HR-MAS NMR Spectroscopy Shows Regional Metabolite Variation in Bovine Articular Cartilage

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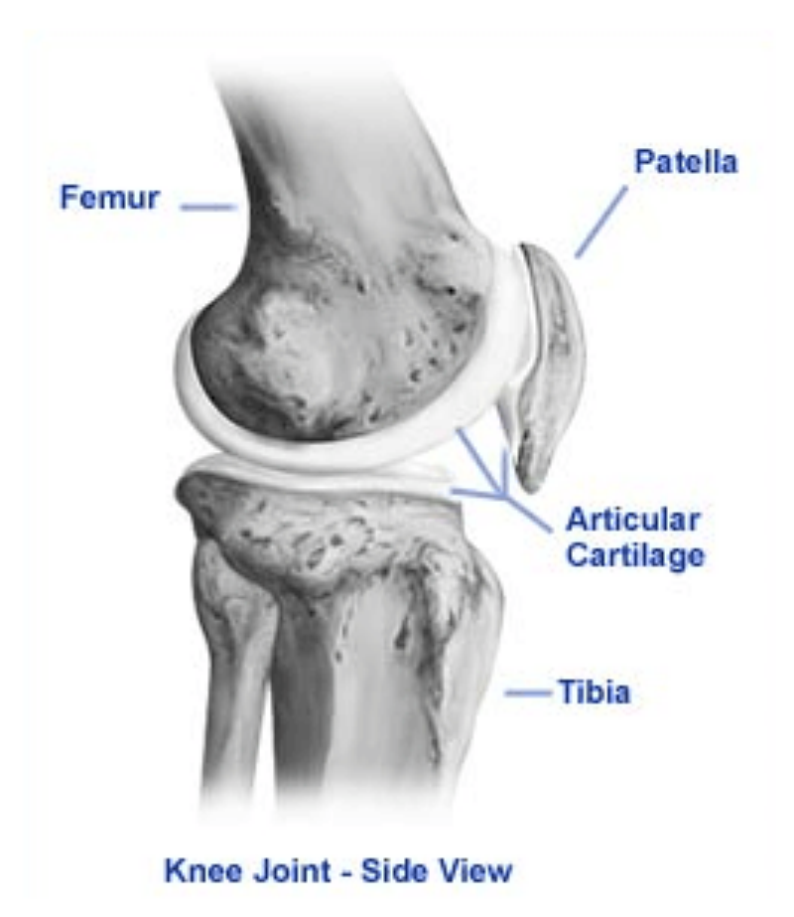
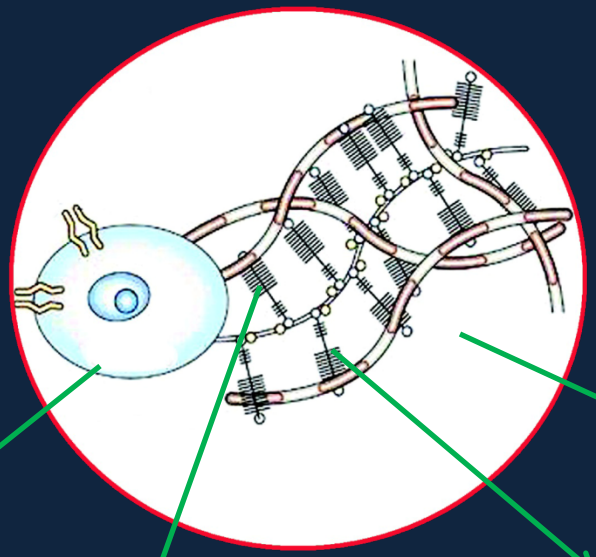
Articular Cartilage



- Thin Layer of hyaline cartilage
- Physiologically isolated
- Functions as a friction free shock absorber.
- Load plays an important role



Chondrocytes (~1%)

Proteoglycans (~15%)

