Noninvasive Quantification of Human Nucleus Pulposus Pressure with Use of T1ρ-Weighted Magnetic Resonance Imaging

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Background: Early diagnosis is a challenge in the treatment of degenerative disc disease. A noninvasive biomarker detecting functional mechanics of the disc is needed. T1ρ-weighted imaging, a spin-lock magnetic resonance imaging technique, has shown promise for meeting this need in in vivo studies demonstrating the clinical feasibility of evaluating both intervertebral discs and articular cartilage. The objectives of the present study were (1) to quantitatively determine the relationship between T1ρ relaxation time and measures of nucleus pulposus mechanics, and (2) to evaluate whether the quantitative relationship of T1ρ relaxation time with the degenerative grade and glycosaminoglycan content extend to more severe degeneration. It was hypothesized that the isometric swelling pressure and compressive modulus would be directly correlated with the T1ρ relaxation time and the apparent permeability would be inversely correlated with the T1ρ relaxation time.

Methods: Eight cadaver human lumbar spines were imaged to measure T1ρ relaxation times. The nucleus pulposus tissue from the L1 disc through the S1 disc was tested in confined compression to determine the swelling pressure, compressive modulus, and permeability. The glycosaminoglycan and water contents were measured in adjacent tissue. Linear regression analyses were performed to examine the correlation between the T1ρ relaxation time and the other measured variables. Mechanical properties and biochemical content were evaluated for differences associated with degeneration.

Results: A positive linear correlation was observed between the T1ρ relaxation time on the images of the nucleus pulposus and the swelling pressure ($r = 0.59$), glycosaminoglycan content per dry weight ($r = 0.69$), glycosaminoglycan per wet weight ($r = 0.49$), and water content ($r = 0.53$). No significant correlations were observed between the T1ρ relaxation time and the modulus or permeability. Similarly, the T1ρ relaxation time, swelling pressure, glycosaminoglycan content per dry weight, and water content were significantly altered with degeneration, whereas the modulus and permeability were not.

Conclusions: T1ρ-weighted magnetic resonance imaging has a strong potential as a quantitative biomarker of the mechanical function of the nucleus pulposus and of disc degeneration.

Clinical Relevance: Several in vivo studies have previously demonstrated the clinical feasibility of using T1ρ-weighted imaging to evaluate both intervertebral discs and articular cartilage. Its application for the diagnosis of disc degeneration looks promising.

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The function of the intervertebral disc is mechanical; it supports and distributes large loads, permits spine motion, and dissipates energy. The swelling pressure of the nucleus pulposus is a particularly important property. Pressurization within the nucleus pulposus is generated by the negatively charged proteoglycans that are entrapped within a loose type-II-collagen network and create a fixed charge density, attracting and binding water. Pressurization of the nucleus pulposus enables the disc to absorb and transmit the compressive spinal loads, maintains disc height, and prevents large deformations within the low-load neutral zone[1-3]. During the early stages of disc degeneration, the proteoglycans and associated glycosaminoglycans in the nucleus pulposus break down, reducing the pressure within the nucleus pulposus[4-7].

A major challenge in treating degenerative disc disease is the need to diagnose the disorder early and accurately. Early-stage diagnosis is essential to a positive prognosis, particularly as emerging treatment modalities aimed at restoring mechanical function, such as nucleus pulposus replacement, total disc replacement, cell or growth factor injection, or gene therapy, are translated to the clinical setting. A noninvasive biomarker detecting functional mechanics of the disc would facilitate early-stage diagnosis.

Conventional magnetic resonance imaging techniques are not sensitive enough to detect early stages of disc degeneration[8]. Other techniques, including delayed gadolinium-enhanced and sodium magnetic resonance imaging, may have sufficient sensitivity but there are also substantial limitations with regard to implementation of these methods[9-11]. T1ρ-weighted imaging is a spin-lock magnetic resonance imaging technique that shows promise as a noninvasive biomarker, as several in vivo studies have demonstrated the clinical feasibility of evaluating both intervertebral disc and articular cartilage with this modality[12-15]. The spin-lock technique was first employed by Redfield in 1955 and allows for a lower Larmor frequency[16]. The lower Larmor frequency increases the sensitivity of T1ρ-weighted imaging, permitting the detection of low-frequency physicochemical interactions between water and extracellular matrix molecules[17,18]. In articular cartilage, the T1ρ relaxation time is inversely correlated with proteoglycan content[19,20]. With degeneration of articular cartilage, the proteoglycan content decreases, the matrix molecules and water become less restricted, and the T1ρ relaxation time increases. The T1ρ relaxation time was also found to be inversely correlated with the compression modulus of articular cartilage in an interleukin-1β-treated degeneration model[20]. In contrast, the T1ρ relaxation time in human discs was demonstrated to be directly correlated with both the degenerative grade and the sulfated-glycosaminoglycan content[18], although the study was limited to a maximum degenerative grade of 3 (out of 5) and limited to sixteen disc samples. The potential for correlations between the mechanical function and T1ρ-weighted imaging measurements of the intervertebral disc remains unknown. If they are correlated, T1ρ-weighted imaging could serve as a biomarker for mechanical function of the disc.

