OsteoArthritis and Cartilage (2003) **11,** 709–715

doi:10.1016/S1063-4584(03)00155-9

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

^{1}H magnetic resonance spectroscopy of nanomelic chicken cartilage: effect of aggrecan depletion on cartilage T_2

T. J. Mosher M.D.†*, Q. Chen Ph.D.‡ and M. B. Smith Ph.D.†

†*Penn State Center for NMR Research, Department of Radiology, The Penn State University College of Medicine, Hershey, PA 17033, USA*

‡*Laboratory of Cell and Molecular Biology, Department of Orthopedics, Brown Medical School and Rhode Island Hospital, Providence, RI 02903, USA*

Summary

Objective: To determine the effect of proteoglycan depletion on cartilage proton magnetic resonance (MR) spectroscopy T_2 using nanomelic chicken cartilage, a genetic mutant that completely lacks aggrecan.

Design: Proton MR spectroscopic T_2 measurements of normal embryonic and nanomelic femoral epiphyseal cartilage were obtained using a 96-echo pulse sequence with inter-echo delay times increased logarithmically over the TE period of 60 μs to 1.7 s. The relative intensity and distribution of cartilage T_2 components were determined by fitting signal decay curves to a multi-exponential function. The number of T_2 components in the signal decay curves was determined by the degree of freedom limited r^2 of the fit.

Results: For normal fetal chicken cartilage, 97.6±0.2% (mean±95% confidence interval) of the total signal comprises a long T_2 component (179.1±1.3 ms) with a relatively small short T_2 component (0.5±0.4 ms). The T_2 distribution for nanomelic cartilage is more heterogeneous with four components identified: two short T_2 components (0.5±0.02 and 7.3±0.6 ms), a large intermediate component (56.4±5.6 ms), and a broadly distributed long component (137.5±16.6 ms). In nanomelic cartilage there is greater heterogeneity of cartilage T_2 indicating greater variation in water proton mobility and exchange of water with the extracellular matrix.

Conclusion: Absence of aggrecan in the extracellular cartilage matrix produces greater heterogeneity in cartilage T_2, but will not increase T_2 as has been previously reported with degenerative change of the collagen matrix.

Key words: Cartilage, Magnetic resonance imaging, MRI-T_2, Proteoglycans.

Introduction

Although the primary clinical application of magnetic resonance imaging (MRI) in evaluation of articular cartilage has been identification of pathologic surface lesions, several research techniques have been proposed that are sensitive to structural and biochemical changes in the extracellular cartilage matrix occurring in early, pre-clinical stages of cartilage damage[1–3]. These techniques have the potential to characterize cartilage damage that precedes loss of articular cartilage identified with anatomic imaging. Such information would be useful in evaluation of new chondroprotective agents. Additionally these techniques provide novel information that may lead to a better understanding of cartilage physiology.

The unique sensitivity of the MRI signal to the macromolecular environment of tissue water makes it a valuable tool to study the interaction of cartilage water with the solid extracellular matrix. This interaction is integral to the functional properties of cartilage. Articular cartilage is 70–75% water with the remainder of the solid extracellular matrix primarily consisting of Type II collagen and proteoglycans. The major proteoglycan, aggrecan, binds to hyaluronic acid to form large proteoglycan aggregates, which through binding of water are essential in regulating the mechanical properties of articular cartilage. Early structural damage to cartilage causes fragmentation and ultimately loss of the proteoglycan aggregates. The sensitivity of MRI to water/proteoglycan interactions may provide a valuable non-invasive marker to study early structural changes in cartilage and lead to better understanding of osteoarthritis.

Several MRI techniques have been developed that appear sensitive and specific to changes in proteoglycan concentration. These include delayed Gd-DTPA$^{(-2)}$ enhanced MRI of cartilage (dGEMRIC)[4], T1ρ-weighted MRI[3], and sodium MRI[5]. The MRI transverse relaxation time (T_2) is a physical time constant reflecting loss of coherent transverse magnetization, which is sensitive to the macromolecular environment of tissue water. Although cartilage T_2 is sensitive to structural cartilage damage[1], an elevated T_2 is primarily correlated with decreased collagen concentration and fiber orientation[6–9], and increased cartilage water content[10,11]. In studies using enzymatic degradation of cartilage proteoglycans there was little change in T_2 with depletion of proteoglycan[5,12,13]. This is somewhat

Source of funding: TJM and QC acknowledge research grant support from the Arthritis Foundation and NIH.