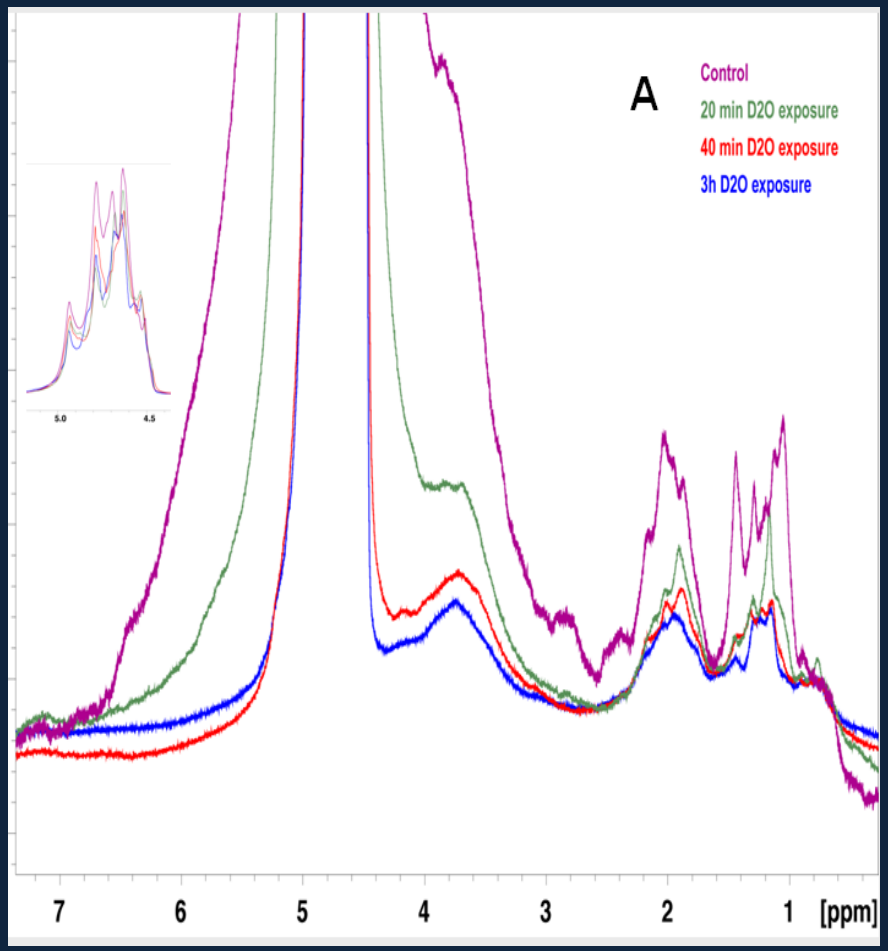
Collagen (~15%)

Water (~70%)

