Jaakko Niinimäki

DIFFUSION-WEIGHTED MRI AND DELAYED CONTRAST ENHANCEMENT OF DEGENERATED INTERVERTEBRAL DISC
DIFFUSION-WEIGHTED MRI AND DELAYED CONTRAST ENHANCEMENT OF DEGENERATED INTERVERTEBRAL DISC

Academic dissertation to be presented with the assent of the Faculty of Medicine of the University of Oulu for public defence in Auditorium 7 of Oulu University Hospital, on 11 September 2009, at 12 noon

OULUN YLIOPISTO, OULU 2009
Abstract

Magnetic resonance imaging (MRI) provides methods to study the microstructure and functional properties of tissues that can be utilized to acquire information about the degenerative processes in the spine. The purpose of the current study was to evaluate the value of diffusion-weighted MRI and quantification of delayed gadolinium enhancement in assessing intervertebral disc degeneration.

An experimental degeneration model was used to evaluate the sensitivity of diffusion-weighted MRI and T2 relaxation time measurements in detecting early degenerative changes in the disc. In six pigs, an annular disc lesion was induced surgically, after which the discs were repeatedly MR imaged for up to eight weeks. T2 relaxation time of the lesioned discs decreased postoperatively, whereas apparent diffusion coefficient (ADC) initially increased, but at eight weeks decreased when compared to the control discs.

The value of ADC in degeneration of human discs was evaluated by imaging 228 voluntary middle-aged men. ADC values of the three lowest lumbar intervertebral discs were measured and disc degeneration was visually graded. The reduction in ADC between visually normal and moderately degenerated discs was 4%, whereas severely degenerated discs showed 5% higher ADC values than normal discs. T2 signal intensity of the discs was significantly correlated with the ADC values. Because of a considerable overlap between ADC values of normal and degenerated discs the clinical relevance of the ADC measurements of lumbar intervertebral discs remains questionable.

A method to quantify delayed enhancement of the nucleus pulposus after intravenous gadolinium contrast agent injection was developed to evaluate the diffusion of small solutes into the disc. Twenty male volunteers were imaged in order to correlate the measured change in the T1 relaxation rate with visually evaluated degenerative changes. The percentual change of T1 relaxation rate for individual discs was up to 126%, and a positive trend was observed between the delayed enhancement and the disc degeneration grades.

In order to study the factors that determine the intensity of delayed enhancement, T1 relaxation rate measurements were further correlated with lumbar artery stenosis, bone marrow changes adjacent to endplates, endplate defects, and ADC of the disc. Lumbar artery stenosis and ADC values of the discs were not correlated with enhancement, while disc space narrowing and the presence of degenerative endplate changes had a strong correlation, suggesting an important role for the endplate in maintaining the integrity of the disc.

Keywords: diffusion-weighted MRI, disc degeneration, gd-DTPA enhanced MRI, intervertebral disc, magnetic resonance angiography, magnetic resonance imaging
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Jaakko Niinimäki
### Abbreviations

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tr>
<td>ADC</td>
<td>apparent diffusion coefficient</td>
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<tr>
<td>AF</td>
<td>annulus fibrosus</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>ceMRA</td>
<td>contrast-enhanced magnetic resonance angiography</td>
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<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
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<td>CT</td>
<td>computed tomography</td>
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<tr>
<td>dGEMRIC</td>
<td>delayed gadolinium enhanced MRI of cartilage</td>
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<td>DSA</td>
<td>digital subtraction angiography</td>
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<tr>
<td>DW</td>
<td>diffusion-weighted</td>
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<tr>
<td>DW-EPI</td>
<td>diffusion-weighted echo-planar imaging</td>
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<td>DWI</td>
<td>diffusion-weighted imaging</td>
</tr>
<tr>
<td>effTE</td>
<td>effective echo time</td>
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<tr>
<td>FDG</td>
<td>$[^{18}F]2$-fluoro-2-deoxy-D-glucose</td>
</tr>
<tr>
<td>FLAIR</td>
<td>fluid attenuated inversion recovery</td>
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<tr>
<td>FOV</td>
<td>field of view</td>
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<td>frFSE</td>
<td>fast recovery fast spin echo</td>
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<tr>
<td>FSE</td>
<td>fast spin echo</td>
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<tr>
<td>FSPGR</td>
<td>fast spoiled gradient echo</td>
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<tr>
<td>FT</td>
<td>full-thickness lesion</td>
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<td>Gd</td>
<td>gadolinium</td>
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<td>GAG</td>
<td>glycosaminoglycan</td>
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<tr>
<td>HIZ</td>
<td>high intensity zone</td>
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<tr>
<td>HSD</td>
<td>honestly significant difference</td>
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<tr>
<td>IDD</td>
<td>intervertebral disc degeneration</td>
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<td>L</td>
<td>lumbar disc level</td>
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<td>LBP</td>
<td>low back pain</td>
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<td>MR</td>
<td>magnetic resonance</td>
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<td>MRA</td>
<td>magnetic resonance angiography</td>
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<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>N</td>
<td>number of patients</td>
</tr>
<tr>
<td>NEX</td>
<td>number of excitations</td>
</tr>
<tr>
<td>NP</td>
<td>nucleus pulposus</td>
</tr>
<tr>
<td>NSF</td>
<td>nephrogenic systemic fibrosis</td>
</tr>
<tr>
<td>P</td>
<td>statistical significance</td>
</tr>
<tr>
<td>R1</td>
<td>longitudinal relaxation rate, $1/T_1$</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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<tr>
<td>ROI</td>
<td>region of interest</td>
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<td>S</td>
<td>sacrum</td>
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<td>SD</td>
<td>standard deviation</td>
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<td>SE</td>
<td>spin echo</td>
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<tr>
<td>SL</td>
<td>superficial lesion</td>
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<tr>
<td>SNR</td>
<td>signal-to-noise ratio</td>
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<tr>
<td>T</td>
<td>tesla</td>
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<tr>
<td>T1</td>
<td>longitudinal relaxation time</td>
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<tr>
<td>T2</td>
<td>transverse relaxation time</td>
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<tr>
<td>TE</td>
<td>echo time</td>
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<tr>
<td>TI</td>
<td>inversion time</td>
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<tr>
<td>TR</td>
<td>repetition time</td>
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List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals.


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1 Introduction

Lumbar back pain (LBP) is a very common problem. The Health 2000 Survey in Finland reported the life-time cumulative occurrence of back pain among 18- to over 80-year-old respondents to be 76.7% among men and 75.8% among women. The prevalence of reported back pain during the past month was 28.2% in men and 33.1% in women, and chronic low-back syndrome was clinically diagnosed in 11% of the participants over 30 years of age (Heistaro et al. 2007). LBP is a benign condition that typically heals in a few weeks. In a systematic review, 82% of individuals who were initially off work due to LBP returned to work in one month (Pengel et al. 2003). However, there is still a high probability that LBP becomes chronic (Airaksinen et al. 1999). Lumbar spine problems also have a huge socioeconomic impact. In Finland, the estimated costs caused by back pain in 2005 due to surgery, drugs, physiotherapy, rehabilitation, sick leave and disability came to nearly 500 million euros (Pohjolainen & Seitsalo 2006).

LBP often originates from an intervertebral disc (Vanharanta 1999). Radial tears from the centre of the disc (nucleus pulposus) to the capsular rim (annulus fibrosus) are often painful and seem to precede intervertebral degeneration (Boos et al. 2002). However, radiological findings of lumbar intervertebral degeneration may not be associated with pain (Boden et al. 1990) and their importance is often overemphasized. For this reason, more sophisticated imaging methods need to be evaluated and developed to better understand the association of spinal imaging findings with clinical symptomatology (Haughton 2004). Quantitative and functional magnetic resonance imaging (MRI) could add information to anatomic imaging, constituting an anticipated imaging method of this kind.

Quantification of MR properties of tissues, such as T1 and T2 relaxation times and apparent diffusion coefficient (ADC), can be used in vivo to acquire quantitative data related to the biochemical composition and microstructure of the disc. Similarly, tracking the transport of gadolinium contrast agent from the circulation to the disc yields functional information about the nutritional pathway of the disc. These methods could provide us new ways to study back problems (Boos et al. 1997, Antoniou et al. 2004, Urban & Winlove 2007) and could bring us closer to the primary mechanisms of LBP.

The purpose of this study was, first, to study the early changes of intervertebral disc degeneration using quantitative magnetic resonance (MR) methods (ADC and T2 relaxation time measurements) in an experimental stab wound model. Second, the aim was to study the association between ADC in...
lumbar discs and visually evaluated degeneration in adults, and third, to develop a method to quantify the delayed enhancement of the nucleus pulposus in vivo using a gadolinium-based contrast agent and T1 relaxation time measurements and to correlate it with visual disc degeneration. Finally, the aim was to study the nutritional pathway of the disc by evaluating the enhancement of the disc in comparison to lumbar artery stenosis, endplate changes and diffusivity of water in the disc.
2 Review of the literature

2.1 Anatomy of the lumbar spine

The spinal column is formed embryonally from the central notochord and surrounding mesenchyme that begins to segment at around the fifth to sixth gestational week. Primitive vertebral segments are divided by sclerotomic fissures around which the intervertebral discs are formed. The central nucleus of the disc is formed from the notochord and outer annular structures from the mesenchyme. Vertebral bodies are formed by fusion of the upper and lower parts of two primitive segments (Keyes & Compere 1932, Epstein 1969, Gray et al. 1973). The five lumbar vertebrae differ from the thoracic and cervical vertebrae. They are larger and heavier and they lack costal facets (Gray et al. 1973). The vertebral bodies are united by anterior and posterior longitudinal ligaments and by intervertebral discs (Figure 1) forming joints classed as symphyses (fibrocartilagenous joint). Vertebral arches are connected by several ligaments and synovial apophyseal joints (Coventry et al. 1945a, Gray et al. 1973).

Fig. 1. Simplified illustration of one lumbar discovertebral segment.
2.1.1 Anatomy of the intervertebral disc

The intervertebral discs are the chief connections between vertebral bodies. Each disc consists of a gel-like inner core, the nucleus pulposus (NP), which is enveloped by an outer fibrous laminated portion, the annulus fibrosus (AF) (Figure 2) (Gray et al. 1973, Gan et al. 2003). The arrangement of the fibres increases towards the periphery of the disc, where they pass obliquely in spherical fashion from one endplate to the other (Coventry et al. 1945a) forming 15 to 25 distinct lamellae (Cassidy et al. 1989). The lamellae are thinner in the outer annulus fibrosus and there are fewer lamellae in the posterior AF than in the lateral or anterior margins (Marchand & Ahmed 1990). Collagen fibres of the lamellae are inclined with respect to the vertical axis. From the edge of the disc inward to the nucleus, this interlamellar angle decreases from 62° to 45° (Cassidy et al. 1989). The nucleus pulposus contains randomly organized collagen fibres that are embedded in highly hydrated gel (Eyre & Muir 1976, Inoue 1981). The boundary between the annulus fibrosus and the nucleus pulposus is very distinct in children, but in adults the boundary becomes a less obvious transitional zone (Coventry et al. 1945a, Gray et al. 1973).

Fig. 2. Illustration of the structure of the intervertebral disc.
2.1.2 Biochemical properties of the intervertebral disc

The main macromolecular components of the disc are collagen fibres that offer tensile strength and proteoglycan aggregates that resist compressive forces (Figure 3) (Roughley 2004, Raj 2008). About 70% of the dry weight of the AF is composed of collagens and 5% of proteoglycans. The corresponding proportions in the NP are 20% and 15% (Eyre & Muir 1976, Eyre & Muir 1977). Interwoven type I and II collagens provide the bulk of the tissue fabric; however, collagen phenotypes V, VI, IX, XI, XII and XIV also co-exists (Eyre et al. 2002). The most abundant proteoglycan in the disc is aggrecan with numerous glycosaminoglycans (GAG) (chondroitin sulphate and keratin sulphate chains) bound to the same core protein (Bushell et al. 1977, Antoniou et al. 1996). GAGs are negatively charged and they bind water, giving discs their hydrostatic pressure. The water content of NP is 70–80% and that of AF 65% (Eyre 1979). Aggrecans bind to hyaluronan, forming huge aggregates, and these lead to the hydrated gel-like structure in the disc that is resistive to compressive forces. The cells of the disc are mainly fibrocartilage-type chondrocytes, sometimes situated in a capsule within the matrix. The cells are highly vacuolated and these vesicles are also filled with proteoglycan aggrecans (Gan et al. 2003). Maximum cell density in the disc seems to be regulated by nutritional constraints, the centre of the disc being the most hypocellular (Horner & Urban 2001).

Fig. 3. A line drawing of the biochemical composition of the disc.
2.1.3 Anatomy of the endplate

The endplates are at the cranial and caudal ends of each disc. They separate the vertebral bone from the disc itself and prevent the highly hydrated nucleus from bulging into the adjacent vertebrae (Coventry et al. 1945a, Moore 2000, Moore 2006). The endplate contains both hyaline cartilage and fibrocartilage; the latter borders the endplate close to the nucleus pulposus and is not always considered part of the endplate. The amount of fibrocartilage in the endplate increases with age (Standring & Gray 2008). Cartilage endplate occupies the central 90% of the interface between the disc and the vertebral body and is surrounded by a ring of bone that forms via the epiphysis fusing with the vertebral body. The cartilage of the endplates locks into the osseous vertebral endplates via the calcified cartilage, with few, if any, collagen fibres crossing the boundary, whereas the annulus fibrosus anchors directly into the adjacent vertebral bone via collagen fibres (Inoue 1981, Roberts et al. 1989, Roberts et al. 2006). The cartilage endplate functions in early life as a growth plate for the adjacent vertebral body and at birth, the endplates have large vascular channels running through them. Soon after birth, these channels fill in with extracellular matrix and almost disappear by the age of ten. During maturation the endplates ossify and decrease in thickness and diameter. In adults the endplate is usually less than 1 mm thick (Coventry et al. 1945b, Boos et al. 2002, Roberts et al. 2006). Mechanically, the centre of the endplate is weakest (Grant et al. 2001).

