The Stress and Strain State of the Annulus under Damaging Loading Conditions
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INTRODUCTION
Loading the intervertebral disc in flexion combined with compression is associated with distortions to the annulus fibrosus (AF) and in acute cases the complete rupture of the posterior AF [1,2]. However, little is known about the damaging stress and strain states of the AF that are induced in this deformation. To address this issue, bovine caudal motion segments were loaded in flexion combined with axial compression (flex+comp) and the biaxial strain on the posterior AF surface was measured. AF stress was determined by recreating the strain state on a planar AF section. In addition, residual strain and stress within an intact, unloaded AF were measured.

METHODS
Bovine caudal discs were obtained from approximately one-year-old animals (Martin’s Custom Butchering, Wakarusa, IN). Extraneous soft tissues were resected from whole tails and the two largest motion segments were isolated. In preparation for strain measurements, gauge section markers were applied to the posterior AF and were augmented by a speckle pattern. The reference configuration for strain measurements was defined by the unloaded, intact motion segment, which was imaged. Specimens were installed in a custom fixture and the vertebral bodies were embedded in body filler for flex+comp testing. A bending moment of 7.5 Nm for human lumbar motion segments [3] was scaled for each specimen to reflect the size difference between human and bovine discs. An axial compressive load was offset from the motion segment centroid by 20 mm, and was applied in a linear ramp over 30 sec, followed by a 30 sec hold time (Model 8821, Instron Corp., Canton, MA). Circumferential and axial strains of the outer posterior AF were measured optically (Vic-2D, Correlated Solutions, Canton, MA). Specimen hydration was maintained by wrapping a 0.15 M saline-soaked gauze pad around the AF except the gauge section area.

Afterwards, the motion segment was removed from the test fixture and a planar posterior section was excised using a diamond saw. The AF residual circumferential and axial strains due to contraction upon sectioning were optically measured with the planar section on a saline-lubricated plastic surface. Planar sample preparation was completed by trimming the AF to a constant thickness and removing bone to form a +shaped specimen [4]. Specimen height and width were measured with dial calipers, and thickness was measured with a custom resistance micrometer [5]. Sandpaper and plastic mesh were attached to the AF as grips. The specimen equilibrated in a 0.15 M saline bath for 30 minutes. The AF specimen was manually displaced in a custom planar-biaxial test machine (TestResources, Shakopee, MN) until zero strain was reached. The specimen was then manually displaced until it reached the flex+comp strain state. The stress was measured following a 60 minute relaxation step.

We previously proposed a nonlinear hyperelastic strain energy function for the AF that has additive model components representing the proteoglycan matrix, the collagen fibers, and the crosslinks between fibers [6]. Using this strain energy function with the flex+comp strain, the fraction of strain energy contributed by each component of the tissue was calculated.

RESULTS
The flex+comp strains in the intact motion segment were approximately equal in magnitude but opposite in sign (Table 1). The planar AF specimen contracted after sectioning; therefore, the residual strains were negative (Table 1). In the reference configuration, the AF was nearly in a state of equi-biaxial tension, on average (Fig 1). The stresses in flex+comp were higher than in the reference configuration, with a significant difference between the axial stresses. The axial flex+comp stress was significantly higher than the circumferential stress (Fig 1). In the flex+comp deformation, the crosslinks stored the largest portion of the strain energy, followed by the fibers and the matrix (Fig 2).

Table 1 AF surface strain with respect to an intact, unloaded motion segment.

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<th>Circ (std)</th>
<th>Ax (std)</th>
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<tr>
<td>Residual Strain</td>
<td>-0.0065 (0.0136)</td>
<td>-0.0034 (0.0230)</td>
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<tr>
<td>Flex+Comp Strain</td>
<td>-0.1087 (0.0227)</td>
<td>0.1051 (0.0293)</td>
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Figure 1 AF reference configuration stress and stress resulting from a motion segment loaded in flexion combined with compression. (*significant difference from reference configuration axial stress and from circumferential flexion combined with compression stress. Significance tested by paired t-tests, p ≤ 0.05.)

Figure 2 Fraction of strain energy resisted by the matrix, fibers, and crosslinks when loading a motion segment in flexion combined with compression.

DISCUSSION
Consistent with the expectation that the AF in an unloaded disc is in a state ofbiaxial tension due to the pressurized nucleus, our results show that these stresses are positive and nearly identical in the circumferential and axial directions. These results will be used to apply tissue-level constitutive models of the AF to the whole disc.

In flex+comp, the stress state was also biaxial but the axial stress was much larger than the circumferential stress, suggesting that axial loading may be a damaging stress state. The strain energy fraction for the flex+comp deformation indicates that the collagen crosslinks, and not the collagen fibers, resist the largest portion of the load, which may indicate an important role for intralamellar collagen crosslinks. In the future, these data will be used to further study AF damage mechanics.

SIGNIFICANCE
We measured the strain and stress states of the annulus fibrosus in the damaging deformation of flexion combined with compression. Results may be used to better understand the evolution of annulus damage that leads to disc herniation.

ACKNOWLEDGMENTS
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REFERENCES