

MR Spectroscopy in Intervertebral Disc and Correlation with Biochemical Analysis

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Introduction Intervertebral disc degeneration (IVDD) is a leading cause of lumbar spine-related lower back pain, a problem that affects 60 to 80% of aging Americans. It is thought the degenerative process starts with proteoglycan (PG) breakdown in the extracellular matrix (ECM) of the nucleus pulposus, followed by tissue dehydration and the reduction of its mechanical properties. Clinically, disc degeneration in a patient with lower back pain is diagnosed solely based on morphology using techniques such as discography, Computed Tomography and Magnetic Resonance Imaging (MRI). However, these techniques fail to detect early degenerative processes of the disc or identify subjects with painful degenerative disc disease and metabolic changes of the disc matrix. Magnetic Resonance Spectroscopy (MRS) is a non-invasive spectroscopic technique that delivers a biochemical and metabolic representation of the tissue in addition to the anatomic information classically derived from MRI. The feasibility of applying MRS to detecting disc degeneration had been reported previously [1]. The goal of this study was to investigate the correlation between biochemical assays of the intervertebral disc of cadaveric spine specimens and bovine specimens with the MRS findings.

Method Fourteen bovine caudal discs and 18 fresh-frozen human intervertebral discs from 5 cadaveric spine specimens (ages 70-92, 1 male and 4 females) were imaged on a 3 Tesla GE Excite Signa whole body MR scanner (General Electric Medical Systems, WI) using an 8 channel phase array knee coil (GE). The single voxel spectra were obtained by a point-resolved spectroscopy (PRESS) spin echo sequence with a three-pulse chemical shift selective (CHESS) saturation sequence for water suppression. The imaging parameters were: TE/TR = 28/1700 ms, 1024 data points, 384 repetitions, total imaging time 11 min 29 sec. A $4 \times 16 \times 14 \text{ mm}^3$ voxel was placed at the center of the disc. The bovine discs were pre-scanned. Then 7 discs were injected with papain and 7 discs were kept as controls. Both papain-injected and control discs were kept in the 4 °C refrigerator for 4-6 days and were scanned again to examine the degeneration that was introduced by papain injection. The human cadaver discs were scanned once without any treatments. After imaging, the specimens were dissected and a 25 mg tissue sample of the nucleus pulposus was obtained by punch biopsy (Sklar Instruments, West Chester, PA). The biopsy punches were taken at central location of nucleus pulposus on intervertebral discs where spectra were acquired. The sample tissues were digested in papain and used for biochemical analysis. PG content was measured using a dimethylmethylene blue assay.

The acquired MRS data were combined and corrected with respect to frequency, phase and baseline distortion. Four spectral regions were identified in each acquired spectrum based upon previous literature [2]: the carbohydrate (Carb) region (3.50-4.20ppm) associated with the breakdown of collagen and chondroitin sulfate, the choline head group region (Cho) (3.15-3.30ppm), the N-acetyl region (N-acetyl) (1.90-2.10ppm) associated with proteoglycan (PG) and the lactate region (Lac) (1.15-1.40ppm). A peak-fitting program developed in-house [3] was then applied to provide robust and reliable estimation of the metabolite concentrations. Peak areas were used in this study for the analysis of relative peak intensities. Three peak area ratios (N-acetyl/Lac, N-acetyl/Cho, N-acetyl/Carb) were computed for each spectrum acquired from the cadaveric human discs. All post-processing was performed on a Sun workstation (Sun Microsystems, Palo Alto, CA). The Pearson's coefficient (R) was used to evaluate the strength of correlation of MRS findings to the PG content.

Results Significant decrease of N-acetyl peak height was observed in the papain-injected bovine disc 4-6 days after injection (Fig. 1a and 1b). In comparison, no significant differences were found in the control bovine disc between the two time points (Fig. 1c and 1d). Analysis of the PG content of the nucleus pulposus of cadaveric discs revealed significant correlations between N-acetyl/Lac and PG content ($R = 0.83, P < 0.0001$) as well as between N-acetyl/Cho and PG content ($R = 0.64, P = 0.0039$) (Fig. 2a and 2c). Significant correlation was also found between N-acetyl/Lac ($R = 0.76, p = 0.0015$) and PG content, but not between N-acetyl/Cho and PG content ($R = 0.45, p = 0.13$) (Fig. 2b and 2d) in bovine discs. No significant correlations were found between N-acetyl/Carb and PG content in both cadaveric and bovine discs.