2.2 Nutrition of the intervertebral disc

After the vascular channels in the cartilaginous endplate disappear by the end of childhood, the intervertebral disc becomes the largest avascular tissue in the human body. Due to the avascular nature of the intervertebral disc nutrients move from the capillaries that supply the vertebra to the cells of the nucleus pulposus primarily by diffusion (Urban et al. 1977, Urban et al. 1978, Urban et al. 2004). The disc cells obtain their energy through glycolysis, which requires both glucose and oxygen.

2.2.1 Vascular supply of the intervertebral disc

Healthy intervertebral disc of an adult human is avascular apart from the outermost regions of the annulus fibrosus. In normal discs, capillaries are found
only at the margin of the annulus fibrosus and in the vertebral marrow spaces (Maroudas et al. 1975). This capillary network is responsible for the supply of nutrients and the removal of wastes. The arterial supply of the intervertebral disc is derived from the abdominal aorta. Almost invariably, four artery pairs leaving the aorta supply blood to the first four lumbar bodies whereas the fifth lumbar-segment is supplied by the middle sacral artery and branches of the iliolumbar arteries. Each artery forms two sets of branches. The first set penetrates vascular foramina of the vertebral body and forms vertebral capillaries that penetrate the subchondral plate to terminate in loops at the bone-cartilage endplate junction (Figure 1), and the second forms fine vertical networks on the surfaces of anterior longitudinal ligament and discs (Ratcliffe 1982, Kauppila 1994, Gilchrist et al. 2002, Grunhagen et al. 2006). The main route of nutrient supply into the nucleus is via the vertebral capillaries through the endplate (Holm et al. 1981).

### 2.2.2 Diffusion

Since the intervertebral disc is an avascular structure, the transfer of nutrients to disc cells and the removal of waste products rely upon diffusion. Diffusion is movement of unbound particles and is caused by random displacements of molecules due to thermal motion (Brownian motion). The magnitude of diffusion is dependent on the temperature and possible thermal and concentration gradients. Self-diffusion, such as diffusion of free water molecules, can take place under equilibrium, whereas chemical diffusion occurs in the presence of chemical potential which results in the transport of molecules, such as nutrients or contrast agent, along the concentration gradient. In living tissue diffusion is always restricted by permeable or impermeable barriers and macromolecules (Hazlewood & Nicholson 1995, Le Bihan & Basser 1995, Buxton 2002). In the intervertebral disc these barriers are made up by cell boundaries, proteoglycan aggregates and collagen fibrils.

### 2.2.3 Metabolism

The main energy supply of disc cells is provided by glycolysis. Disc cells cannot survive long without glucose; however, oxygen is not necessary for cell viability (Horner & Urban 2001). At low oxygen levels the production of macromolecules, such as sulphated glycosaminoglycans, is severely inhibited, which may increase the rate of matrix breakdown and cell death, eventually leading to the changes we
20
recognize as disc degeneration (Urban et al. 1977, Grunhagen et al. 2006). Glucose consumption leads to the production of lactic acid. Accumulation of lactic acid in the disc causes a decrease in extracellular pH levels, which leads first to a decrease in energy consumption and matrix production of cells, and later, if pH decreases further (pH < 6.3), cells begin to die (Horner & Urban 2001, Grunhagen et al. 2006).

2.3 Intervertebral disc degeneration (IDD)

Deterioration of the osseous and soft tissue structures of the spine is a normal consequence of the ageing process and can be predisposed to or accelerated by a variety of developmental and acquired factors (Coventry et al. 1945b, Modic 1999). Degeneration of the intervertebral disc begins early; by the end of the second decade of life unequivocal findings of tissue degradation can be observed in the disc. Clefts and radial tears are already seen in the disc in the age group from 11 to 16 years (Coventry et al. 1945b, Boos et al. 2002). Almost all signs associated with disc degeneration, such as loss of disc height, annular tears, marginal osteophytes, disc bulging, and endplate irregularities are common in adults (Battie et al. 2004, Battie & Videman 2006).

2.3.1 Aetiological factors

Traditionally, disc degeneration has been associated with biomechanical factors causing structural disruption and cell-mediated changes in disc composition (Kimura et al. 2001, Battie et al. 2004, Liang et al. 2008). The biomechanical theory is supported by the higher prevalence of degenerative changes in the spine at L4-S1 levels carrying most of the load (Jensen et al. 1994). It has also been suggested that even minor damage to a vertebral body endplate may lead to progressive structural changes in the adjacent intervertebral discs (Adams et al. 2000). The association between degeneration and trauma (Kerttula et al. 2000b) may be due to injuries to the endplate, supporting the biomechanical theory. However, biomechanical factors seem to explain only a small part of disc degeneration. In studies of twin pairs mechanical loading beyond normal daily activities explained only 2% of IDD (Battie et al. 1995a, Battie et al. 1995b).

Disc degeneration may also be explained by nutritional factors, as the vitality of disc cells is dependent on the diffusion of oxygen and glucose into the disc (Urban et al. 1977, Grunhagen et al. 2006). An association between nutrient
supply of the disc and degeneration has been inferred by the temporal order of changes; vascular changes in lumbar arteries seem to occur before degeneration of the disc in the lumbar spine and they seem to be associated with LBP (Kauppila 1997, Kauppila et al. 1997). The difference in the prevalence of degenerative changes between sexes has been explained by the same theories. Degenerative changes are more common in men than in women, possibly due to higher mechanical stress and longer nutritional pathways (Miller et al. 1988).

In addition to biomechanical and nutritional causes there seems to be a strong genetic predisposition to IDD. Heritability studies in twins have shown that genetic factors explain up to 75% of lumbar disc degeneration (Battie et al. 1995a, Battie et al. 1995b, Sambrook et al. 1999, Battie et al. 2004), which has motivated the search for degeneration genes. In recent studies, several candidate genes have been linked to disc degeneration. At present, COL9A2 (Annunen et al. 1999) has been most extensively studied although several other genes, such as the vitamin D receptor and matrix metalloproteinase-3 genes, are known to be associated with lumbar disc disease (Zhang et al. 2008). Unfortunately, the biological mechanisms of IDD are still largely unknown.

**2.3.2 Morphological and biochemical changes**

There are large changes in the appearance of the adult disc with increasing age; the nucleus becomes less hydrated and more collagenous. It becomes discoloured, changing from white to yellow-brown in colour (Urban et al. 2000). With age, the boundary between annulus and nucleus becomes increasingly blurred and the annular rings thicken and appear more disorganized (Coventry et al. 1945b). Moreover, changes in disc degeneration include some or all of the following: real or apparent desiccation, fibrosis, narrowing of the disc space, diffuse bulging of the annulus beyond the disc space, extensive fissuring (i.e., numerous annular tears) and mucinous degeneration of the annulus, defects and sclerosis of the endplates, and osteophytes at the vertebral apophyses (Modic & Ross 2007). The matrix of the intervertebral disc is under constant change: during the growth phase matrix molecules are synthesized and type II collagen is actively denaturated, during the maturation and ageing phase both synthetic activity and denaturation are decreased, and during the degeneration phase synthesis of type I procollagen and denaturation of type II is increased (Antoniou et al. 1996). Biochemically, disc degeneration starts with proteoglycan degradation as the ratio of proteoglycan aggregates relative to total proteoglycan within the disc decreases...
with age (Johnstone & Bayliss 1995), leading to reduced capacity of NP to bind water (Antoniou et al. 1996).

2.3.3 Degenerative disc disease

Degenerative disc disease can be defined as a clinical syndrome characterized by manifestations of disc degeneration and symptoms thought to be related to these changes (Fardon 2001, Fardon & Milette 2001, Adams & Roughley 2006). Lumbar back pain is a very common problem and strongly associated with radiological signs of intervertebral disc degeneration (Modic 1999, Vanharanta 1999, Luoma et al. 2000). Around 75% of Finnish people suffer from LBP during their lifetime and one in every three Finns has had back pain during the past month (Heistaro et al. 2007). However, disc degeneration is not synonymous with lumbar back pain since degenerative morphological changes in discs are also very common in people without back pain (Jensen et al. 1994, Battie & Videman 2006). Although the term “spondylosis deformans” has been used for changes in the disc associated with a normal ageing process and the term “intervertebral osteochondrosis” for changes that are possibly the consequences of a clearly pathological process (Fardon & Milette 2001), at present there are no clear markers, either morphological or biochemical, distinguishing ageing from pathological changes in the disc (Antoniou et al. 1996, Urban et al. 2000).

Experimental animal models of disc degeneration

Intervertebral disc degeneration animal models are used to provide basic scientific data that help establish biologic plausibility and can address temporality, specificity, and dose-response issues (Lotz 2004). Experimental animal models can be classified as mechanical (alteration of the magnitude or distribution of forces on the normal joint) or structural (injury or chemical alteration). A commonly used structural model of intervertebral disc degeneration is surgical lesion to the annulus fibrosus (Osti et al. 1990) or endplate (Holm et al. 2004). The annular lesion is thought to initiate a degenerative cascade by causing acute herniation and subsequent nuclear depressurization. Shortly after trauma there is a precipitous loss of proteoglycan and water content that is partially recovered within the first couple of weeks. However, there is a subsequent, gradual loss over time that leads to nuclear fibrous replacement due to metaplasia of annular cells into chondrocyte-like cells and increased collagen production (Kääpä et al. 1994a, 1994b, 1995).
Kääpä et al. 1994b, Kääpä et al. 1995). Typically, biochemical reference is used to evaluate the degeneration, but also imaging methods such as MRI can be used to obtain information about the degenerative process before killing the animals (Lappalainen et al. 2002).

2.4 Conventional imaging in intervertebral disc degeneration

2.4.1 Radiography

Wilhelm Conrad Röntgen’s discovery of “New Kind Of Rays” in 1895 made plain radiographs of spine possible (Hesselink 1988). Conventional plain radiography, with or without flexion and extension images, has long been the primary imaging modality of the lumbosacral spine despite the availability of modern imaging methods (Kormano 1989). Although plain radiography depicts bony changes accurately, its value in diagnosing intradiscal changes is limited, showing only disc space narrowing and calcifications and gas in the disc (Deyo et al. 1990), all late manifestations of degenerative disease. However, standing full-spine radiographs are used increasingly for evaluation of the sagittal balance of the spine, especially in planning of corrective spinal surgery due to degenerative changes in the lumbar spine (Vialle et al. 2005, Barrey et al. 2007, Lund 2007).

Myelography

Lumbar myelography is a radiographic examination of the lumbar spinal canal with intrathecal injection of contrast medium. Myelography with radio-opaque contrast media was discovered accidentally in 1922, when Sicard and Forestier inadvertently introduced iodized poppy-seed oil (Lipiodol) into the subarachnoid space (Hesselink 1988). Before the advent of computed tomography (CT) imaging it was the only imaging method showing the compromising effect of bony and soft tissue changes in the spinal channel, and even today it is used to show those changes in standing posture. When compared to previous-generation CT-technology, the sensitivity of myelography exceeded that of CT (82% vs. 73%) in diagnosing lumbar disc herniation, but its specificity was lower (67% vs. 77%) (Schipper et al. 1987). CT and myelography can be combined (CT-myelography) and three-dimensional myelographic images can also be obtained with flat panel detector-equipped angiographic devices (Kardaun et al. 1989,
Bartynski & Lin 2003). CT-myelography can be performed under axial loading with special devices (Willén et al. 1997); however, the effect of flexion and extension cannot be evaluated with such a device. Although MR imaging is a superb method in the search for degenerative disc disease and disc protrusion, conventional myelography is still a crucial supplemental examination that may be necessary to confirm degenerative root impingement in the lateral recess as the cause of radiculopathy (Bartynski & Lin 2003).

**Discography**

Lumbar discography, originally introduced by Lindblom in 1948 (Hesselink 1988), is a diagnostic procedure in which lumbar discs are punctured under imaging guidance for the instillation of contrast agent. The resulting discogram gives information about the degeneration of the NP and shows possible radial tears of AF. Because MRI is a sensitive method showing degenerative changes in the disc, discography is presently used mainly to determine whether a particular disc is painful and to show the presence of annular tears. Although the pain-evaluating feature of discography has been challenged (Carragee et al. 2000b, Carragee et al. 2006), authors of recent systematic reviews find it a useful imaging and pain evaluation tool in identifying a subset of patients with chronic spinal pain secondary to intervertebral disc disorders with test specificity of 0.94 and a false-positive rate of 6% (Buenaventura et al. 2007, Wolfer et al. 2008). It is nevertheless an invasive investigation and it poses a risk to patient for both LBP (Carragee et al. 2000a) and intradiscal infection at around 1:400 risk per patient (Willems et al. 2004). The use of discography should therefore be limited to a subset of patients who have persistent LBP but in whom non-invasive tests fail to provide sufficient diagnostic information and to patients in whom lumbar spinal fusion is being considered (Guyer & Ohnmeiss 1995).

