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# In vivo Sub-regional dGEMRIC Analysis and Contrast Distribution in Clinical Studies of Human Knee Cartilage

Zana K Hawezi



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DOCTORAL DISSERTATION

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<p>Title and subtitle</p> <p>In vivo Sub-regional dGEMRIC Analysis and Contrast Distribution in Clinical Studies of Human Knee Cartilage</p>		
<p><b>Abstract</b></p> <p><b>Aims:</b> This work was carried out to investigate whether considering cartilage depth <i>in vivo</i> dGEMRIC would provide additional information on the molecular content and changes in normal and diseased cartilage.</p> <p><b>Methods:</b> Study I was a longitudinal study on 23 healthy volunteers. Study II was a case-control study on 9 sedentary individuals and 8 elite runners. Study III was a longitudinal study on 30 patients with a history of medial meniscectomy, they were divided into three groups according to self-reported changes in level of physical activity. MRI measurements were performed in femoral knee cartilage pre- &amp; post-injection of Gd-DTPA<sup>2</sup>. Depth-wise dGEMRIC analysis was performed at 1–8 times p.i. depending on the study group.</p> <p><b>Results:</b> Studies I and II: T<sub>1</sub> before contrast was higher in the superficial region than in the deep regions of the cartilage. Gd-DTPA<sup>2</sup> uptake was significantly slower in the deep region than in the superficial region of the cartilage. Gd concentration in the superficial region was independent of cartilage thickness. A trend was seen towards lower Gd concentration in the superficial region of weight-bearing cartilage in elite runners, than in sedentary individuals. In Study III, those who decreased their physical activity showed a significant decrease in dGEMRIC index in the medial weight-bearing cartilage.</p> <p><b>Conclusions:</b> The higher pre-contrast T<sub>1</sub> in the superficial region than in the deep region is an indication of a higher water content in superficial cartilage. The uptake of contrast was found to be from the superficial region of the cartilage, with diffusion into the deeper parts, and this affects the interpretation of bulk dGEMRIC measurements. The Gd concentration in the superficial layer supports dGEMRIC findings that cartilage has an adaptive capacity to exercise. Decreasing physical activity leads to a decrease in GAG content in the cartilage. Variation in cartilage thickness is a source of error in dGEMRIC studies that should be considered.</p>		
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*To the people of Kurdistan*

It is not a question of whether we are descended from animals or not, rather are we animals or not?



# Abbreviations

BMI	Body mass index ( $\text{kg}/\text{m}^2$ )
dGEMRIC	Delayed gadolinium-enhanced MRI of cartilage
GAG	Glycosaminoglycan
Gd-DTPA <sup>2-</sup>	Gadolinium diethylene triamine pentaacetic acid
Eq	Equation
MRI	Magnetic resonance imaging
OA	Osteoarthritis
p.i.	Post injection
RF	Radio frequency
ROI	Region of interest
T <sub>1</sub>	T <sub>1</sub> -relaxation time
T <sub>2</sub>	T <sub>2</sub> -relaxation time
T <sub>1 pre</sub>	T <sub>1</sub> -relaxation time of cartilage before contrast injection
T <sub>1Gd</sub>	T <sub>1</sub> -relaxation time of cartilage after saturation with Gd-DTPA <sup>2-</sup>
T <sub>1Gd correct</sub>	T <sub>1Gd</sub> after correction for BMI
dGEMRIC <sub>corr</sub>	dGEMRIC index corrected for cartilage thickness





# Papers Included in this Thesis

- I. In vivo Transport of Gd-DTPA<sup>2-</sup> in Human Knee Cartilage Assessed by Depth-wise dGEMRIC Analysis**  
*Z.K. Hawezi, E. Lammentausta, J. Svensson, L.E. Dahlberg and C.J. Tiderius*  
J Magn Reson Imaging. 2011 Dec;34(6):1352-8
  
- II. Can Cartilage Adaptive Capacity Studies Be Improved by Sub-regional dGEMRIC Analysis?**  
*Z. K. Hawezi, C. J. Tiderius, E. Lammentausta, J. Svensson, Lars E. Olsson and L. E. Dahlberg*  
Submitted to J Magn Reson Imaging
  
- III. Sub-regional dGEMRIC Analysis in Patients at Risk of OA Provides Additional Information on Activity-Related Changes in Cartilage Structure**  
*Z. K. Hawezi, E. Lammentausta, J. Svensson, E.M. Roos, L. E. Dahlberg, and C. J. Tiderius*  
Submitted to Osteoarthritis and Cartilage