*Address correspondence and reprint requests to: Timothy J. Mosher, M.D., Department of Radiology, MC H066, The Milton S. Hershey Medical Center, 500 University Drive, Hershey, PA 17033, USA. Tel: 1-717-531-4566; Fax: 1-717-531-8486; E-mail: tmosher@psu.edu

Received 18 December 2002; revision accepted 25 June 2003.

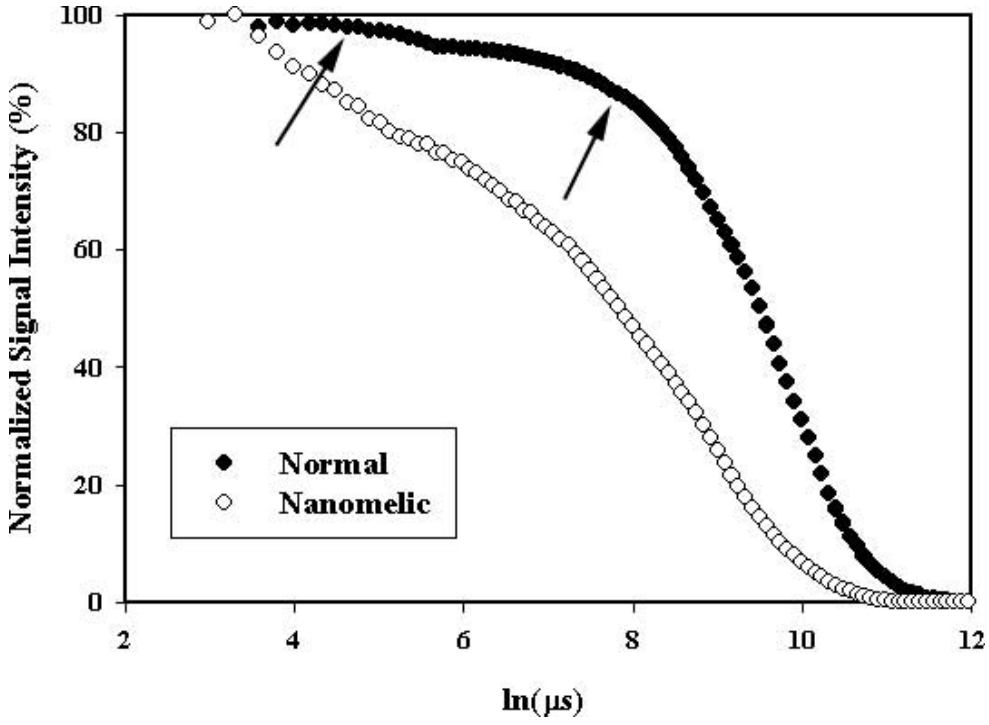

Fig. 1. Signal decay curves for normal and nanomelic embryonic chicken cartilage. Normalized signal intensity is plotted as a function of the natural logarithm of the echo time (μs). For normal cartilage two distinct asymptotic points are identified indicative of two differing T_2 populations (arrows). No resolvable asymptotic point is demonstrated in the signal decay curve of nanomelic cartilage indicative of a more heterogeneous, multi-exponential T_2 decay process.

unexpected given the fundamental role of proteoglycans in regulating tissue water mobility and content. Enzyme degradation studies are limited in that removal of proteoglycan is typically incomplete and non-specific, resulting in degradation of other protein components of the cartilage matrix such as collagen, and smaller non-aggregating proteoglycans. The purpose of this study was to evaluate the effect of proteoglycan depletion on cartilage T_2 using nanomelic chicken cartilage, a genetic mutant that does not contain aggrecan. Because, the remaining elements of the extracellular matrix are intact[14], this unique tissue provides a natural system to specifically evaluate the effect of aggrecan on cartilage T_2.

Methods

Nanomelic chicken embryos were obtained from fertilized eggs supplied through the University of Connecticut (Storrs, CT), following an 18-day incubation period. Under a dissecting microscope, distal femoral epiphyseal cartilage from each leg was harvested from normal (n=3) and nanomelic (n=3) chickens, and placed in phosphate balanced saline at room temperature.