The objectives of this study were twofold: (1) to quantitatively determine the relationship between T1ρ relaxation time and the mechanics of the nucleus pulposus, and (2) to evaluate whether the quantitative relationship of the T1ρ relaxation time with the degenerative grade and the glycosaminoglycan content extends to more severe degeneration. With human disc degeneration, the swelling pressure, compressive modulus, and proteoglycan content decrease[4,21,22], the permeability increases[21], and the T1ρ relaxation time decreases[18]. Therefore, it was hypothesized that the isometric swelling pressure and compressive modulus would be directly correlated with the T1ρ relaxation time and the apparent permeability would be inversely correlated with the T1ρ relaxation time.

Materials and Methods

Eight fresh-frozen cadaver lumbar spine sections (mean age of the donors at the time of death, 51.8 years; range, fifteen to seventy-nine years) were imaged on a 1.5-T whole-body clinical magnetic resonance scanner (Sonata; Siemens Medical Solutions, Malvern, Pennsylvania) as previously described[18]. Briefly, a series of sagittal plane T2 and T1ρ-weighted images were acquired with a field of view of 28 × 28 cm, a slice thickness of 4 mm, an acquisition matrix of 256 × 256, an echo time of 3000 msec, a repetition time of 12 msec, a spin-lock pulse time of 15 to 75 msec in five increments of 15 msec each, and a spin-lock pulse amplitude of 500 Hz. The T1ρ values were calculated, with use of a custom-written MATLAB (The MathWorks, Natick, Massachusetts) program, on a pixel-by-pixel basis by fitting intensity data of the spin-lock images at each spin-lock pulse time to the following exponential function: $S(TSL) = S_0 e^{-(TSL/T_{1\rho})}$ (Fig. 1). Mean T1ρ values were calculated from a manually selected 5-mm-diameter circular region of interest in the center of the nucleus pulposus. The T2-weighted images were used to assess each disc's degenerative grade according to the classification system described by Pfirrmann et al.[23]. Non-degenerated discs were defined as discs with a grade of <2.5 (fifteen discs), and degenerated discs were defined as discs with a grade of ≥2.5 (eighteen discs).

The L1 through S1 discs were isolated with sharp dissection at the superior and inferior end plates. Seven discs were too degenerated to dissect and test, resulting in thirty-three discs for testing. A 7-mm-diameter punch-biopsy specimen was removed from the center of the nucleus pulposus. A freezing-stage (model BFS-30; Physitemp, Clifton, New Jersey) sledge microtome (model SM2400; Leica, Nussloch, Germany) was then used to section each sample to a uniform thickness. Thickness was measured in triplicate with use of a micro-laser sensor (LM10 Micro Laser Displacement Sensor; Aromat, New Providence, New Jersey). The average thickness (and standard deviation) of the thirty-three samples was 2.55 ± 0.14 mm. The samples were frozen at −20°C prior to testing.

A 4.37-mm-diameter circular punch was used to remove cylindrical samples from the sectioned 7-mm-diameter biopsy specimen of each disc. The samples were mechanically tested in confined compression as previously described[21]. The sample

