Journal of Biomechanics 47 (2014) 2983–2988

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Effect of microgravity on the biomechanical properties of lumbar and caudal intervertebral discs in mice

Jeannie F. Bailey [a,c], Alan R. Hargens [b], Kevin K. Cheng [a], Jeffrey C. Lotz [a,*]

[a] Orthopaedic Surgery, University of California, San Francisco, CA, USA
[b] Orthopaedic Surgery, University of California, San Diego, CA, USA
[c] Anthropology and Orthopaedics & Sports Medicine, University of Washington, Seattle, WA, USA

ARTICLE INFO

Article history:
Accepted 4 July 2014

keywords:
Microgravity
Intervertebral disc
Disc degeneration
Compressive creep
Disc herniation

ABSTRACT

Prolonged exposure to microgravity has shown to have deleterious effects on the human spine, indicated by low back pain during spaceflight and increased incidence of post-spaceflight herniated nucleus pulposus. We examined the effect of microgravity on biomechanical properties of lumbar and caudal discs from mice having been on 15-day shuttle mission STS-131. Sixteen C57BL/C mice (spaceflight group, $n=8$; ground-based control group, $n=8$) were sacrificed immediately after spaceflight. Physiological disc height (PDH) was measured in situ, and compressive creep tests were performed to parameterize biomechanical properties into endplate permeability (k), nuclear swelling pressure strain dependence (D), and annular viscoelasticity (G). For caudal discs, the spaceflight group exhibited 32% lower PDH, 70% lower D and crept more compared to the control mice ($p=0.03$). For lumbar discs, neither PDH nor D was significantly different between murine groups. Initial modulus, osmotic pressure, k and G for lumbar and caudal discs did not appear influenced by microgravity ($p>0.05$). Decreases in both PDH and D suggest prolonged microgravity effectively diminished biomechanical properties of caudal discs. By contrast, differences were not noted for lumbar discs. This potentially deleterious interaction between prolonged weightlessness and differential ranges of motion along the spine may underlie the increased cervical versus lumbar disc herniation rates observed among astronauts.

1. Introduction

The intervertebral disc is a viscoelastic structure that integrates with rigid spinal vertebrae to support compressive load and provide flexibility. The disc's anatomical composition includes a thick, collagenous annulus fibrosus which functions as a ligament attaching to the circumference of the adjacent vertebral endplates, and by doing so, retains the proteoglycan-rich nucleus pulposus. Nucleus proteoglycans are hydrophilic and osmotically attract water to facilitate the swelling that supports compressive loads.

The human intervertebral disc biomechanically responds to a diurnal cycle of loading and unloading (Adams and Hutton, 1983; Adams et al., 1990; Botsford et al., 1994). When loaded by muscle and gravity forces, the applied stress exceeds the disc swelling pressure and fluid is slowly expelled from the disc. This water is typically re-imbibed once load is reduced during rest. Movement of water is accompanied by changes in disc height, intradiscal pressure (Wilke et al., 1999), and biomechanical properties (Adams et al., 1990). The time-depend change in disc height in response to a compressive stress is well-characterized by a three-parameter solid mathematical model (Burns et al., 1984), or similarly, a fluid transport model based on assumptions of endplate permeability, annulus viscoelasticity, and nuclear swelling (Cassidy et al., 1990).

Exposure to microgravity causes a reduction in disc compressive loading in humans. Associated disc water shifts and atrophy of spine-stabilizing muscles causes loss of spinal curvature and lengthening of the vertebral column to more than double the diurnal values (LeBlanc et al., 2000; Lee et al., 2003; Sayson and Hargens, 2008). Astronauts experience increased episodes of low back pain during spaceflight (Wing et al., 1991) and a heightened incidence of herniated nucleus pulposus (HNP) upon return to gravity (Johnston and Campbell, 2010). This elevated risk of post-spaceflight disc herniation is significantly greater for the cervical spine (21 times that of the incidence among a control population) than the lumbar spine (2 to 3 times that of controls) (Johnston and Campbell, 2010). An explanation for the increased susceptibility of cervical discs to post-spaceflight HNP may be due to their increased range of motion (ROM) relative to lumbar discs (White and Panjabi, 1990). The deleterious effects of movement, rather than load,

* Corresponding author at: 513 Parnassus Ave, 11th Floor S1157, San Francisco, CA 94143-0514, USA. Tel.: +415 476 7881; fax: +415 476 1128.
E-mail address: Jeffrey.Lotz@ucsf.edu (J.C. Lotz).

http://dx.doi.org/10.1016/j.jbiomech.2014.07.005
0021-9290/

may implicate microgravity-triggered injury mechanisms that could form the basis for in-flight and post-spaceflight countermeasures.

Given the experimental constraints associated with spaceflight studies, rodents are a standard mammalian model for assessing the physiologic effects of prolonged microgravity. Likewise, the rodent caudal (tail) disc is commonly used as a model for the human intervertebral disc. However, the extent murine discs experience diurnal fluctuations that may be exacerbated by microgravity has not been investigated. Yet, studies in rats have demonstrated that two weeks in microgravity degrades disc cellularity, and that this degradation is significantly greater than that observed in tail-suspended ground controls. (Pedrini-Mille et al., 1992; Maynard, 1994). Further, these adverse consequences to disc cellularity and biomechanics are measurable after as little as five days (Sinha et al., 2002). However, no studies have compared the effect of microgravity between rodent caudal and lumbar discs. Rodent lumbar and caudal discs differ functionally and some argue the rodent lumbar disc is a better functional representation of the human lumbar disc (Smit, 2002; Elliot and Sarver, 2004).