Each of the six nanomelic and six normal cartilage specimens were independently measured with magnetic resonance (MR) spectroscopy measurements approximately 1 h after harvesting cartilage. Cartilage samples were rinsed with saline and blotted dry to remove adherent fluid. Samples were placed in a 5-mm×15-mm NMR tube, sealed, and positioned within a solenoid coil in an Oxford 1.9 T magnet interfaced to a Tecmag Taurus console (Tecmag, Houston, Texas). Signal decay curves were generated using a 96-echo pulse sequence that sampled a single point at the center of the spin–echo. The inter-echo delay was incremented logarithmically from 60 μs to 79 ms with a TR of 2 s and 512 signal averages. With these inter-echo delays, the 96 data points were acquired over a TE interval of 60 μs to 1.7 s. The T_2 measurements were performed at 25°C. The logarithmic echo train was chosen to minimize radiofrequency heating of the tissue while sampling a broad range of the T_2 decay curve. A separate experiment was performed to insure that the sample temperature did not increase by more than 0.5°C during the high radio frequency duty cycle of the experiment as heating could alter cartilage T_2 through desiccation and protein denaturation.

Using commercially available software (TableCurve, Jandel Scientific Software, San Rafael, CA), signal decay curves were fit to multi-exponential equations using a non-linear least squares fit where the number of exponential components ranged from one to five. The number of T_2 components in the signal decay curves was determined by the degree of freedom limited r^2 of the fit.

Following the T_2 measurement, water content was determined by desiccation to constant weight[15].

Results

As demonstrated in signal decay curves illustrated in Fig. 1, there are distinct differences in T_2 decay of nanomelic and normal cartilage. For all samples nanomelic cartilage demonstrated more rapid T_2 decay than normal cartilage. When signal intensity is plotted as a function of the natural logarithm of TE, two asymptotic points are

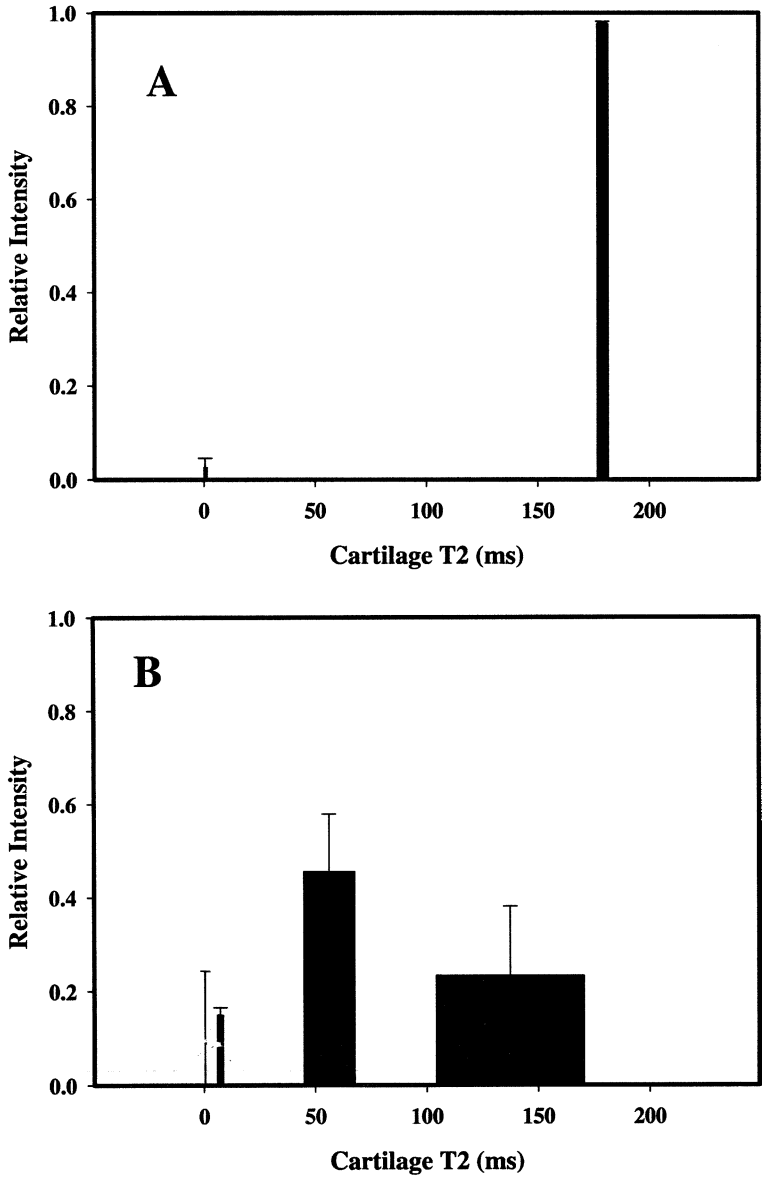

Fig. 2. Relative intensity and distribution of cartilage T_2 components for normal (A) and nanomelic cartilage (B). Vertical error bars represent the superior limit of the 95% confidence interval (CI) for determination of relative signal intensity for the T_2 component. The width of the bars corresponds to the 95% CI for the distribution of T_2 value. Two distinct T_2 components are identified in normal cartilage. The four T_2 components observed in nanomelic cartilage demonstrate a substantially broader distribution of T_2 decay.

