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Intervertebral disc magnetic resonance image: correlation with gross morphology and biochemical composition

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The magnetic resonance image, gross morphology, and biochemical composition of the intervertebral disc nucleus pulposus (NP), anulus fibrosus (AF) and cartilaginous end-plates (CEP) from two groups of three human lumbar spines were compared. Group I consisted of all healthy discs from young donors (Grade 1) and group II was comprised of discs that had undergone degeneration and age-related changes (average Grade 4). The gross morphological changes in the individual disc tissues associated with ageing/degeneration were consistent with specific changes in the characteristics of the magnetic resonance image. In particular, the mid-nuclear band of decreased magnetic resonance signal intensity seen in Grade 4 discs was associated with the appearance of clefts and fissures as well as a region of mucinous infiltration. The results of the biochemical analysis suggest that the changes in signal intensity are not due merely to changes in water content, but are also associated with changes in proteoglycan content. The changes associated with ageing/degeneration in the magnetic resonance image of the disc were related to a decrease in the proteoglycan content of the AF and NP. The water content of the NP also decreased. There was no clear association between the biochemical composition of the CEP and the magnetic resonance image. These results demonstrate that magnetic resonance imaging is an effective technique for evaluating subtle morphological changes in the intervertebral disc tissues and may be a sensitive indicator of the proteoglycan content of the AF and NP.
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KEY WORDS: magnetic resonance imaging, anatomy and histology, biochemistry, proteoglycans, intervertebral disc, ageing, degeneration.

La résonance magnétique nucléaire, la morphologie globale et la composition biochimique du noyau gélatineux (nucleus pulposus) du disque intervertébral, de l'anneau fibreux périphérique (AF) et du plateau vertébral cartilagineux de deux groupes de trois colonnes lombaires humaines ont été comparées. Le Groupe I était constitué des disques sains de jeunes donneurs (Catégorie 1); le Groupe II était constitué de disques en état de dégénérescence ou qui subissaient des changements en rapport avec l'âge (la moyenne étant de Catégorie 4). Les changements morphologiques globaux associés au vieillissement ou à la dégénérescence des tissus des disques correspondaient à des changements spécifiques dans les caractéristiques de résonance magnétique nucléaire. En particulier, la bande nucléaire moyenne d'intensité du signal de résonance magnétique décroissante constatée chez les disques de la Catégorie 4 a été associée à l'apparition de fentes et de fissures ainsi qu'à une région d'infiltration muqueuse. Les résultats de l'analyse biochimique suggèrent que les variations d'intensité du signal ne sont pas seulement dues aux variations de la teneur en eau; elles pourraient également être associées aux variations de la teneur en protéoglycane. Les changements associés au vieillissement ou à la dégénérescence dans la résonnance magnétique nucléaire du disque ont été mis en relation avec la baisse de teneur en protéoglycane de l'anneau fibreux périphérique et du noyau gélatineux. La teneur en eau du noyau gélatineux a aussi diminué. Aucune relation claire n'a pu être établie entre la composition biochimique du plateau vertébral cartilagineux et la résonnance magnétique nucléaire. Ces résultats démontrent que la résonnance magnétique nucléaire constitue un moyen technique efficace pour évaluer les changements morphologiques subtils dans les tissus des disques intervertébraux et peut être considérée comme un indicateur sensible de la teneur en protéoglycane de l'anneau fibreux et du noyau gélatineux.
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MOTS CLÉS : résonance magnétique nucléaire, anatomie et histologie, biochimie, protéoglycanes, disque intervertébral, vieillissement, dégénération.

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Introduction

Magnetic resonance (MR) represents a significant advance in imaging the spine with high resolution. It is the first imaging modality that can distinguish the anatomical components of the intervertebral disc and thus permit separate analysis of the nucleus pulposus (NP), anulus fibrosus (AF) and cartilaginous end-plates (CEP).[1,2] Previous studies have demonstrated that MR imaging can readily distinguish normal disc tissues from those having undergone mild degenerative changes.[3,4,5] Such early changes are not detectable, even indirectly, with plain film radiography, tomography or CT scanning. The MR imaging technique is apparently non-invasive and therefore provides a useful means for studying the course of disc degeneration *in vivo*.

