weightbearing, may have maintained the torsional stiffness during unloading.

While the hindlimb unloaded rat served to simulate reduced spinal loading, the cellular and functional disparities need to be kept in mind when interpreting the consequences of any applied load. Despite the limitations associated with using a quadruped to simulate the effects of unloading and loading human IVD, when normalized by the geometry, biomechanical properties of rodent lumbar IVD are similar to those of human IVD [25,27,39]. Moreover, upright posture in humans induces discal bending with the anterior region of the IVD loaded in tension and the posterior region in compression [40]. Surprisingly, a rat disc may be loaded in a similar fashion as suggested by thinner collagen fibers suited for recoverable creep in the posterior region [4]. While hindlimb unloading produces high tension on the tail, the induced mechanical environment of the lumbar disc remains unclear and may account for the disparity noted between the hypotrophy of IVD with hindlimb unloading [22] and hypertrophy with human spinal unloading [11]. Furthermore, the relatively young age of the rodents may have influenced the response to loading and it is entirely possible that older rodents and humans respond differently. Lastly,

while CT is commonly used to quantify muscle volume and disc height, MRI and histology will be required to further characterize the structural and functional changes induced by unloading and the vibratory intervention.

Low-level, high-frequency mechanical signals partially maintained the function and morphology of a spinal motion segment, and prevented the loss in the relationship between IVD mechanics and morphology during spinal deconditioning. Clinically, vibrations mitigate the increased incidence of back pain and normalize IVD morphology during prolonged bed rest and suggest a putative link [2]. The inability of the signal to fully protect the IVD and muscle from the detrimental effects of immobilization may suggest that efficacy could be improved by the incorporation of complementary mechanical stimuli [19,41]. However, the complimentary stimulus must be applied with caution to not exacerbate pain [19] since deconditioned discs are mechanically vulnerable to damage [7]. Further, it is possible that contractions from the spinal muscles indirectly influenced maintenance of the IVD [42]. Nevertheless, the results highlight the fundamental physiologic relevance of low-level mechanical signals at high frequencies to maintain mechanical tissue function during deconditioning [43]. Whether vibratory signals can be used clinically to prevent or treat muscle atrophy, back pain or disc degeneration during conditions of reduced dynamic loading or immobilization requires further investigation.

Acknowledgments

This work was kindly supported by NIH, NASA, Alliance for Graduate Education and the Professoriate, and NASA-Harriett G. Jenkins Pre-doctoral and W. Burghardt Turner Fellowships.

REFERENCES

1. Wilke HJ, Neef P, Caimi M, Hoogland T, Claes LE. New in vivo measurements of pressures in the intervertebral disc in daily life. *Spine*. 1999; 24:755–62. [PubMed: 10222525]
2. LeBlanc 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; 73:2172–8. [PubMed: 1474100]
3. Macias BR, Cao P, Watenpaugh DE, Hargens AR. LBNP treadmill exercise maintains spine function and muscle strength in identical twins during 28-day simulated microgravity. *J Appl Physiol*. 2007; 102:2274–8. [PubMed: 17395762]
4. Pedrini-Mille A, Maynard JA, Durnova GN, et al. Effects of microgravity on the composition of the intervertebral disk. *J Appl Physiol*. 1992; 73:26S–32S. [PubMed: 1526953]
5. Iatridis JC, Mente PL, Stokes IA, Aronsson DD, Alini M. Compression-induced changes in intervertebral disc properties in a rat tail model. *Spine*. 1999; 24:996–1002. [PubMed: 10332792]
6. Sayson JV, Hargens AR. Pathophysiology of low back pain during exposure to microgravity. *Aviat Space Environ Med*. 2008; 79:365–73. [PubMed: 18457293]
7. Johnston SL, Campbell MR, Scheuring R, Feiveson AH. Risk of herniated nucleus pulposus among U.S. astronauts. *Aviat Space Environ Med*. 2010; 81:566–74. [PubMed: 20540448]
8. Wing P, Tsang I, Gagnon F, Susak L, Gagnon R. Diurnal changes in the profile shape and range of motion of the back. *Spine*. 1992; 17:761–6. [PubMed: 1502639]
9. LeBlanc AD, Evans HJ, Schneider VS, Wendt RE 3rd, Hedrick TD. Changes in intervertebral disc cross-sectional area with bed rest and space flight. *Spine*. 1994; 19:812–7. [PubMed: 8202800]
10. Mimura M, Panjabi MM, Oxland TR, Crisco JJ, Yamamoto I, Vasavada A. Disc degeneration affects the multidirectional flexibility of the lumbar spine. *Spine*. 1994; 19:1371–80. [PubMed: 8066518]
11. Belavy DL, Armbrrecht 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; 36:137–45. [PubMed: 20595922]
12. Belavy DL, Bansmann PM, Bohme G, et al. Changes in intervertebral disc morphology persist 5 mo after 21-day bed rest. *J Appl Physiol*. 2011; 111:1304–14. [PubMed: 21799122]

