Detecting Early Cartilage Degradation by Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy in a Mechanical Injury Model

Maegha R. Singh[1], Ping Yu[2], Derek D. Cissell[1]
[1]J.D. Wheat Veterinary Orthopedic Research Laboratory, School of Veterinary Medicine, University of California, Davis
[2]Nuclear Magnetic Resonance CORE Facility, Office of Research, University of California, Davis

Introduction

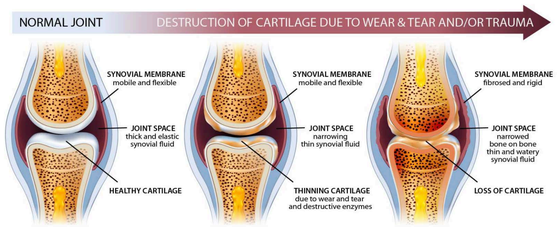

Fig 1. Progression of cartilage injury in a joint as seen during osteoarthritis (OA)[1]

Cartilage loss associated with OA detected late stage by radiography or magnetic resonance imaging (MRI)[2]

Proton magic angle spinning nuclear magnetic resonance spectroscopy ([1]H MAS NMR) detects biochemical changes that occur with cartilage degradation[2]

Previous MAS NMR studies of end stage osteoarthritic cartilage show:

- Lower levels of N-acetyl correlated with loss of glycosaminoglycans (GAGs)
- Lower levels of amino acids glycine and alanine correlated with collagen loss[2]

NMR May Show Biochemical Differences Between Treatments

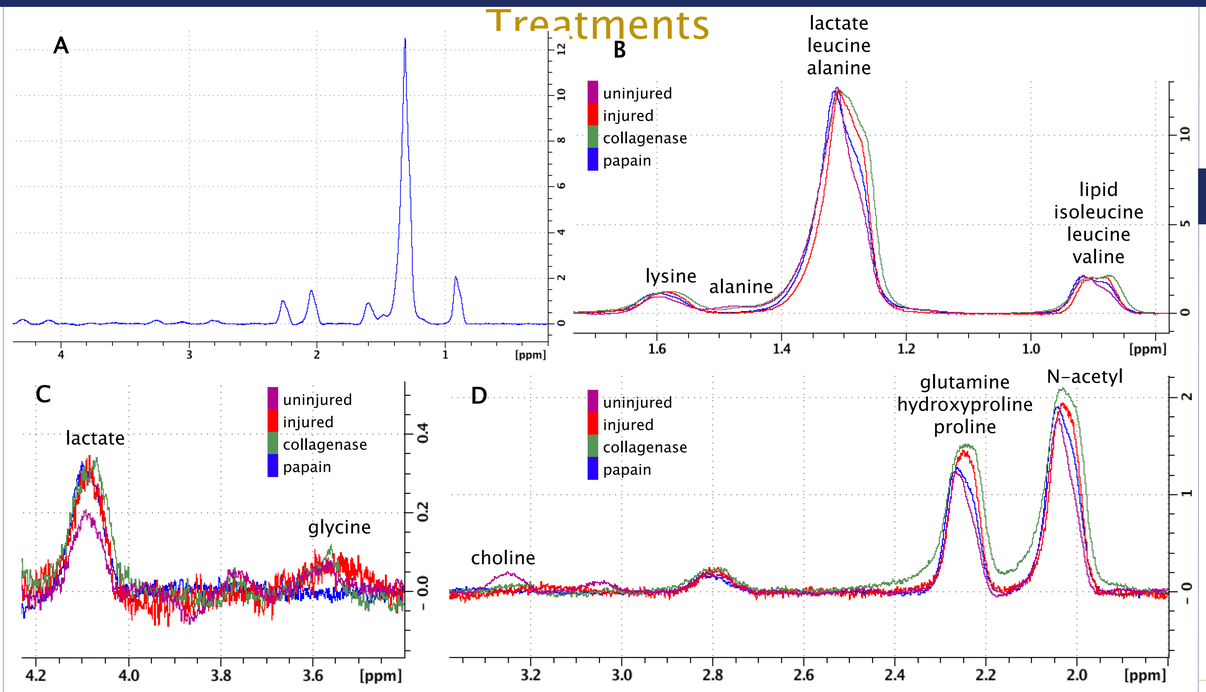

Fig 2. (A) NMR spectrum of uninjured juvenile bovine cartilage. (B-D) Magnified NMR spectra highlighting differences between experimental groups (n=1 per treatment group)

