High Strain Magnitude Cyclic Loading Does Not Affect Ultimate Strength of the Annulus Fibrosus

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INTRODUCTION: Low back pain (LBP) is experienced at a disproportionate rate by individuals exposed to cyclic loading of the lumbar spine, such as military helicopter pilots ([1]). This unique loading profile of both low and high magnitude cyclic loads likely contributes to annulus fibrosus (AF) degradation, a known cause of LBP ([2]). To prevent or limit AF degradation in these populations, protocols and technologies can be developed to reduce the mechanical burden individuals endure. However, to properly design these mitigation strategies, it is necessary to understand how the properties of AF tissue change in response to elevated mechanical loading conditions. Thus, we aimed to quantify mechanical properties of the AF before and after elevated mechanical loads are applied. We hypothesized that in response to elevated mechanical loads, the elastic response, viscoelastic response, and ultimate strength of the AF will degrade relative to the magnitude and duration of applied mechanical loads.

METHODS: Fresh porcine cervical spines were acquired from a local abattoir (Johnsonville, LLC) and refrigerated until the day of testing with all specimens tested within five days postmortem. On the day of testing, AF specimens from C3/4 to C6/7 intervertebral discs (IVDs) were dissected with a scalpel from the outermost layers of the anterior AF regions. Test specimens were oriented in the circumferential direction relative to the axial direction of the cervical spine and hydrated in a bath of physiologic saline for approximately one hour to ensure uniform hydration levels. Specimen length, width, and thickness were measured using ImageJ Software (Version 1.53) following hydration and prior to testing. A fine metal mesh was secured to each end of the specimen to prevent slippage during tensile testing and the specimen was mounted in the load frame (ElectroForce 3200, TA Instruments) using grips with knurled contact surfaces. During testing, specimens were submerged in a saline bath heated to a constant temperature of 37°C to avoid temperature-based effects. Force and displacement were measured via a 45-N loadcell (TA Instruments, New Castle, DE) and a linear variable differential transformer (LVDT) internally within the load frame, respectively, at a rate of 1 kHz. Force was converted into stress using the mid-length cross sectional area of the specimen as measured prior to the initiation of the loading protocol and displacement was converted into strain based on the length of the specimen at the 0.1-N pre-load step in the loading protocol.4

Mechanical change from cyclic loading was assessed with a 3-step experimental protocol: **(1) Pre-Damage**: (1A) Pre-load specimen to 0.1 N; (1B) Precondition; (1C) step and hold for 20 minutes at four discrete strain magnitudes (4%, 8%, 12%, 16%); and (1D) 100 tensile cycles to 11% strain at 5 Hz. **(2) Damage:** Specimens were exposed to one damage protocol with a specific strain magnitude for 400-6400 tensile loading cycles at 1.3-5 Hz (Baseline – zero damage loading cycles, 11% Strain for 1600 Cycles, 11% Strain for 6400 Cycles, 28% Strain for 400 Cycles, 28% Strain 1600 for Cycles, 44% Strain for 6400 Cycles). **(3) Post-Damage:** (3A) Step and hold for 20 minutes at four discrete strain magnitudes (4%, 8%, 12%, 16%); (3B) 100 tensile cycles to 11% strain at 5Hz; (3C) Quasi-static distraction to failure. All cyclic loading steps (1D, 2, 3B) were strain rate matched at 1.12 s⁻¹. Tissues were allowed to fully recover between each loading step. Average change in strain energy density (SED) from pre- to post-damage was calculated to quantify changes in the elastic response of the tissue. Strain energy density for each loading hysteresis was the difference between the area under the loading and unloading stress strain curve for each loading cycle (100 pre-damage, 100 post-damage). Relaxation stress delta (delta = maximum stress – minimum stress) of each incremental strain hold was calculated and compared pre- to post-damage to quantify changes in the viscoelastic response of the tissue. Finally, the linear modulus of each specimen was calculated as the slope of the linear region of the stress-strain curve during quasi-static distraction to failure, the ultimate stress and strain for each specimen were calculated to be the point at which peak stress before failure was reached, and the SED of the failure curve was calculated as the area under the stress-strain curve. To determine what effects the prescribed damage strain magnitude and number of damage loading cycles had on the mechanical properties, two factor ANOVA tests were performed with the factors of (1) strain magnitude and (2) number of cycles during the damage protocol.

RESULTS: No changes in SED and relaxation stress delta were present between steps 1 and 3 in the baseline group, indicating their elastic and viscoelastic properties were not altered by the pre-damage and post-damage loading protocols. Ultimate properties from the baseline group were comparable with findings from our previous study measuring the elastic and ultimate properties in healthy AF tissue. Specimens exposed to damage cycles had dose dependent changes in the elastic and viscoelastic response that were each significantly dependent on strain magnitude ($p<0.001$) and number of damage cycles ($p<0.001$). Interestingly, during quasi-static distraction to failure, the linear modulus (17.5 ± 10.4 MPa) ($p_{strain}=0.5$, $p_{cycles}=0.3$), ultimate stress (5.1 ± 1.7 MPa) ($p_{strain}=0.7$, $p_{cycles}=0.5$), ultimate strain (0.55 ± 0.1) ($p_{strain}=0.7$, $p_{cycles}=0.9$), and SED (1.2 ± 0.6 MPa) ($p_{strain}=0.5$, $p_{cycles}=0.3$) were not significantly affected by damage protocol. These findings indicate that the mechanical response during low strain magnitudes is significantly affected, while the high strain mechanical response remains relatively unaffected. This statement is further supported by the stress response in the toe region (0-20% strain) of the stress-strain curve during quasi-static distraction to failure. It was determined that the SED of the toe region was significantly dependent on both the strain magnitude ($p= 0.01$) and number of damage cycles ($p=0.04$). Specimens exposed to 44% strain for 6400 cycles were affected the most and had a SED of 0.002 ± 0.002 MPa, 98.2% less than the baseline group.

DISCUSSION: This study aimed to characterize changes in AF mechanical properties due to cyclic loading. It was determined that increasing the number and strain magnitude of damage cycles significantly affected the mechanical response at low strain magnitudes (0-20%). However, the linear modulus and ultimate stress and strain were unaffected, regardless of the damage protocol, suggesting the mechanical response at high strain magnitudes is maintained, regardless of the loading profile, within the bounds of damage testing incorporated here. This finding suggests a temporary instability of the IVD after cyclic loading, as shown by the reduced elastic and viscoelastic response, but the overall integrity of the IVD is maintained. These findings correlate with studies reporting military helicopter pilots experience acute lumbar back pain immediately after flights which then dissipates after some duration of recovery time devoid of spinal loads ([3]). While this issue is obviously multifactorial, the mechanical change of the IVD at low strain magnitudes could temporarily increase annulus laxity, putting more stress on the surrounding apophyseal joints. This increased stress state would exist until the AF recovers which could contribute to temporary, post-flight, lumbar back pain experienced by pilots. These findings can be used to better inform the design of future technologies and exposure protocols aimed at reducing the mechanical load on the lumbar spine to prevent or limit LBP and AF degeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: Increasing the magnitude and duration of mechanical loading significantly affected the mechanical response of AF tissue at low strain magnitudes (0-20%), but not the elastic and ultimate mechanical properties. These findings indicate annulus laxity that may contribute to IVD instability occurs immediately after cyclic loading, but the overall strength of the tissue is maintained, suggesting the mechanical response of the tissue can be restored after a period of physiologic recovery.

REFERENCES: [1] Yang et al., Front Public Health, 2021; [2] Adams et al., Spine, 2006; [3] Gaydos et al., Aviat. Sp. Environ. Med., 2012