### 2.4.2 Computed tomography

The introduction of computed tomography by Hounsfield in 1973 began a new chapter in spine imaging (Hesselink 1988). After development of whole body systems CT was soon able to provide high-resolution images with good soft-tissue contrast and it largely replaced invasive myelography as the method of choice to rule out or localize lumbar disc herniations (Hesselink 1988). When compared to MRI, which has even better soft-tissue contrast, in detection of
herniations they both have similar diagnostic performance, with a sensitivity of 88%–94% and a specificity of 57%–88% (Modic et al. 1984, Thornbury et al. 1993, van Rijn et al. 2006). However, in the evaluation of lumbar nerve root compression CT seems to be less reliable than MRI (van Rijn et al. 2006). Because CT-examinations have better availability and are less costly, it is justifiable to use CT instead of MRI at least in the setting of acute radicular pain (Thornbury et al. 1993). The main drawback of CT is the rather large radiation dose even though it can be reduced 35% with modern multi-detector CT-devices without affecting diagnostic performance of herniation diagnostics (Bohy et al. 2007).

### 2.4.3 Magnetic resonance imaging

Lauterbur and Mansfield shared the Nobel Prize in Physiology or Medicine in 2003 for their discoveries concerning magnetic resonance imaging (Riederer 2004). Lauterbur’s idea from 1973 was developed into a clinical imaging method which has since the early 1980s allowed specific determination of the nature of disc protrusions and other related degenerative soft tissue abnormalities in the spine (Modic et al. 1984, Edelman et al. 1985). Comparison with radiographs, high-resolution CT scans, and myelograms showed that MR was the most sensitive imaging method for identification of disc degeneration and disc space infection (Modic et al. 1984). Herniation, stenosis of the canal, and scarring can be identified as accurately with MR as with CT or myelography (Modic et al. 1984). Magnetic resonance offers the most complete evaluation of specific degenerative disorders including degenerative facet disease, spondylolysis, spondylolisthesis, spontaneous lumbar epidural haematomas, and juvenile discogenic disease (Gundry & Fritts 1997).

In Finland, MRI at 1.5 tesla is currently the most common radiological examination after plain radiography in imaging the degenerative spine, with more than 19,000 examinations performed each year (Tenkanen-Rautakoski 2006). The T1- and T2-weighted images in 3–4 mm sections in sagittal and axial plane still serve as fundamental sequences (Modic et al. 1984). Fast spin echo (FSE) images, when compared with spin echo (SE) imaging, provide superior image contrast and signal-to-noise (SNR) ratio at a reduced imaging time (Constable et al. 1992, Ross et al. 1993) and have therefore largely replaced spin echo sequences. However, the drawback of reduced imaging time in FSE imaging is “anomalously high signal intensity of fat” and blurring if long echo train lengths are used.
The value of T1-weighted images in depicting discogenic changes in the spine may possibly be further improved by using T1-weighted fluid-attenuated inversion recovery (FLAIR) sequence instead of T1-weighted FSE (Erdem et al. 2005). T2-weighted fat-suppressed images or post-contrast fat-suppressed T1-weighted images may yield additional information in problematic cases by detecting bone marrow oedema and showing changes in enhancement after injection of paramagnetic contrast agent due to inflammatory or infectious diseases (Mulholland & McCall 1996). Contrast enhancement is particularly important in interpretation following discectomy since it facilitates differential diagnosis between disc herniation and post-surgical fibrosis. However, contrast enhancement may not necessarily be beneficial following other procedures (Bradley 1999, Babar & Saifuddin 2002). Gradient-recalled echo techniques have also been proposed for spine imaging because of their fast imaging times and better spatial resolution (Murayama et al. 1990, Georgy & Hesselink 1994), but they have not replaced the spin echo-based techniques.

Field strength does not seem to have any effect on the sensitivity of detecting signal intensity loss of the disc. However, low-field imaging provides inferior spatial resolution to high-field imaging and therefore image quality below 0.04 tesla is inadequate in detecting structural changes (Tertti 1991). Low-field imaging already at 0.2 tesla field strength has been shown to have convincing reliability and repeatability in the evaluation of structural parameters of the lumbar spine (Solgaard Sorensen et al. 2006). Increasing field strength to 3 tesla increases SNR, which can be utilized to increase either spatial resolution or imaging speed. However, higher field strength also introduces challenges, such as increase in chemical shift, pulsatile flow, and susceptibility artefact and decrease in fluid contrast due to lengthened T1 relaxation times (Shapiro 2006).

Since the spine is the major weight-bearing organ in the body, MRI imaging in supine posture may not reveal dynamic changes of the spine that are only visible under the pressure of physiological body weight. This limitation of the MRI has been addressed by using axial loading devices (Willén et al. 1997) or upright MRI scanners (Niggemann et al. 2009), the latter also allowing imaging in flexion and extension positions.

**MRI findings in disc degeneration**

In magnetic resonance imaging, disc degeneration is manifested by T2-weighted signal intensity loss, disc space narrowing, presence of fissures, fluid, vacuum
changes and calcification within the intervertebral disc, marrow signal changes, osteophyotsis and disc herniation (Modic & Ross 2007). The hallmark of disc degeneration on MRI is T2-weighted signal intensity loss in nucleus pulposus due to desiccation of disc (Modic & Herfkens 1990, Schiebler et al. 1991, Mulholland & McCall 1996). Signal loss is also a more sensitive indicator of lumbar disc degeneration than loss of lumbar disc height (Luoma et al. 2001). In early MRI literature, the term “intranuclear cleft” was used to describe normal fibrous transformation of nucleus pulposus showing as a dark horizontal line in the disc (Schiebler et al. 1991). The presence of this line in normal discs of adults has been suggested to be useful in discriminating normal discs from pathologically bright discs due to inflammation (Aguila et al. 1985). In addition, true clefts in more degenerated discs have been found in histological studies (Boos et al. 2002). These voids are probably filled with fluid (Boos et al. 2002) or gas (vacuum phenomenon) in vivo (Berns et al. 1991). Although fluid in voids is easily depicted on MRI, calcifications and intradiscal gas can be difficult to differentiate (Berns et al. 1991).

Defects, or tears, of the annulus fibrosus can be classified as peripheral, circumferential or radiating (Osti et al. 1992). Discs that show annular defects on MRI also have a typically decreased signal on the T2-weighted images, and annular defects commonly associate with bulge or protrusion. Such defects are also frequently asymptomatic (Jensen et al. 1994) and therefore they are considered degenerative changes. However, the tears are clearly related to LBP, as the absence of tears is more common in asymptomatic individuals (Videman & Nurminen 2004). High-intensity zone (HIZ) is an area of high signal intensity on T2-weighted magnetic resonance images of the disc, which may reflect fissure or tear of the outer annulus (Fardon & Milette 2001). True tear can actually be shown in 90% of the discs with HIZ in discography (Aprill & Bogduk 1992). Although HIZs were originally considered to be strongly associated with pain, the clinical importance of HIZs is not that straightforward, as HIZs do not either change or improve spontaneously in a large proportion of cases over time, and there does not seem to be a statistical correlation between HIZ changes and change in a patient’s symptoms (Mitra et al. 2004).

Disc herniation occurs either due to volume loss in the NP or due to structural changes in the AF causing the displacement of disc material beyond the limits of the intervertebral space (Fardon 2001, Fardon & Milette 2001). Because the term herniation has been used variably by different professionals and individuals, the nomenclature for lumbar disc pathology has been standardized by a task force of
radiologists, neuroradiologists, orthopaedists and neurosurgeons and there are exact definitions and preferred terms for different types of herniations (Milette 2000, Fardon 2001, Fardon & Milette 2001). If the herniation comprises less than 25% of the disc circumference it is called “focal”, between 25 and 50% it is “broad-based”, and if more than that, it is called “disc bulge”. Herniations can be further divided into protrusions or extrusions based on the ratio of the diameter of the herniated material to the diameter of the base of the herniation; if the base of the herniation is “narrower” than the mass of herniated material in any plane (usually sagittal) the herniation is called extrusion, otherwise it is called protrusion (Milette 2000). If the extrusion is not connected to the disc, it is called sequestration. If the herniation of disc material occurs through the endplate into the vertebral body it is called intravertebral, more commonly known as Schmorl’s node (Resnick & Niwayama 1978, Park et al. 2007). In acute phase of Schmorl’s node there is increased T2-weighted and decreased T1-weighted signal in the vertebral bone marrow adjacent to the node, reflecting bone oedema around the endplate fracture (Park et al. 2007). Occasionally this enhancing bone marrow reaction may cover almost the entire vertebra (Stabler et al. 1997) and may therefore require differential diagnostic workup to rule out infection and neoplasms. In histologic examination enhancing bone marrow adjacent to Schmorl’s nodes has been shown to include fibrous granulation tissue and capillary proliferation (Stabler et al. 1997).

Bone marrow signal intensity changes adjacent to the endplates have also been observed without intravertebral herniations in the presence of degenerative disc disease (de Roos et al. 1987, Modic et al. 1988b, Kuisma et al. 2006). These so-called Modic changes have been classified as Type 1 (increased T2-weighted and decreased T1-weighted signal), Type 2 (increased T2-weighted and increased T1-weighted) or Type 3 (decreased T2-weighted and decreased T1-weighted signal). If the changes present signal characteristics of two types, they are called mixed types (Braithwaite et al. 1998). Type 1 changes are considered active changes whereas Type 2 and Type 3 changes are considered to be more stable. Although Type 1 changes typically convert to Type 2, conversion from Type 2 to Type 1 is also possible (Kuisma et al. 2006); this may indicate that there is a superimposed process, such as continued or accelerated degeneration or vertebral osteomyelitis (Modic 2007). Bony sclerosis has generally only been associated with type 3 changes (Modic & Ross 2007), but this may be due to the fact that low signal sclerosis is easily superimposed by fat signal on MRI. In CT-
densitometric measurements, an increase of bone density can be observed in all
types (Kuisma et al. 2008).

Osteophytes of the vertebral endplates are a less prominent finding on MRI
than in plain radiography or CT since they are primarily shown as signal voids on
MRI. Although a distinction has been made between claw osteophytes and
traction osteophytes, indicative of spinal instability, they may both coexist in a
single vertebral body and appear to represent different stages of the same
pathologic process (Pate et al. 1988). Osteophytes are also heritable in the lumbar
spine (Sambrook et al. 1999).

2.4.4 Contrast-enhanced MR angiography of lumbar arteries

Lumbar artery stenosis has been shown to associate with lumbar disc
degeneration (Kauppila et al. 1994, Kauppila et al. 1997), and MR angiography
(MRA) can be used to evaluate lumbar arteries in this setting (Kurunlahti et al.
2004). Although MR imaging can take advantage of spatial misregistration of a
signal due to flow in vessels to provide angiographic images without contrast
agents, the spatial resolution of contrast-enhanced MR angiography (ceMRA) is
superior to non-enhanced MRA techniques (Bosmans et al. 2001). With the
modern MR scanners and imaging sequences the diagnostic accuracy of ceMRA
is approaching that of CT angiography and digital subtraction angiography (DSA)
in the detection of arterial stenoses (Nael et al. 2007a, Nael et al. 2007b) and its
role in diagnostic imaging is therefore increasing. When the lumbar arteries are
imaged for research purposes the invasiveness of DSA and radiation dose of CT
angiography makes them less suitable for volunteer study population, and ceMRA
can thus be considered the gold standard in such imaging purposes.

2.4.5 Other imaging modalities

Nuclear imaging methods are used in spine imaging in detecting neoplastic and
inflammatory diseases (Hesselink 1988). Currently they do not have a role in
imaging degenerative changes in disc (Mulconrey et al. 2006), but in problematic
cases FDG positron emission tomography can be used to differentiate
degenerative endplate changes from infectious abnormalities (Stumpe et al. 2002).
Sonography can also depict the normal lumbar intervertebral disc and
degenerative changes in appropriate subjects (Tervonen et al. 1991, Kakitsubata
et al. 2005); however, it does not have a role in clinical spine imaging.
2.5 Quantitative imaging of the intervertebral disc

Although the current non-invasive imaging techniques in clinical use provide detailed anatomic information about the intervertebral disc, they are seldom able to distinguish incidental findings from symptomatic ones, or age-related degeneration from degenerative disc disease. At present there are no clear markers, either morphological or biochemical, distinguishing ageing from pathological changes in the disc (Antoniou et al. 1996, Urban et al. 2000). The challenge for modern spine imaging is to develop more sophisticated imaging methods to characterize the changes in disc that correlate with clinical symptoms (Haughton 2004).

MR imaging can also be used for observer-independent and quantitative analysis of images by calculation of different MR parameters. Quantitative MR measurements, such as T1 and T2 relaxation time and apparent diffusion coefficients, have been suggested to be more sensitive methods than conventional MR imaging (Boos et al. 1997, Antoniou et al. 2004, Antoniou et al. 2006) in imaging degenerative changes in disc and may therefore provide a way to improve our understanding of spine imaging and clinical symptomatology (Haughton 2004, Majumdar 2006).