13. O'Connell GD, Malhotra NR, Vresilovic EJ, Elliott DM. The effect of nucleotomy and the dependence of degeneration of human intervertebral disc strain in axial compression. *Spine*. 2011; 36:1765–71. [PubMed: 21394074]
14. Hargens AR, Mahmood M. Decreased swelling pressure of rat nucleus pulposus associated with simulated weightlessness. *Physiologist*. 1989; 32:S23–4. [PubMed: 2727097]
15. Yasuoka H, Asazuma T, Nakanishi K, et al. Effects of reloading after simulated microgravity on proteoglycan metabolism in the nucleus pulposus and annulus fibrosus of the lumbar intervertebral disc: an experimental study using a rat tail suspension model. *Spine*. 2007; 32:E734–40. [PubMed: 18245988]
16. Parry DA, Barnes GR, Craig AS. A comparison of the size distribution of collagen fibrils in connective tissues as a function of age and a possible relation between fibril size distribution and mechanical properties. *Proc R Soc Lond B Biol Sci*. 1978; 203:305–21. [PubMed: 33395]
17. Sinha RK, Shah SA, Hume EL, Tuan RS. The effect of a 5-day space flight on the immature rat spine. *Spine J*. 2002; 2:239–43. [PubMed: 14589473]
18. Kawano F, Wang XD, Lan YB, et al. Hindlimb suspension inhibits air-righting due to altered recruitment of neck and back muscles in rats. *Jpn J Physiol*. 2004; 54:229–42. [PubMed: 15541201]
19. Belavy 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:E121–31. [PubMed: 18317179]
20. Holguin N, Muir J, Rubin C, Judex S. Short applications of very low-magnitude vibrations attenuate expansion of the intervertebral disc during extended bed rest. *Spine J*. 2009; 9:470–7. [PubMed: 19356986]
21. Holguin N, Uzer G, Chiang FP, 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; 111:1846–53. [PubMed: 21960658]
22. Holguin N, Judex S. Rat intervertebral disc health during hindlimb unloading: brief ambulation with or without vibration. *Aviat Space Environ Med*. 2010; 81:1078–84. [PubMed: 21197851]
23. Judex S, Luu YK, Ozcivici E, Adler B, Lublinsky S, Rubin CT. Quantification of adiposity in small rodents using micro-CT. *Methods*. 2010; 50:14–9. [PubMed: 19523519]
24. Sarver JJ, Elliott DM. Mechanical differences between lumbar and tail discs in the mouse. *J Orthop Res*. 2005; 23:150–5. [PubMed: 15607887]
25. Elliott DM, Sarver JJ. Young investigator award winner: validation of the mouse and rat disc as mechanical models of the human lumbar disc. *Spine*. 2004; 29:713–22. [PubMed: 15087791]
26. Boxberger JI, Sen S, Yerramalli CS, Elliott DM. Nucleus pulposus glycosaminoglycan content is correlated with axial mechanics in rat lumbar motion segments. *J Orthop Res*. 2006; 24:1906–15. [PubMed: 16865712]
27. Espinoza Orias AA, Malhotra NR, Elliott DM. Rat disc torsional mechanics: effect of lumbar and caudal levels and axial compression load. *Spine J*. 2009; 9:204–9. [PubMed: 18495544]
28. O'Connell GD, Vresilovic EJ, Elliott DM. Comparison of animals used in disc research to human lumbar disc geometry. *Spine*. 2007; 32:328–33. [PubMed: 17268264]
29. O'Connell GD, Jacobs NT, Sen S, Vresilovic EJ, Elliott DM. Axial creep loading and unloaded recovery of the human intervertebral disc and the effect of degeneration. *J Mech Behav Biomed Mater*. 2011; 4:933–42. [PubMed: 21783103]
30. Overholser BR, Sowinski KM. Biostatistics primer: part 2. *Nutr Clin Pract*. 2008; 23:76–84. [PubMed: 18203967]
31. Panjabi MM. The stabilizing system of the spine. Part II. Neutral zone and instability hypothesis. *J Spinal Disord*. 1992; 5:390–6. discussion 7. [PubMed: 1490035]
32. Meakin JR, Redpath TW, Hukins DW. The effect of partial removal of the nucleus pulposus from the intervertebral disc on the response of the human annulus fibrosus to compression. *Clin Biomech (Bristol, Avon)*. 2001; 16:121–8.
33. Keller TS, Spengler DM, Hansson TH. Mechanical behavior of the human lumbar spine. I. Creep analysis during static compressive loading. *J Orthop Res*. 1987; 5:467–78. [PubMed: 3681521]