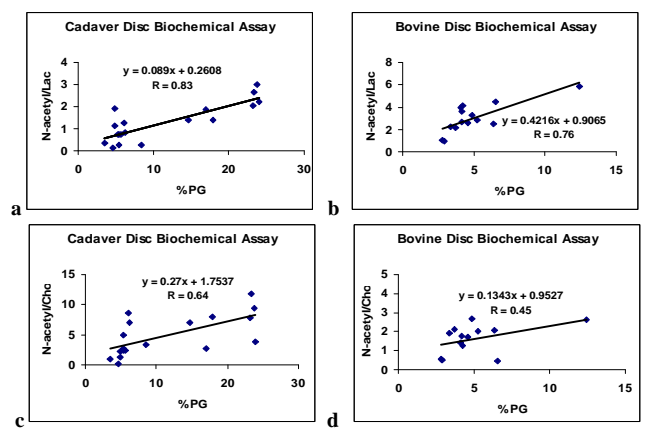
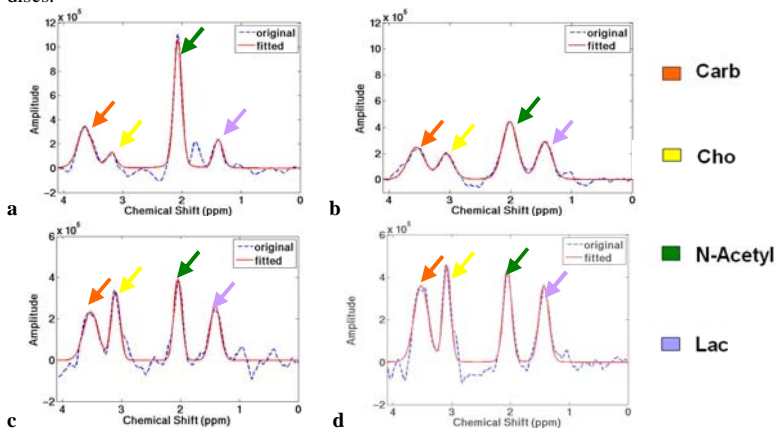


Fig. 1. MR spectra acquired in a bovine disc before (a) and 4-6 days after (b) papain injection versus the spectra acquired in a control bovine disc at the same time points (c, d).

Fig. 2. Biochemical analysis on cadaver human discs and bovine discs in relation to MRS derived ratios.

Discussion This study demonstrates that Magnetic Resonance Spectroscopy of the intervertebral discs can be successfully used to non-invasively detect biochemical and metabolic changes in the tissue. The reduction in the N-acetyl peak height and area observed in our study agrees with previous studies [2], indicating a decrease in PG content of the nucleus pulposus with degeneration.

In a recently published study, Keshari et al found an increase in lactate content of discs in patients with degenerative disc disease and low back pain [4]. Intervertebral discs are avascular structures; cells rely on nutrient diffusion from nearby capillaries through significant thicknesses of matrix and bony endplates and use anaerobic glycolysis for ATP production. If nutrient diffusion into the nucleus pulposus is challenged in any way, anaerobic glycolysis increases, creating more lactate and decreasing the pH, in turn activating tissue matrix metalloproteinases which break down the ECM [4]. The ratio of N-acetyl/Lac was severely decreased in degenerated discs in our study, which, while is in part due to increased anaerobic glycolysis post-mortem, may also indicate a possible barrier to nutrient diffusion as a result of the degenerative changes occurring in the discs. The N-acetyl/Lac ratio also strongly correlated to the proteoglycan content of the tissue, indicating a possible role for PG and Lactate spectroscopy as a non-invasive tool to detect early disc degeneration.

Overall, Combining anatomic information using traditional spine imaging with the new metabolic and biochemical information from MRS has the potential to non-invasively detect disc degenerative processes early in the process and to help guide better prevention or treatment planning for the clinician and the patient.

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References

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