At present, it is uncertain which components of the individual disc tissues are responsible for generating the MR image and thus the significance of any alterations in disc MR image are largely ill-defined. To elevate MR analysis of the disc from an empirical level to that of a valuable analytical tool, will require correlation of MR images with known disc parameters.

The gross morphological features of the human intervertebral disc have been well-described. Recently, a comprehensive grading scheme has been developed which describes five stages of morphological change associated with disc ageing and/or degeneration.[6] The disc tissues are assessed individually such that no one component disproportionately influences the overall assessment. This technique has proven useful in the study of the pathogenesis of disc degeneration as it provides a mechanism whereby meaningful interexaminer comparison of experimental results can be achieved.

An additional level at which the human intervertebral disc has recently been characterized is through biochemical analysis. The components of the disc's extracellular matrix are chiefly responsible for its functional capacity.[7] It is now known that by the time the earliest morphological changes can be detected, substantial and widespread changes have taken place at the biochemical level, in all disc tissues.[8,9,10] Recent studies in this and other laboratories have determined that it is the proteoglycan component of the disc's extracellular matrix that is chiefly affected.[8,11]

The current investigation compares the MR image of the disc at varying stages of ageing/degeneration with both the morphological appearance (i.e. disc grade) and the proteoglycan content of the individual disc tissues. The results of this study will advance our understanding of the true nature of alterations in the MR image of the disc that are associated with ageing and/or degeneration.

Methods

Analytical methods

All chemicals used were either of analytical reagent grade or the best commercial grade available. The total proteoglycan content was estimated as the total hexuronate content and was assayed using the carbazole-borosulphuric acid reaction[12] with a sodium glucuronate monohydrate standard (Corn Products Refining Co., New York, N.Y., U.S.A.).

Collection and grading of intervertebral discs

Six lumbar spines free of the posterior elements were obtained within 48 hours of death from the morgues of the Vancouver General and University Hospital – U.B.C. sites. The spines studied were from donors who had died suddenly and were free of disease affecting the spine. Immediately after dissection, MR images of the specimens were obtained in the mid-sagittal plane using the Picker Vista 2000 MR Proton Tomograph operating at 0.15T. Spin echo pulse sequences were used with a repeat time (TR) of 1916 ms or 2916 ms and an echo time (TE) of 40 ms. The spines were then wrapped in impermeable plastic and aluminum foil prior to freezing at −80°C. Each of the vertebral columns studied was cut, whilst frozen, in the mid-sagital plane using a band saw, rinsed in tepid water with brushing to remove bone fragments, and photographed. The spines were stored at −80°C until needed.

Discs were assessed on the basis of gross morphology using the grading scheme developed by Thompson et al.[6] Morphological grades of I to V were assigned blindly by an orthopaedic surgeon, two rheumatologists, a pathologist and the author; a grade of I corresponded to a healthy, non-degenerate disc; grades II to V described increasingly severe degeneration. Two groups of spines were studied. One group from donors aged 16–21, contained only healthy (Grade 1) discs, while the second group comprised of donors aged 76 to 84, contained discs having undergone advanced degenerate change (average Grade 4).

Dissection of disc tissues and determination of water content

At each level of the spine, the anulus fibrosus (AF), nucleus pulposus (NP) and cartilaginous end-plates (CEP) were carefully removed with a scalpel. The individual disc tissues were pooled for each spine. Each tissue pool was weighed, sectioned at 20 μm using a cryostat, then freeze-dried. Water content was estimated as the difference between fresh weight of the tissue following dissection and the dry weight after freeze-drying.