identified in the T_2 decay curves of normal cartilage, indicative of two distinguishable T_2 components with differing T_2 times. No resolvable asymptotic point is observed in the nanomelic chicken cartilage indicating a broad distribution of T_2 components. The relative intensity and distribution of cartilage T_2 components for normal and nanomelic cartilage are presented in Fig. 2. For normal fetal chicken cartilage, 97.6±0.2% (mean and 95% confidence interval) of the total signal comprises a long T_2 component (179.1±1.3 ms), with a relatively small short T_2 component (0.5±0.4 ms). The T_2 distribution for nanomelic cartilage is more heterogeneous with four components identified: two short T_2 components (0.5±0.02 and 7.3±0.6 ms), a large intermediate component (56.4±5.6 ms), and a smaller broadly distributed long T_2 component (137.5±16.6 ms).

Water content was similar for normal and nanomelic cartilage (86.5 vs 85.0%, respectively).

Discussion

AGGRECAN AND NANOMELIC CARTILAGE

In articular cartilage the main proteoglycan is aggrecan, with smaller concentrations of leucine-rich proteoglycans associated with the collagen fibril matrix[16]. Aggrecan is a large proteoglycan containing a core protein with glycosylated side chains, primarily keratan sulfate, and chondroitin sulfate. The core protein contains three globular domains (G_1, G_2, and G_3), and two extended domains where side chains are attached. Once secreted from the

chondrocyte, the G_1 domain of aggrecan binds to hyaluronan to form large proteoglycan aggregates. Within vertebrate species, the compositions of globular domains of aggrecan are highly conserved, with greater variation observed in the extended regions of the protein. A single gene encodes the core protein of aggrecan. In nanomelic cartilage a single point mutation of this gene leads to a shortened core protein that is neither secreted from the chondrocyte nor processed into the aggrecan proteoglycan[17]. As a result no aggrecan is present in the cartilage of the nanomelic chicken embryo.

Nanomelia is a lethal mutation characterized by shortened and malformed limbs[18]. Histologically nanomelic chondrocytes are normal, however, due to a reduction in the extracellular space, the relative chondrocyte volume is greater than that of normal cartilage. There is also a proportionate increase in density of the Type II collagen fibrils[19]. Although nanomelic cartilage lacks aggrecan, it does contain the other small non-aggregating proteoglycans[20].

EFFECT OF AGGRECAN DEPLETION ON CARTILAGE T_2

Several studies using either enzymatically degraded or osteoarthritic cartilage specimens found that cartilage T_2 is insensitive to change in proteoglycan concentration. In a study comparing the effect of proteoglycan depletion on sodium and proton (^{1}H) MRI, Borthakur and colleagues found cartilage ^{1}H T_2 did not correlate with the degree of proteoglycan loss[5]. A similar lack of correlation between T_2 and proteoglycan content was observed in proteoglycan depleted cartilage treated with trypsin[21]. Regatte *et al.* found no significant change in cartilage T_2 after enzymatic degradation of cartilage proteoglycans in the uncompressed state, however, if proteoglycan depleted cartilage was placed under compression there was a statistically significant decrease in T_2 compared to normal cartilage[12]. This is likely due to greater extrusion of water from the more compressible aggrecan depleted cartilage. In a study evaluating the effect of proteoglycan depletion on cartilage ^{1}H relaxation properties, Toffanin and coworkers found extraction of proteoglycan and calcium ions from cartilage plugs had little effect on cartilage T_2[13].