Future Directions

- Obtain additional NMR spectra from larger sample size
- Quantify differences in NMR spectra
- Compare NMR to current MRI based methods of cartilage evaluation

Treatments Alter Cartilage Properties

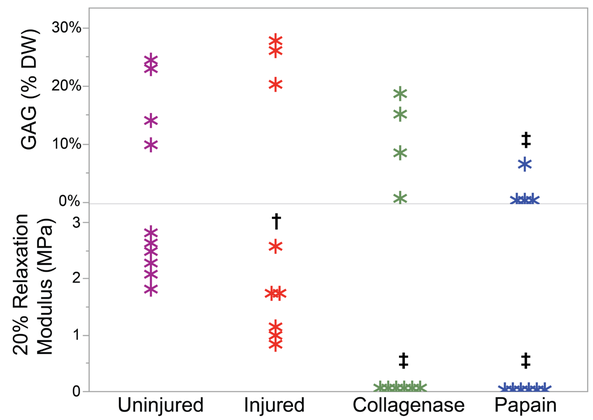

Fig 3. Papain reduces GAG and all treatments reduce compressive stiffness compared to controls.

† = $p < 0.05$
‡ = $p < 0.01$

References
[1] Trackener. "Equine Arthritis." 18 Apr 2018.
[2]Shet, Keerthi, et al. "High-Resolution Magic Angle Spinning NMR Spectroscopy of Human Osteoarthritic Cartilage." NMR in Biomedicine. 18 Aug 2011.

Hypothesis & Aims

Aims

- Use a bovine *ex vivo* mechanical model to simulate early cartilage injury
- Detect biochemical changes seen in early cartilage degradation by measuring NMR spectral peaks in mechanically injured cartilage explants compared to uninjured and enzymatically digested cartilage

Hypothesis

- [1]H MAS NMR spectra of mechanically injured cartilage will differ from uninjured cartilage with decreases in spectral peaks of N-acetyl, alanine, and glycine associated with changes in cartilage composition and stiffness

Methods: Preparation and Analysis of Cartilage Explants

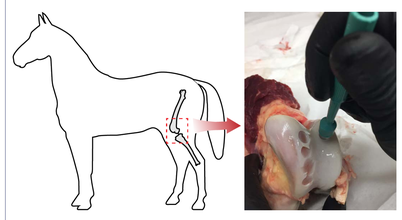

Fig 4. Cartilage explants harvested from bovine and equine stifles *post mortem*.

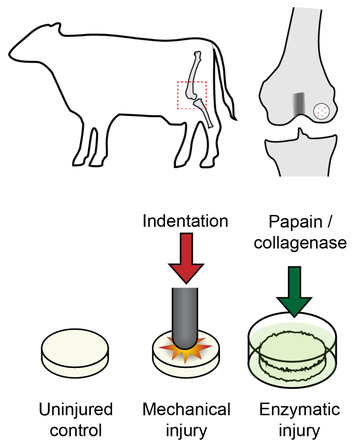

Fig 5. Explants subjected to mechanical or enzymatic injury to induce cartilage degradation

Sample Preparation

- **Mechanical injury**: Applied 50% strain with 5 mm diameter spherical indenter tip at strain rate of 500% per second. Cultured explants for 7 days after injury.
- **Enzyme digestion**: Incubated explants at room temperature for 20.5 hours in phosphate buffered saline with 1 mg/mL of collagenase or 0.5 mg/mL of papain.

Sample Analysis

- **[1]H MAS NMR**: Homogenized explants, then acquired NMR spectra on Bruker AVANCE 11.74 T spectrometer with 4 mm MAS zirconia rotor spinning at 2.5 kHz. Applied presaturation pulse sequence for water suppression.
- **Mechanical testing**: Assessed compressive viscoelastic properties of 3 mm sub-samples by stress relaxation testing in unconfined compression.
- **Biochemical testing**:
  Weighed portions of cartilage before and after 72 hours of lyophilization to determine water content. Digested lyophilized samples with papain for biochemical analysis. Quantified sulfated GAG content by dimethylene blue binding assay and total collagen by hydroxyproline assay.

Acknowledgements: Research supported by the University of California, Davis Center for Equine Health, which receives funds from the State of California Pari-Mutuel Fund and contributions from private donors. Student support was provided by the Boehringer Ingelheim Veterinary Scholars Program and the UC Davis STAR program. Thank you to Kat Morucci, Leticia Flores Mercado, Danielle Tran, Alice Wong, Dustin Leale, Chrisoula Toupadakis, and the members of VORL for your support!