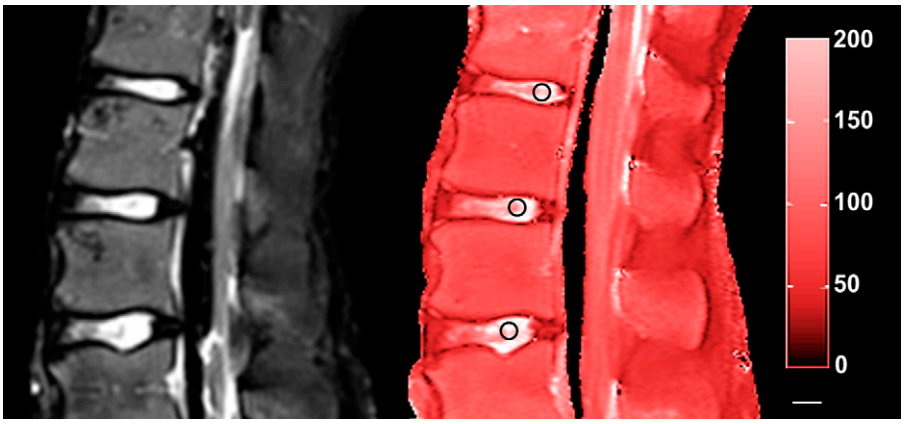

Fig. 1

Representative T2-weighted (left) and T1ρ-weighted (right) images of the spine of a fifteen-year-old male donor. The average degenerative grade is 1.43. The 5-mm-diameter circular regions of interest where the T1ρ relaxation times were determined are denoted by the black circles. Scale bar (small, horizontal white bar) = 10 mm. The numbers on the right of the red bar indicate the T1ρ relaxation time.

was placed into the test chamber, and the porous platen was lowered at 10 μm/sec with a linear stepper motor until a contact load of 0.20 N was reached. The chamber was then filled with 0.15-M phosphate buffered solution. After a five-minute wait period, a 1% compressive strain at a rate of 0.25 μm/sec was applied, followed by a three-hour hold. The isometric swelling pressure was determined from the equilibrium stress at the end of that three-hour hold. Following the isometric swelling test, a 5% compressive strain at a rate of 0.25 μm/sec was applied, followed by a two-hour hold to measure relaxation. The data acquired during the stress relaxation test were analyzed with use of the linearly elastic, isotropic biphasic theory[24]. Compressive modulus was calculated from a direct analysis of the relaxation data, and apparent permeability was calculated from a fit to a forward finite-difference approximation of the biphasic theory[21,25].

Following testing, adjacent thickness-matched nucleus pulposus tissue from the original 7-mm specimens were used to determine water and sulfated-glycosaminoglycan content. The wet weight of each tissue sample was first recorded in triplicate. Samples were then lyophilized, dry weight was recorded in triplicate, and the percentage water content was calculated. To determine sulfated-glycosaminoglycan content, 5-μL aliquots were pipetted into a ninety-six-well plate and were analyzed with a microplate reader (Synergy HT Multi-Mode Microplate Reader; BioTek Instruments, Winooski, Vermont) and the 1,9-dimethylmethylene blue assay[26]. The sulfated-glycosaminoglycan content was normalized to dry weight and wet weight.

TABLE I Correlations Between T1ρ Relaxation Times and Mechanical and Biochemical Parameters in Non-Degenerated and Degenerated Discs

Parameter	Non-Degenerated Discs*	Degenerated Discs*	Correlation with T1ρ Relaxation Time (R)
Degenerative grade	1.9 ± 0.5	3.6 ± 0.7†	−0.83†
T1ρ relaxation time *(msec)*	124 ± 38	40 ± 18†	
Swelling pressure *(MPa)*	0.142 ± 0.077	0.048 ± 0.037†	0.59‡
Compressive modulus *(MPa)*	1.42 ± 0.69	1.04 ± 0.62	0.20
Apparent permeability *($\times 10^{-15}$ m^4/N-s)*	0.47 ± 0.26	0.71 ± 0.34	−0.25
Sulfated-glycosaminoglycan content *(μg/mg)*			
Per dry weight	457 ± 193	269 ± 142†	0.69‡
Per wet weight	87 ± 24	70 ± 28	0.49‡
Water content *(%)*	79.8 ± 6.9	71.6 ± 8.4†	0.53‡

*The values are given as the mean and standard deviation. †$P < 0.05$ for the difference between the non-degenerated and degenerated discs. ‡$P < 0.05$ for the significance of the correlation.