2.5.1 T1, T2 and T1ρ relaxation time measurements

The flexibility of MRI stems from the variability of relaxation times of proton nuclei in anatomic tissues and from the variability of different relaxation mechanisms between and within tissues that can be measured. The contrast in MR images between tissues depends on these relaxation times and on operator-controlled parameters, such as repetition time (TR) and echo time (TE), that define the time intervals of radiofrequency pulses and signal acquisition from the tissue (Buxton 2002). If a series of images is collected with varying T1- or T2-weighting, the (T1 or T2) relaxation time constants can be calculated by exponential fitting on all pixels from the same anatomic location in the obtained images. Measurement results of relaxation times in intervertebral discs were reported as early as in 1984 by Modic and colleagues (Modic et al. 1984). The reproducibility of relaxation time measurements has also been reported to be good; intra- and interobserver variation in measurements is less than 10% (Boos & Boesch 1995). Measurements are typically performed from regions of interest, but also visual inspection of relaxation time maps that are calculated from pixel-by-
pixel measurements can be performed, which may be helpful in measuring relaxation times in different regions of the disc and in evaluating measurement artefacts (Chatani et al. 1993, Boos & Boesch 1995).

Spin-lattice relaxation in the rotating frame, which will relax with time constant $T_{1p}$, is a more recently invented relaxation mechanism that can be used to provide a contrast which is sensitive to very slow molecular motion. The spin-lattice relaxation occurs after the application of a spin-lock pulse after magnetization has been flipped into the transverse plane; the resulting $T_{1p}$-weighting provides a $T_2$-like contrast with the advantage of increased dynamic range and increased tissue contrasts based on variations in protein content (Wang et al. 2007). $T_{1p}$ relaxation time mapping has been used to map the distribution of proteoglycans in cartilage (Akella et al. 2001). The method is also applicable in the intervertebral disc. In recent studies a significant correlation has been reported between $T_{1p}$ and disc degeneration grade and sulfated glycosaminoglycan content in the NP (Auerbach et al. 2006, Johannessen et al. 2006).

2.5.2 Sodium MRI

Conventional MRI relies on imaging proton nuclei in water, but MRI can also be performed on physiologically more relevant sodium nuclei ($^{23}$Na). The advantage of sodium is that it is an abundant, physiologically relevant, and MR-observable cation that is highly sensitive to changes in GAG (Gray et al. 2008). Sodium-MRI has been applied to measure proteoglycan in articular cartilage (Reddy et al. 1998, Borthakur et al. 2002) and it can also be used to study proteoglycan degradation in the intervertebral disc (Insko et al. 2002). However, the signal in sodium-MRI is significantly lower than in conventional proton MRI, and the technique also requires instrumentation modifications (Wang et al. 2007).

2.5.3 Measurements of Apparent Diffusion Coefficients

Diffusion-weighted (DW) MR imaging (DWI) yields information about the diffusion movement of water protons. DWI gives an estimate of this movement in the form of apparent diffusion coefficients (ADC) by measuring the decrease in signal (Figure 4) due to moving protons (Buxton 2002). In biological tissues DWI measures restricted movement of unbound water molecules. If the time allowed for diffusion is long enough, water molecules encounter obstacles that restrict the diffusion and therefore the measured ADC is smaller than that of freely moving
water (ADC of water at 37 °C = 0.003 mm²/s). Restricted diffusion measures hence give an estimate of the microstructure and the pore size of the examined tissue. Due to T2 decay, the signal in diffusion imaging decreases with longer diffusion times, and therefore diffusion times between 10 and 100 ms are usually chosen. In NP the diffusion movement is restricted by cell boundaries, proteoglycan aggregates and collagen fibrils.

Diffusion images from the spine can be obtained using several different sequence variations. A diffusion-weighted single-shot spin-echo echo-planar imaging sequence (DW-EPI) has the advantage of short acquisition time, which reduces artefacts due to bulk motion (Kerttula et al. 2000a, Kurunlahti et al. 2001). Variations of this, multishot spin-echo echo-planar imaging (Kealey et al. 2005) and a single-shot dual-spin-echo echo-planar imaging (Beattie et al. 2008) acquisitions have also been used. The drawback of echo planar imaging is its susceptibility to magnetic field homogeneity variations which may introduce artefacts in the images. The acquired signal is also low, which leads to low resolution and relatively thick sections. Line scan diffusion imaging is another approach to obtain DW images. It produces good-quality images with less artefacts than DW-EPI (Bammer et al. 2003). However, the imaging times are considerably longer than with DW-EPI sequences (over 6 minutes vs. less than one minute). Somewhat shorter imaging times (2 min 30 sec) have been reported using a two-dimensional fast advantage spin-echo technique, with the drawback of blurring in images (Tokuda et al. 2007).

Several researchers have recently published results suggesting an association between disc degeneration and reduction in apparent diffusion coefficients (Table 1). In a study combining data from 39 patients and five volunteers at 1.5 T a 9% reduction in ADC was observed (Kealey et al. 2005), and in another study of thirty volunteers at 3 T a 19% reduction (Beattie et al. 2008) was observed in degenerated discs compared to normal ones. In addition, decreased ADC values have been reported in discs adjacent to vertebral fractures one year or later after trauma (Kerttula et al. 2001). Efforts have also been made to correlate the change in ADC with biochemical alterations in the intervertebral disc. In one study with human lumbar specimens, a decrease in glycosaminoglycan and water content in the nucleus pulposus correlated with a reduction in ADC (Antoniou et al. 2004). The association of lumbar artery stenosis or occlusion and reduction in ADC in the disc has also been reported (Kurunlahti et al. 2001, Tokuda et al. 2007).
Fig. 4. Principle of diffusion-weighted pulse sequence. Diffusion gradient with strength (G) and duration (δ) dephases spins according to their position and another gradient after diffusion pulse interval or diffusion time (Δ), rephases spins that have remained in the same relative position. Spins that are moved are therefore not rephased and do not have the same phase, which results in a smaller signal.

Table 1. Previously reported in vivo ADC values of normal and degenerated human intervertebral discs.

<table>
<thead>
<tr>
<th>N</th>
<th>Reference</th>
<th>Normal Discs Mean ± SD (x10^{-3} mm²/sec)</th>
<th>Degenerated Discs Mean ± SD (x10^{-3} mm²/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>Kealey et al. 2005</td>
<td>2.27 ± 0.58</td>
<td>2.06 ± 0.47</td>
</tr>
<tr>
<td>30</td>
<td>Beattie et al. 2008</td>
<td>1.92 ± 0.14</td>
<td>1.50–1.60 ± 0.05–0.38</td>
</tr>
<tr>
<td>34</td>
<td>Kerttula et al. 2000a</td>
<td>1.7 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Kurunlahti et al. 2001</td>
<td>1.41–1.61 ± 0.16–0.39</td>
<td>0.90–1.42 ± 0.20–0.86</td>
</tr>
<tr>
<td>15</td>
<td>Bammer et al. 2003</td>
<td>1.65 ± 0.21</td>
<td></td>
</tr>
</tbody>
</table>

N = number of studied individuals

2.5.4 Tracer studies

The first studies on nutrient transfer into the disc in vivo were carried out using fluorescent or radioactive tracers. Tracers were injected in the animal, and the animal was later killed and the disc examined (Urban et al. 1978, Roberts et al. 1996, Urban et al. 2004). In recent years, MRI has been applied to study nutrition transfer by measuring the enhancement of the intervertebral disc after

dGEMRIC

A gadolinium contrast agent tracing technique called delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) has recently been developed for the quantitative analysis of glycosaminoglycans within cartilaginous tissue (Bashir et al. 1997). This technique utilizes the negative charge of the paramagnetic gadolinium-based contrast agent, gadopentetate dimeglumine (Gd-DTPA\(^2\)), which after reaching equilibrium in studied tissue is assumed to distribute inversely to the concentration of negatively charged GAGs. Delayed gadolinium enhancement has primarily been utilized in studying GAGs in cartilage (Burstein et al. 2001, Nieminen et al. 2002, Williams et al. 2004), but its feasibility has also been shown in studying the GAG contents of the intervertebral disc (Vaga et al. 2008).
3 Purpose of the study

The purpose of this study was to evaluate the value of diffusion-weighted MRI and quantification of delayed gadolinium enhancement in assessing intervertebral disc degeneration. The particular aims were to

1. study the early changes of intervertebral disc degeneration using quantitative MR methods (ADC and T2 relaxation time measurements) with an experimental annular stab wound degeneration model;
2. determine the relation of ADC to degenerative changes in disc morphology and signal intensity in conventional MR imaging in a large and homogenous group of adult volunteers;
3. develop a method to quantify the delayed enhancement of the nucleus pulposus in MR imaging using a non-ionic contrast agent and T1 relaxation time measurements before and 90 minutes after intravenous contrast agent injection, and to correlate the changes in the relaxation rate with visual disc degeneration;
4. evaluate the role of lumbar artery stenosis, bone marrow changes adjacent to endplates, endplate defects and ADC of the disc in determining the delayed post-contrast enhancement of the intervertebral disc using MRI.
4 Materials and methods

4.1 Study population

4.1.1 Experimental model (I)

In six female juvenile domestic pigs (weighing 18 – 24 kg) a superficial stab incision was made into the anterolateral annulus fibrosus of the discs at L2–L3 using the retroperitoneal approach. Similarly, a full-thickness incision reaching the nucleus pulposus was made at L4–L5.

Experimental study protocol (I)

The first MR imaging was performed immediately after the operation. Further MR imaging was planned every week postoperatively, but because pig A died after the third and pig B after the second MR imaging, possibly because of anaesthesia complication (autopsy was not performed), the remaining animals had two weeks of rest before the second postoperative MR imaging. The animals (C to F) were therefore imaged immediately after surgery and then after 2, 3 and 4 weeks. Pigs C and D were killed after the last imaging in the scanner room 4 weeks after the surgery using an overdose of pentobarbitone. Pigs B, C and D had immediate post mortem MR imaging for ADC measurements. Pigs E and F were killed 8 weeks after the lesion surgery, their lumbar spines were removed in animal laboratory and after labelling disc levels with surgical sutures imaged en block at 1.5 T. The blocs were wrapped in plastic foil and stored at –70°C for biochemical analysis. The study protocol was approved by Oulu University Board for the Care and Use of Laboratory Animals and the county veterinary surgeon.

Biochemical evaluation of the disc preparations (I)

The deep-frozen lumbar spine blocks of pigs E and F were thawed and the disc levels identified based on the surgical suture markings. The discs were carefully opened with a surgical scalpel by cutting along the endplate and visually evaluated before sampling of the nucleus pulposus for the biochemical analysis. The disc samples were weighed, freeze-dried, and reweighed to estimate the water content. The proteoglycans were extracted from the minced disc samples
with 4 M GuHCl in 50 mM sodium acetate supplemented with protease inhibitors. The uronic acid content was analysed according to a method described by Blumenkranz and Asboe-Hansen (Blumenkrantz & Asboe-Hansen 1973).

4.1.2 Adult volunteers (II, III, IV)

The study population of study II consisted of 228 male volunteers with a mean age of 47 years (range 36–56 years). They included 159 train engineers working for the Finnish state railways and 69 paper mill and chemical factory workers from the same geographical region. The study population was collected to study the effect of whole-body vibration and genetics on IDD (Virtanen et al. 2007), degenerative endplate changes (Kuisma et al. 2007), and quantitative imaging methods in disc degeneration (current study). A subgroup of this population formed the study population of studies III and IV and consisted of 20 paper mill and chemical factory workers with a mean age of 49 years (range 40–56 years). All study members participated on a voluntary basis and received no compensation. The study was approved by the local Ethics Committee, and informed consent was obtained from all subjects.

4.2 MR imaging methods

4.2.1 Protocols for anatomical imaging (I–IV)

In study I, MR imaging of pigs was performed under general anaesthesia with mechanically assisted ventilation using a 1.5-T scanner (GE Signa Infinity Twinspeed, GE Medical Systems, Milwaukee, Wisconsin, USA) with a non-phased-array general-purpose flexible coil (GPFLEX, GE Medical Systems, Milwaukee, Wisconsin, USA). T2-weighted fast recovery fast spin-echo (frFSE) images in sagittal (repetition time 3960 ms, effective echo time (effTE) 118 ms, number of excitations (NEX) 4, field of view (FOV) 250 × 250 mm, matrix 448 × 224, slice thickness 3 mm) and transverse plane (TR/effTE 5160/106, NEX 4, FOV 180 × 180 mm, matrix 256 × 160; slice thickness 3 mm) were obtained for visual analysis.

In studies II–IV, two 1.5T scanners (GE Signa Infinity TwinSpeed and GE Signa HDx, Milwaukee, Wisconsin, USA) with a phased array spine coil (USA Instruments, Aurora OH, USA) were used for anatomical imaging. Two scanners
were used to keep the workflow fluent and to keep the time between scans constant (study III and IV). Examination included T2-weighted frFSE sagittal images (effTE/TR 116/3960 ms, NEX 4, FOV 28 cm, matrix 448 × 224, slice thickness 4 mm with a 1 mm section gap). Also T1-weighted fluid-attenuated inversion recovery (T1-FLAIR) images were obtained (TR 1809/18 ms, inversion time (TI) 660 ms, NEX 4, FOV 28 cm, matrix 448 × 192, 4 mm thick slices with intersection gap of 1-mm).

4.2.2 Bolus MRA (IV)

The intravenous bolus of non-ionic contrast agent, gadodiamide (Gd-DTPABMA, Omniscan, GE Healthcare Inc, Princeton NJ, USA), with a dosage of 0.2 mM/kg was injected with remote-controlled automated injector followed by 40 mL of saline flush. Coronal 3-D fast-spoiled gradient echo (FSPGR) was used to obtain contrast-enhanced MR angiogram (TR/TE 6.6/1.2, flip angle 45, NEX 1, FOV 35 cm, matrix 256 × 128, 3.4 mm thick slices with overlap of 1.7 mm). The scanning was started when the bolus was visually detected in the abdominal aorta (fluoro trigger).