Preparation of purified proteoglycan

Proteoglycan (PG) was extracted from the dry residue under dissociative conditions by suspension in 10 ml/g tissue of 4*M*-guanidinium HCl containing 0.05*M* sodium acetate buffer, pH 5.8, and protease inhibitors, and shaking gently for 48 hours at 4°C.[13] The protease inhibitors added immediately prior to the beginning of the extraction, were 6-aminocaproic acid (100 m*M*) for cathepsin D activity against proteoglycans, benzamidine-HCl (1 m*M*) for trypsin-like activity,[13] disodium EDTA (10m*M*) for metalloproteinases, phenylmethylsulphonyl fluoride in methanol (1 m*M*) for serine-dependent proteases and N-ethylmaleimide (10 m*M*) for thiol proteases. The pH of 5.8

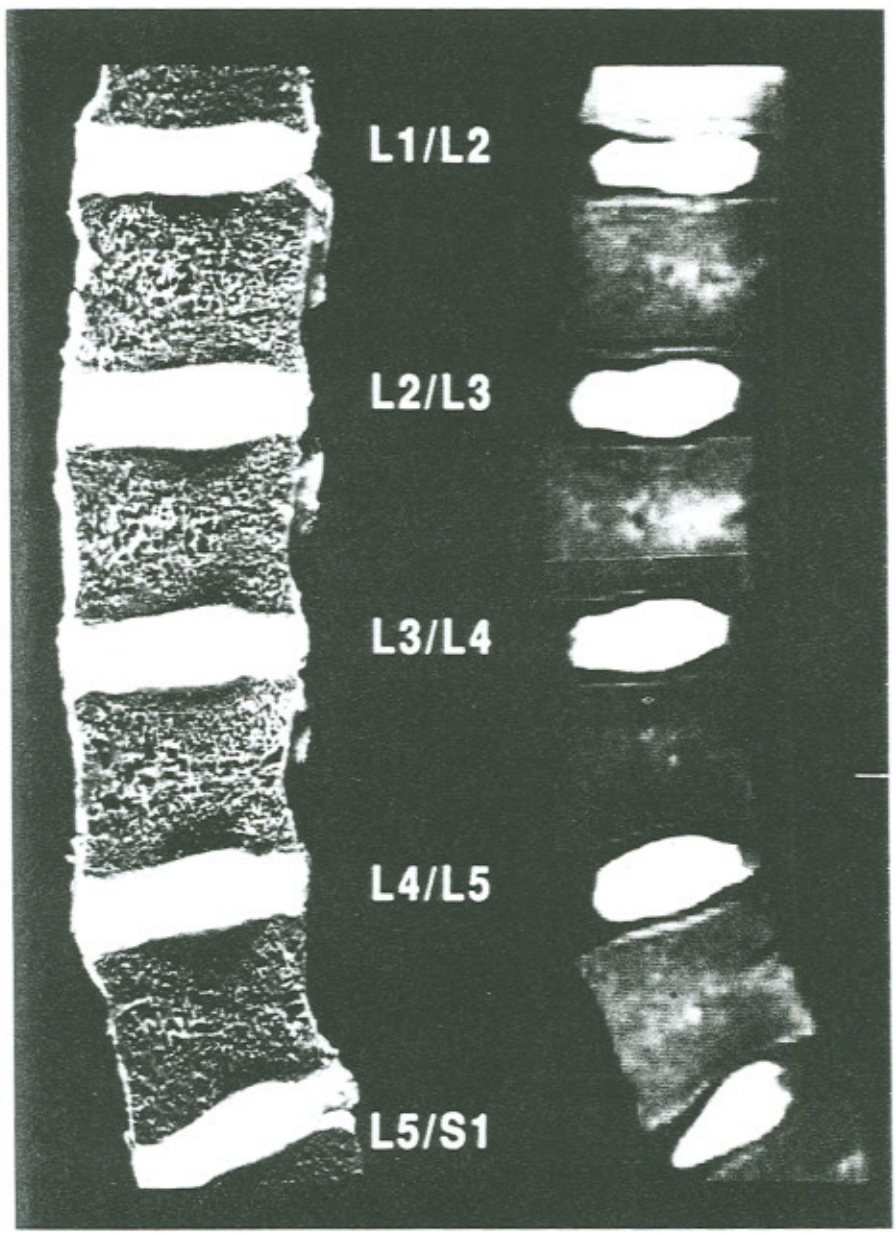

Figure 1. Magnetic resonance image and gross morphology of Grade 1 discs. Photograph (left) and MRI (right) of mid-sagittal section of the lumbar spine from a 16-year-old male. The L1/L2 to L5/S1 discs are shown and were each assigned a grade of 1.

was above the optimum pH for acid proteases and below that for neutral proteases[13] and also close to optimal for extraction of proteoglycan.[14] After extraction, the suspension was centrifuged at 15,000g for 60 min and the supernatant decanted.