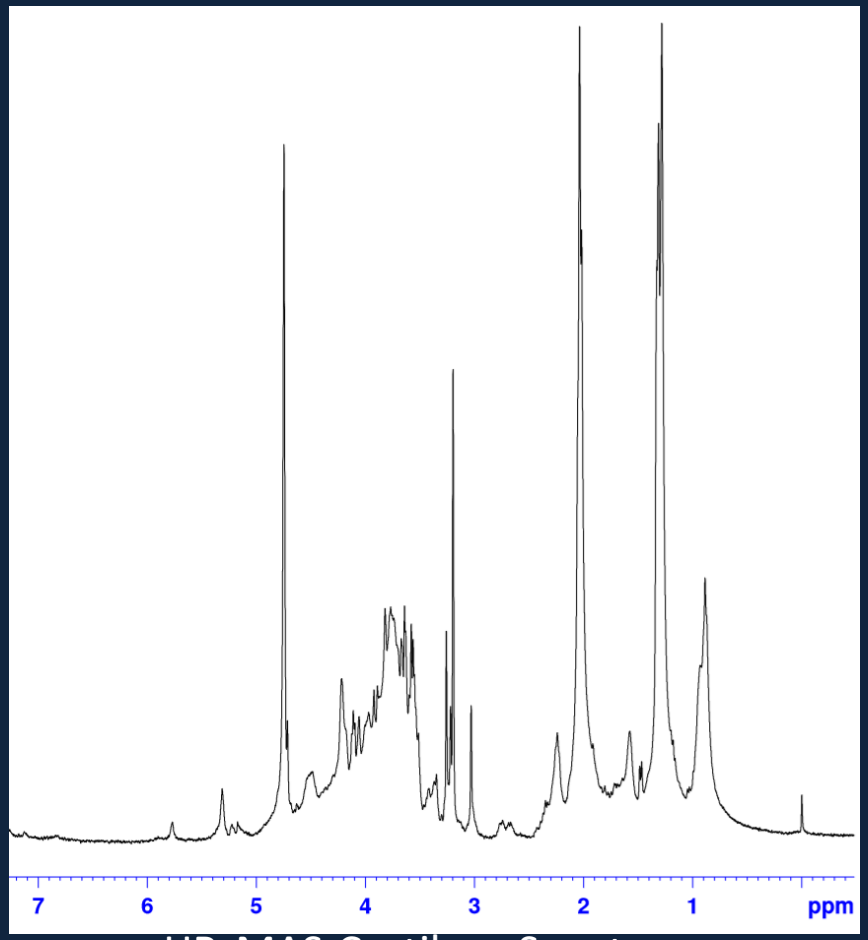
ECM

HR-MAS NMR Spectroscopy

- Solution state NMR is not suited for solids
  - Exhibit broad NMR signals due to anisotropic interactions (dipolar coupling, chemical shift anisotropy (CSA), and quadrupolar interactions)
- all anisotropic interactions in solids have a orientation component that scales as $(3\cos^2\theta-1)$.
- sample is spun at the "magic angle" ($\theta = 54.7$ degree) w.r.t external magnetic field to minimise interactions
- Hybrid between solid state and classical solution state NMR



MAS Cartilage Spectrum



HR-MAS Cartilage Spectrum

Principal Component Analysis

- Most commonly used metabolomic analytical method
  - linear combination of original data describing the variation in the samples
- Dimension reduction technique
  - defines relationship within samples (Scores plots)
  - defines relationship between variables (loading Plots)

Research Article

NMR IN BIOMEDICINE

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High-resolution magic angle spinning NMR spectroscopy of human osteoarthritic cartilage

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technical notes Journal of proteome research

Longitudinal Profiling of Articular Cartilage Degradation in Osteoarthritis by High-Resolution Magic Angle Spinning ^{1}H NMR Spectroscopy: Experimental Study in the Meniscectomized Guinea Pig Model

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AIM

Determine differences in metabolic profile between load bearing and non-load bearing region of the articular cartilage

Cartilage Preparation

- Bovine knee joints acquired from local abattoir
- ~ 15 mg of tissue removed from the desired locations.
- Tissue placed in 4mm HR-MAS insert containing D_2O-PBS (20µL, pH 7.4)
- Total of 32 samples from four joints

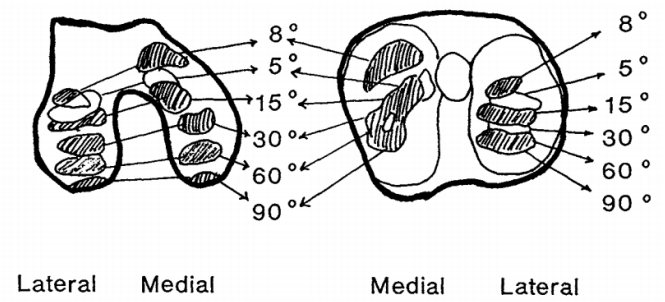
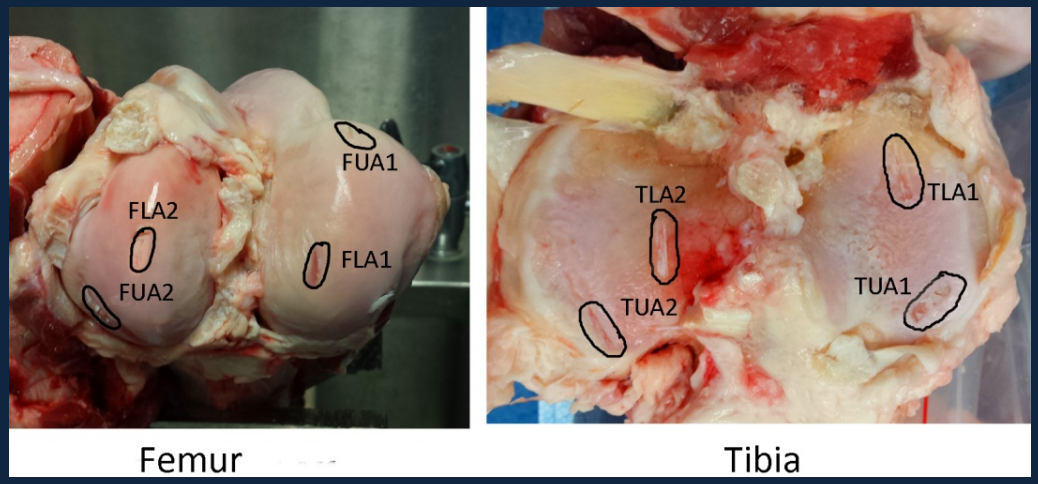


FIGURE 20.9 Tibio-femoral contact area as a function of knee flexion angle. *Source:* Iseki F, Tomatsu T. 1976. The biomechanics of the knee joint with special reference to the contact area. Keio J Med 25:37. With permission.