Additional studies have shown perturbation of cartilage collagen rather than proteoglycan is the key determinant of cartilage T_2. In a study comparing loaded and unloaded porcine cartilage from the humerus, Fragonas and coworkers found that cartilage T_2 was strongly influenced by collagen content, but not by proteoglycans[6]. In evaluation of human cartilage specimens obtained from osteoarthritic knees, Mylnarik *et al.* found no significant difference in T_2 between normal regions and sites with reduced proteoglycans[7]. However, elevated T_2 was longer in sites of surface fibrillation suggesting damage of the collagen network was the cause of increased cartilage T_2. In an MRI study evaluating the effect of skeletal maturation on T_2 mapping of rat patellar cartilage, Watrin and coworkers found collagen content was a greater determinant of MRI signal intensity, than was proteoglycan concentration[8]. Nieminen and coworkers found no significant change in cartilage T_2 following treatment with chondroitinase ABC to remove proteoglycans, however, there was a significant increase in T_2 following treatment with collagenase[9]. Several investigators have demonstrated a strong inverse correlation between collagen fiber anisotropy measured with polarized light microscopy and cartilage T_2[22–24]. These reports have led to the conclusion that cartilage T_2 is primarily a measure of collagen network integrity, and is relatively insensitive to loss of proteoglycan.

A limitation of studies using enzymatic depletion of proteoglycans is incomplete and non-specific removal of aggrecan from the extracellular matrix. The intact collagen matrix provides steric hindrance to large enzymatic molecules and prevents their diffusion into deeper layers of cartilage. Also, proteases such as trypsin are non-specific, and will also degrade the collagen matrix. Similar limitations apply to the use of osteoarthritic samples, which have depletion, and fragmentation of aggrecan as well as alteration in structure and composition of the collagen network. The non-specific manipulation of the extracellular matrix makes it difficult to identify a specific effect of aggrecan on cartilage T_2. This limitation is overcome with the use of nanomelic cartilage, in which there is complete and specific absence of aggrecan from the extracellular matrix.

Because nanomelia is a lethal mutation with death occurring near 18 days gestation, embryonic rather than mature cartilage must be studied. This has important ramifications when comparing results to other studies using cartilage obtained from skeletally mature animals. First, the water content of both normal (86.5%) and nanomelic (85.0%) embryonic chick cartilage is greater than the 70–75% typically observed in mature cartilage[16]. Because cartilage T_2 increases with increasing water content[11], it is anticipated that longer T_2 values will be observed in embryonic cartilage. Also, studies evaluating the effect of developmental age on cartilage T_2 have shown age related changes in both proteoglycan content, and organization of the collagen network that produce longer bulk cartilage T_2 in cartilage of skeletally immature animals[8,25]. Our value of 179.1±1.3 ms for the bulk T_2 of normal embryonic chick cartilage is consistent with results of these prior studies. Second, the embryonic specimens contain a mixture of epiphyseal and articular cartilage. Although there are differences in composition of the extracellular matrix of these tissues, Dardzinski *et al.* have demonstrated that T_2 mapping of epiphyseal cartilage is very similar to that previously observed in adult articular cartilage[26]. Intrinsic differences in the extracellular matrix of immature epiphyseal and articular cartilage preclude direct extrapolation of these results to mature articular cartilage.

In comparing the T_2 of aggrecan deficient nanomelic cartilage with normal embryonic cartilage two observations are made. First, T_2 values for nanomelic cartilage are statistically significantly less than that observed for major T_2 component of normal embryonic cartilage. Second, the variation in T_2 values observed in nanomelic cartilage is substantially greater than that observed in normal embryonic cartilage. These observations provide insight on the role of aggrecan in organizing cartilage water, and the effect of aggrecan on cartilage T_2.

For nanomelic cartilage the long T_2 components had T_2 values of 56.4±5.6 and 137.5±16.6 ms; less than the value of 179.1±1.3 ms observed in age matched normal cartilage. Two factors may contribute to this observation. First, the water content of nanomelic cartilage was 1.5% less than that observed in normal cartilage. In prior studies on mature cartilage, Lusse *et al.* observed a linear dependence of T_2 on articular cartilage with T_2 increasing approximately 2 ms for a 1% increase in water content[11]. Thus it seems unlikely that water content alone would account for shorter T_2 of nanomelic cartilage. More likely the shorter T_2 value reflects the greater concentration of Type II collagen in the extracellular matrix of nanomelic cartilage[19]. Prior studies have demonstrated an inverse

correlation of T_2 and collagen concentration in tissue[9,27], and collagen solutions[28]. The shorter T_2 of the collagen rich nanomelic cartilage is consistent with previous magnetization transfer[29] and T_2 dispersion studies[30] of collagen solutions that demonstrate significant chemical exchange between the hydration layer and bulk water, providing an efficient mechanism for T_2 relaxation. Additionally, since a balance of aggrecan and non-aggregating proteoglycans influences collagen fibrillogenesis[31], the lack of aggrecan production in nanomelia will likely alter structural organization of the Type II collagen matrix differently from that observed in normal cartilage.