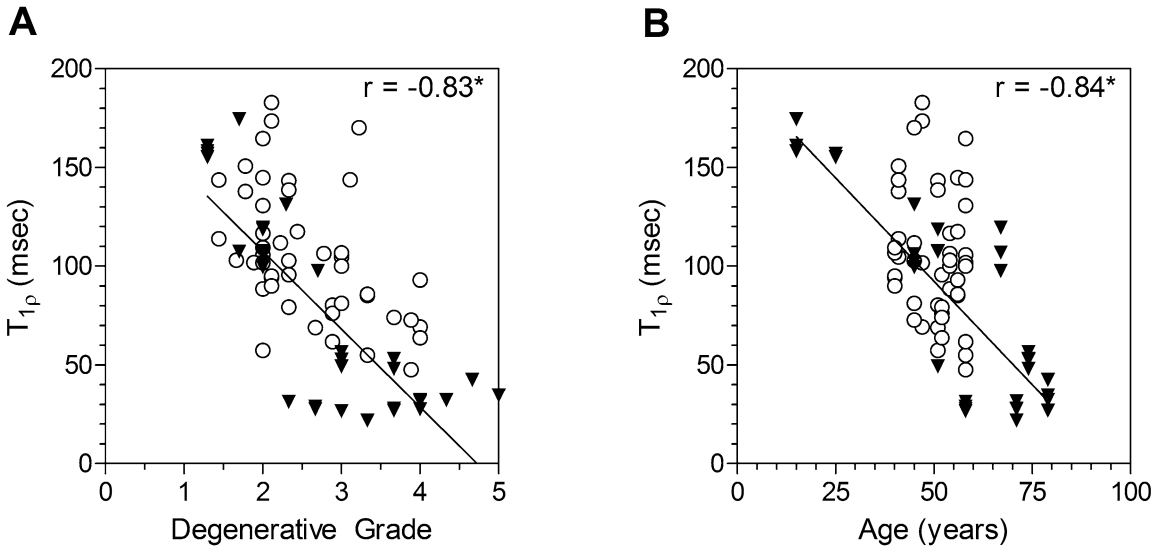

Fig. 2

Correlation between T1ρ relaxation time and degenerative grade (A) and between T1ρ relaxation time and age (B). The asterisks denote significance ($p < 0.05$). The T1ρ relaxation times in this in vitro study (filled triangles) were within the range of values in a previous in vivo study of ten asymptomatic forty to sixty-year-old subjects (open circles)[13].

Sample size was based on a power analysis, with a power of 0.8 and a level of significance of 0.05, in which we used data from our previous study demonstrating significant differences in sulfated-glycosaminoglycan content with degeneration[21]. Linear regression analyses were performed among the following variables: degeneration (degenerative grade, age, and T1ρ relaxation time), mechanical characteristics (swelling pressure, compressive modulus, and apparent permeability), and biochemical composition (water content and sulfated-glycosaminoglycan content) with use of GraphPad Prism software (GraphPad Software, San Diego, California). A significance level of 0.05 was used. The correlation was considered strong when the r value was >0.7, moderate if it was ≥0.5 but <0.7, and weak if it was ≤0.5[27]. Analysis of variance with a Bonferroni post hoc test was also used to determine significant differences in mechanical characteristics and biochemical composition between non-degenerated and degenerated discs.

Results

Twenty-six discs were imaged, tested mechanically, and analyzed biochemically. An additional seven discs were imaged and analyzed biochemically but were too severely degenerated to be tested mechanically. The degenerative grades for the thirty-three discs ranged from 1.3 to 4.7, with a mean grade (and standard deviation) of 2.7 ± 1.0. (1.9 ± 0.5 for the fifteen non-degenerative discs and 3.6 ± 0.7 for the eighteen degenerated discs) (Table I). The imaging and biochemistry data for sixteen of the thirty-three discs have been previously reported[18].

T1ρ relaxation times ranged from 22 to 195 msec (Fig. 1). The average imaging time was approximately thirty-five min-