(Joint-Articulating Surface Motion, Kenton R. Kaufman, Kai-Nan An in Biomedical Engineering Fundamentals, CRC Press 2006)

~15 mg of tissue



Regions measured from one joint.

Femur	
FLA1	loaded region 1
FLA2	loaded region 2
FUA1	unloaded region 1
FUA2	unloaded region 2

Tibia	
TLA1	loaded region 1
TLA2	loaded region 2
TUA1	unloaded region 1
TUA2	unloaded region 2

HR-MAS Spectra

Acquisition

- 9.4T Bruker Avance III spectrometer equipped with 4mm HR-MAS probe operating at 400MHz for ^{1}H, D_2O for field frequency lock
- Sample spun at 4KHz;
- 1D-NOESY pulse sequence with water pre-saturation to minimize broad base line components arising from macro molecules
  - Parameters : 32k data points, spectral width 6kHz, total scans 512, acquisition time 2.5s, relaxation delay 2s: total measurement time 40 min.

HR-MAS Spectra

Processing

- Baseline corrected using polynomial function in Topspin.
- Referenced to strong (*N*-acetyl group) signal at 2.04 ppm.
- Spectra overlaid to check for consistency of baseline and peak alignment using Amix.
- Spectra divided into ‘buckets’ of 0.04 ppm
- Region between 4.7 ppm and 5.2 ppm (residual water signal) excluded.

Multivariate Analysis

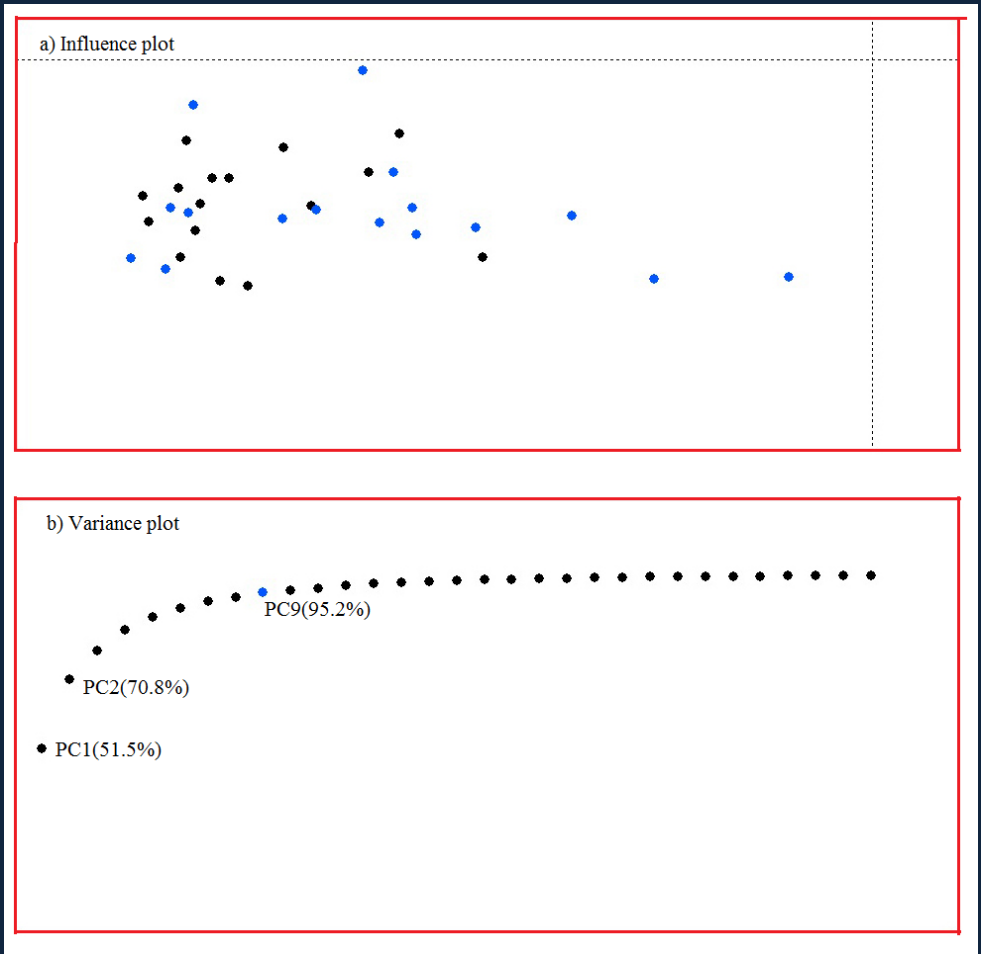
- An unsupervised Principal component analysis was performed using with Pareto scaling applied to the data (AMIX software, Bruker).
- The confidence level was set at 95%

