Spaceflight Decreases Bending Strength and Alters Failure Mode in Murine Spinal Segments

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Introduction: The effects of microgravity during spaceflight negatively impact the human spine and lead to increased risk for low back pain and injury. Intervertebral disc herniation risk is increased 4.3-fold following spaceflight [1]. Animal models are often used to study the effects of spaceflight on the spine, and compressive creep properties have been shown to decrease in mice post-spaceflight [2]. However, uniaxial compression alone does not cause disc herniation. Rather, hyper-flexion is a significant contributor to herniation, i.e. during heavy lifting in a flexed posture [3]. Due to the observed increase in herniation risk following spaceflight, we hypothesized that the bending strength of discs is decreased under microgravity conditions. The effects of spaceflight on spine bending properties have not previously been studied.

The specific aims of the current study were to determine: (1) mechanical bending properties; and (2) locations of bending failure in murine spinal segments following spaceflight. We hypothesized that biomechanical bending properties of post-flight mouse discs would be diminished and that specimens would fail within the annulus fibrosus or at the annular insertion to the vertebra.

Methods: The fourteen animals used for this study were obtained through NASA’s Biospecimen Sharing Program (BSP). Six mice were sent on a 30-day microgravity Bion M1 mission and acclimated to 1-G for 12 hours before they were sacrificed, while eight mice remained on the ground for the 30 days in standard vivarium cages. BSP personnel sacrificed mice and dissected and flash froze tissues. We received isolated frozen tails from the six post-spaceflight mice (flight, n=6) and eight ground vivarium controls (control, n=8).

The C3-C4 motion segment was isolated from each tail. The ventral side of each motion segment was marked with tissue dye to track orientation. Surrounding ligaments and soft tissues were removed to isolate the discs and vertebrae, and each specimen was radiographed to estimate disc height and cross-sectional area. Specimens were kept hydrated with phosphate buffered saline throughout tissue preparation. Each motion segment was placed in a custom 3D-printed four-point bending device such that the plane of bending coincided with the ventral-dorsal plane. This bending device had stainless steel rods inserted through the top and bottom pieces, perpendicular to the plane of bending, to create the four contact points (Fig. 1). The lower supports of the bending system were spaced 7.5 mm apart, while the upper supports were spaced 2.5 mm apart and attached to the moving cross-head of a materials test system (ElectroForce 3200; Bose, Eden Prairie, MN). Specimens were preloaded between 0 and 5 mm at a rate of 0.05 mm/sec for 5 cycles, and then loaded to failure at 0.006 mm/sec. Ramping was terminated after failure, characterized by a drop in force of 1.5 N. From force-displacement data, the toe region was defined as the displacement corresponding to an applied force of 1 N, the stiffness was defined as the slope of the most linear region, and the strength was defined as the maximum load
before failure (Fig. 1). Paired t-tests were used to assess differences in failure strength, failure displacement, toe region length, and disc height between the control and flight groups. Failed motion segments (n = 4 control, n = 3 flight) were fixed in their bent position, decalcified, and processed for histology. Sections were stained with a tri-chrome Mallory-Heidenhain stain containing aniline blue, orange G, and acid fuchsin and analyzed for failure location. Polarized light microscopy was used to visualize collagen fibers near failure locations.

**Results:** Spaceflight reduced bending strength by 17% (6.27 ± 0.94 vs. 7.53 ± 1.13 N, p < 0.05) and reduced toe region by 32% (0.53 ± 0.13 vs. 0.77 ± 0.21 mm, p < 0.05) with no significant effect on bending stiffness (p > 0.25). Disc height was reduced by 16% in flight mice (0.25 ± 0.03 vs. 0.29 ± 0.04 mm, p = 0.054). Results are summarized in Fig. 2.

The two modes of failure identified by histology were: (1) separation within the growth plate; and (2) annulus avulsion at the disc-vertebra junction (Fig. 3). Post-flight mouse spinal segments tended to fail within the growth plate (2/3) while control spinal segments tended to fail at the disc-vertebra junction (3/4). Polarized light microscopy showed annulus collagen fibers inserting into the cartilage endplate but not continuing through the endplate to the subchondral bone.

**Discussion:** The reduction in bending strength following spaceflight supports our hypothesis that reduced bending properties may be an important factor for explaining microgravity-associated increased herniation risk. The reduction in toe region is consistent with reports of decreased range of motion in astronauts, and the shortened disc height is consistent with prior studies investigating post-flight mice [2]. All specimens failed at soft-hard tissue interfaces between cartilage and bone, presumably due to stress concentrations caused by abrupt changes in material properties at these junctions. The increased likelihood of post-flight mice to fail at the growth plate before the disc-vertebra junction suggests a decrease in growth plate integrity, and may explain the reduction in bending strength without a corresponding reduction in stiffness.

The results of this study motivate future work investigating the underlying biochemical and structural mechanisms of growth plate failure in flight mice. Previous work suggests that chondrocyte polarity and TGF-β family signaling are essential for growth plate health [4,5]. A thorough investigation of these traits in post-flight mice may illuminate effects of microgravity that compromise murine growth plate integrity.

A limitation of animal models used in shuttle missions is the lack of clarity on whether post-flight changes are caused by the same mechanisms as in human astronauts. Microgravity likely has different effects on murine spines due to anatomical and physiological differences. The growth plate separations observed in this study are not relevant to adult humans, but may provide insight for mechanisms of vertebral growth plate fractures observed in children before growth plate closure. Interestingly, mice have been observed using their tails to move about inflight enclosures [2], which may be a confounding variable in accelerating disc degeneration. It should also be noted that the flight mice in this study acclimated to 1-G for 12 hours before they were sacrificed, and the effects on tissue properties during this period are unknown. Despite these limitations, this study provides the first analysis of bending properties of intervertebral discs following spaceflight and provides insight into possible mechanisms for increased herniation risk following spaceflight.
Significance: Intervertebral disc herniation risk is increased over 4-fold following spaceflight. Here we show the first evidence that bending properties of spinal segments are decreased following spaceflight, which may contribute to increased herniation risk.