4.2.3 Analyses of anatomic images and MRA (I–IV)

In study I, areas of the central high-signal nucleus pulposus of the discs were measured in the sagittal T2-weighted images by a radiologist (J.N.) in blinded fashion. The same radiologist also visually evaluated the images and recorded herniations of NP.

In studies II and IV, degeneration of lumbar discs L3–L4, L4–L5, and L5–S1 was classified according to Pfirrmann’s grading system (Pfirrmann et al. 2001) by a radiologist (J.N.) and a fourth-year medical student (A.K.). The grading takes into account disc signal, morphology and height (the grading is shown in Table 2 and examples from the studied population in Figure 5). In cases of disagreement a consensus reading was performed. T2 signal intensities of the disc centres and cerebrospinal fluid (CSF) were measured and the ratio between them (T2/CSF-ratio) was calculated. The medical student measured all the cases and the radiologist measured 20 randomly selected cases for inter-observer reliability analysis. Height and width of the discs were measured from sagittal T2-weighted images by the radiologist and a width-to-height ratio (W/H ratio) was calculated. In study III, the same radiologist evaluated degeneration grading of the discs and
the presence of HIZs and radial tears, and in study IV the presence of endplate defects.

Table 2. Visual intervertebral degeneration grading (Pfirrmann et al. 2001).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Discs exhibiting homogenous signal that is hyperintense or isointense to cerebrospinal fluid, clear distinction between annulus and nucleus, normal height</td>
</tr>
<tr>
<td>II</td>
<td>Discs with hyperintense signal and inhomogeneous structure and clear distinction between annulus and nucleus, normal height</td>
</tr>
<tr>
<td>III</td>
<td>Discs with intermediate signal and inhomogeneous structure and blurriness between nucleus and annulus, height may be slightly decreased</td>
</tr>
<tr>
<td>IV</td>
<td>Discs with hypointense signal and inhomogeneous structure and distinction between nucleus and annulus is lost, height may be moderately decreased</td>
</tr>
<tr>
<td>V</td>
<td>The same as Grade IV but with collapsed disc space</td>
</tr>
</tbody>
</table>

Fig. 5. Examples of T2-weighted MR images of adult lumbar discs graded visually in degeneration grades II–V. Grade I (not shown) represents a healthy adolescent disc.
In study IV, degenerative bone marrow changes adjacent to endplates (Modic changes) were evaluated by two radiologists (M.K. and R.O) as previously described (Modic et al. 1988a, Modic et al. 1988b, Kuisma et al. 2007). The patency of first to fourth lumbar artery pairs from MR angiograms was evaluated by a radiologist and a medical student (J.N. and A.K.). Each lumbar artery was evaluated as one unit from the ostium to the periphery and graded as: grade 0 (normal or narrowing < 50% of the artery diameter) or grade 1 (at least 50% reduction in diameter). In case of disagreement, a consensus reading was performed.

4.2.4 T2 relaxation time measurements (I)

The T2 relaxation time in study I was measured using a sagittal four-echo spin-echo sequence (TR/TE 6000/25, 50, 75 and 100, NEX 1, FOV 250 × 125 mm, matrix 256 × 128, slice thickness 5 mm). The mean T2 values of the ROIs placed in the NP of the discs were calculated from four-echo SE measurement by a least-squares fitting routine using FuncTool Performance software (GE, Milwaukee, Wisconsin, USA).

4.2.5 T1 relaxation time mapping (III–IV)

For the T1 relaxation time measurements of lumbar discs a series of T1-weighted sagittal single-slice inversion-recovery fast spin-echo measurements were conducted (TR 3400, TI 100, 200, 400, 800, 1600 and 3200, NEX 2, FOV 20 cm, matrix 256 × 256, slice thickness 4-mm) using two 1.5T scanners (GE Signa Infinity TwinSpeed and GE Signa HDx, Milwaukee, Wisconsin, USA) with the Torso Array coil (GE, Milwaukee, Wisconsin, USA). This was followed by an intravenous injection of 0.2 mM/kg of non-ionic GdDTPA-BMA, gadodiamide (Omniscan), and a repeat of the T1 measurement series 90 ± 2 minutes after the injection. T1 relaxation time maps were fitted using FuncTool Performance software. For each disc, T1 relaxation times were averaged from ROIs that were placed centrally in the disc. Absolute change (ΔR1, where R1 = 1/T1) and percentual change (ΔR1%) were calculated to evaluate the enhancement of the discs.
4.2.6 Diffusion-weighted imaging (I–II, IV)

In study I, a diffusion-weighted single-shot spin-echo echo-planar imaging sequence in the sagittal plane was used to obtain the images for ADC measurements (TR/effTE 6000/64.6–72.2, NEX 1, FOV 250 × 125 mm, matrix 256 × 128, slice thickness 10 mm). Images were obtained with two diffusion-weighting factors, $b_0$ and $b_1$, where $b_0 = 0$ and $b_1 = 300, 400, 500$ and $600$ s/mm$^2$. The diffusion-encoding gradients were applied sequentially in three orthogonal directions. In order to study the accuracy of the apparent diffusion coefficient determinations, each acquisition was repeated twice. The b-scale was calibrated by measuring the known self-diffusion coefficient of pure water and cyclohexane (Tofts et al. 2000).

In studies II and IV, a DW-EPI sequence (TE/TR 71/6000, FOV 250 × 125 mm, matrix of 256 × 128, NEX 1, one 10 mm thick section) of the lumbar spine was performed with a non-phased-array general-purpose flexible coil (GPFLEX, GE Medical Systems, Milwaukee, Wisconsin, USA) in three orthogonal directions with a b value of 500 s/mm$^2$. An isotropic ADC map was calculated from the orthogonal images, and ROI measurements of ADC values from lumbar discs L3–L4, L4–L5, and L5–S1 were obtained with FuncTool Performance software. ROIs were placed centrally in the high signal area of the disc in the DW-EPI image ($b = 0$ s/mm$^2$) and then copied exactly to the same position on the ADC map by two research assistants trained specifically for the task. Both readers measured an equal number of cases, and for inter-observer reliability analysis a set of 20 randomly selected cases was mixed with both readers’ cases.

4.3 Statistical methods

In study I, the significance of differences before and after operation was tested using paired t-test. For study II, the parameters for sample size estimation (Eng 2003) were extracted from previous studies (Kerttula et al. 2000a, Kerttula et al. 2001, Kurunlahti et al. 2001, Bammer et al. 2003, Kealey et al. 2005). For $0.10 \times 10^{-3}$ mm$^2$/s minimum expected difference between normal and degenerated discs with standard deviation of $0.40 \times 10^{-3}$ mm$^2$/s and with 95% confidence interval, the required number of ADC measurements would be 245. The inter-observer agreement of the visual degeneration grading and T2 signal intensity and ADC measurements was estimated by calculating the intra-class correlation coefficient (ICC, single measurements). Values in the range of 0–0.2 indicate slight, 0.2–0.4
fair, 0.4–0.6 moderate, 0.6–0.8 substantial, and 0.8–1.0 almost perfect agreement (Landis & Koch 1977). Pearson’s correlation coefficient was used to correlate the ADC, T2/CSF ratio and W/H ratio with each other. Analysis of variance (ANOVA) with post-hoc Tukey’s honestly significant difference (HSD) test was used to test the difference of ADC values in different degeneration grades.

In study III, the Wilcoxon signed rank test was used to examine the differences of disc enhancement. The associations between visual degeneration score and T1 measurements and disc height measurements were derived using Mann-Whitney U-test. The influence of disc height on the enhancement of the discs was analysed using linear regression analysis.

In study IV, Pearson’s correlation analysis was used to assess the association between ADC, height and enhancement (ΔR1) of the disc. The differences in ΔR1 in different degeneration groups were evaluated using Kruskal-Wallis test, and after that between separate groups using Mann-Whitney U-test. The determinants of enhancement were evaluated using Student’s t-test for binomial determinants and linear regression for normally distributed determinants. Univariate analysis of variance was used for a multinomial model.

Statistical analyses were performed by using SPSS for Windows versions 11–13 (SPSS Inc, Chicago, IL). P-values less than 0.05 were considered statistically significant.
5 Results

5.1 ADC and T2 relaxation time in experimentally induced disc degeneration (I)

In visual analysis of the six operated pigs, in all the discs with a full-thickness stab incision there was a focal high-signal area visible immediately adjacent to the disc, presumably herniated nucleus pulposus material. The measured area in sagittal plane of those discs diminished 30% (p < 0.05) in two weeks, after which no significant change was noted.

The ratio between measured ADC from full-thickness lesioned discs and control discs (pigs C-F) increased from 1.03 to 1.12 in four weeks (p < 0.05). The harvested spines of pigs E and F that were imaged eight weeks after the operation showed again a reduced ratio (Table 3). The measured absolute ADC values of the NP of the discs did not change significantly during the study period. The mean T2 relaxation times of the nucleus pulposus of the discs with full-thickness incisions were reduced in four weeks from 154 ± 18 ms to 120 ± 17 ms (p < 0.005). The mean T2 relaxation time of untouched discs was 154 ± 17 ms. There were no significant ADC or T2 relaxation time changes in the superficially incised or untouched discs.

Biochemical analysis of full-thickness lesioned discs (pigs E and F) showed a decrease in the water content and uronic acid content of injured discs when compared to control discs. The water content of the lesioned discs was on average 91%, whereas it was 93% in control discs. The corresponding uronic acid contents were 94 µg/mg and 107 µg/mg.
Table 3. Average apparent diffusion coefficients at 1.5-T of the pigs in vivo, post mortem and in vitro imaging after the annular lesion surgery. Standard deviation is calculated from eight separate ADC\textsubscript{mean} determinations.

<table>
<thead>
<tr>
<th>Pig</th>
<th>ROI</th>
<th>Average ADC\textsubscript{mean} of nucleus pulposus (10\textsuperscript{-3} mm\textsuperscript{2}/s)</th>
<th>Post op.</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>post mortem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>in vivo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A\textsuperscript{1}</td>
<td>SL ROI</td>
<td>2.15 ± 0.14</td>
<td>2.14 ± 0.13</td>
<td>2.20 ± 0.15</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Control ROI</td>
<td>2.16 ± 0.11</td>
<td>2.18 ± 0.14</td>
<td>2.30 ± 0.13</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FT ROI</td>
<td>2.20 ± 0.08</td>
<td>2.30 ± 0.13</td>
<td>2.19 ± 0.11</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FT/control-ratio</td>
<td>1.02</td>
<td>1.06</td>
<td>0.95</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B\textsuperscript{2}</td>
<td>SL ROI</td>
<td>2.10 ± 0.14</td>
<td>2.19 ± 0.15</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.94 ± 0.09</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Control ROI</td>
<td>2.29 ± 0.14</td>
<td>2.09 ± 0.16</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2.03 ± 0.10</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FT ROI</td>
<td>2.31 ± 0.15</td>
<td>2.35 ± 0.12</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2.13 ± 0.10</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FT/control-ratio</td>
<td>1.01</td>
<td>1.12</td>
<td>1.05</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C\textsuperscript{3}</td>
<td>SL ROI</td>
<td>1.88 ± 0.10</td>
<td>—</td>
<td>1.91 ± 0.16</td>
<td>1.80 ± 0.15</td>
<td>1.85 ± 0.08</td>
<td>1.86 ± 0.07</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Control ROI</td>
<td>2.04 ± 0.07</td>
<td>—</td>
<td>1.94 ± 0.12</td>
<td>1.85 ± 0.13</td>
<td>1.91 ± 0.11</td>
<td>1.96 ± 0.11</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FT ROI</td>
<td>2.00 ± 0.12</td>
<td>—</td>
<td>2.11 ± 1.0</td>
<td>1.91 ± 0.08</td>
<td>1.99 ± 0.06</td>
<td>2.01 ± 0.12</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FT/control-ratio</td>
<td>0.98</td>
<td>1.09</td>
<td>1.03</td>
<td>1.04</td>
<td>1.03</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>D\textsuperscript{4}</td>
<td>SL ROI</td>
<td>1.98 ± 0.09</td>
<td>—</td>
<td>2.01 ± 0.17</td>
<td>1.76 ± 0.09</td>
<td>1.78 ± 0.10</td>
<td>1.95 ± 0.13</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Control ROI</td>
<td>1.95 ± 0.11</td>
<td>—</td>
<td>2.13 ± 0.19</td>
<td>1.81 ± 0.10</td>
<td>1.94 ± 0.10</td>
<td>2.00 ± 0.13</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FT ROI</td>
<td>2.03 ± 0.17</td>
<td>—</td>
<td>2.23 ± 0.21</td>
<td>1.99 ± 0.08</td>
<td>2.10 ± 0.10</td>
<td>2.20 ± 0.09</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FT/control-ratio</td>
<td>1.02</td>
<td>1.05</td>
<td>1.05</td>
<td>1.06</td>
<td>1.10</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>E\textsuperscript{1,4}</td>
<td>SL ROI</td>
<td>1.91 ± 0.12</td>
<td>—</td>
<td>1.76 ± 0.09</td>
<td>1.84 ± 0.13</td>
<td>2.03 ± 0.09</td>
<td>1.95 ± 0.14</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Control ROI</td>
<td>2.06 ± 0.12</td>
<td>—</td>
<td>1.91 ± 0.11</td>
<td>1.89 ± 0.11</td>
<td>2.09 ± 0.15</td>
<td>2.01 ± 0.14</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FT ROI</td>
<td>2.08 ± 0.10</td>
<td>—</td>
<td>2.08 ± 0.13</td>
<td>1.95 ± 0.08</td>
<td>2.16 ± 0.14</td>
<td>1.81 ± 0.10</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FT/control-ratio</td>
<td>1.01</td>
<td>1.09</td>
<td>1.03</td>
<td>1.03</td>
<td>0.90</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>F\textsuperscript{3,4}</td>
<td>SL ROI</td>
<td>1.64 ± 0.07</td>
<td>—</td>
<td>1.94 ± 0.18</td>
<td>1.84 ± 0.09</td>
<td>1.80 ± 0.12</td>
<td>1.74 ± 0.07</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Control ROI</td>
<td>1.83 ± 0.07</td>
<td>—</td>
<td>1.98 ± 0.15</td>
<td>1.84 ± 0.09</td>
<td>1.88 ± 0.15</td>
<td>1.79 ± 0.11</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FT ROI</td>
<td>2.01 ± 0.10</td>
<td>—</td>
<td>2.09 ± 0.12</td>
<td>2.11 ± 0.11</td>
<td>2.21 ± 0.22</td>
<td>1.81 ± 0.06</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FT/control-ratio</td>
<td>1.10</td>
<td>1.10</td>
<td>1.14</td>
<td>1.17</td>
<td>1.01</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Imaging one week after lesion surgery. Pig died after third imaging in vivo
\textsuperscript{2} Imaging one week after lesion surgery. Pig died after second imaging in vivo
\textsuperscript{3} Imaging two weeks after lesion surgery
\textsuperscript{4} Immediate post mortem imaging in vitro four weeks after last imaging in vivo
SL = superficial lesion; FT = full thickness lesion; ROI = region of interest
5.2 Association between ADC and visual disc degeneration on MRI among adult volunteers (II)