34. Holguin N, Muir J, Rubin C, Judex S. Re: short applications of very low-magnitude vibrations attenuate expansion of the intervertebral disc during extended bed rest. *Spine J.* 2010; 10:364–5.
35. Moriggi M, Vasso M, Fania C, et al. Long term bed rest with and without vibration exercise countermeasures: effects on human muscle protein dysregulation. *Proteomics.* 2010; 10:3756–74. [PubMed: 20957755]
36. Cassidy JD, Yong-Hing K, Kirkaldy-Willis WH, Wilkinson AA. A study of the effects of bipedism and upright posture on the lumbosacral spine and paravertebral muscles of the Wistar rat. *Spine (Phila Pa 1976).* 1988; 13:301–8. [PubMed: 3388116]
37. Belavy 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; 109:1801–11. [PubMed: 20864564]
38. Bogduk, N. *Clinical Anatomy of the Lumbar Spine and Sacrum.* Elsevier, Churchill Livingston; London: 2007.
39. Beckstein JC, Sen S, Schaer TP, Vresilovic EJ, Elliott DM. Comparison of animal discs used in disc research to human lumbar disc: axial compression mechanics and glycosaminoglycan content. *Spine.* 2008; 33:E166–73. [PubMed: 18344845]
40. Wang S, Xia Q, Passias P, Wood K, Li G. Measurement of geometric deformation of lumbar intervertebral discs under in-vivo weightbearing condition. *J Biomech.* 2009; 42:705–11. [PubMed: 19268946]
41. Cao P, Kimura S, Macias BR, Ueno T, Watenpaugh DE, Hargens AR. Exercise within lower body negative pressure partially counteracts lumbar spine deconditioning associated with 28-day bed rest. *J Appl Physiol.* 2005; 99:39–44. [PubMed: 15761083]
42. Cochrane DJ, Loram ID, Stannard SR, Rittweger J. Changes in joint angle, muscle-tendon complex length, muscle contractile tissue displacement, and modulation of EMG activity during acute whole-body vibration. *Muscle Nerve.* 2009; 40:420–9. [PubMed: 19618430]
43. Fritton SP, McLeod KJ, Rubin CT. Quantifying the strain history of bone: spatial uniformity and self-similarity of low-magnitude strains. *J Biomech.* 2000; 33:317–25. [PubMed: 10673115]

Figure 1.

Change of intervertebral disc (IVD) height from baseline of age-matched (AC, n=7), hindlimb unloaded, with or without brief weightbearing (HU±SC, n=14) and hindlimb unloaded with brief weightbearing and 90 Hz oscillations (HU+90, n=7); mean+SD. The introduction of 90 Hz mechanical signals to upright posture maintained the normal change in IVD height. †: *significant difference vs. AC*, ‡: *significant difference vs. HU±SC*; $p < 0.05$

Figure 2.

Nucleus pulposus area of age-matched (AC, n=7), hindlimb unloaded with or without brief weightbearing (HU±SC, n=14), and hindlimb unloaded, with brief weightbearing and 90 Hz oscillations (HU+90, n=7) IVD; mean±SD. Compared to AC, IVD of hindlimb unloaded rats, with or without upright loading, had a smaller nucleus pulposus area. The application of 90 Hz vibrations maintained normal nucleus pulposus area. †: *significant difference vs. AC*, ‡: *significant difference vs. HU+SC*; p < 0.05

Figure 3.

Compressive, tensile and neutral zone modulus of the L4–L5 segment of age-matched (AC, n=7), hindlimb unloaded with or without brief weightbearing (HU±SC, n=14) and hindlimb unloaded with, brief weightbearing and 90 Hz oscillations (HU+90, n=7) rats; mean±SD. Compared to AC, HU±SC segments had weaker neutral zone moduli and greater compressive moduli. HU+90 segments had greater neutral zone moduli and smaller compressive moduli than HU±SC segments. †: *significant difference vs. AC*, ‡: *significant difference vs. HU±SC*; $p < 0.05$

Figure 4.

Change in paraspinal muscle area at L4 and L5 of age-matched (AC, n=7), hindlimb unloaded with or without brief weightbearing (HU±SC, n=14) and hindlimb unloaded with brief weightbearing and 90 Hz oscillations (HU+90, n=7) animals; mean±SD. Unloading with and without upright loading increased the psoas area at L4 and L5. At L4, paraspinal area of HU±SC was less than in AC rats. At L5, paraspinal area of HU+90 was less than in HU±SC and AC rats. *: *significant difference vs. baseline of same animal*, †: *significant difference vs. AC*, ‡: *significant difference vs. HU±SC*; $p < 0.05$

Figure 5.