Caution must be used in extrapolating this result to degenerative changes in human cartilage. The high density of collagen fibrils in nanomelic cartilage is unlikely to be representative of collagen content in osteoarthritic cartilage, where in later stages of damage there is a loss of collagen as well as proteoglycan. Furthermore the multi-exponential nature of the T_2 decay curve for nanomelic cartilage decreases accuracy in T_2 determination. The ability to resolve different T_2 populations using discrete exponential analysis is dependent on SNR and number of acquired data points[32]. Prior studies on analysis of relaxation properties in trabecular bone, have demonstrated limitations in interpretation of multicomponent relaxation data analyzed with discrete exponential analysis[33]. Given these limitations, the T_2 values obtained from nanomelic cartilage are susceptible to error and should not be interpreted in terms of discrete components of water protons, but rather as indicative of greater variability in T_2 decay. These qualitative differences observed in nanomelic cartilage can provide information on the role of aggrecan in organization of cartilage water and influence on cartilage T_2.

The most striking difference between normal and nanomelic cartilage is the distribution of T_2 values. In normal cartilage, two distinct components are resolved in the T_2 decay curves. For the longer T_2 component there is little variation in T_2 values as indicated by the narrow distribution of T_2 values. Much greater heterogeneity in T_2 is observed in nanomelic cartilage where four components are resolved in the T_2 decay curves. Although four T_2 components are resolved, given the shape of the signal decay curves it is likely that far more components are present that are below the limits of resolution for the experimental technique. Our results suggest the major effect following loss of aggrecan from the cartilage matrix is not a change in magnitude of T_2 but rather increased variation in cartilage T_2. Interestingly, in an early report by Gahunia *et al.* correlating T_2 with histologic damage in an animal model, it was observed that there was a trend toward increased variability in T_2 with increasing severity of OA[34]. Our results suggest this increased variation in T_2 is a result of aggrecan depletion.

Unlike most tissues that demonstrate multi-exponential T_2 behavior, normal cartilage displays a relatively narrow distribution of T_2 values. In prior studies of excised bovine cartilage plugs Henkleman and coworkers demonstrated a bimodal histogram of cartilage T_2 centered near 20 and 55 ms[32]. Lusse *et al.* identified three T_2 components in cartilage[10]. A short T_2 component of approximately 1 ms was assigned to water inside collagen fibrils, and a minor long T_2 component ranging from 150 to 300 ms attributed to free water adsorbed on the cartilage surface. It is unlikely that these components contribute significantly to the cartilage MRI signal observed in clinical imaging or T_2 mapping where TE values are generally greater than 10 ms. The major T_2 component had values in the range of 5–150 ms and was attributed to water within the proteoglycan matrix[10]. As these are bulk T_2 measurements they do not account for the additional spatial variation in cartilage T_2 that occurs as a function of depth from the articular surface[35]. In clinical T_2 mapping studies, which do not detect signal from components with T_2 values <1 ms, T_2 decay curves within a small region of cartilage fit well to a single exponential equation[35]. When accounting for this inherent spatial variation, the uniformity of cartilage T_2 suggests normal cartilage water is highly organized, with little variation in water proton mobility and exchange with the macromolecular environment. As indicated by the greater heterogeneity of cartilage T_2 in nanomelic cartilage, this organization is lost when aggrecan is no longer present. This supports the theory that hydrophilic aggrecan serves to organize tissue water[36].

CLINICAL IMPLICATIONS

Prior studies have indicated that cartilage T_2 is sensitive to early forms of cartilage degeneration[1]. However, it is likely that degenerative changes of specific components of the extracellular matrix affect cartilage T_2 differently. As indicated by prior *ex vivo* studies, degradation of the collagen matrix, or an increase in cartilage water content will elevate T_2[7,9,11]. Loss of anisotropy in the cartilage matrix, which has been shown to occur very early in the degenerative process[37,38] appears to be highly correlated with elevated cartilage T_2[24]. Our results indicate that isolated depletion of aggrecan will not increase cartilage T_2 values, however, it may lead to greater variability in T_2. Different methods of data acquisition or analysis such as T_2 histograms, or cartilage T_2 texture mapping may provide a means for measuring this increased randomness of cartilage T_2. Alternatively cartilage T_2 mapping could be combined with a technique sensitive to proteoglycan loss such as dGEMRIC[4] to provide complementary diagnostic information[7].