- Integral values of peaks defined by the first three PCs tabulated
- Two-tailed, paired sample Students t test (significance level $\alpha = 0.05$)

Results and Discussion

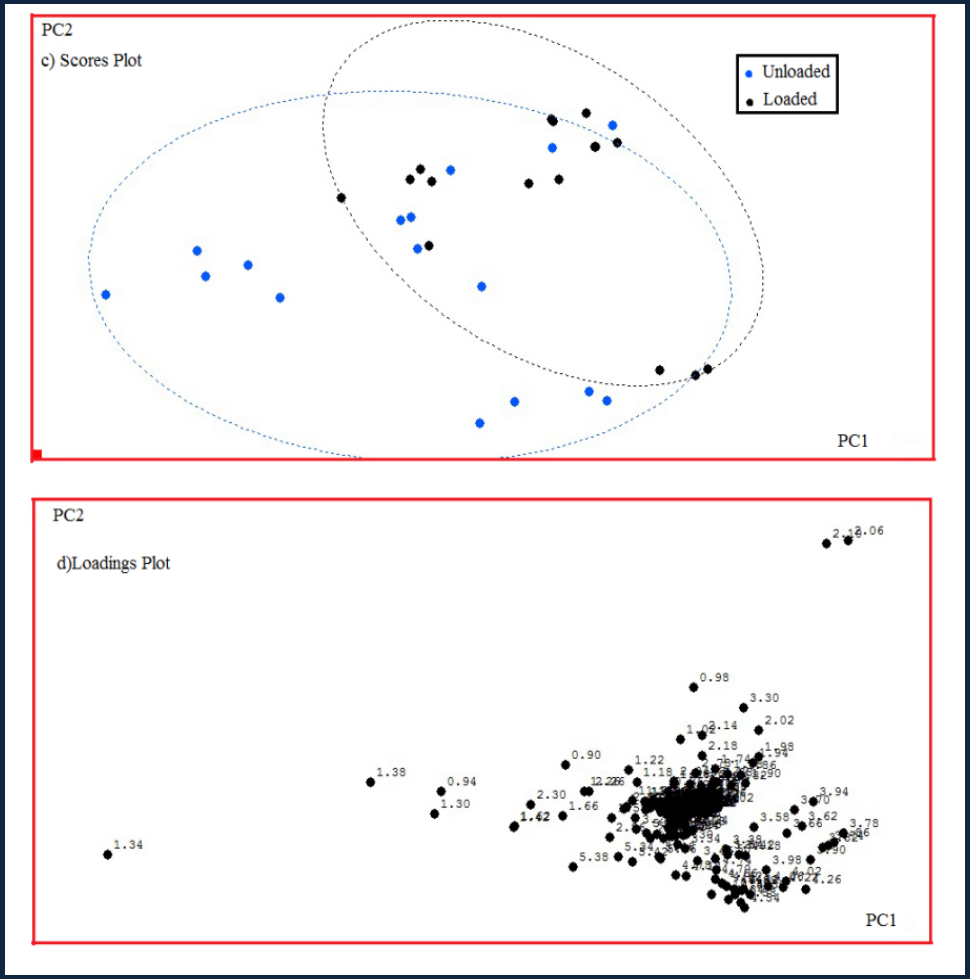
(a) The influence plot: shows that there are no samples falling beyond the projected space of 95% CI

(b) The variance plot shows the percentage of contribution to the variance by each PC.



(c) scores plot : shows the relationship between samples with Hotellings test 80% CI. The unloaded sample clustering shows a wider distribution compared to the loaded samples.

(d) loadings plot: the buckets responsible for the variance PC1 and PC2 are distributed orthogonally.