5.2.1 Distribution of degenerative changes

Among the studied 684 discs no grade I discs were observed, and all grade II discs were reclassified as grade III in consensus reading. At L3–4 level 60% of the discs were grade III, compared to 30% at L5–S1 level. Fifty-nine percent of the collapsed (grade V) discs were at L5–S1. The distribution of T2/CSF ratio and W/H ratio according to anatomic levels and degeneration grades is shown in Figure 6. The age of the subjects was negatively correlated with the T2/CSF ratio (Pearson correlation \( r = -0.21, P < 0.01 \)) and positively correlated with the W/H ratio (Pearson correlation \( r = 0.14, P < 0.01 \)).

![Fig. 6. Boxplots showing width-to-height ratio and ratio of measured T2 signal intensity of disc to cerebrospinal fluid vs. visual degeneration grades according to lumbar levels.](image)

5.2.2 Apparent diffusion coefficient measurements

All in all, 530 ADC measurements of 684 discs were obtained. The percentage of missing ADC measurements was 12% at L3–4, 23% at L4–L5, and 32% at L5–S1. The non-measured discs had significantly lower T2/CSF ratios at all levels (Pearson correlation \( r = -0.40, P < 0.01 \)) as shown in Table 4. There was no
significant correlation between the age of the participants and the measured ADC values.

Table 4. Mean T2/CSF ratios divided according to visual degeneration grading from discs with and without ADC measurements. Data are means ± standard deviations, the number of measurements is shown in parentheses.

<table>
<thead>
<tr>
<th>ADC</th>
<th>Visual Degeneration Grades</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>III</td>
<td>IV</td>
</tr>
<tr>
<td>Measured</td>
<td>0.22 ± 0.06 (255)</td>
<td>0.12 ± 0.04 (258)</td>
</tr>
<tr>
<td>Non-measured</td>
<td>0.20 ± 0.06 (20)</td>
<td>0.11 ± 0.03 (88)</td>
</tr>
<tr>
<td>All</td>
<td>0.22 ± 0.06 (275)</td>
<td>0.12 ± 0.04 (346)</td>
</tr>
</tbody>
</table>

Table 5. Mean ADC values divided according to visual degeneration grades at L3–4 to L5–S1. Data are means (x10^{-3} mm²/sec) ± standard deviations, the number of measurements/all discs is shown in parentheses.

<table>
<thead>
<tr>
<th>Intervertebral Disc Level</th>
<th>Visual Degeneration Grades</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>III</td>
<td>IV</td>
</tr>
<tr>
<td>L3–4</td>
<td>2.02 ± 0.23 (128) *</td>
<td>1.91 ± 0.27 (71)</td>
</tr>
<tr>
<td>L4–5</td>
<td>2.13 ± 0.26 (63) *</td>
<td>1.98 ± 0.30 (107)</td>
</tr>
<tr>
<td>L5–S1</td>
<td>2.03 ± 0.33 (64)</td>
<td>1.98 ± 0.32 (81)</td>
</tr>
<tr>
<td>All</td>
<td>2.05 ± 0.27 (255)</td>
<td>1.96 ± 0.30 (259)</td>
</tr>
</tbody>
</table>

* significant difference between groups (ANOVA, post hoc Tukey’s HSD p < 0.05)

5.2.3 Association between ADC and degenerative changes

The results of ADC measurements are shown in Table 5. In grade III degeneration mean ADC at L4–5 was significantly higher than at L3–4, otherwise no significant association between ADC and anatomic levels was observed. ADC values in grade IV disc degeneration were significantly lower than in grades III and V, whereas the T2/CSF ratios of grade IV were significantly lower than in grade III, but equal to grade V. There was a statistically significant correlation between ADC and the T2/CSF ratio (Pearson correlation r = 0.26, P < 0.01), but not between ADC and the W/H ratio.
5.3 Association between visual degeneration and delayed gadolinium enhancement of discs (III)

In study III, altogether 93 discs were analysed. A positive trend was observed between the change in the T1 relaxation rate (∆R1, where R1 = 1/T1) and the degeneration grading. A statistically significant difference in the ∆R1 was observed between all except visual degeneration grades II and III (Mann-Whitney U test, p < 0.0001–0.02). A statistically significant correlation between disc height and ∆R1 was observed (R² = 0.458, p < 0.0001) (Table 6 and Figure 7). No difference was observed in the ∆R1 between discs with and without high-intensity zones or radial tears (Mann-Whitney U test, p = 0.40).

Table 6. Results of T1 measurements before (T1) and 90 minutes after (T1Gd) intravenous contrast injection; percentual change in relaxation rates (∆R1%) and ROI sizes are presented. Values are presented as mean ± standard deviation.

<table>
<thead>
<tr>
<th>Degeneration grade</th>
<th>N</th>
<th>T1 (ms) ± SD</th>
<th>T1Gd (ms) ± SD</th>
<th>∆R1 (ms⁻¹) ± SD</th>
<th>∆R1% ± SD</th>
<th>ROI size (mm²) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>19</td>
<td>885.8 ± 72.2</td>
<td>823.9 ± 76.3</td>
<td>0.088 ± 0.069</td>
<td>7.8 ± 5.6</td>
<td>46.3 ± 1.5</td>
</tr>
<tr>
<td>III</td>
<td>28</td>
<td>816.0 ± 94.7</td>
<td>770.4 ± 101.2</td>
<td>0.077 ± 0.098</td>
<td>6.3 ± 7.6</td>
<td>45.8 ± 1.7</td>
</tr>
<tr>
<td>IV</td>
<td>42</td>
<td>715.8 ± 100.1</td>
<td>594.8 ± 130.3</td>
<td>0.339 ± 0.345</td>
<td>24.3 ± 25.6</td>
<td>45.7 ± 1.8</td>
</tr>
<tr>
<td>V</td>
<td>4</td>
<td>716.1 ± 84.8</td>
<td>458.4 ± 103.1</td>
<td>0.852 ± 0.403</td>
<td>60.2 ± 28.2</td>
<td>32.7 ± 6.9</td>
</tr>
</tbody>
</table>

$\Delta R1 = \Delta R1gd - \Delta R1$, where $R1 = 1/T1$, $\Delta R1% = ((R1 – R1gd) / (R1gd)) * 100$

![Enhancement ratios (∆R1) vs. disc height. The decrease in disc height explained approximately 46% (R² = 0.458, p < 0.0001) of the differences in the enhancement.](image-url)
5.4 Roles of lumbar artery stenosis, endplate changes and the ADC in determining gadolinium enhancement of discs (IV)

5.4.1 Prevalence of imaging findings

The prevalences of Modic changes, endplate defects, disc degeneration grades and narrowing of lumbar arteries at different lumbar levels of twenty male volunteers in study IV are summarized in Table 7. Modic changes were most common in the lower lumbar levels whereas endplate defects were more evenly distributed. Thirteen of the 20 volunteers studied had at least one endplate defect, and 8 of 31 defects appeared at L1–2 and L2–3 levels, at which there were no Modic changes. Thirteen of the volunteers studied had at least one narrowed lumbar artery. There were no totally occluded arteries in the study population.

Table 7. Prevalences of degenerative bone marrow changes adjacent to endplates (Modic changes), endplate defects, visual disc degeneration grade (Pfirrmann et al. 2001), and status of segmental lumbar arteries at different lumbar disc levels in 20 male subjects. Data are presented as number of cases.

<table>
<thead>
<tr>
<th>Grading</th>
<th>Lumbar level</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L1–2</td>
<td>L2–3</td>
</tr>
<tr>
<td>Modic changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1/2</td>
<td>0 0</td>
<td>1 4</td>
</tr>
<tr>
<td>Type 2</td>
<td>0 0</td>
<td>1 0</td>
</tr>
<tr>
<td>Endplate defects</td>
<td>3 5</td>
<td>9 7</td>
</tr>
<tr>
<td>Degeneration grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>11 9</td>
<td>5 2</td>
</tr>
<tr>
<td>III</td>
<td>7 9</td>
<td>12 4</td>
</tr>
<tr>
<td>IV</td>
<td>2 2</td>
<td>3 12</td>
</tr>
<tr>
<td>V</td>
<td>0 0</td>
<td>0 2</td>
</tr>
<tr>
<td>Narrowed arteries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>1 4</td>
<td>4 4</td>
</tr>
<tr>
<td>Both</td>
<td>0 2</td>
<td>3 3</td>
</tr>
</tbody>
</table>

5.4.2 ADC measurements

Table 8 summarizes the results of ADC measurements from 85 out of 100 discs according to visual degeneration grades. The ADC in z-direction (z is the superior-inferior direction and the direction of the main magnetic field) was on average higher than the ADC in other directions (P < 0.001).
Table 8. Distribution of ADC results in degeneration groups. Values are presented as mean (×10\(^{-3}\) mm\(^2\)/s) ± SD.

<table>
<thead>
<tr>
<th>Degeneration grade</th>
<th>N</th>
<th>ADC(_x) ± SD</th>
<th>ADC(_y) ± SD</th>
<th>ADC(_z) ± SD</th>
<th>ADC(_{mean}) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>26</td>
<td>1.54 ± 0.38</td>
<td>1.57 ± 0.37</td>
<td>1.83 ± 0.30</td>
<td>1.65 ± 0.29</td>
</tr>
<tr>
<td>III</td>
<td>35</td>
<td>1.46 ± 0.38</td>
<td>1.52 ± 0.27</td>
<td>1.70 ± 0.31</td>
<td>1.55 ± 0.26</td>
</tr>
<tr>
<td>IV</td>
<td>23</td>
<td>1.69 ± 0.42</td>
<td>1.57 ± 0.38</td>
<td>1.70 ± 0.29</td>
<td>1.65 ± 0.31</td>
</tr>
<tr>
<td>V</td>
<td>1</td>
<td>1.65</td>
<td>2.06</td>
<td>1.85</td>
<td>1.85</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>1.55 ± 0.40</td>
<td>1.56 ± 0.33</td>
<td>1.74 ± 0.30</td>
<td>1.62 ± 0.28</td>
</tr>
</tbody>
</table>

N = number of ADC measurements
ADC\(_{mean}\) = (ADC\(_x\) + ADC\(_y\) + ADC\(_z\)) / 3

5.4.3 Post-contrast enhancement and associations

Disc height was strongly associated with the enhancement of the disc (R\(^2\) = 0.46, P < 0.001). Endplate defects and Modic changes were also significantly associated (P < 0.001) with the enhancement (Table 9). In the multivariate analysis, disc space narrowing (F = 42.7, P < 0.001) was the strongest determinant of enhancement, followed by the presence of Modic changes (F = 9.0, P = 0.003) and endplate defects (F = 4.2, P = 0.043). Narrowing of lumbar arteries was not associated with enhancement (F = 0.13, P = 0.88). ADC in the disc did not correlate with enhancement, either, the correlation of ADC mean being 0.01 (P = 0.921) and correlations at X, Y and Z directions being 0.10 (P = 0.37), 0.01 (P = 0.94) and −0.10 (P = 0.39), respectively.

Table 9. Enhancement of the discs in the presence of adjacent Modic changes and endplate defects. Values are mean ∆R1 (×10\(^{-3}\) ms\(^{-1}\)) ± SD, the number of cases is shown in parentheses.

