Intervertebral disc fluid content increases with treadmill exercise in a rat model

*Lyla A. Handoklow[1], *Christine Park[2,3], Stephanie J. Blocker[2], Wyatt M. Austin[2], Thien Pham[4], Charles C.Y. Huang[4], Ana Chee[1], John T. Martin[1,2]
*co-first; 1 Rush University, Chicago, IL; 2 Duke University, Durham, NC; 3 University of Washington, Seattle, WA; 4 University of Miami, Miami, FL

INTRODUCTION: The intervertebral discs of the lumbar spine naturally degenerate with age as evidenced by altered disc composition, structure, and function. The discs are the largest avascular tissues in the body and as a result disc cells that are distant from the vertebral vasculature rely on indirect diffusion and convection of oxygen and glucose from the vertebrae to maintain metabolic processes. Our global hypothesis for this work is that impaired fluid transport (and subsequently nutrient transport) underly the degeneration process and strategies to increase transport may delay or reverse disc degeneration. In previous work, mechanical loading of the spine impacted disc health, where static bending loads induce a degenerative response and dynamic loads in a physiologic range induce an anabolic response[1,2]. Dynamic loading appears to be important to disc health, as dynamic disc loading causes an increase in small molecule uptake suggesting that the concentration of glucose, a critical nutrient for disc cell metabolism, may be increased with dynamic loading[3]. In these previous studies, disc were loaded using external mechanical loading devices that were surgically affixed to adjacent vertebrae. One way to induce dynamic mechanical loads *naturally* is through exercise and recent work suggests that exercise is beneficial to the spine[4] and can even reverse surgically-induced disc degeneration[5]. However, the mechanism by which this occurs is not clear. We hypothesize that exercise improves transport of fluid and nutrients into the intervertebral disc and this drives disc anabolism. In this study, we investigated how disc fluid composition is related to age and disc degeneration in a rat model and determined acute effects of exercise on disc fluid transport. We used magnetic resonance imaging (MRI) to measure how disc fluid composition changes with age as a marker of degeneration and then before and after treadmill walking as a measure of exercise-induced fluid transport. Our hypotheses were that 1) disc fluid decreases with age and 2) disc fluid increases with exercise.

METHODS: This study was approved by the Duke Health animal care committee. ***Study 1:*** *Effect of age on disc fluid composition* Fischer 344 rats from 10-week (n=8, 4M/4F, "young"), 6-9 month (n=8, 4M/4F, "adult"), and 20-month (n=8, 4M/4F, "advanced") age groups were imaged by MRI and evaluated histologically. *MRI Analysis* MRI was performed at 7T (Bruker Avance) using a surface array coil. Rats were anesthetized via isoflurane for the duration of the imaging experiment. Disc geometry was measured in 3D using a sagittal T2 Turbo RARE sequence (TE = 12.3 ms, TR = 3000 ms, voxel dim. = 0.15 x 0.15 x 0.5 mm, scan time = 16:40). Disc boundaries on each slice were manually segmented and compiled in 3D. Mean disc height was calculated as the distance between the centroids of superior and inferior surfaces. Disc fluid composition was measured in a 2D mid-sagittal slice using a T2 mapping sequence (TE = 10-60 ms, TR = 300 ms, voxel dim. = 0.25 x 0.25 x 1 mm, scan time = 4:00). T2 signal intensity at each echo was fit to a single-term exponential decay to calculate T2 relaxation time. Discs were manually segmented, and mean T2 relaxation time was calculated for the whole disc. *Histological Analysis* Bone-disc-bone segments were isolated from the rat lumbar spine, decalcified, fixed in formalin, and embedded in paraffin. Discs were sectioned at 8 µm, stained with hematoxylin and eosin, and whole-slide brightfield images were captured at 400x (Leica Aperio AT2). ***Study 2:*** *Effect of exercise on disc fluid composition and glucose uptake* 10-week Fischer 344 rats (n=8, 4M/4F, "young") were imaged by MRI before and after treadmill exercise. Rats were acclimated to the treadmill over 5 sessions where speed and duration gradually increased to 4 m/min at 60 minutes in the final session. *MRI Analysis* Rats were imaged by MRI before and after treadmill exercise (4 m/min, 60 min) for disc geometry using a T1 FLASH sequence (TE = 2.5 ms, TR = 180 ms, pixel spacing = 0.15 x 0.15 x 0.5 mm, scan time = 4:22) and for disc fluid composition using a T2 mapping sequence (TE = 10-150, TR = 300 ms, spacing = 0.25 x 0.25 x 1 mm, scan time = 4:00). Between the end of the treadmill walk and the start of the post-exercise T2 map 15±2 min elapsed on average. *Statistical Analysis* We used linear mixed effects models to account for correlations within individual rats. We evaluated the relationship between age and T2 relaxation time as well as the relationship between exercise and T2 relaxation time, controlling for spinal level.