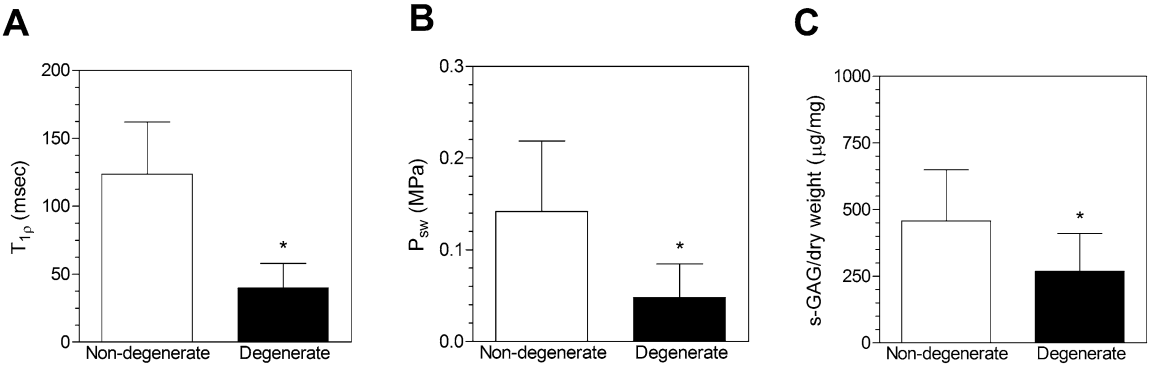

Fig. 3

Comparison of T1ρ relaxation time (A), swelling pressure (P_{sw}) (B), and sulfated-glycosaminoglycan content (s-GAG) per dry weight between non-degenerated and degenerated discs (C). The asterisks denote significance ($p < 0.05$). The biochemistry and T1ρ data for sixteen of the thirty-three discs were previously reported[18].

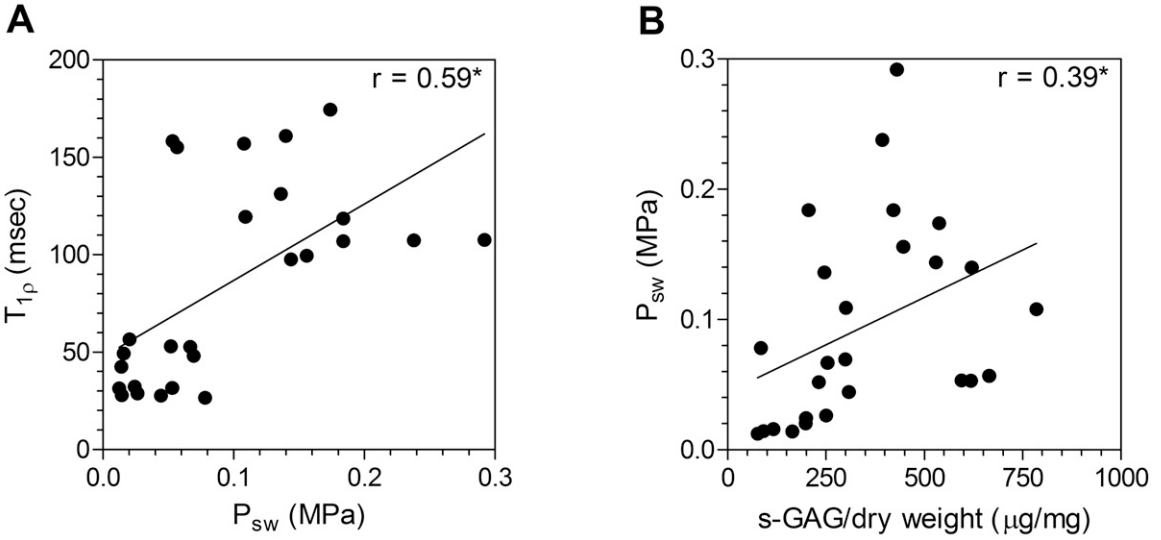

Fig. 4

Correlation between T1ρ relaxation time and swelling pressure (P_{sw}) (A) and between swelling pressure and sulfated-glycosaminoglycan content (s-GAG) per dry weight (B). The asterisks denote significance ($p < 0.05$).

utes. The T1ρ relaxation time was strongly correlated with the degenerative grade ($r = -0.83$, $p < 0.05$) and age ($r = -0.84$, $p < 0.05$) (Fig. 2), and it was significantly higher for the fifteen non-degenerated discs than it was for the eighteen degenerated discs (Fig. 3, *A*; Table I).

The swelling pressure as measured with confined compression testing was directly correlated with the T1ρ relaxation time ($r = 0.59$, $p < 0.05$) (Fig. 4, *A*; Table I) and the sulfated-glycosaminoglycan content per dry weight ($r = 0.39$, $p < 0.05$) (Fig. 4, *B*). Additionally, the swelling pressure in the thirteen non-degenerated discs in which it was tested was nearly three times as high as that in the thirteen degenerated discs in which it was tested (Fig. 3, *B*; Table I). The compression modulus and apparent permeability were not correlated with the T1ρ relaxation time (modulus: $r = 0.20$, $p > 0.05$; permeability: $r = -0.25$, $p > 0.05$) and did not differ significantly between the non-degenerated and degenerated discs (Table I).