<table>
<thead>
<tr>
<th>Modic change</th>
<th>Endplate defect</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td></td>
<td>0.13 ± 0.17 (62)</td>
<td>0.29 ± 0.39 (21)</td>
</tr>
<tr>
<td>Mixed type 1/2</td>
<td></td>
<td>0.18 (1)</td>
<td>0.93 ± 0.22 (6)</td>
</tr>
<tr>
<td>Type 2</td>
<td></td>
<td>–</td>
<td>0.40 ± 0.13 (3)</td>
</tr>
</tbody>
</table>

5.5 Reliability of MRI measurements and visual evaluation

In studies I and III, there was only one reader and repeated readings were not performed. In study IV, the evaluation of Modic changes and narrowing of lumbar
arteries was performed by two readers and in cases of disagreement a consensus reading was performed. In study II, inter-observer agreement of visual degeneration grading between the two readers was substantial (684 graded lumbar levels, ICC single measures mean 0.67, SD 0.05). The mean agreement of ADC measurements between the two readers was almost perfect (52 repeated ADC measurements, ICC single measures mean 0.84, SD 0.07). For T2 signal intensity measurements of disc centres and CSF the mean agreement between the two operators was almost perfect (80 repeated signal intensity measurements, ICC single measures mean 0.86, SD 0.10).
6 Discussion

The purpose of the current study was to evaluate the value of diffusion-weighted imaging and delayed diffusion of gadolinium in assessing degenerative changes in the intervertebral disc.

First, the sensitivities of T2 relaxation time and apparent diffusion coefficient measurements were investigated in early disc degeneration induced by experimental stab wound injury in six pigs. As expected, T2 relaxation time of the injured discs was significantly reduced, presumably due to desiccation. A new observation in this study was the increased ADC values in injured discs four weeks after the operation. After eight weeks the ADC values were again lower than in control discs.

Secondly, the value of apparent diffusion coefficient measurements in intervertebral disc degeneration was evaluated in 228 middle-aged male volunteers. Reduction in ADC was observed in moderately degenerated discs when compared to normal discs. However, severely degenerated discs showed 5% higher ADC values than normal discs, presumably due to free water in the cracks and fissures of those discs. The difference in ADC between anatomic levels suggested by some earlier studies was not confirmed in this study. Because there is considerable overlap between ADC values of normal and degenerated discs, both on the basis of this and previous studies, ADC measurements of intervertebral discs seem to have limited clinical value.

Thirdly, a method to quantify the delayed gadolinium enhancement of nucleus pulposus was developed. In this study delayed gadolinium enhancement of disc was evaluated for the first time using T1-mapping and quantification. The method is promising and strong correlations were observed between visual degeneration scoring and post-contrast T1 measurements.

Finally, the changes associated with disc degeneration that may have an effect on the nutrition of the disc, and therefore determine the delayed gadolinium enhancement of the disc, were studied in 20 male volunteers. Changes that were correlated with gadolinium enhancement were lumbar artery narrowing, Modic changes, endplate defects, and apparent diffusion coefficient in the nucleus pulposus. Increased enhancement of a degenerated disc was mostly associated with disc space narrowing and the presence of degenerative endplate changes and endplate defects, addressing the importance of integrity of endplate in controlling the diffusion of small solutes into the disc.
6.1 Imaging of disc degeneration

Lumbar back pain is a very common problem (Heistaro et al. 2007) and strongly associated with radiological signs of intervertebral disc degeneration (Modic 1999, Vanharanta 1999, Luoma et al. 2000). When degenerative changes in the disc are associated with symptoms the condition is often called degenerative disc disease in clinical terminology (Fardon 2001, Fardon & Milette 2001, Adams & Roughley 2006). However, degenerative morphological changes in discs are also very common in people without back pain (Jensen et al. 1994, Battie & Videman 2006) and at present there are no clear markers, either morphological or biochemical, distinguishing ageing from pathological changes in the disc (Antoniou et al. 1996, Urban et al. 2000). The challenge for modern spine imaging is to develop more sophisticated imaging methods to characterize the disc changes that correlate with clinical symptomatology (Haughton 2004).

Current imaging methods easily depict endplate fractures, radial fissures, and herniations that are unambiguous markers of impaired disc function and at least somewhat related to pain. However, such structural failures are irreversible since adult intervertebral discs have limited healing potential. If the emerging treatment options of degenerative disc disease, such as nucleus replacement, gene therapy and growth factor therapy (Boden et al. 2004, Vadala et al. 2007) are found to be beneficial there may be a need for more sensitive imaging methods that may be beneficial in the evaluation and follow-up of such therapies.

In this study the usability of quantitative imaging methods, primarily diffusion-weighted imaging and delayed gadolinium enhancement of disc, were evaluated using both experimental models and human volunteers.

6.2 Methodological considerations

6.2.1 Experimental stab wound model (I)

The sensitivity of imaging methods in early degeneration is difficult to evaluate in humans, since the initiation of the degenerative process is not usually evident and the morphological changes typically evolve slowly. Therefore, experimental animal models are used to address the temporality of initiating event (experimental lesion) and subsequent degenerative changes in the disc in a controlled environment. A commonly used experimental animal model of intervertebral disc degeneration is a surgical injury to the annulus fibrosus (Osti et
al. 1990), in which the annular lesion is thought to initiate a degenerative cascade by causing acute herniation and subsequent nuclear depressurization. Although quantitative MR measurements, such as T2 relaxation time and apparent diffusion coefficients, have been suggested to be sensitive methods in imaging early degenerative changes in the disc (Kerttula et al. 2000a, Kerttula et al. 2001, Kurunlahti et al. 2001), early changes after stab incision injury have not been previously documented with quantitative MR imaging, and therefore this experimental study (I) was conducted.

As expected, herniation of the nucleus pulposus adjacent to the incision of the disc and a subsequent decrease of 30% in the mid-slice sagittal area of the nucleus pulposus was observed at two weeks after annulus-penetrating experimental stab wound. One disc with superficial incision showed similar findings, suggesting that the incision penetrated the annulus fibrosus, while the rest of the superficially incised or untouched discs showed no visual alterations. It was also expected that the measured in vivo T2 relaxation time of the discs with full-thickness lesion decreased, assumingly reflecting desiccation of the disc. The T2 relaxation time four weeks after the lesion surgery was on average 73% of that of the control discs.

Significant changes in the absolute ADC values were not observed, whereas the ratio between ADC measured from full-thickness lesioned discs and control discs increased 9% during the first four weeks, followed by a decrease by 9.5% from the initial level by eight weeks after operation. The shrinking of NP, dehydration and changes in uronic acid content in biochemical analysis were in concordance with degenerative changes reported in earlier studies using the stab wound model (Kääpä et al. 1994a, Kääpä et al. 1994b, Kääpä et al. 1995, Lappalainen et al. 2002).

The early increase of ADC in vivo after disc injury was an interesting observation that has not been documented earlier in the literature. A similar result has been observed in vitro after enzymatic degradation of the bovine NP either by collagenase or by trypsin and hyaluronidase; targeting the proteoglycan and/or hyaluronan integrity (Antoniou et al. 2006). Because diffusion-weighted MR provides information about the freedom of motion of water molecules and therefore yields indirect information about the microstructure of the examined tissue, the changes in microstructure that modify the diffusivity may not be related to the visual MR imaging. Theoretically, ADC may therefore be sensitive to early degenerative changes. However, the changes in diffusivity of water in more progressed degeneration in humans seems to be opposite, as decreased ADC
values have been reported (Kerttula et al. 2000a, Kurunlahti et al. 2001, Bammer et al. 2003, Kealey et al. 2005, Beattie et al. 2008). This may suggest that ADC first increases as proteoglycan aggregates, i.e. boundaries of water diffusion, degrade and decreases later along with desiccation of the nucleus pulposus due to reduced capacity of NP to bind water (Antoniou et al. 1996). This may explain the finding of relative initial increase in ADC four weeks postoperatively.

The main problem of experimental animal models is that the results cannot be generalized to humans. The response to trauma of the intervertebral disc may be different in animals and humans, and the experimental stab wound model or enzymatic degradation model do not resemble normal events that initiate degeneration in the human intervertebral disc, either. Different experimental injury models, such as endplate injury (Holm et al. 2004, Cinotti et al. 2005) or blocking of the nutritional pathway to the intervertebral disc (Hutton et al. 2004), may initiate degeneration through different processes, and it is not known whether ADC changes would be observed in those models.

Diffusion-weighted MR imaging is prone to errors caused by bulk movement and requires fast imaging sequences. The drawbacks of applying fast sequences, such as single-shot spin-echo EPI used in this study, are increased susceptibility to artefacts and reduced SNR. However, susceptibility artefacts were not detected in the visual evaluation of images. To evaluate the effect of physiological motion in vivo measurements were compared with post-mortem measurements of three animals, and no significant differences in ADC values were found. However, although the physiological motion was minimized by imaging animals under general anaesthesia and by wrapping animals with supporting pillows, the error levels of in vivo T2-measurements compared to in vitro measurements were slightly higher (Figure 4 in original article of study I), which can partly be explained by the errors caused by physiological movements. Another possible source of error in T2 relaxation time and ADC measurements is the manual placement of ROIs, and care was therefore taken in ROI placements. The thickness of the intervertebral disc of the animals was approximately 3 mm, and to avoid the partial volume effect a 1 mm² voxel size was used and the ROIs were accurately placed only on the nucleus pulposus. Automated or semi-automated ROI placement, based e.g. on signal intensity analysis, might reduce the risk of ROI misplacements; however, the workstation used in this study did not provide such tools.
6.2.2 Cross-sectional cohort (II–IV)

Cross-sectional cohorts are used primarily to determine prevalence of diseases (Mann 2003), or in the case of radiology, prevalence of imaging findings. Because the prevalence of incidental degenerative changes in lumbar discs is very high – at least two thirds of asymptomatic people have degenerative changes (Jensen et al. 1994) – population-based cohorts are suitable for studying imaging methods of intervertebral degeneration. By selecting the study population from a distinct geographic area and from similar occupational groups, the possible confounding effects of different aetiologies can be diminished. The study population for studies II–IV consisted of train engineers and paper mill and chemical factory workers from the same region, thus forming a homogenous study group.

A major problem in the current cross-sectional study is the lack of gold standard. Although widely used visual degeneration grading (Pfirrmann et al. 2001) was applied in the current study, there is still a lack of correlation with histology or biochemistry, and diffusion imaging could therefore only be correlated to morphological changes or other MR measurements. During the study, the degeneration grading turned out to be ambiguous. For example, in the age group studied the measured signal intensity of discs was always clearly hypointense, less than 50%, to CSF and thus the literal criteria of isointensity to CSF were not met. Therefore in study II, when signal intensity measurements of disc and CSF were available, both normal and slightly degenerated discs were classified in consensus reading as grade III. However, when the grading was used without signal intensity measurements, the comparison was performed against readers’ image of normal disc, which resulted also in grade II discs (studies III and IV). The inter-observer agreement of visual degeneration grading between two readers was only substantial (684 graded lumbar levels, ICC single measures mean 0.67, SD 0.05). Due to these problems in the grading system, in study IV it was decided to measure separately the signal intensity and height and width of the disc, and in these measurements there was almost perfect inter-reader repeatability.

Although T2-weighted fast-spin-echo pulse sequence is widely used in spine imaging, other pulse sequences, such as Short Tau Inversion Recovery (STIR) or T2 relaxation time quantification, might detect degenerated discs more accurately. Imaging at 3 tesla could also be more sensitive to early degenerative changes and could therefore serve as better reference to diffusion measurements. However, due
to the large sample size in this study the weaker sensitivity of the sequences and field strength should not have obscured the results.

6.3 Diffusion-weighted imaging of degenerative discs (II)

The mean ADC values of this study accord with previous studies (see Table 1). In three studies, however, the average ADC values of normal discs were somewhat lower. The difference may be explained by possible partial voluming in ROI measurements due to use of the axial plane for diffusion-weighted imaging in two studies (Kerttula et al. 2000a, Kurunlahti et al. 2001), and to a different pulse sequence (line scan diffusion imaging) for obtaining ADC values in one study (Bammer et al. 2003). Significant differences in measured ADC values between visual lumbar disc degeneration grades were observed in concordance with previous studies. In the study population, the mean reduction of ADC between grades III and IV was 4%. However, the mean ADC value in grade V discs was 5% higher than in grade III discs and 10% higher than in grade IV discs.

Increased ADC values in severely degenerated discs (grade V) have also been observed previously by other researchers (Chiu et al. 2001, Kurunlahti et al. 2001) and they have been explained by cracks and cavitations in the degenerated nucleus pulposus filled with freely moving liquid. This explanation seems plausible, since the discs with successful ADC measurements showed higher T2/CSF ratio in this study than the non-measured discs (Table 4), indicating overrepresentation of collapsed but high-signal discs among the ADC measurements. The divergent results from severely degenerate discs in some previous reports (Anontiou et al. 2004, Kealey et al. 2005) may be due to exclusion of collapsed discs, varying methodology of degeneration grading, or different study populations.

The dependence of ADC values on anatomical disc level was less evident in this study than in previous studies. However, the previous results are also contradictory, showing the highest ADC values of normal-appearing discs in either cephalad (Kealey et al. 2005) or caudal discs (Kerttula et al. 2000a, Kurunlahti et al. 2001). Only the three lowest lumbar discs were measured because degenerative findings are more common at those levels and because the general-purpose flexible coil produces the best signal for a volume of about 15 cm. Only in grade III degeneration could a statistically significant difference be detected between the L3–4 and L4–5 levels (Table 5), but overall there was no association between anatomical level and ADC values.
6.3.1 Technical aspects

With the current technology it is not easy to measure diffusivity in non-homogenous regions in vivo. Despite the good repeatability of ADC measurements in study II there is obvious internal inaccuracy in the diffusion imaging in the current and previous studies that manifests as large standard deviations (see Table 1 and Table 5). The inaccuracy can partly be explained by noise internal to DW imaging but also by the ROI measurements. Large ROIs average the results from significantly different areas of histology and biochemistry (nucleus pulposus and annulus fibrosus) including different concentrations of collagen, proteoglycans, hydration, and possibly calcifications, which may introduce e.g. susceptibility artefacts.