The extract was dialysed for 24 hours at 4°C against 8 vol. 0.5 M sodium acetate, pH 5.8 containing protease inhibitors to reduce the guanidine-HCl concentration to 0.5 M (associative conditions). Hyaluronate (Healon, Pharmacia, Uppsala, Sweden) equivalent in hexuronate to 2% of the proteoglycan hexuronate was added to assure maximum re-aggregation of the PG. The PG was prepared by cesium chloride density gradient centrifugation[15] using a starting density of 1.42 g/ml at 100,000g at 10°C for 72 hours. The tubes were cut into five sections of equal volume (A1 to A5 from bottom to top) and the densities, in g/ml, were determined by weighing a measured volume. Each fraction was dialyzed for 24 hours at 4°C against 0.05 M sodium acetate buffer, pH 5.8. The dialyzed A1 fraction, containing the bulk of PG, was freeze-dried and stored at −80°C until required (usually less than 7 days).

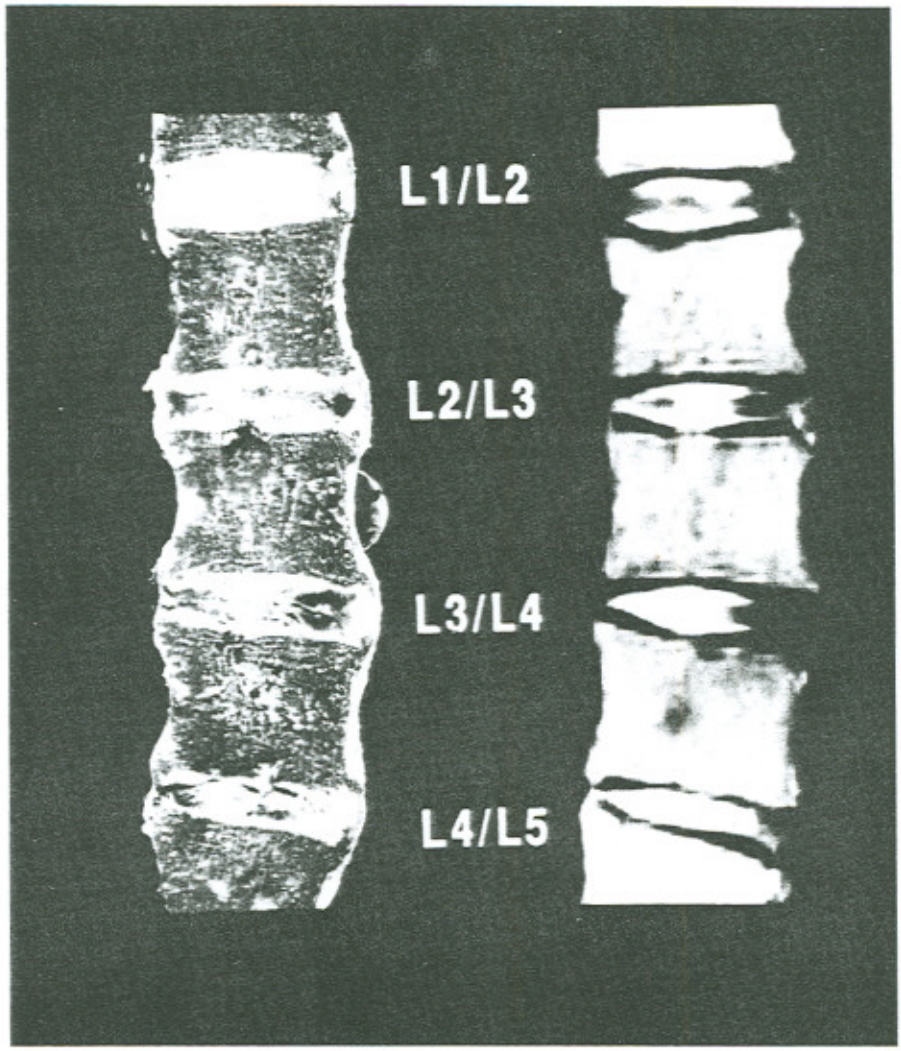

Figure 2. Magnetic resonance image and gross morphology of Grade 4 discs. Photograph (left) and MRI (right) of mid-sagittal section of the lumbar spine from a 84-year-old male. The L1/L2 to L4/L5 discs are shown and were each assigned a grade of IV. Note that the L5/S1 disc was not obtained from this dissection, but was present in all of the other spines used.