Conclusion

In nanomelic cartilage there is greater heterogeneity of cartilage T_2 indicating greater variation in the interaction of water with other components of the extracellular matrix. We hypothesize that in the absence of aggrecan, extracellular Type II collagen produces heterogeneous T_2 shortening of cartilage water confirming results of prior solution studies. These results suggest loss of proteoglycan, which occurs with advanced osteoarthritis, may lead to greater heterogeneity in cartilage T_2, but unlike degenerative change of the collagen matrix, will not increase T_2.

References

1. Mosher TJ, Dardzinski BJ, Smith MB. Human articular cartilage: influence of aging and early symptomatic degeneration on the spatial variation of T_2—preliminary findings at 3 T. Radiology 2000;214(1): 259–66.
2. Bashir A, Gray ML, Hartke J, Burstein D. Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. Magn Reson Med 1999;41(5): 857–65.

3. Akella SV, Regatte RR, Gougoutas AJ, Borthakur A, Shapiro EM, Kneeland JB, *et al.* Proteoglycan-induced changes in T1rho-relaxation of articular cartilage at 4T. Magn Reson Med 2001;46(3):419–23.
4. Bashir A, Gray ML, Boutin RD, Burstein D. Glycosaminoglycan in articular cartilage: in vivo assessment with delayed Gd(DTPA)(2–)-enhanced MR imaging. Radiology 1997;205(2):551–8.
5. Borthakur A, Shapiro EM, Beers J, Kudchodkar S, Kneeland JB, Reddy R. Sensitivity of MRI to proteoglycan depletion in cartilage: comparison of sodium and proton MRI. Osteoarthritis Cartilage 2000;8(4): 288–93.
6. Fragonas E, Mlynarik V, Jellus V, Micali F, Piras A, Toffanin R, *et al.* Correlation between biochemical composition and magnetic resonance appearance of articular cartilage. Osteoarthritis Cartilage 1998;6(1): 24–32.
7. Mlynarik V, Trattnig S, Huber M, Zembsch A, Imhof H. The role of relaxation times in monitoring proteoglycan depletion in articular cartilage. J Magn Reson Imaging 1999;10(4):497–502.
8. Watrin A, Ruaud JP, Olivier PT, Guingamp NC, Gonord PD, Netter PA, *et al.* T_2 mapping of rat patellar cartilage. Radiology 2001;219(2):395–402.
9. Nieminen MT, Toyras J, Rieppo J, Hakumaki JM, Silvennoinen J, Helminen HJ, *et al.* Quantitative MR microscopy of enzymatically degraded articular cartilage. Magn Reson Med 2000;43(5):676–81.
10. Lusse S, Knauss R, Werner A, Grunder W, Arnold K. Action of compression and cations on the proton and deuterium relaxation in cartilage. Magn Reson Med 1995;33(4):483–9.
11. Lusse S, Claassen H, Gehrke T, Hassenpflug J, Schunke M, Heller M, *et al.* Evaluation of water content by spatially resolved transverse relaxation times of human articular cartilage. Magn Reson Imaging 2000;18(4):423–30.
12. Regatte RR, Kaufman JH, Noyszewski EA, Reddy R. Sodium and proton MR properties of cartilage during compression. J Magn Reson Imaging 1999;10(6): 961–7.
13. Toffanin R, Mlynarik V, Russo S, Szomolanyi P, Piras A, Vittur F. Proteoglycan depletion and magnetic resonance parameters of articular cartilage. Arch Biochem Biophys 2001;390(2):235–42.
14. Goetinck PF. Proteoglycans in development. Curr Top Dev Biol 1991;25:111–31.
15. Venn M, Maroudas A. Chemical composition and swelling of normal and osteoarthrotic femoral head cartilage. I. Chemical composition. Ann Rheum Dis 1977;36(2):121–9.
16. Hardingham T. Proteoglycans and glycosaminoglycans. In: Seibel M, Robins S, Bilezikan JP, editors. Dynamics of Bone and Cartilage Metabolism. New York: Academic Press; 1999. p. 71–81.
17. Li H, Schwartz NB, Vertel BM. cDNA cloning of chick cartilage chondroitin sulfate (aggrecan) core protein and identification of a stop codon in the aggrecan gene associated with the chondrodystrophy, nanomelia. J Biol Chem 1993;268(31):23504–11.
18. Landauer W. Nanomelia, a lethal mutation of the fowel. J Hered 1965;56:131–8.
