INTRODUCTION
Degeneration of the intervertebral disc (IVD) is characterized by a loss of cellularity, degradation of the extracellular matrix, and the subsequent morphological changes and alterations in biomechanical properties [1]. One of the changes that can occur in the IVD matrix is the accumulation of molecular crosslinks known as advanced glycation end-products (AGEs). AGEs form through non-enzymatic glycation (NEG) that post-translationally modifies the amino residues of the structural components of the IVD matrix including collagen and aggrecan [2]. The increased accumulation AGEs has been implicated in reduced height [3], the loss of tissue water content [4], and a stiffening of the matrix and reduced strain energy [5] of the intervertebral disc. Because of the multi-faceted role of AGEs in degenerative disc disease (DDD), a non-invasive method to detect levels of AGEs within the IVD may improve the ability to diagnose the early onset of disc degeneration, and identify the “the disc at risk”. Magnetic resonance imaging (MRI) is a useful clinical tool for noninvasively determining the morphology of the IVD. MRI techniques such as measurement of T2 relaxation time have been shown to correlate with water content [6]. Since we have previously demonstrated that AGEs can modulate water content in the IVD [4], we sought to determine whether the molecular-level changes mediated by AGEs in the intervertebral disc are detectable by MRI-based T2 relaxation mapping.

MATERIALS AND METHODS
Sample preparation
Two sheep spines were obtained from Colorado State, and 18 lumbar and thoracic intervertebral discs were removed using a surgical scalpel. The discs were further divided into the annulus fibrosis (AF) and nucleus pulposus (NP), and allocated to undergo non-enzymatic glycation. Using a previously established in vitro ribosylation procedure [7], the tissues were subjected to a 0, 2, 4, 6, 8 day incubation period in a ribose-rich solution. The samples were then stored in a 0.15M PBS solution with enzymatic inhibitors.

T2 Relaxation Magnetic Resonance Imaging
Imaging was performed on a clinical 3.0 T GE Excite MRI scanner (GE Medical Systems, Milwaukee, WI) using a 7-turn solenoidal small animal coil built in-house. Spin echo T2-Weighted images were acquired using a 4 cm x 2 cm field of view, 256 x 128 matrix, 1 mm slice thickness and 2000 ms repetition time (TR). Individual echo times (TE) were varied at intervals of 20 ms between 20 and 160 ms for samples. Diffusion weighted imaging was acquired using an in-house Spin Echo-DWI sequence with a TR of 750 ms, a TE of 100 ms and DWI gradients in the x-direction of 935 s/mm2. Histogram analysis of the AF and NP MRI data used in-house software written using IDL 7.0 (ITT Visual; Boulder, CO). Gaussian distributions were used to fit the voxel distributions to yield estimates of the means for apparent diffusion coefficients (ADC) and T2 values.

Statistical Analyses
The general linear model (GLM) was used to determine the effects of in vitro ribosylation on T2 relaxation times and ADC values. Pearson’s correlations were used for determining the specific relationships between MRI parameters and incubation time. Statistical analyses were conducted using MiniTab (MiniTAB, PA).

RESULTS
In vitro ribosylation significantly altered the mean peak T2 relaxation times in the nucleus pulposus (p=0.011; GLM), but not in the annulus fibrosis samples (Fig. 1). Furthermore, the mean T2 values of the NP samples significantly decreased (p=0.001) with increasing periods of incubation time (Fig. 2). The apparent diffusion coefficient revealed that consistent with tissue composition, NP tissues were more hydrated than AF tissue. The in vitro ribosylation altered the ADC in both the NP and AF (p=0.046; GLM).

DISCUSSION
Although NEG does not structurally alter the IVD tissue, the accumulation of AGEs have been shown to modulate water content [4] and alter tissue stiffness [5]. Since MRI relaxation times can provide surrogate measures of tissue hydration [6], the observed changes in the T2 relaxation times in NP and AF suggest that the AGEs-mediated changes in water content of the tissue may be detectable by non-invasive biomedical imaging. Thus MRI imaging may be a useful adjunct to quantify levels of AGEs in vivo. The decreases of the T2 relaxation time with reducing water content as mediated by the accumulation of AGEs are consistent with the trends observed in the pathological degeneration of the IVD [8]. The loss of water content within the NP has been shown to initiate a cascade of events that can result in disc degeneration [6]. Although the mechanism is unknown, the accumulation of AGEs may adversely accelerate this process by competitively inhibiting water content. Since AGEs may play a significant role in the pathogenesis of DDD, the ability to detect the molecular level changes due to NEG may provide therapeutic insights and disease mechanisms of disc degeneration.

REFERENCES

ACKNOWLEDGEMENTS
We would like to acknowledge Kim Menges and Dr. Krishna Juluru, M.D. for valuable assistance.

Alterations in Magnetic Resonance Imaging T2 RELAXATION TIMES OF THE INTERVERTEBRAL DISC Due to Non-enzymatic Glycation
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Poster No. 1565 • 56th Annual Meeting of the Orthopaedic Research Society