Table 1
Comparison of disc gross morphology and magnetic resonance image

Average Disc Grade	Morphology*	MR Image Characteristics
1	Bulging gelatinous nucleus; discrete fibrous anular lamellae; thick, uniform hyaline end-plates; rounded vertebral body margins	Uniform, high signal intensity with clear demarcation of nucleus and anulus; smooth end-plate and vertebral body margins
4	Clefts in nucleus parallel to end-plates; focal disruptions of anulus; irregular, thinned end-plates; osteophytes less than 2mm	Loss of disc height; horizontal band of decreased signal intensity; no clear demarcation of nucleus and anulus; irregular end-plates and sub-chondral bone

* From Thompson et al.[6]

Results

Figures 1 and 2 illustrate the typical gross morphological appearance and the MR image of lumbar spines containing healthy (Grade 1) and degenerate (Grades 4 and 5) intervertebral discs, respectively. The L5/S1 disc was not available for study in the spine shown in Figure 2. As listed in Table I, the comparison of healthy and degenerate discs revealed readily discernable differences in the morphological appearance of the individual disc tissues. In the advanced stages of degeneration, the well-demarcated, gelatinous NP and discrete fibrous lamellae of the AF present in Grade 1 discs, became infiltrated with mucinous material and traversed by large fissures and clefts. With degeneration, the CEP had their uniform, thick hyaline appearance replaced by thinned, irregular fibrocartilage. In addition, there was diffuse sclerosis through the region of the junction of CEP and subchondral bone.

The MR image of the healthy intervertebral disc demonstrated high signal intensity from the NP and a low signal intensity from the AF. While the CEP were not visualized as well, a smoothly contoured, homogenous region was detectable overlying an even surface of subchondral bone. With ageing and degeneration, the signal intensity from the NP decreased. A horizontally-oriented band of low signal intensity was present through the mid-zone of the nucleus and inner two thirds of the anulus. The CEP region had an irregular appearance with a number of focal defects and the surfaces of the subchondral bone appeared roughened.

The biochemical composition changed markedly as the disc underwent ageing/degeneration. The water content of the NP from the Grade 4 discs was significantly less than that of the Grade 1 discs ($p = 0.038$). There was no significant difference in the water content of the other disc tissues, although in the case of the AF, the difference in water content approached significance ($p = 0.061$). As illustrated in Figure 4, the proteoglycan