Biochemical assays showed the sulfated-glycosaminoglycan content per dry weight to be correlated with the T1ρ relaxation time ($r = 0.69$, $p < 0.05$) (Fig. 5, Table I) and to be 70% higher in the non-degenerated discs ($p < 0.05$) (Fig. 3, *C*; Table I). The T1ρ relaxation time was moderately correlated with the water content ($r = 0.53$, $p < 0.05$) (Fig. 5, Table I) and the sulfated-glycosaminoglycan content per wet weight ($r = 0.49$, $p < 0.05$).

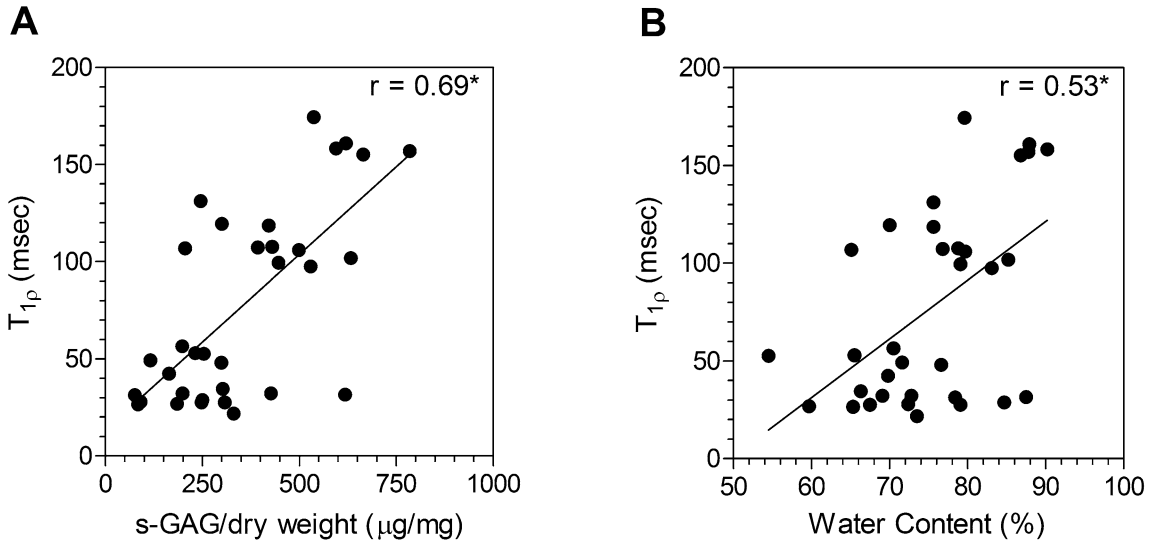

Fig. 5

Correlation between T1ρ relaxation time and sulfated-glycosaminoglycan content (s-GAG) per dry weight (A) and between T1ρ relaxation time and water content (B). The asterisks denote significance ($p < 0.05$).

The water content was 11% higher in the non-degenerated discs ($p < 0.05$) (Table I), whereas the sulfated-glycosaminoglycan content per wet weight did not differ significantly between the non-degenerated and degenerated discs ($p > 0.05$) (Table I).

Discussion

This study demonstrated that, as we had hypothesized, isometric swelling pressure is directly correlated with findings on T1ρ-weighted imaging. However, the compressive modulus and permeability were not correlated with the T1ρ values, which was not consistent with our hypotheses. Overall, the findings suggest that T1ρ-weighted magnetic resonance imaging may be a valuable quantitative biomarker of nucleus pulposus mechanical function and proteoglycan composition for the diagnosis of disc degeneration. The linear correlation between swelling pressure and T1ρ relaxation time was the primary finding of this study. The mechanical function of the nucleus pulposus depends on its swelling pressure, the mechanism through which the disc supports axial loads. Although the primary utility of T1ρ-weighted magnetic resonance imaging will remain the detection of early degenerative changes in swelling pressure and proteoglycan content, this study extended the previous observation that T1ρ relaxation time is correlated with disc proteoglycan content over a broader range of degeneration and a larger sample size[18]. However, across the spectrum of degeneration, there may be several other biochemical and structural changes within the disc that affect the T1ρ relaxation time or that are not detected by T1ρ-weighted imaging, so the application of this biomarker should not be extrapolated beyond the specific properties quantified in this study. The lack of correlations of T1ρ relaxation time with permeability and compression modulus were not surprising considering previous observations of moderate-to-weak correlations between proteoglycan content and these mechanical properties[21]. With degeneration, the modulus and permeability of the nucleus pulposus changed by 50% in a previous study[21]. In this study, these properties were not significantly affected by degeneration, although their values and trends with degeneration were consistent with those in previous reports[21]. In contrast, the swelling pressure changes by a factor of two to three with degeneration. Thus, although T1ρ-weighted imaging may not be sensitive enough to detect changes in modulus and permeability with degeneration, these properties are probably not as important in the degenerative process as is swelling pressure.

