MR Spectroscopy In Intact and Degenerated Bovine Intervertebral Disc

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Introduction Intervertebral Disc Disease (IVDD) afflicts nearly 12 million people in the United States and is a leading cause of lumbar spine-related lower-back pain [1]. Conventional magnetic resonance imaging (MRI) has a critical role in detection of late-stage degenerative changes (such as changes in disc morphology, height, bulge and herniation). Recently, other MRI methods have been proposed to detect early degenerative changes in the matrix content of the disc, including delayed gadolinium-enhanced MRI, diffusion imaging, T1rho and T2 imaging. High-resolution magic-angle spinning (HR-MAS) spectroscopy has been used to correlate *in vitro* disc degeneration with the changes of concentration of the metabolites in disc [2]. However, no research work has been done to use non-invasive MR spectroscopy to estimate the concentration of metabolites in disc. The goals of this study were to (i): test the feasibility of MRS in quantification of the concentration of metabolites in the intervertebral disc and to (ii) examine the changes in concentration of metabolites as a result of papain-induced degeneration of bovine intervertebral discs *in vitro*.

Method Ten fresh-frozen bovine discs 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. 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 = 35/2000 msec, 1024 data points, 256 repetitions, total imaging time 9 mins. The enzymatic degradation of the disc was achieved with papain (Sigma P-4762). Each disc was injected with 0.1 mL of papain solution containing 2mg papain (14U/mg), 0.1 M sodium phosphate (Aldrich 342483), 0.05 M EDTA (Sigma E6758) and 0.01 M L-Cysteine hydrochloride (Sigma C1276), following the formulation used by Bradford et al [3]. The discs were scanned at 5 different time points: before papain injection, 4 hours, 24 hours, 2 days and 4 to 5 days after papain injection (the time point of 2 days after papain injection of two discs were missing, the time point of 4 hours of the other two discs were missing). The data acquired from multiple channels of the coil were combined and the ratios of spectroscopic peak height of different metabolites were calculated. A paired students t-test was performed and the result was considered significant if $P < 0.05$. All post-processing was performed on a Sun workstation (Sun Microsystems, Palo Alto, CA).

Results Four spectral regions were identified as follows based upon previous literature [2]: the carbohydrate (Carb) region, the choline head group (Cho), the N-acetyl region (N-acetyl) and the lipid and lactate region (Lac+Lip). Four peak height ratios (N-acetyl/Lac+Lip, N-acetyl/Cho, Cho/Carb, N-acetyl/Carb) were computed for each spectrum acquired at different time points of all bovine discs. Figure 1 shows typical spectrum acquired before and 24 hours after papain injection, with an increase in the choline head group resonances. The peak ratio of the metabolites before papain injection was set to 1.0, the peak ratios acquired at rest time points were calculated as the ratios of the first time point. Among the calculated ratios, the N-acetyl/Cho ratio indicates an increasing trend with time (Fig. 2a), while the Cho/Carb ratio demonstrates a decreasing trend with time (Fig. 2b). No trend was observed for the other two ratios (N-acetyl/Lac+Lip and N-acetyl/Carb). Significant differences were found between the ratios acquired before and 24 hours after papain injection ($P = 0.0011$ for N-acetyl/Cho and $P < 0.001$ for Cho/Carb) and the ratios acquired 24 hours and 4-5 days after papain injection ($P = 0.023$ for N-acetyl/Cho and $P < 0.009$ for Cho/Carb).

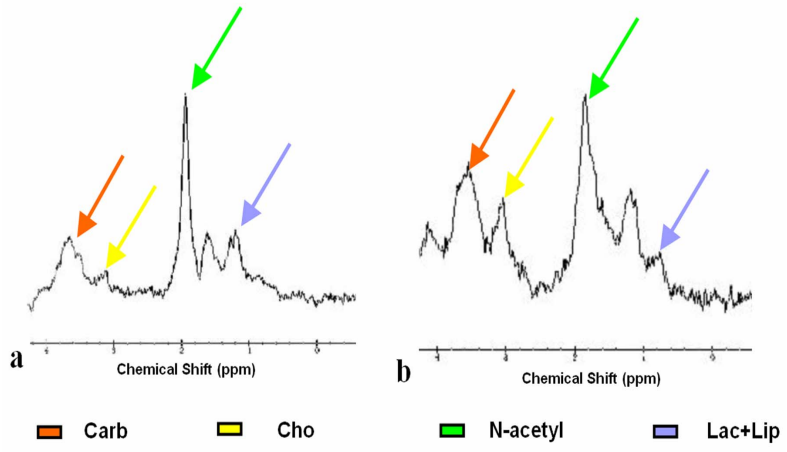

Fig. 1. Representative spectrum acquired before (**a**) and 24 hours after (**b**) papain injection.

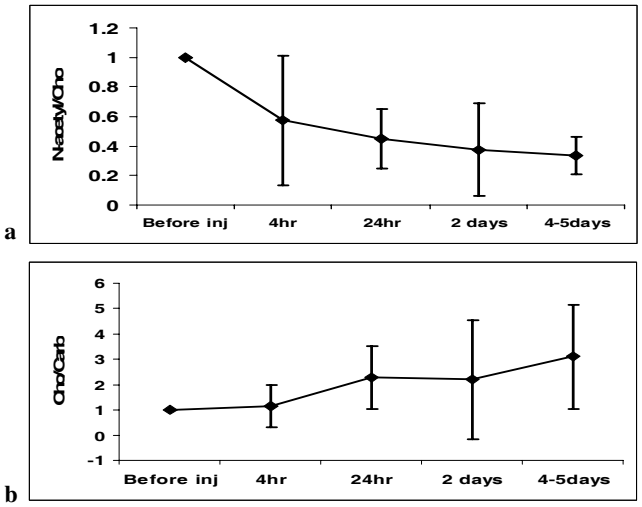

Fig.2. Computed N-acetyl/Cho ratios (**a**) and Cho/Carb ratios (**b**) at different time points.

Discussion The study demonstrated that single voxel spectroscopy can be successfully applied to detect changes of the metabolites in intervertebral discs non-invasively. The decrease of N-acetyl/Cho and the increase of Cho/Carb with disc degeneration are in agreement with previous studies [2]. That no significant spectroscopic changes were observed at 4 hours after papain injection is probably due to the short time period allowed for the papain solution to achieve detectable degradation of the disc. The lactate peak may contain substantial lipid contamination, and measurement of the N-acetyl/Lac+Lip ratio may be considerably prone to errors due to volume selectivity of the RF pulses in the sequence. This might contribute to the fact that no significant difference was found for this ratio before and after the papain injection. In summary, single voxel spectroscopy of the intervertebral discs is a promising approach for the non-invasive detection of early IVDD.

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[3] Bradford, DS., et al. Spine. 1984: 9: 135-147.