Regressions between neutral zone modulus and change in posterior disc height of age-matched (AC, n=7), hindlimb unloaded with or without brief weightbearing (HU±SC, n=14), and hindlimb unloaded with brief weightbearing and 90 Hz oscillations (HU+90, n=7) rats. Low-intensity vibrations prevented a decoupling of the relationship observed in hindlimb unloaded rats. *: *significant regression*; $p < 0.05$

Table 1

Structural mechanical properties of the L4-L5 motion segment of BC, AC, HU±SC and HU+90 rats

Group	S _{COM} [N/mm]	S _{TEN} [N/mm]	S _{NZ} [N/mm]	Com ROM [mm]	S ₁ [N/mm]	S ₂ [N/mm]	S ₃ [N/mm]	K _{NZ} [N/mm°]	K [N/mm°]
BC	97±5	41±5	4.6±2.6	0.31±0.26	34±5	17±2	466±40	0.30±0.11	1.1±.4
AC	89±11	40±3	6.8±3.6	0.21±0.06	39±4	18±1	447±41	0.31±0.05	1.6±.3 [*]
HU±SC	99±11	37±5	2.3±1.8 [†]	0.28±0.11	36±7	18±1	534±59 [†]	0.23±0.03 [†]	NA
HU+90	82±13 [‡]	40±5	6.714.3 [‡]	0.28±0.13	38±6	17±2	447±50 [‡]	0.20±0.05 [‡]	1.4±.2

Data are presented as mean±SD; n=7 for baseline (BC), age-matched (AC) and hindlimb unloaded 90 Hz oscillations (HU+90); n=14 for hindlimb unloaded with or without brief weightbearing (HU±SC).

NA – Not pooled due to significant difference between HU (1.3±0.3) and HU+SC (1.7±0.2)

^{*} AC vs. BC

[†] significant difference vs. AC

[‡] significant difference vs. HU±SC; p<0.05

Table 2

Material mechanical properties and time constants of the L4–L5 motion segment in BC, AC, HU±SC and HU+90 rats

Group	Com ROM [mm/mm]	S ₁ [MPa]	S ₂ [MPa]	S ₃ [MPa]	τ ₁ [s]	τ ₂ [s]	G _{NZ} [kPa/°]	G [kPa/°]
BC	0.27±0.19	3.1±0.6	1.7±0.4	41.8±5.1	16.3±2.4	792±74	19.1±6.3	80±22
AC	0.24±0.04	3.3±0.5	1.6±0.2	38.4±5.7	19.8±2.5 [*]	768±76	18.5±3.0	105±26
HU±SC	0.36±0.17	3.6±0.9	1.6±0.2	48.0±7.2 [†]	18.9±4.2	900±55 [†]	15.9±3.0	NA
HU+90	0.31±0.15	3.5±1.0	1.7±0.2	43.4±4.5	19.5±4.4	884±81 [†]	14.2±3.5	100±12

Values are presented as mean±SD. Sample size for HU±SC was n=14 and n=7 for all other groups was

Note η=S_i. τ_i

NA – Not pooled due to significant t-test; values for HU and HU+SC were 97±13 and 112±24.

^{*} significant difference between AC vs. BC

[†] significant difference vs. AC, p<0.05

Table 3Muscle volume between L4 and L5 of AC, HU±SC and HU+90 rats over 4 weeks [cm³]

Time	AC	HU±SC	HU+90
0 week	91.4±2.3	93.4±2.8	92.7±3.2
4 week	94.5±2.4 *	95.7±3.3 *	95.7±2.9 *
<i>Relative Change [%]</i>	<i>3.7 ± 2.1</i>	<i>1.9 ± 1.1</i>	<i>3.2 ± 2.1</i>

Data are presented as mean±SD; n=7 for age-matched (AC) and hindlimb unloaded with brief 90 Hz oscillations (HU+90); n=14 for hindlimb unloaded with or without brief weightbearing (HU±SC).

* significant difference vs 0 week, p<0.05

Table 4Baseline psoas and paraspinal muscle area of AC, HU±SC and HU+90 rats [cm²]

Muscle-Location	AC	HU±SC	HU+90
Psoas-L4	0.62±0.04	0.65±0.05	0.68±0.05
Psoas-L5	0.84±0.09	0.81±0.07	0.84±0.07
Paraspinal-L4	2.67±0.18	2.67±0.16	2.63±0.19
Paraspinal-L5	2.57±0.10	2.39±0.24	2.44±0.24

Values are presented as mean±SD; n=7 for age-matched (AC) and hindlimb unloaded with brief 90 Hz oscillations (HU+90); n=14 for hindlimb unloaded with or without brief weightbearing (HU±SC)