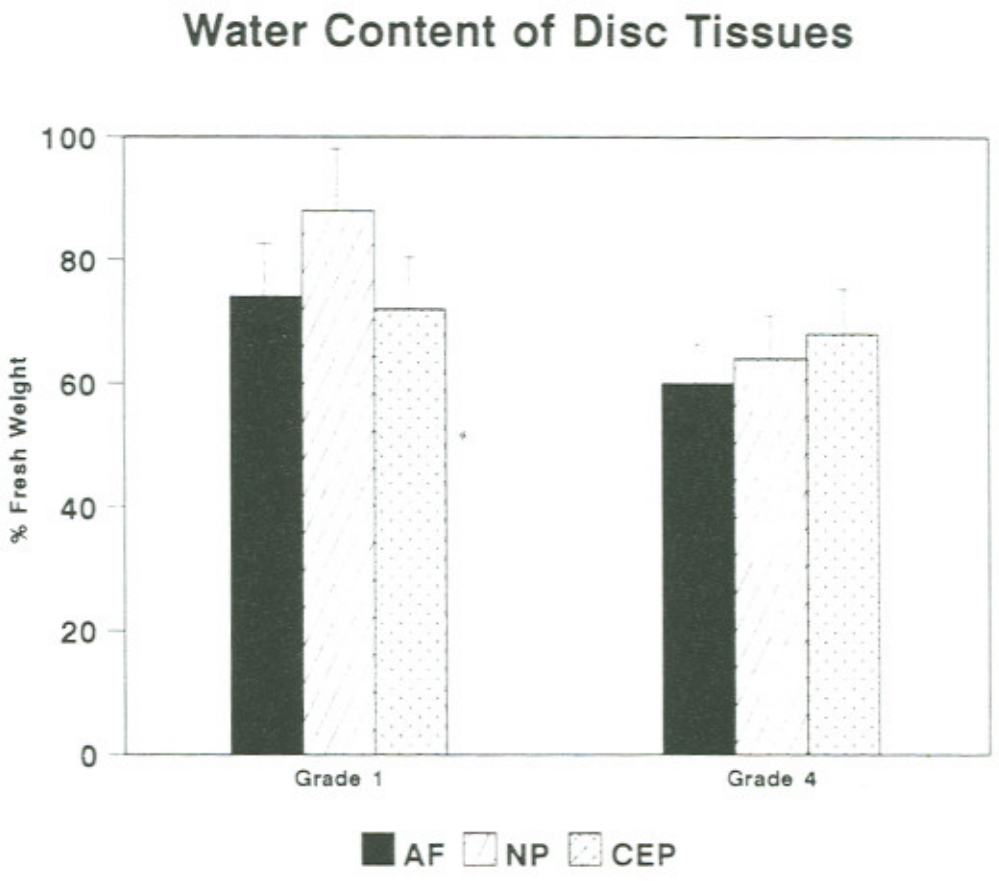

Figure 3. Water content of intervertebral disc tissues. The water content is expressed as a percentage of the fresh tissue weight. All values are averages of data obtained from three spines. For the purposes of statistical analysis, discs from healthy young spines were assigned to group I, while disc tissues from spines having undergone degeneration and age-related (Grades 4/5) changes were assigned to group II.

