Effect of Cyclic Loading on The MR Properties, Compressive Properties, And Biochemical Content of Collagenase-Treated Nucleus Pulposus

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Introduction: Changes in the biochemical composition and structure of the intervertebral disc (IVD) have been widely reported with age and disc degeneration [1]. Evaluation of these changes in the IVD hinges on the ability to objectively and non-invasively assess the disc matrix composition and integrity. To this end, quantitative MR analysis, using the relaxation times T1, T2 and T1rho, the magnetization transfer (MTR), and the apparent diffusion coefficient (TrD), can be used to correlate MR signal to disc tissue degeneration. Using targeted enzyme digestion, our previous work demonstrated quantitative MRI (qMRI) reflects the composition, and mechanical properties of disc tissue, the structural integrity of the nucleus pulposus (NP) matrix, and that these MRI parameters are affected by IVD loading [2-4]. There is very limited information on interaction between loading and enzymatic digestion on qMRI parameters, composition, and mechanical properties, particularly as it relates to collagen integrity, which is known to be affected in human disc degeneration.

The purpose of this study was to assess the biochemical and mechanical properties of collagenase-treated discs under 16-hour physiological load using qMRI. We hypothesized collagenase digestion would disrupt collagen integrity of the disc in a manner that would be detectable by quantitative MRI, and enhanced by mechanical loading.

Materials and Methods: Experimental Groups: Bovine caudal segments (n=18; 2-3 years-old) as 3-disc segments were injected in the NP with either 5 mg of type IA bacterial collagenase (Sigma-Aldrich Canada) in 40 μl Tris with CaCl2 buffer or with buffer only. The 3-disc segments were placed in bags containing saline solution and antibiotics and were kept at 37°C throughout the experiment. The segments were subjected to either 16h of cyclic compression loading (50N–300N–50N at 1Hz) or were left unloaded for 16h. The segments were then paraffin embedded for MRI. MRI Procedure: The MR examinations were carried out in a 1.5T whole-body Siemens’ Avanto system using the standard circularly polarized head coil. T1, T2, MTR, and TrD were measured as described previously [3]. For T1rho, a series of T1rho-weighted images were acquired using a self-compensating turbo-spin-echo sequence [5] with TE/TR of 3000 ms/12 ms, six TSL of 10-80 ms, and BSL set at 500 Hz. Biochemical Composition: The discs were dissected and the NP tissue was processed for biochemical and mechanical analyses. NPs were analyzed for contents of water, glycosaminoglycan (GAG), total collagen, and denatured collagen [2,3]. Mechanical Testing Procedure: The portions of NP tissue for mechanical analysis were immediately frozen on dry ice and kept at −80°C until analysis. Each NP tissue plug of 5-mm diameter and 2-mm thickness was prepared using a cryostat and biopsy punch, and tested under confined compression as previously described [3]. Swelling pressure, compressive modulus HA, and hydraulic permeability k were obtained using a ramp stress-relaxation experiment. Statistical Analysis: ANOVAs were used to evaluate the effects of loading and collagenase treatment on the MR parameters, biochemical properties, and mechanical variables (P ≤ 0.05).

Results: Digestion of the NP tissue with collagenase led to a significant increase in denatured collagen content (Fig. 1A), but not total collagen content, and this was not affected by loading. Surprisingly, collagenase had no effect on the MR parameters (as shown for T1rho in Fig. 1B). As expected, loading affected the MR parameters; T1, T2, T1rho (Fig. 1B), and TrD decreased with load, while MTR increased. Treatment of NP with collagenase tended to increase its compressive modulus (Fig. 1C). Results also suggest that loading tended to affect the compressive properties of the NP tissue.

Discussion: This study examined changes in IVD biochemical, quantitative MR and mechanical properties in response to collagenase digestion. Importantly, collagenase induced an increase in denatured collagen but had no effect on qMRI parameters. This contrasts with other studies where T1, T2 and MTR were dependent on collagen degradation [2]. This difference may relate to the fact that in this study disc segments were placed in saline solution (an open system) while previously, disc segments were wrapped in paraffin, suggesting that swelling and water content effects are important parameters to control. The effect of load on the compressive properties and qMRI parameters is consistent with our previous trypsin experiments [4]. Importantly, there was an apparent interaction between collagenase digestion and mechanical loading conditions for both qMRI and mechanical parameters, indicating a very specific relationship between mechanical loading, biochemical composition, and mechanical behaviors that is altered when collagen or proteoglycan content or integrity is disrupted. We conclude that mechanical loading if the IVD can modulate matrix composition and structure in a complex manner that may be of particular significance in disc degeneration. Improved understanding of this interaction may allow development of an objective, accurate, non-invasive diagnostic tool (qMRI) in the detection and quantification of matrix (composition and integrity) and biomechanical changes in early IVD degeneration.