Factors Influencing Quantitative Magnetization Transfer (QMT) Parameters of Articular Cartilage

Pouria Mossahebi[1], Rajeev Chaudhary[1], Nade Sritanyaratana[1], Walter F Block[1,2], Alexey A Samsonov[3], and Richard Kijowski[3]

[1]*Biomedical Engineering, University of Wisconsin, Madison, WI, United States,* [2]*Medical Physics, University of Wisconsin, Madison, WI, United States,* [3]*Radiology, University of Wisconsin, Madison, WI, United States*

Introduction: Quantitative magnetization transfer (qMT) imaging can be used to probe macromolecular tissue composition typically non-accessible by conventional MRI techniques [1]. Cross-relaxation imaging (CRI), an efficient qMT approach, yields unique measures including the fraction of protons bound to macromolecules (*f*), the cross-relaxation (exchange) rate between water protons and bound protons (*k*), and the transverse relaxation time of macromolecular bound protons (T_2^B) [2] and has been applied for qMT parameter mapping in human patellar cartilage in vivo at 3.0T with a scan time under 30 minutes [3]. Several studies reported that qMT parameters are correlated with macromolecular content in ex-vivo cartilage samples [4, 5] and change with age and activity level in human patellar cartilage in-vivo [3]. However, the mechanisms responsible for changes in qMT parameters with cartilage degeneration remain unknown. Thus, this study was performed to investigate the factors leading to changes in qMT measures of cartilage in controlled conditions including phantom experiments and an ex-vivo bovine cartilage degradation model.

Method: *Phantom Studies*: Phantom solutions containing 0% to 25% collagen and proteoglycan (PG) were prepared by dissolving bovine Achilles tendon type 1 collagen and bovine trachea chondroitin sulfate (Sigma-Aldrich, St. Louis, MO) respectively in saline. The second set of phantom solutions containing 20% collagen were prepared in the same manner and heated at temperatures ranging between 20ºC and 100ºC for 30 minutes. This heating protocol was shown in previous studies to cause incremental increases in denaturation of type 1 collagen solution starting at threshold temperatures between 50ºC and 60ºC [6]. *Ex-Vivo Bovine Cartilage Study*: A fresh bovine patella specimen was excised from a skeletally mature cow. A transverse cut was made through the cartilage and bone separating the patellar specimen into superior and inferior halves which allowed one portion of the cartilage to be immersed in 25 mg/ml of trypsin solution and the other portion to be immersed within saline solution for 24 hours with antibiotics added to both solutions. *Data Acquisition*: All phantoms and the bovine patellar cartilage specimen before and after enzymatic degradation were placed in a container filled with saline solution and imaged on a 3.0T scanner (Discovery MR750, GE Healthcare; Waukesha, WI) using an single-channel wrist coil. For the phantom study, variable flip angle (VFA) SPGR data were acquired at excitation flip angles (FA) α=[4,6,15,25,35,50]˚ and MT-weighted SPGR data were acquired at α=15˚ for several off-resonance frequencies Δ=[2.5,4,6,8,10,12,14,18,20,22] kHz and MT saturation powers α_{MT}=[850,1300]˚ with TR/TE=37/2.1ms and 1.5×1.5×2 mm^3 voxel size. For the bovine cartilage study, VFA data were acquired at FAs α=[4,10,20,30]˚ and MT-weighted data were acquired (α=13˚, Δ(kHz)/α_{MT}(˚)=2/750,2/1550,5/750,11/1350,13/1250,13/1350,20/1550,21/950) with TR/TE=41/3.8ms and 0.5×0.5×3 mm^3 voxel size. Spatially varying flip angle (B1) map was measured by AFI [6] for subsequent flip angle corrections. *Data Processing:* The qMT processing workflow was implemented according to [8]. All processing has been done by in-house-written C and MATLAB (MathWorks, Natick, MA) software using nonlinear least squares fitting.

Results: As shown in the first row of Figure 1, there was a large incremental increase in *f* and *k* with increasing collagen concentration. At the same time, the level of *f* and *k* associated with PG was minor compared with that of collagen at all concentrations, although some minimal incremental increase was observed in both *f* and *k* with increasing PG concentration. No significant changes in T_2^B were observed with increasing collagen concentrations. The dependence of qMT parameters on denaturation temperature is shown in the second row of Figure 1. There was an incremental decrease in *f* and *k* and incremental increase in T_2^B with increasing temperature above the critical temperature threshold of 50ºC when collagen denaturation begins. As shown in Figure 2, trypsin induced PG loss in bovine cartilage resulted in no change in *f* (average slice *f* of 14% for both degraded and control cartilage for baseline and post-treatment scans) but a decrease in *k* (average slice decrease in *k* of 0.64s^{-1} for degraded cartilage and increase in *k* of 0.09 s^{-1} for control cartilage between baseline and post-treatment scans) and an increase in T_2^B (average slice increase in T_2^B of 0.71 µs for degraded cartilage and increase in T_2^B of 0.09 µs for control cartilage between baseline and post-treatment scans).