19. Pennypacker JP, Goetinck PF. Biochemical and ultrastructural studies of collagen and proteochondroitin sulfate in normal and nanomelic cartilage. Dev Biol 1976;50(1):35–47.
20. Primorac D, Stover ML, Clark SH, Rowe DW. Molecular basis of nanomelia, a heritable chondrodystrophy of chicken. Matrix Biol 1994;14(4):297–305.
21. Regatte RR, Akella SV, Borthakur A, Kneeland JB, Reddy R. Proteoglycan depletion-induced changes in transverse relaxation maps of cartilage: comparison of T_2 and T1rho. Acad Radiol 2002;9(12):1388–94.
22. Xia Y, Moody JB, Burton-Wurster N, Lust G. Quantitative in situ correlation between microscopic MRI and polarized light microscopy studies of articular cartilage. Osteoarthritis Cartilage 2001;9(5): 393–406.
23. Xia Y, Moody JB, Alhadlaq H, Hu J. Imaging the physical and morphological properties of a multi-zone young articular cartilage at microscopic resolution. J Magn Reson Imaging 2003;17(3):365–74.
24. Nieminen MT, Rieppo J, Toyras J, Hakumaki JM, Silvennoinen J, Hyttinen MM, *et al.* T_2 relaxation reveals spatial collagen architecture in articular cartilage: a comparative quantitative MRI and polarized light microscopic study. Magn Reson Med 2001;46(3): 487–93.
25. Reibetanz U, Hanke G, Dartsch D, Labadie C, Reinert T, Butz T, *et al.* Age-dependent collagenous network structures of articular cartilage (Abstract). International Society of Magnetic Resonance in Medicine (ISMRM). Honolulu, HI: ISMRM; 2002. p. 1842.
26. Dardzinski BJ, Laor T, Schmidthorst VJ, Klosterman L, Graham TB. Mapping T_2 relaxation time in the pediatric knee: feasibility with a clinical 1.5-T MR imaging system. Radiology 2002;225(1):233–9.
27. Scholz TD, Fleagle SR, Burns TL, Skorton DJ. Tissue determinants of nuclear magnetic resonance relaxation times. Effect of water and collagen content in muscle and tendon. Invest Radiol 1989;24(11): 893–8.
28. Cole WC, LeBlanc AD, Jhingran SG. The origin of biexponential T_2 relaxation in muscle water. Magn Reson Med 1993;29(1):19–24.
29. Kim DK, Ceckler TL, Hascall VC, Calabro A, Balaban RS. Analysis of water-macromolecule proton magnetization transfer in articular cartilage. Magn Reson Med 1993;29(2):211–5.
30. Zhong JH, Gore JC, Armitage IM. Relative contributions of chemical exchange and other relaxation mechanisms in protein solutions and tissues. Magn Reson Med 1989;11(3):295–308.
31. Kuijer R, van de Stadt RJ, de Koning MH, van Kampen GP, van der Korst JK. Influence of cartilage proteoglycans on type II collagen fibrillogenesis. Connect Tissue Res 1988;17(2):83–97.
32. Henkelman RM, Stanisz GJ, Kim JK, Bronskill MJ. Anisotropy of NMR properties of tissues. Magn Reson Med 1994;32(5):592–601.
33. Fantazzini P, Garavaglia C, Guglielmi G. Continuous distribution analysis of marrow ^{1}H magnetic resonance relaxation in bone. Magn Reson Imaging 2001;19(2):227–31.
34. Gahunia HK, Lemaire C, Babyn PS, Cross AR, Kessler MJ, Pritzker KP. Osteoarthritis in rhesus macaque knee joint: quantitative magnetic resonance imaging tissue characterization of articular cartilage. J Rheumatol 1995;22(9):1747–56.
35. Dardzinski BJ, Mosher TJ, Li S, Van Slyke MA, Smith MB. Spatial variation of T_2 in human articular cartilage. Radiology 1997;205(2):546–50.

36. Muir H. Proteoglycans as organizers of the intercellular matrix. Biochem Soc Trans 1983;11:613–22.
37. Dunham J, Chambers MG, Jasani MK, Bitensky L, Chayen J. Changes in the orientation of proteoglycans during the early development of natural murine osteoarthritis. J Orthop Res 1990;8(1):101–4.
38. Dunham J, Shackleton DR, Nahir AM, Billingham ME, Bitensky L, Chayen J, *et al.* Altered orientation of glycosaminoglycans and cellular changes in the tibial cartilage in the first two weeks of experimental canine osteoarthritis. J Orthop Res 1985;3(3):258–68.