To assess microgravity's relative effects on lumbar and caudal intervertebral discs, we quantified changes in physiological disc height and biomechanical properties of tissues from mice that had returned from 15-day NASA shuttle mission, STS-131, as compared to those of ground-based controls. Since tail discs have a significantly greater ROM as compared to lumbar discs, we hypothesized that microgravity would have a more pronounced detrimental effect among the caudal discs.

2. Materials and methods

Experimental procedures were approved by the Institutional and Animal Care and Use Committee at the National Aeronautics and Space Administration (NASA) and followed the Guide for the Care and the Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication no. 85-23, revised 1996).

2.1. Animals

NASA's Biospecimen Sharing Program (BSP) maintained mice before, during, and after spaceflight. BSP personnel sacrificed mice three hours after landing and control mice were sacrificed 48 h later. After sacrifice, tissues were harvested and flash frozen using liquid nitrogen (−196 °C). Fully intact spines and tails of 16 female, 16-week-old C57BL/C mice were received by our lab 48 h after the landing of the 15-day NASA STS-131 shuttle mission. Mice exposed to microgravity for the duration of the flight represented the spaceflight group ($n=8$). Age-matched, C57BL/C mice maintained on-ground represented the control group ($n=8$). Both groups were exposed to the same environmental parameters (temp, humidity, light cycle, food, water) with gravity or lack thereof being the primary environmental difference. Caudal motion segments (C9/C10) from eight subjects ($n=4$ control mice; $n=4$ spaceflight mice) and lumbar motion segments (L4/L5) from sixteen subjects ($n=8$ control mice; $n=8$ spaceflight mice) were harvested and tested. The tail and spine specimens were thawed immediately prior to mechanical testing (for the first time since animal sacrifice and tissue harvesting), and consequently, all specimens experienced only one freeze/thaw cycle between sacrifice and mechanical testing.

2.2. Physiological disc height (PDH)

Once thawed, specimens were then radiographed (Faxitron) prior dissection for a measure of PDH. Radiographs where imaged at 40 × magnification (Nikon Eclipse E800) and measured with image analysis software (SPOT Advanced, SPOT Imaging Solutions, Sterling Heights, MI). Motion segments were then dissected and further prepared by removing extraneous soft-tissue, as well as, posterior elements from the lumbar specimens.

Due to considerable morphological differences between the murine lumbar and caudal disc spaces, separate methods were chosen for measuring disc height. Lumbar disc height was measured by averaging the lateral borders from a coronal view, as well as, the anterior and posterior edges from a lateral view (Fig. 1a; method adapted from previous study (Elliott and Sarver, 2004)). Because caudal disc spaces are more symmetrical, disc height was measured by averaging three points across the diameter of the disc: one height from the mid-diameter point and two heights from halfway in between the midline and diameter edges (Fig. 1b).

2.3. Mechanical testing

The dissected motion segments were then fixed into custom fixtures and mounted into the mechanical testing instrument (Bose Electroforce 3200, Bose Corporation, Eden Prairie, MN) (Fig. 1c). To determine the disc cross-sectional area and initial testing height, a radiograph of the specimen secured between to fixture was taken prior to testing (Fig. 1a and b). During testing, the sample was submerged in room-temperature phosphate buffered saline to ensure the tissue hydration was stable throughout the entire test. Compressive creep testing recorded the tissue deformation under a constant load. Testing included five cycles of compressive creep loading at 0.5 MPa for 20 min and recovery at 0.1 MPa for 40 min (load profile on Fig. 1c). The first four cycles were used for preconditioning and the last cycle was used for data collection and analysis.

Lastly, a displacement controlled stress-relaxation test was administered to determine initial nuclear swelling pressure (P_{osm}). Discs were swelled (40 min) to the height and displacement position of the end of the recovery period of fourth, and final, creep recovery. We calculated the change displacement occurring between the fourth creep recovery to the initial point that 0.5 MPa is applied

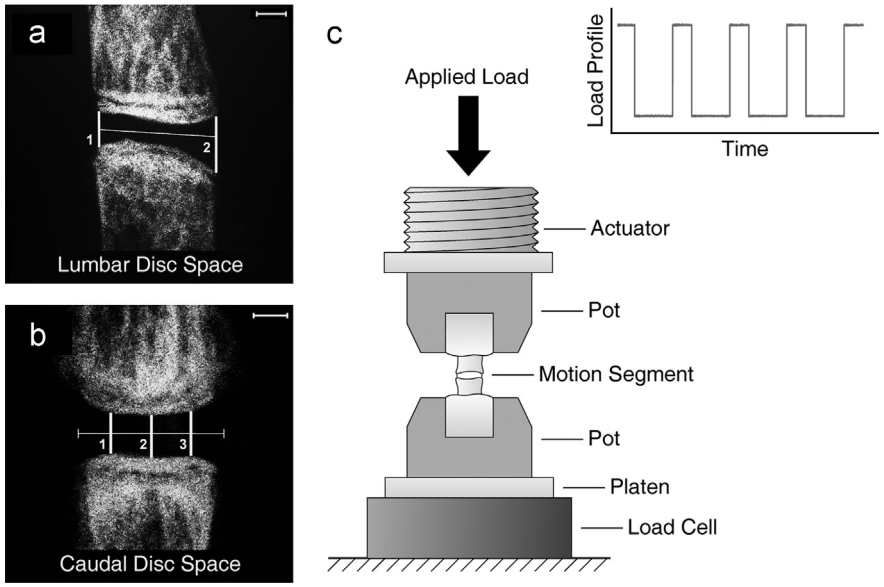

Fig. 1. Methods overview: Fig. 1a and b depicts the methods used for measuring disc height and cross-sectional disc area in lumbar and caudal murine discs. Fig. 1c shows a schematic of the testing apparatus used for compressive creep testing, along with a plot demonstrating the profile for load input in the top-right corner.