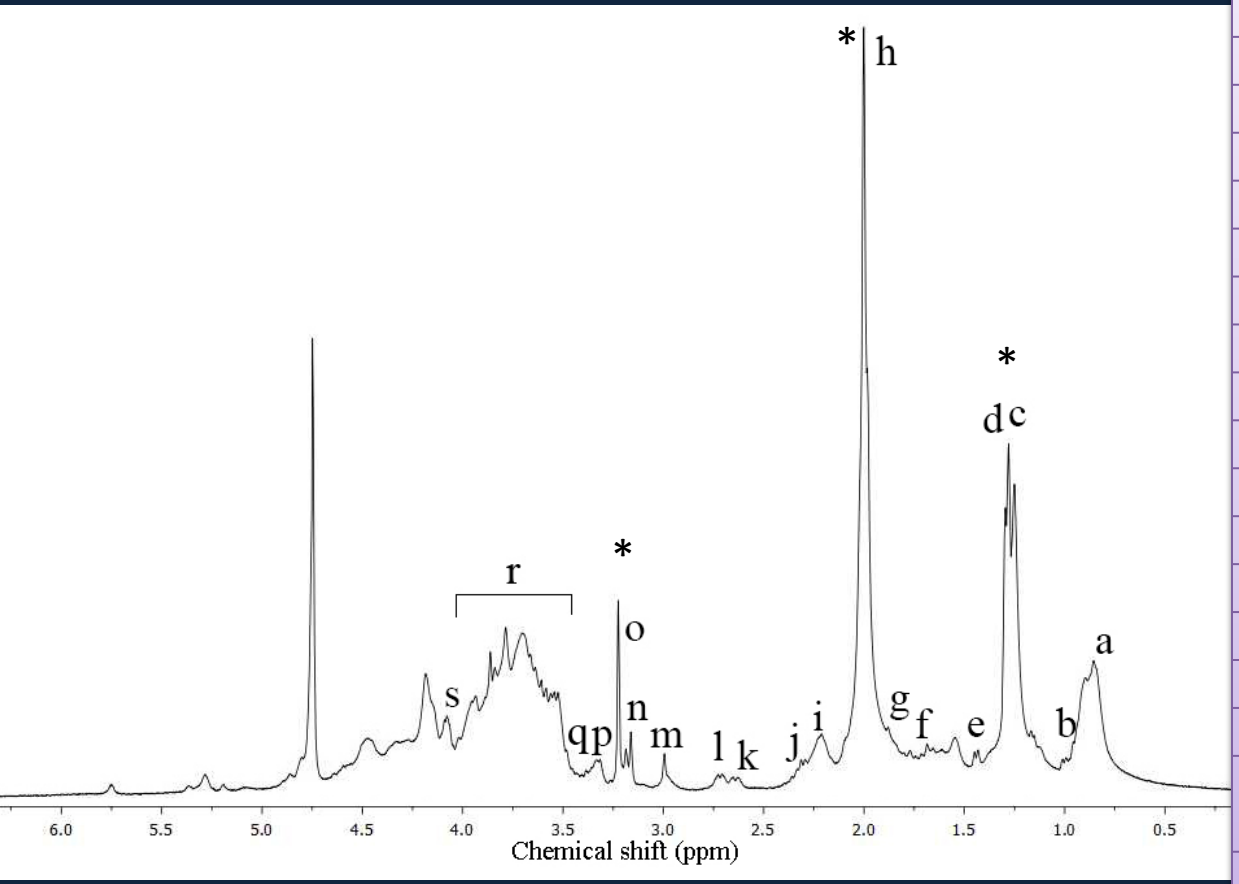


Metabolite Identification

Identifying buckets associated with principle components

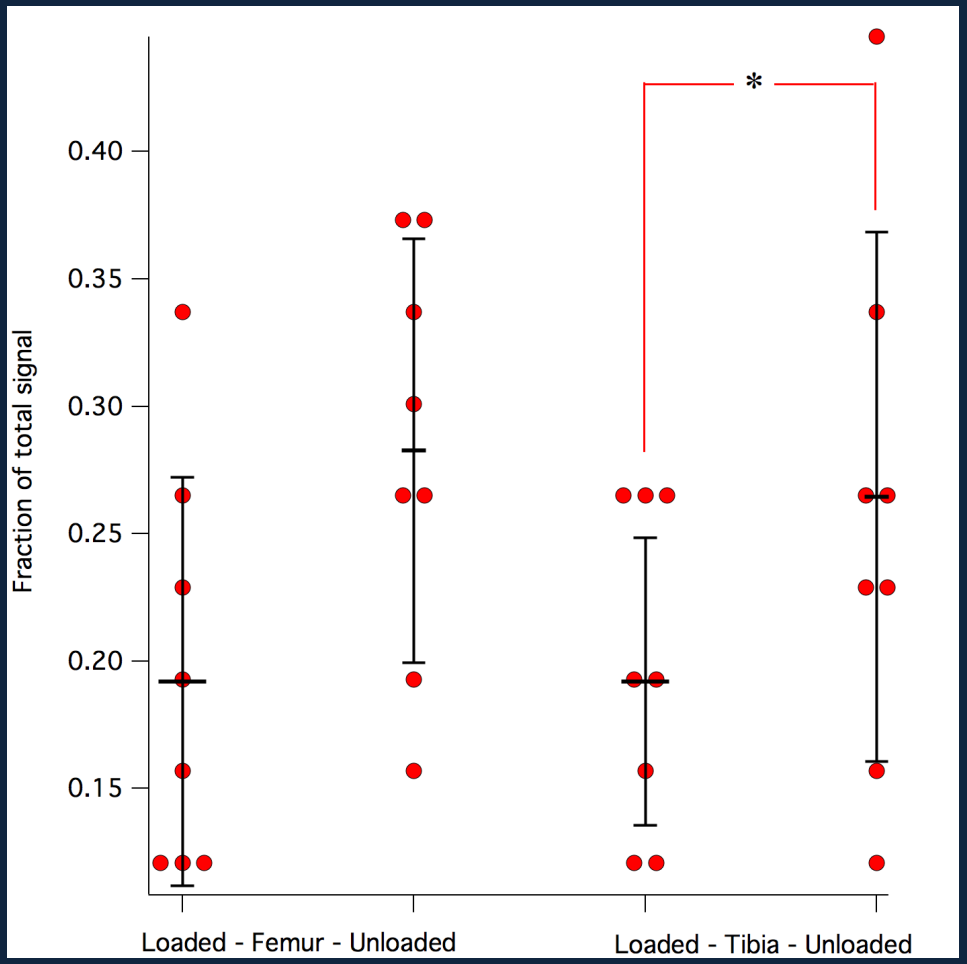
- AMIX SBASE metabolite database
- Previously published literature
- Human Metabolome Database

Representative 1D-NOESY spectra with corresponding labeled metabolite peaks



	Metabolites
a	CH_3 - amino acids (collagen) & lipids
b	Valine
c-d	Lactate, Threonine
e	Alanine
f	Isoleucine
g	Lysine
h	*N*-acetyl group
i	Glutamine
j	Glutamate
k	Proline
l	Hydroxyproline
m	Lysine
n	Choline
o	Phosphocholine
p	Hydroxyproline
q	Glycine
r	Resonances 3.5-4.0 attributed to carbohydrates
s	Lactate methyne