Discussion: Our study suggests that *f* is primarily influenced by the content of structurally intact collagen as *f* significantly increased with increasing collagen solution concentration and fragmentation of collagen solution due to thermal denaturation resulted in a decrease in *f*. The minimal influence of PG content on *f* is illustrated in both the phantom studies in which there was only a negligible increase in *f* with increasing PG solution concentration and the bovine cartilage study in which *f* did not change with PG loss due to trypsin degradation. Factors leading to changes in *k* and T_2^B appear to be more complex. Both thermal denaturation of collagen solution and loss of PG within trypsin degraded bovine cartilage resulted in a decrease in *k* and increase in T_2^B. Fragmentation of collagen, which decreases proton binding sites on the macromolecule, and loss of bulk water from cartilage due to decreased PG content may reduce the exchange rate between mobile and collagen-bound protons. Likewise, fragmentation of collagen due to denaturation may increase mobility of the molecules and thereby increase T_2^B. The decreased organization of the cartilage matrix due to PG loss may increase spin diffusion of collagen-bound protons, the primary mechanism defining T_2 relaxation time in the semisolid fraction [9], and thereby also increase T_2^B. However, exact mechanisms of the observed dependencies require further investigation.

Our results showing strong association of *f* with collagen content differ from those of previous studies which have shown that *f* is correlated with PG content in human cadaveric cartilage [3] and that trypsin degradation of bovine cartilage leads to a decrease in *f* [4]. However, some previous studies have used qMT fitting models which fixed T_2^B. Such constraint may cause significant bias in the measurement of other qMT parameters if significant variability of T_2^B is present The high sensitivity of T_2^B to denaturation of collagen solution and trypsin degradation of bovine cartilage noted in our study suggests that T_2^B may change significantly with cartilage degeneration, and, hence, should be estimated along with the other qMT parameters. Another potential source of discrepancy is the differences in the measurement and estimation approaches. In conclusion, our study has shown that while some qMT measures may have utility for direct assessment of collagen content (*f*), multiple factors may be responsible for changes in qMT parameters at different stages of cartilage degradation. The observed trends and unique sensitivity of qMT measures to macromolecules, however, suggest that a multivariate analysis involving all qMT parameters may be the most specific method to analyze the complex changes which occur during cartilage degeneration.

References: [1] Henkelman R. NMR Biomed, 2001. [2] Yarknykh V, NI, 2004. [3] Sritanyaratana N. ISMRM Abstract #1382, 2011. [4] Stikov, N. MRM, 2011. [5] Henkelman R. MRM, 2002. [6] Harel A. JMRI, 2008. [7] Yarnykh V. MRM, 2007. [8] Mossahebi P. et al. ISMRM 2012, 0741. [9] Edzes H. Nature, 1977.

Acknowledgements: Support for the study was provided by NIAMS U01 AR059514, NINDS R01 N5065034, and GE Healthcare.

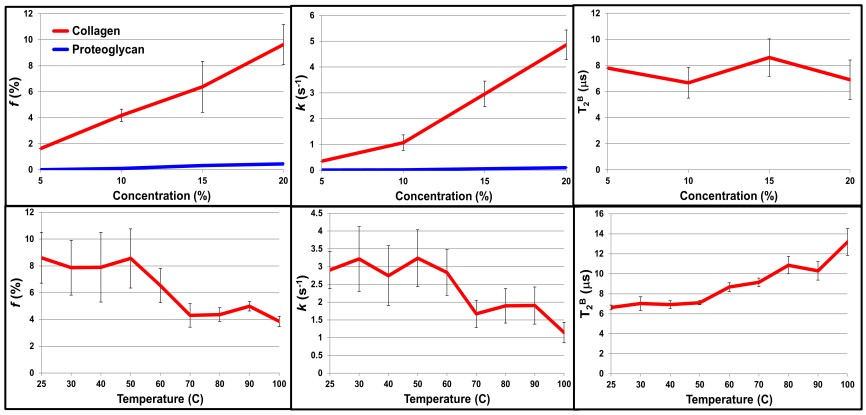

Figure 1.

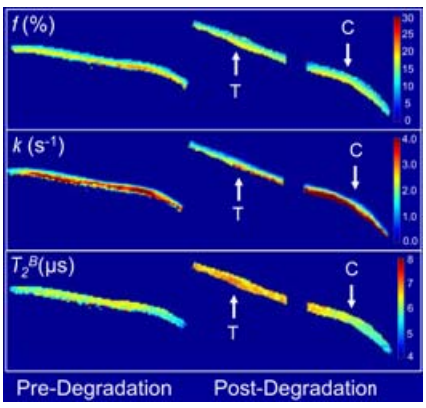

Figure 2.