An intriguing observation is that, while disc and articular cartilage contain similar constituents and undergo similar degenerative processes, these two tissues have opposite correlations between T1ρ relaxation time and proteoglycan content. The correlation is positive in disc tissue and negative in articular cartilage[19,20]. The strong direct correlation of T1ρ relaxation time with proteoglycan content in the disc suggests that, as proteoglycan content decreases with degeneration, the water and extracellular matrix molecules within the nucleus pulposus become more restricted, resulting in a lower T1ρ relaxation time (and also a lower swelling pressure). However, in articular cartilage, as the proteoglycan decreases with degeneration the water and extracellular matrix molecules become less restricted, resulting in a higher T1ρ relaxation time. While T1ρ relaxation time is believed to be primarily related to interactions between water and proteoglycans in the tissue, it is not known how other macromolecules may also contribute to T1ρ relaxation. The mechanisms driving the opposing T1ρ-relaxation-time responses associated with disc and articular cartilage degeneration may be related to compositional differences during the degenerative processes[18]. Furthermore, it is likely that intrinsic differences between these tissues with regard to structural and functional properties, such as the containment of the nucleus pulposus and the increased fibrosis and cross-linking with degeneration, can be attributed to the opposing responses in terms of T1ρ relaxation time. Specific factors that lead to the differences in correlations observed for disc and articular cartilage remain to be determined.

While this study was limited to cadaver human spines, the measured T1ρ relaxation times were consistent with the values measured in vivo in ten asymptomatic forty to sixty-year-old subjects[13]. Several preliminary studies have demonstrated this feasibility of in vivo T1ρ-weighted imaging of articular cartilage[28-30] and the spine[13,14]. This study was also limited to two-dimensional magnetic resonance scans acquired at the midsagittal section of the disc, with which it would be difficult to register locations across sequential imaging sessions in an in vivo longitudinal study. Work to develop a pulse sequence to acquire three-dimensional T1ρ maps is ongoing[31]. While T2-weighted images were used to determine the degenerative grades, we did not attempt to correlate T2 relaxation time with disc mechanics or composition in this study. T1ρ has a larger dynamic range than T2, which will be needed to translate this modality into clinical use[13,18].

Correlations in this study were not adjusted for intercorrelation among discs within the same spine. Although some changes may be similar from one adjacent disc to the next, the discs were treated as independent levels within the spine. With the addition of an adjustment for intercorrelations within the same spine, the correlations of T1ρ relaxation time with the degenerative grade, age, and swelling pressure remain significant (degenerative grade: $r = -0.83$, $p < 0.05$; age: $r = -0.73$, $p < 0.05$; swelling pressure: $r = 0.65$, $p < 0.05$). Sulfated-glycosaminoglycan content per dry weight also remains significantly correlated with swelling pressure ($r = 0.38$, $p < 0.05$). The adjusted correlations of T1ρ relaxation time with sulfated-glycosaminoglycan content per dry weight or water content are not significant. Increasing the number of spines to account for this adjustment may result in significant correlations. Thus, adjustment of the correlations did not affect the main finding of this study.

In conclusion, this study demonstrated a positive linear correlation between the findings on T1ρ-weighted imaging of the nucleus pulposus and both swelling pressure and proteoglycan content. Thus, T1ρ-weighted imaging has potential as a quantitative biomarker for mechanical function of the nucleus

pulposus and disc degeneration. Future work should include a longitudinal in vivo study of symptomatic patients to build on the previous in vivo study of normal volunteers[13]. The application of T1ρ-weighted magnetic resonance imaging could also be investigated as a guide for selection of treatment, to quantify the state of the nucleus pulposus following herniation and/or discectomy, and to evaluate emerging technologies developed to treat early degeneration, including prosthetic replacement or biological and cell-based therapies. ■

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