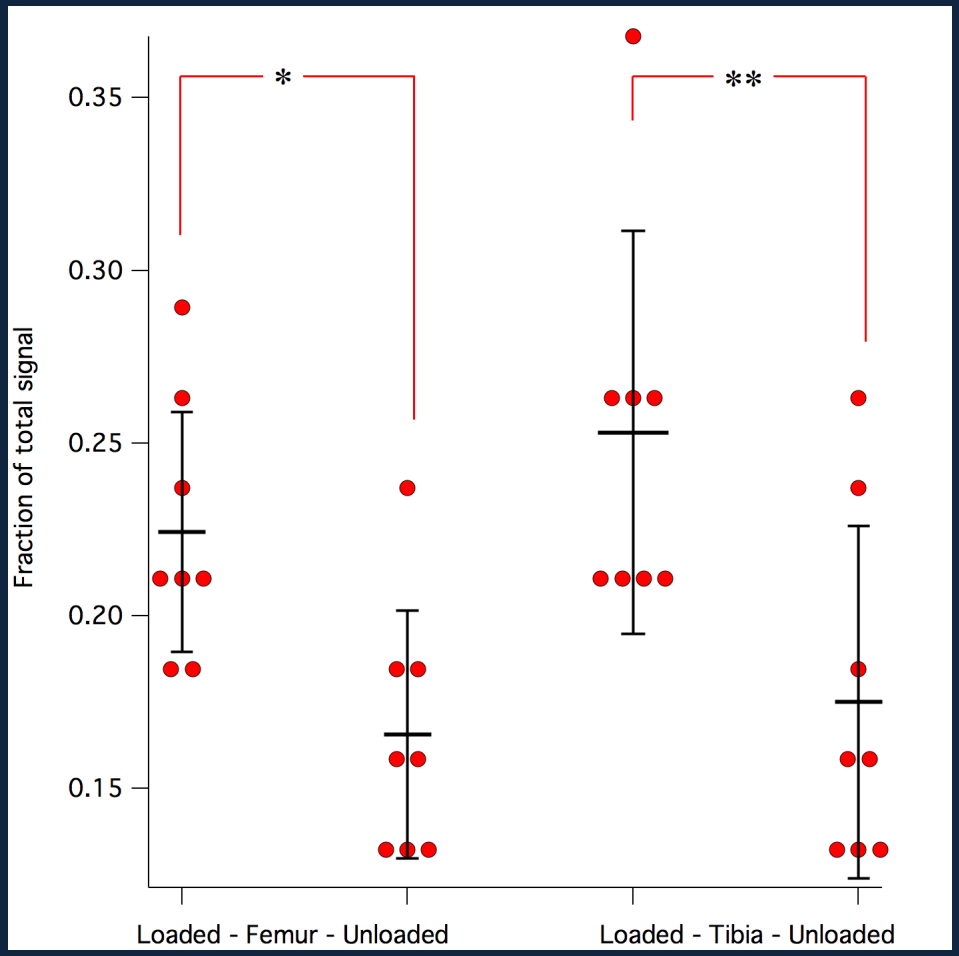
Lactate/threonine



Tibia loaded *cf* unloaded

**p = 0.003.

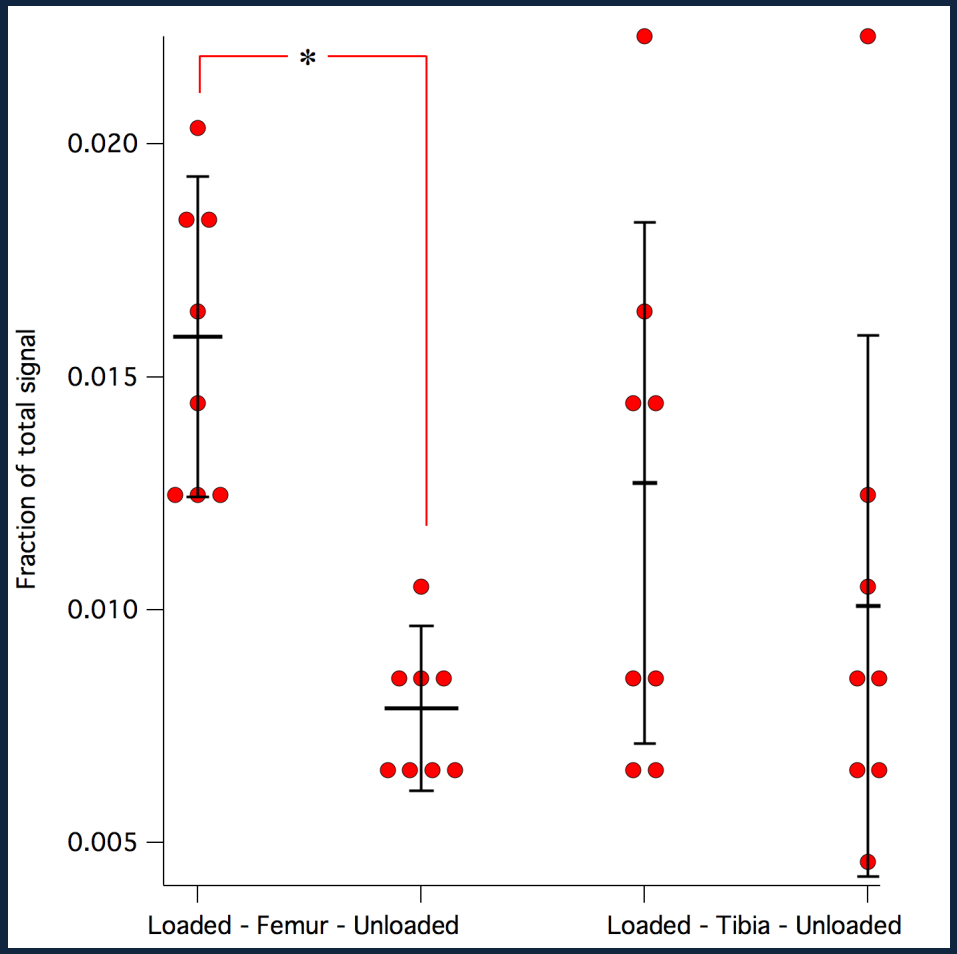
N-Acetyl group



N-acetyl group:

Femur and tibia loaded *cf* unloaded
*p = 0.001, **p = 0.04.

Phosphocholine



Femur loaded *cf* unloaded
*p = 0.0025.

Conclusion

HR-MAS NMR spectroscopy can detect significant differences between the metabolite profiles of load bearing and non-load bearing regions of healthy bovine articular cartilage

Thank You