HR-MAS Spectral Analysis of Osteoarthritic Cartilage

S. M. SIDDIQUI[1,2], R. IMAN[2], J. WOOTEN[2], J. KURHANEWICZ[2], M. RIES[3], AND X. LI[2]

[1]UNIVERSITY OF CALIFORNIA, BERKELEY, BERKELEY, CA, UNITED STATES, [2]RADIOLOGY, UNIVERSITY OF CALIFORNIA, SAN FRANCISCO, SAN FRANCISCO, CA, UNITED STATES, [3]ORTHOPAEDIC SURGERY, UNIVERSITY OF CALIFORNIA, SAN FRANCISCO, SAN FRANCISCO, CA, UNITED STATES

INTRODUCTION

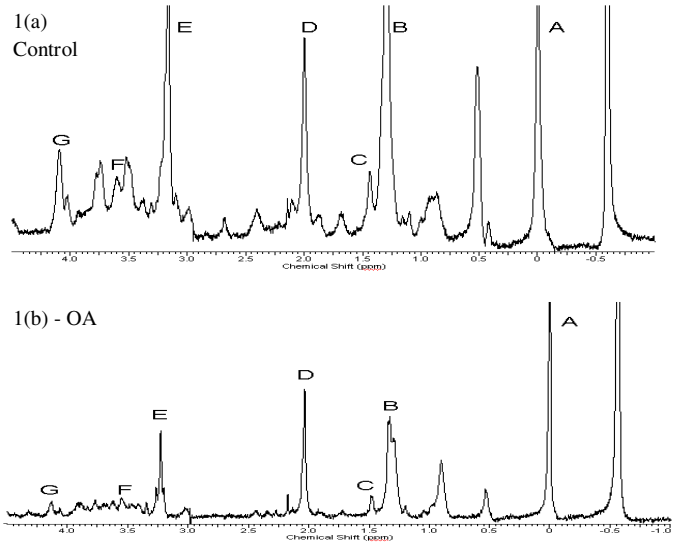
Osteoarthritis (OA) is a multifactorial disease that is characterized primarily by the progressive loss of articular cartilage. The progression of the disease is marked by the loss of collagen and proteoglycan (PG) content of the cartilage. Current radiological imaging techniques are limited to observing morphological changes that occur at relatively late stages of the disease [1]. However, the biochemical composition of cartilage changes even in early stages of the disease. With the development of high-resolution magic angle spinning (HR-MAS) NMR spectroscopy, metabolites of intact tissues can be analyzed non-destructively at high resolutions comparable to the spectra of tissue extract solutions. A few previous studies have used HRMAS spectroscopy to identify the spectral markers in enzyme-degraded bovine cartilage [1,5]. The goal of this study is to determine whether there are any significant, detectable changes in the spectra of healthy and osteoarthritic human cartilage using HRMAS spectroscopy.

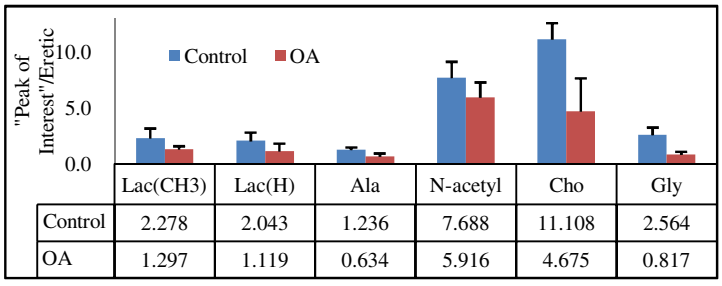
METHODS

Nine OA cartilage samples were harvested from 6 patients who underwent Total Knee Arthroplasty (TKA) surgeries and 5 healthy (control) samples were extracted from NDRI cadavers using 3.5mm biopsy punches from the femoral condyle and tibia. The samples were flash frozen at -80^{O}C for storage. They were scanned in a 500 MHz Varian INOVA spectrometer in a 30 µL zirconium rotor. The reference used was 0.75 mass % 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid (TSP in D_2O). The 1D HR-MAS NMR spectra were acquired at temperature =1^{O}C, spin rate = 2,250 Hz, spectral width = 20,000 Hz, 90^{O} pulse width using two sequences: (1) eretic and (2) Carr-Purcell-Meiboom-Gill (CPMG) sequence with a 144 ms acquisition time to suppress macromolecules with broad spectral peaks. The total acquisition time was 35:38 minutes. The data was processed using ACD Labs 1D NMR processer (ver. 8.0). The 1D FID's were apodized with an exponential function, Fourier transformed, phase corrected, baseline corrected, and referenced to TSP at 0 ppm. The integrals of several peaks were calculated (Figure 1). The ratios of the particular markers (Figure 2) to eretic were compared between healthy and osteoarthritic cartilage. A two-tailed T-Test was performed to determine whether the two groups had statistically significant differences.

RESULTS

Figure 1 shows two spectra representative of healthy (control) cartilage (1a) and osteoarthritic cartilage (1b). Figure 2 shows the mean and standard deviations of several metabolites. These values are ratios of metabolites to eretic, scaled according to mass. All the ratios in figure 2 were calculated from the data obtained from the CPMG sequence. From figure 2, it can be seen that the ratios of all the metabolites studied increase in OA samples compared to the controls.





	Lac(CH3)	Lac(H)	Ala	N-acetyl	Cho	Gly
Control	2.278	2.043	1.236	7.688	11.108	2.564
OA	1.297	1.119	0.634	5.916	4.675	0.817

Figure 1: CPMG sequence for 1(a) one control sample and 1(b) one osteoarthritic sample. Labeled peaks are as follows : A—TSP (0 ppm), B—Lactate(1.33 ppm), C—Alanine(1.47 ppm), D—N-acetyl (2.03 ppm), E—Choline (3.22 ppm), F—Glycine(3.55 ppm), G—Lactate (4.12 ppm). The N-acetyl peak is a marker for PG [2], found in Keratan Sulfate (KS) and Chondroitin Sulfate (CS), constituents of PG. Alanine and Glycine are amino acids that constitute about 44% of collagen fibers [2].

Figure 2: Mean ratios of all metabolites was lower in OA samples compared to the control samples . The changes in Ala, Cho, and Gly were statistically significant.

DISCUSSIONS

From figure 1 we can qualitatively see that the spectrum of osteoarthritic cartilage (1b) is very different than that of healthy cartilage (1a). The OA sample has less broad peaks, especially at the 3.0 to 4.0 region (CH_2 resonances of CS). This is because as the cartilage degenerates, the large macromolecules break down into smaller, more mobile components, improving the spectrum's resolution. The decrease in the amount of all metabolites (labeled A-G) can also be observed.

The N-acetyl decreases in OA compared to control. As the amount of N-acetyl is directly related to the amount of PG, this result agrees with previous works that have shown that the PG content decreases as OA progresses [1, 4]. However, more samples will be needed to determine whether these differences are significant. Choline, a component of cell membranes, decreases significantly from control to OA patients [5]. As there are no other significant sources of choline in cartilage, this suggests that the cellularity of osteoarthritic cartilage is very different than healthy cartilage. Future work can focus on correlating choline content with cell apoptosis assays or histology of osteoarthritic cartilage.

The results also show that Gly and Ala also decrease in osteoarthritic cartilage. This suggests that collagen is being depleted from the cartilage as OA progresses. Although a T-test suggests these results are statistically significant, Ala is also a byproduct of the catabolism of Lactate, so further work must be done to quantify the amount of Ala from collagen compared to that from other sources [5].

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Funded by the UCSF Radiology Seed Grant and the NIH K25.