INTRODUCTION: Gross morphologic, biochemical and histologic grading schemes have been used to characterize degenerative disc disease (1,4,5). The long term goal of this research is to develop an accurate and non-invasive diagnostic tool able to objectively detect early changes in the disc matrix with degeneration. Quantitative Magnetic Resonance Imaging (MRI) allows for an objective and reproducible way of assessing protons and their interaction with matrix macromolecules. In previous studies we showed that T1, T2, and magnetization transfer (MT) correlate to disc biochemical content and grade of disc degeneration (2). We have also shown that the apparent diffusion coefficient (ADC) is not a reflection of the disc’s biochemical subunit content. When the ultrastructural state of the disc is accounted for, the ADC is a direct reflection of matrix integrity. These studies also indicate that the information obtained with ADC can be used in combination with T1, T2 and MT to give us an accurate assessment of the disc matrix composition and structural integrity. Last year, we reported the preliminary results of quantitative MRI changes using directed enzymatic techniques. The effects noted were enzyme and concentration specific (3). Despite these advances, we do not know to what extent collagen and/or proteoglycan degradation affect changes in quantitative MRI values. The aim of this study was to induce the changes seen on quantitative MRI using directed enzymatic degradation and correlate them to a biochemical quantitation of matrix content and integrity. To this end, we injected various concentrations of collagenase and trypsin in the bovine intervertebral disc while performing quantitative T1, T2, MT and ADC assessments.

METHODS: Thirty bovine caudal intervertebral discs were harvested. The discs were injected with buffer solution (inicate the buffer in brackets), 5mg/ml of trypsin or collagenase in the nucleus pulposus region. The specimens were allowed to incubate over 18 hours. Standard SE sequences were used for T1, T2, and MT determination as previously described (2). ADC was determined along the anterior/posterior axis using an SE sequence (TE 25ms, TR 700ms, TD 150ms, Diffusion Gradient 0-24 mT/m). Specimens were then isolated from the nucleus pulposus region. Specimens were treated with alpha-chymotrypsin, and proteinase K to quantitate total type II collagen content and its percent denaturation (1). Proteoglycan, collagen and water content were measured using standard assays (1).

RESULTS: ANOVA analysis of GAG, water and collagen content did not demonstrate a significant change in content with collagenase or trypsin treatment. As expected, percent type II collagen denaturation was significantly increased with collagenase treatment (p<0.001). Our results indicate a significant (p<0.01) drop in T1 with collagenase treatment as compared to the control samples and to the samples treated with trypsin. T2 values also dropped markedly with collagenase treatment (p<0.001) as compared to the control samples and to the samples treated with trypsin. A significant (p<0.05) increase was noted in the MT signal of trypsin treated samples. As expected, percent type II collagen denaturation was also significantly altered with collagenase treatment, indicating a facilitation of the proton diffusion through the matrix. The significant alteration induced by collagenase emphasizes the importance that the collagenous lattice has on the MR signal generated. Trypsin mainly acts by cleaving proteoglycan core protein. Trypsin treatment did not significantly alter the matrix integrity to induce a change in the quantitative MR characteristics of the disc, even though PG was cleaved by trypsin, as confirmed by agarose gel electrophoresis. This was an unexpected, yet important finding. It is important to note that cleavage of PG does not change the total fixed-charges present within the nucleus. This controlled alteration of the matrix allows us to develop predictive formulas of matrix content and integrity (as shown in the result section). Further analysis of the role of aggrecan will be analyzed in future work aimed more specific at aggrecan degradation. These findings further our understanding of the effect of matrix components and matrix integrity have on the quantitative MR signal being generated. This represents an advancement in our attempt to further MR’s role as a powerful diagnostic tool of early disc degeneration.

CONCLUSION: These results indicate that enzymatic matrix degradation can be reproducibly quantified using quantitative MR techniques. The effects noted are enzyme specific. Given the constant percentage of water, GAG and collagen content throughout all discs, the change in quantitative MRI signal is a result of the change in matrix integrity induced by these enzymes and not a reflection of content. Collagenase induced a significant alteration in the collagen matrix integrity. This was reflected by the %collagen denaturation and the significant alteration in T1, T2, MT, and ADC values. The drop in T1 and T2 relaxation times imply that spin/spin and spin/lattice proton relaxation is rendered significantly shorter with the denaturation of the collagen matrix. The fact that collagenase alters the MT reinforces the theory that the MT signal is partly due to the hydroxyl / water interaction on the collagen chains. ADC was also significantly altered with collagenase treatment, indicating a facilitation of the proton diffusion through the matrix. The significant alteration induced by collagenase emphasizes the importance that the collagenous lattice has on the MR signal generated. Trypsin mainly acts by cleaving proteoglycan core protein. Trypsin treatment did not significantly alter the matrix integrity to induce a change in the quantitative MR characteristics of the disc, even though PG was cleaved by trypsin, as confirmed by agarose gel electrophoresis. This was an unexpected, yet important finding. It is important to note that cleavage of PG does not change the total fixed-charges present within the nucleus. This controlled alteration of the matrix allows us to develop predictive formulas of matrix content and integrity (as shown in the result section). Further analysis of the role of aggrecan will be analyzed in future work aimed more specific at aggrecan degradation. These findings further our understanding of the effect of matrix components and matrix integrity have on the quantitative MR signal being generated. This represents an advancement in our attempt to further MR’s role as a powerful diagnostic tool of early disc degeneration.

References:

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Quantitative MR Imaging setup used for analysis

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