The effect of random dispersion of measurements can be minimized by increasing the number of measurements from the same individual, either by repeating the imaging or by increasing the number of excitations. The reason why the number of averages was not increased was to avoid bulk motion, which is a known source of error in ADC measurements. Increasing averages would have increased imaging time and the probability of error due to bulk motion. With more modern scanners this problem can be overcome using motion correction algorithms, but unfortunately the equipment used here did not have that option.

Another way to diminish the effect of random dispersion is to increase sample size. In this study there was a large and homogenous study population: the number of subjects studied was 228, whereas in previous studies it ranged from 15 to 44. Therefore measurements of numerous individuals, anatomical levels and different degrees of degeneration could be averaged.

Due to the scanners’ restrictions, a phased array coil could not be used for diffusion imaging. At our institution, there is long experience of diffusion imaging with a general-purpose flexible non-phased array receiver-only RF-coil. Because the coil can be wrapped around the patient’s back it produces a relatively uniform signal throughout the region of interest. However, only a limited area can be measured with the coil, since the magnitude of the ADC values seem to depend on the distance from the coil coverage area. The uniformity of signal was quantified with phantom measurements, showing a decrease in ADC values when moving away from the optimal coverage area of the coil (Figure 8).
6.3.2 Applicability of diffusion imaging to discs

At present, incidental degeneration of an intervertebral disc can be demonstrated by imaging only when new morphological changes appear. Classifying disc degeneration using e.g. the Pfirrmann scale (Pfirrmann et al. 2001) can never be completely objective and therefore quantitative measurements would be a major improvement in the evaluation of intervertebral disc disease (Haughton 2006). ADC values reflect the diffusivity of free water in the measured tissue and thereby yield information about the microstructure of the disc. Changes in microstructure that modify the diffusivity may not be related to the visual changes seen on MR images (Antoniou et al. 2006). In the light of the literature and the
present results, ADC seems to be associated with disc degeneration, which suggests that structural changes in the disc during degeneration are also reflected in the diffusivity of free water. However, there is substantial overlap between normal and abnormal values and the mean standard deviation in current and previous ADC measurements seems to be larger than the difference between degeneration grades. In addition, one study at 3 T reported up to 24% variation in repeated measurements over 4 to 7 weeks, which may suggest that ADC measurements of discs have limited value in longitudinal studies as well (Beattie et al. 2008). Therefore the added value of ADC measurements to morphological imaging in detecting degenerative changes using the current technology is questionable for studying individual patients.

6.4 Diffusion of gadolinium contrast into disc (III–IV)

Nutrition of the disc is dependent on diffusion, and MRI can be used to evaluate the transport of small solutes from systemic blood circulation into intervertebral discs by quantifying the enhancement of the disc after intravenously injected contrast media. Several researchers have studied the enhancement of the disc after intravenous administration of a paramagnetic contrast agent (Ibrahim et al. 1995, Akansel et al. 1997, Nguyen-minh et al. 1997, Nguyen-minh et al. 1998, Rajasekaran et al. 2004); however, the factors that modify the enhancement are still largely unknown. The most comprehensive work so far has documented a 24-hour temporal pattern of diffusion in 10 volunteers and 43 LBP patients (Rajasekaran et al. 2004). The key finding in their work was the introduction of the concept of “an endplate zone” and the description of normal delay of contrast agent diffusion when passing that zone. They also identified changes in diffusion following damage to the endplate zone, and suggested that efforts to understand and prevent disc degeneration should focus on the anatomical and functional characteristics of the endplate zone.

Another interesting recent study on enhancement of disc utilized ionic contrast agent and T1 time mapping (Vaga et al. 2008). The advantage of using a negatively charged contrast agent is that after reaching equilibrium in the tissue being studied it is assumed to distribute inversely to the concentration of negatively charged GAGs, which gives indirect information about the concentration and distribution of GAGs in the disc.
6.4.1 Technical aspects

Uncharged paramagnetic contrast medium was used, because it is known to diffuse more quickly into the intervertebral disc than negatively charged contrast media, which are repelled by negatively charged GAGs (Ibrahim et al. 1995, Vaga et al. 2008). T1 mapping was used, because it allows more reliable quantification and better visualization of enhancement than simple T1-weighted imaging. If the relaxivity rate of the gadolinium complex in the disc was known, the absolute concentrations of the diffused gadolinium could also be estimated. For a single compartment model the change in the R1 relaxivity ($\Delta R_1$) is directly proportional to the paramagnetic contrast agent concentration if a constant relaxivity ($R$) is assumed, i.e. $[\text{GdDTPA} - \text{BMA}] = R \Delta R_1$. The drawback of using T1 mapping is longer imaging time, 20 minutes in this study, which reduces the temporal resolution of the technique, making evaluation of the early enhancement impossible. Considerable variation was measured in the T1 relaxation times in the non-contrast images, in agreement with literature (Boos et al. 1994), and quantification of the enhancement rate thus requires pre- and post-contrast measurements. The post-contrast measurements were timed 90 minutes after the injection of gadolinium because the observed decrease in the T1 relaxation time in a pilot study (data not shown) suggested that the delay between the scans is sufficient for the contrast medium to diffuse into the disc and because a washout of gadolinium from the nucleus pulposus was already observed at three hours after the injection of the contrast agent. Earlier experimental studies with animal models have shown the equilibrium of contrast enhancement in the nucleus pulposus to be reached in 60–100 minutes in both normal and degenerated discs (Ibrahim et al. 1995, Nguyen-minh et al. 1997). In humans, measurable enhancement has been observed 10 minutes after the injection and the equilibrium has been reached between 2 and 6 hours, depending on which area of the disc was being assessed (Akansel et al. 1997, Rajasekaran et al. 2004). With ionic contrast agent a 3.5-hour delay has been used (Vaga et al. 2008). In clinical setting it is favourable to use a delay that allows one-stay measurements.

6.4.2 Determinants of contrast agent diffusion into the disc

The loss of height of the disc had the strongest correlation with increased enhancement of the disc 90 minutes after intravenous gadolinium contrast injection. The height of the disc explained approximately 46% of the
enhancement. The loss of height shortens the distance from the endplate to the
centre of nucleus pulposus, which, if diffusivity remains unchanged, allows the
contrast medium to reach the centre of the disc more rapidly. However, the height
is only one explaining factor and its effect is less evident in normal to moderately
degenerated discs, where it explained approximately 21% of the enhancement.

The second strongest association with enhancement was the presence of bone
marrow changes (Modic changes) adjacent to the vertebral endplates. The
changes presenting Modic type 1 signal had stronger association with the
enhancement than pure type 2 changes. An explanation for this may be the
vascular granulation tissue that has been found in histological examination of
type 1 changes, whereas the fatty degeneration of bone marrow found in type 2
changes is less vascular (Modic et al. 1988b). This theory is also supported by a
recent histological study, where degenerative lesions of the endplate were
reported to be associated with an in-growth of capillary buds into the cartilage
tissue, showing an association between degeneration and increased
vascularization of the peripheral zones of the disc (Nerlich et al. 2007).

Endplate defects were the third strongest determinant of the enhancement in
the current study. They are commonly found in association with Modic changes
(Modic et al. 1988b), but they do not always co-occur. For example, at the L1–2
and L2–3 levels Modic changes are rare whereas small endplate defects (typically
Schmorl’s nodes) are common. In this study 8 of the 31 endplate defects were at
those anatomic levels and they were also associated with increased enhancement
when compared to normal endplates. This may suggest that the integrity of the
endplate has an important role in regulating nutrient transfer to the disc. This
finding is supported by the finding of an abnormally short delay in contrast agent
transfer passing the endplate cartilage reported in a previous study (Rajasekaran
et al. 2004).

Narrowing of the lumbar arteries has been shown to associate with
degenerative changes in the disc (Kauppila & Tallroth 1993, Kurunlahti et al.
2001, Tokuda et al. 2007), which may indicate that reduced blood flow due to
atherosclerotic changes is interfering with the supply of nutrients. However, in the
present study the association between lumbar artery narrowing and changes in
enhancement of the intervertebral disc could not be demonstrated. In this and one
previous study (Rajasekaran et al. 2004) the enhancement in degenerated discs
seems to be more prominent than in normal discs, which may suggest that the
vascular narrowing at the lumbar artery level does not directly reduce the vascular
supply to intervertebral disc but may precede other changes, perhaps in the
endplate or adjacent bone marrow, that lead to disc degeneration. Vascular supply at the lumbar spine area is also well secured since lumbar discs receive blood from four segmental arteries that form anastomosing vertical vascular networks along longitudinal ligaments and around discs (Urban & Winlove 2007). Therefore, even occlusion of a single segmental artery may not significantly alter the nutrient supply to the disc. However, since there were no totally occluded arteries in the studied population, the enhancement of disc could not be evaluated in such a context. Furthermore, the ceMRA used to image lumbar arteries may not depict all ostial narrowings because of its low resolution. However, due to minimal invasiveness, ceMRA can be considered as the gold standard in imaging volunteers.

Since nutrients are transported into normal discs solely by diffusion, the decreased diffusivity of water in the disc could also slow down the diffusion of nutrients. In this study no association between post-contrast enhancement and ADC of the disc was found. However, any mild association may have been obscured by the rather large standard deviation of the ADC in the measurements in combination with a relatively small study population and the measurements of post-contrast T1 relaxation times only 90 minutes after contrast agent injection.

### 6.4.3 Applicability of delayed gadolinium enhancement measurements in imaging of disc

Delayed gadolinium enhancement measurements of the disc provide a functional tool to study the integrity of the vertebral endplate, which may open new paths in spine imaging. However, there are still technical problems with the method. First, the long diffusion times of gadolinium may cause problems in clinical workflow. Non-ionic contrast medium was used because it diffuses relatively fast in the disc and a 90-minute delay after gadolinium injection proved to be sufficient. Negatively charged contrast media diffuse more slowly, but on the other hand they may reveal additional information about the GAG distribution of the disc. Second, due to the 20-minute imaging time the temporal resolution of T1-mapping does not allow evaluation of the early enhancement. This could possibly be overcome with multi-slice or fast 3D T1-mapping (Mamisch et al. 2008) or evaluating early enhancement separately with dynamic single inversion recovery pulse sequence. Third, the safety status of gadolinium contrast agents has recently been undermined. Gadolinium has been related to nephrogenic systemic fibrosis (NSF), which is a very rare but severe adverse effect. NSF has only been
observed in patients with known renal failure and is strongly associated with the use of double-dose (0.2 mmol/kg) gadodiamide, the same agent as used in the current study (Broome et al. 2007, Kuo et al. 2007). In general, gadolinium contrast agents are still considered safe in individuals without known renal failure and therefore they can be used in epidemiological studies as long as the renal function of the individuals studied is not impaired.
7 Summary and conclusions

1. Early traumatic or degenerative changes are detectable with both T2 relaxation time and apparent diffusion coefficient measurements. The results of this study suggest that T2 relaxation time of the lesioned discs starts decreasing within a few weeks after stab wound trauma, whereas ADC of the disc may initially show increased values compared to control discs before it begins to decrease.

2. ADC values in moderately degenerated discs are significantly lower than in normal discs. However, severely degenerated discs may show increased ADC values. T2 signal intensity was the only single measure that correlated with decreased ADC. ADC values were not dependent on the anatomical level as suggested in some previous studies. Because there is considerable overlap between the ADC values of normal and degenerated discs, ADC measurements of intervertebral discs are more suitable for epidemiological studies than for clinical use, at least with current MR technology.

3. A method for T1 relaxation time measurements was developed to quantify delayed enhancement of intervertebral disc after intravenous gadolinium contrast agent injection. The results on change in relaxation rate suggest that moderately and severely degenerated discs enhance more intensively than normal discs at 90 minutes after contrast injection.

4. While lumbar artery stenosis and ADC values of discs did not correlate with the delayed post-contrast enhancement of the intervertebral disc, enhancement was associated with disc space narrowing and the presence of degenerative endplate changes, especially vascularized bone marrow changes and endplate defects, suggesting an important role for the endplate in maintaining the integrity of the disc. However, in clinical setting the value of information about endplate integrity does not justify the extra costs of contrast agent and prolonged imaging time before therapeutic tools against disc or endplate degeneration are available.
References


Original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals.


Reprinted with permission from Taylor and Francis Informa (I), Elsevier (II), John Wiley & Sons, Inc. (III), and ESMRMB (IV).

Original publications are not included in the electronic version of the dissertation.
1005. Iinattiniemi, Sari (2009) Fall accidents and exercise among a very old home-
dwelling population

1006. Westerlund, Tarja (2009) Thermal, circulatory, and neuromuscular responses to
whole-body cryotherapy

ischemic event in general population. A case-control study

1008. Kuisma, Mari (2009) Magnetic resonance imaging of lumbar degenerative bone
marrow (Modic) changes. Determinants, natural course and association with low
back pain

metabolic and epidemiological studies

1010. Löfgren, Johan (2009) Genetic polymorphisms in collectins and Toll-like receptor
4 as factors influencing susceptibility to severe RSV infections and otitis media

and genetically engineered experimental models of cardiac excitation-contraction
coupling

techniques, evaluation of outcomes after complications and biochemical and
histological analyses of collagen type I and III and tenascin-C expression in the
Achilles tendon

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hemorrhage

työhyvinvoinnin ytimenä hoitotyössä

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DIFFUSION-WEIGHTED MRI AND DELAYED CONTRAST ENHANCEMENT OF DEGENERATED INTERVERTEBRAL DISC

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