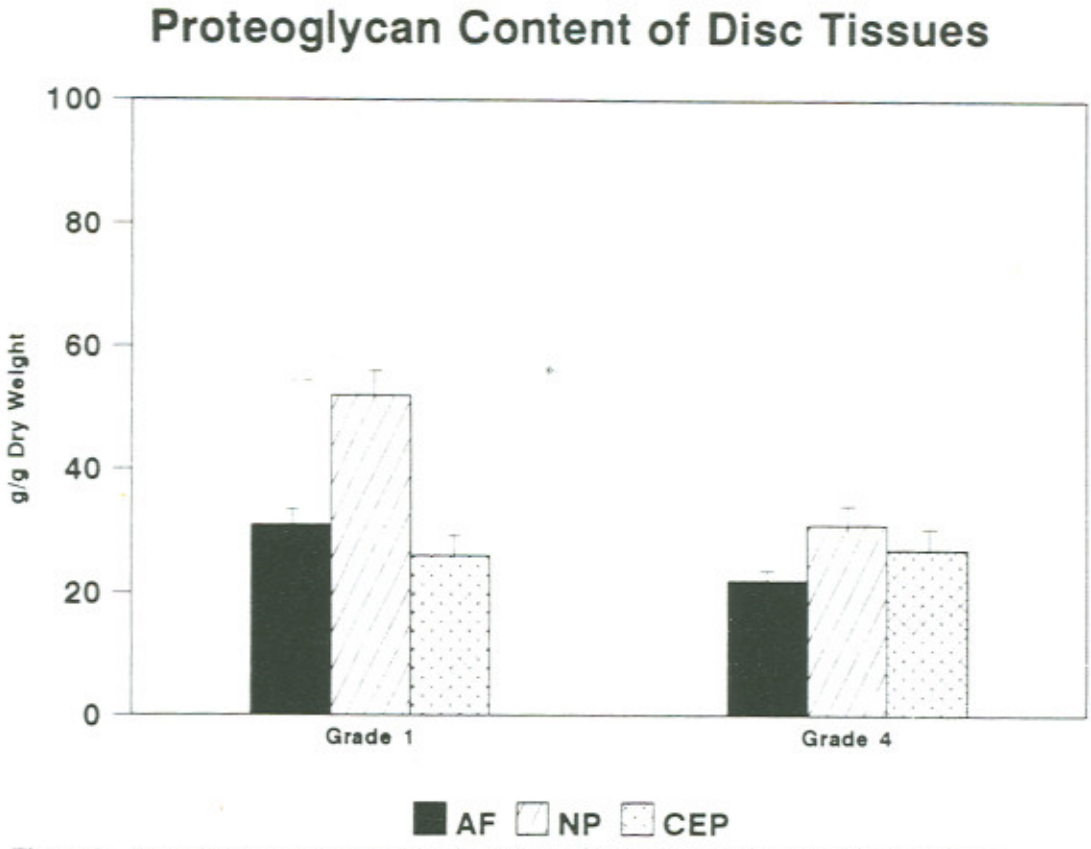

Figure 4. Proteoglycan content of intervertebral disc tissues. The proteoglycan content is expressed as g/g dry tissue weight.

Table 2
Unpaired t-test statistical analysis comparing water content

Tissue	Parameter	Comparison	D.F.	P
NP	Water Content	Between groups	1	0.038
AF	Water Content	Between groups	1	0.061
CEP	Water Content	Between groups	1	0.447

Nucleus pulposus (NP); Anulus fibrosus (AF); Cartilaginous end-plate (CEP).

Table 3
Unpaired t-test statistical analysis comparing proteoglycan content

Tissue	Parameter	Comparison	D.F.	P
NP	Proteoglycan Content	Between groups	1	0.031
AF	Proteoglycan Content	Between groups	1	0.044
CEP	Proteoglycan Content	Between groups	1	0.662

Nucleus pulposus (NP); Anulus fibrosus (AF); Cartilaginous end-plate (CEP).

content of the NP and AF was significantly less in the Grade 4/5 disc ($p = 0.044$ and 0.031, respectively). The greatest loss of proteoglycan associated with ageing/degeneration occurred in the NP (average of 40% decrease), while the AF proteoglycan content was approximately 30% less than that present in healthy discs from young donors. The CEP proteoglycan content did not change significantly in the two groups of discs studied.

Discussion

The potential that MR imaging may demonstrate clinically important degenerative changes in the disc, underscores the importance in understanding the structural and chemical basis for the image. The results of this investigation show that the marked changes in gross morphology that take place as the disc undergoes degenerative change, correlate with specific alterations in the MR image. A horizontal band of decreased signal intensity that bisects the NP and inner AF region (i.e. the "hamburger effect") is consistently present in discs having undergone advanced degeneration. This finding appears to correlate with the infiltration of mucinous material and the development of fissures and clefts in this region of the gross specimens. The MR image also identifies the loss of clear demarcation between the AF and NP that is evident at the gross morphological level. Finally, the irregularities of the CEP and the subchondral bone that accompany the morphogical changes seen with advanced degeneration can be readily seen in the MR image.

The relationship between the MR image and the biochemical properties of the disc has been previously investigated.[5,9] The loss of MR signal intensity seen with advanced degeneration cannot be attributed solely to a decrease in water content, as it has been demonstrated elsewhere that the magnitude of the change in signal intensity is far greater than the magnitude of the change in water concentration.[16] In addition, other studies have reported that the loss of MR signal correlates with both a decrease in NP proteoglycan content[17] and an increase in collagen content.[5] The results of this study show that the loss of MR signal intensity in degenerate discs is accompanied by a significant decrease in water content in the NP and possibly in the AF, but not in the CEP of degenerate discs. Similarily, the proteoglycan content of the AF and NP decrease with degeneration, while the CEP proteoglycan content remains unchanged. These results suggest that while degeneration is associated with a decrease in the absolute amount of CEP tissue, the biochemical composition of the remaining tissue is likely not affected to any great degree. Thus, the changes seen in MR image with advanced degeneration appear to be related chiefly with biochemical changes localized to the NP and AF. The exact nature of these changes is difficult to assess since the decrease in MR signal intensity is most pronounced in one region of the disc (i.e. nucleus and inner anulus); whereas the biochemical analysis reported here for each lumbar spine was derived from a pool of the individual tissues from all of the discs in that spine. The morphological features of this area suggest that it may be particularly susceptible to fissure and cleft formation. A more detailed characterization of the biochemical composition of this low MR signal intensity region of the disc at varying stages of degeneration, is currently underway.