RESULTS: We identified age-related changes in the rat intervertebral disc consistent with mild degeneration. While disc height significantly increased with age (20 months, Δ = 0.1 mm, *P*=0.009), disc T2 relaxation time significantly decreased (6-9 months: Δ = -9.1 ms, *P*=0.009; 20 months, Δ = -13.4 ms, *P*=0.0003). Decreased T2 was apparent in the caudal lumbar levels (L4-L5 to L6-S1) at 6-9 months of age and then across the whole lumbar spine at 20 months. Histological analysis revealed fibrosis of the inner AF and decreased NP size at 20 months, but no apparent changes at 6-9 months. Sixty minutes of treadmill walking caused an increase in disc T2 relaxation time (Post-exercise, Δ = 12.1 ms, *P*=0.03), that was largest in the upper lumbar levels. This change was accompanied by a decrease in disc height of about 5% (Post-exercise, Δ = 0.1 mm, *P*=0.03).

DISCUSSION: We evaluated age-related lumbar intervertebral disc degeneration in Fischer 344 rats. Disc T2 relaxation time decreased in rat discs with age, consistent with age-related disc dehydration typical in humans. In these rats, the first signs of disc degeneration were in the inner annulus fibrosus, where histological staining indicated increased collagen. Rat discs continued to grow through 20 months of age as disc height increased across the lumbar spine. Due to decreased disc hydration but increased disc height, we conclude that Fischer 344 rats experience mild degenerative changes with age. These results are consistent with other murine studies indicating mild age-related degenerative changes in the spine[6] with one notable exception, the age-related diabetic sand rat model, which develops disc height narrow, herniation, osteophytes, subluxation, and other degenerative changes consistent with humans[7].
We confirmed our hypothesis as exercise caused an increase in disc fluid concentration. In the context of previous work, the suspected driver is mechanical loading, though other factors like increased blood flow or fluid concentration due to disc height loss could also be important. Increased fluid transport may be the driver of exercise-induced disc regeneration[4] and future work confirming increased disc cell nutrient uptake and metabolism with exercise is warranted.

CLINICAL SIGNIFICANCE: The intervertebral discs degenerate with age and are implicated in low back pain, the world's leading cause of disability. This work suggests that exercise is a natural way to improve transport into the disc and may be beneficial to long-term disc health and prevent low back pain.

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REFERENCES: 1) Masuoka+ Spine 2007; 2) Capogna+ Eur Spine J 2022; 3) Gullbrand+ Eur Spine J 2015; 4) Belavy+ Sci Rep 2017; 5) Luan+ Int J Mol Sci 2015; 6) Hou+ Arch Gerontol Geriatr 2014; 7) Gruber+ Biotech Histochem 2017.

Figure 1 – **Age-related changes in the rat disc**. Mild age-related degenerative changes were confirmed in the rat using MRI (disc T2) and histological analysis. Disc T2 decreased first in the lower lumbar levels at 6-9 months and the across the lumbar spine at 20 months. Disc height measurement revealed increased disc size with age.

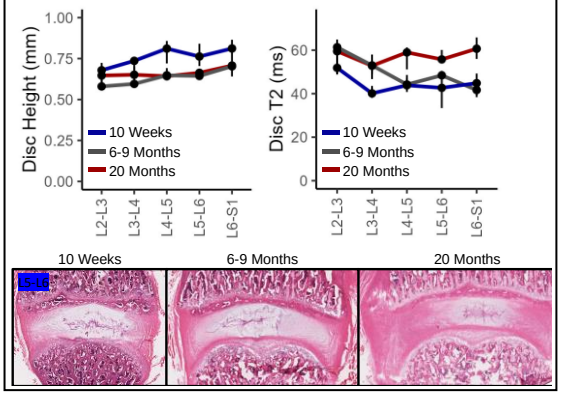

Figure 2 – **Exercise-induced changes in the rat disc**. Exercise caused a significant decrease in disc height and a significant increase in disc T2 at the upper lumbar levels.