The ability to detect and define the early stages of intervertebral disc degeneration may provide clinicians with an opportunity to fundamentally change their approach to the treatment of discogenic back pain. The majority of diagnostic imaging techniques currently in use at best, identify generalized changes in the disc that represent the end stages of the degenerative process. In addition, they have poor predictive value in separating normal individuals from those complaining of low back disorders.[18,19,20,21] MR imaging however, shows promise in being able to identify specific changes taking place in the disc that appear to occur near the onset of the degenerative process. With early identification comes the basis for developing therapeutic interventions designed to arrest the degenerative process in its early stages.

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References

1 Fullerton GD. Magnetic Resonance Imaging. St. Louis: CV Mosby, 1988:36–55.
2 Haughton VM. MR imaging of the spine. Radiology 1988; 166:297–301.
3 Chaftez NI, Genant HK, Moon KL, Helms CA, Morris JM. Recognition of lumbar disc herniation with NMR. Am J Radiol 1983; 141:1153–1156.
4 McCarthy S, Scott G, Majumdar S, Shapiro B, Thompson S, Lnage R, Gore J. Uterine junctional zone: MR study of water content and relaxation properties. Radiology 1989; 171:241–243.
5 Weidenbaum M, Foster RJ, Best BA, Saed-Nejad F, Nickoloff E, Newhouse J, Ratcliffe A, Mow VC. Correlating magnetic resonance imaging with the biochemical content of the normal human intervertebral disc. J Orthop Res 1992; 10:552–6561.
6 Thompson JP, Pearce RH, Schechter MT, Adams ME, Tsang IKY, Bishop PB. Preliminary evaluation of a scheme for grading the gross morphology of the human intervertebral disc. Spine 1990; 15:411–415.
7 Bishop PB. Proteoglycans and degenerative spondylosis. J Manipulative Physiol Ther 1988; 11:36–40.
8 Pearce RH, Grimmer BJ, Adams ME. Degeneration and the chemical composition of the human lumbar intervertebral disc. J Orthop Res 1987; 5:198–207.
9 Bishop PB, Pearce RH. The proteoglycans of the cartilaginous end-plate of the human intervertebral disc change after maturity. J Orthop Res, in press.
10 Eyre D. Biochemistry of the intervertebral disc. Int Rev Connect Tissues Res 1979; 8:227.
11 Eyring EJ. The biochemistry and physiology of the intervertebral disc. Clin Orthop 1969; 67:16–28.

12 Bitter T, Muir HM. A modified uronic acid carbazole reaction. Annal Biochem 1961, 93:440–447.
13 Hardingham TE, Muir HM. The specific interactions of hyaluronic acid with cartilage proteoglycans. Biochem Biophys Acta 1972; 279:401.
14 Hascall VC, Kimura JH. Proteoglycans. Isolation and characterization. In: Structural and contractile proteins. Part A. Extracellular Matrix, pp 769–800. Ed by Cunningham LW and Frederiksen DW. Methods in Enzymology, vol 82. New York: Academic Press: 1982.
15 Heinegard D. Extraction, fractionation and characterization of proteoglycans from bovine tracheal cartilage. Biochem Biophys Acta 1972; 285:181.
16 Modic MT, Masaryk TJ, Ross JS, Carter JR. Imaging of degenerative disk disease. Radiology 1988; 168:177–186.
17 Pearce RH, Thompson JP, Bebault GM, Flak B. Magnetic resonance imaging reflects the chemical changes of ageing/degeneration in the human intervertebral disk. J Rheumatology 1991; (Supplement 27) 18:42–43.
18 Nachemson AL. Advances in low-back pain. Clin Orthop Rel Res 1985; 200:266–278.
19 Holt EP. The question of lumbar discography. J Bone Jt Surg 1968; 50:720–726.
20 Hitselberger WE, Witten RM. Abnormal myelograms in asymptomatic patients. J Neuro 1968; 28:204–206.
21 Wiesel SW, Tsourmas N, Fetter HC, Citrin CM, Patronas N. A study of computer-assisted tomography 1. The incidence of positive cat scans in an asymptomatic group of patients. Spine 1984; 9:549–555.