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# 1 Introduction

## 1.1 Cartilage

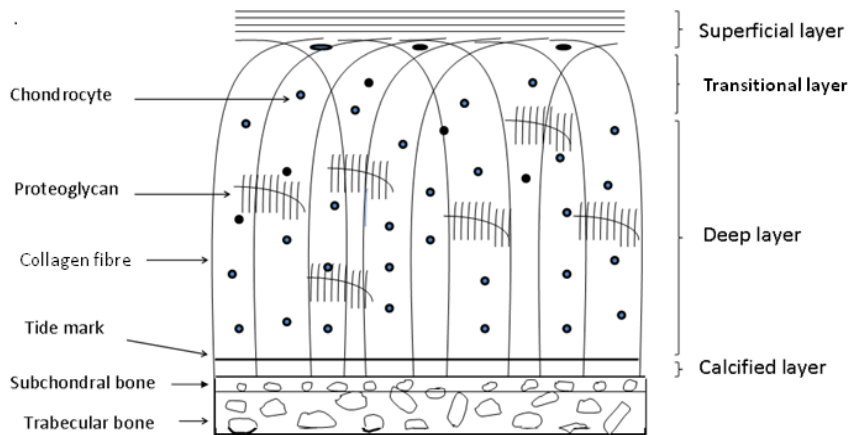
The human body contains three main types of cartilage: elastic (e.g. in the ear and nose), fibrous (e.g. the menisci and intervertebral discs) and hyaline cartilage (joint surfaces). The biomechanical properties of each type of cartilage are strongly influenced by their biochemical composition and the molecular structure of their extracellular matrices, which determine the function of each type of cartilage (1).

## 1.2 Articular Cartilage

Articular cartilage is made up of hyaline cartilage, which is a highly specialized avascular, aneural tissue that provides low friction and load distribution in the joints. It is mainly hypocellular, and chondrocytes constitute about 4% of the wet weight (2). The average thickness of the cartilage in the knee joint is about  $2.0 \pm 0.3$  mm. The articular cartilage under the patella is the thickest, up to 7 mm (1). There is a significant difference in cartilage volume (thickness and surface area) between males and females. These gender differences are mostly due to the difference in joint surface area and, to a lesser extent, to cartilage thickness (1). Cartilage thickness decreases significantly with age, and in the presence of OA (3).

The major components of hyaline articular cartilage are extracellular matrix and solid material. Water constitutes 65 – 85% of the extracellular matrix, and its decreases from the surface to the deep cartilage (2). During motion, the cartilage is compressed, and the interstitial water flows through collagen-proteoglycan pores. This flow induces additional forces within the tissue, and it is important for cartilage physiology (1). Chondrocytes are responsible for making up cartilage matrix molecules, and previous experimental studies have shown chondrocytes to be sensitive to their mechanical environment; different loading in weight-bearing and non-weight-bearing articular cartilage influences the topographical variation of the glycosaminoglycan (GAG) content (4-6).

The solid components consist of collagen (15–20%) and proteoglycans (3–10%) (7). The main kind of collagen in articular cartilage is type II, however, small amounts of types VI, IX, XI, XII, and XIV are also present (1). The main function of collagen is to provide tensile strength to the tissue. Collagen forms fibres in the cartilage, the orientation of which varies according to the loading stress placed on the cartilage. In the superficial layer of articular cartilage, collagen fibres are oriented parallel to the cartilage surface. In the transitional layer, i.e. the layer below this, collagen fibres mostly curve towards the deep region, and are more perpendicular to the cartilage surface. The deep layer of cartilage is firmly bound to the subchondral bone by collagen fibres (Fig. 1.1).



**Figure 1.1** Illustration of the structure of articular cartilage and subchondral bone.

Proteoglycans are large proteins in which at least one GAG molecule is bound. The most abundant proteoglycan in articular cartilage is aggrecan. Aggrecan consists of a core protein to which many GAG side chains are linked; these include chondroitin sulphate and keratin sulphate (1). Both chondroitin sulphate and keratin sulphate are unbranched polysaccharide units. They are highly negatively charged due to the presence of sulphate or carboxyl groups, or both, on many of the sugar residues. These carboxyl and sulphate groups provide the tissue with a high negative charge density that draws cations (mostly  $\text{Na}^+$ ) into the cartilage. These attract water and create a swelling pressure that is counteracted by the collagen network. This interaction is the key mechanism behind the viscoelastic properties of articular cartilage that provide the joint with the necessary resistance to mechanical loading (8).

## 1.3 Permeability

Cartilage is permeable from both the superficial layer and the subchondral bone (8). This permeability is essential for cartilage nutrition and the transport of signal molecules to the chondrocytes, and also for the removal of metabolites at the rates necessary for cell survival. However, the permeability of the articular cartilage varies with depth from the superficial layer to the subchondral bone. It has been reported in previous studies that diffusion is one hundred times higher in the superficial layer than in the deep layer next to the subchondral bone (9,10). Only small molecules can perfuse through the subchondral bone to the cartilage. The transport of small solutes occurs mainly through the pores within the calcified zone in the deep layer of the cartilage. The size and permeability of these pores may be dependent on the degree of mineralization of the calcified region of the cartilage (10).

## 1.4 Mechanical Load and Exercise

*In vivo* studies on human articular cartilage have revealed a decrease in knee cartilage thickness after 6–7 weeks of absence of mechanical stimulation (11). Thickness recovery following 24 months of remobilization was also reported (11). An animal study using beagles, immobilization of articular joints for 11 weeks reduced the GAG content in the cartilage, while remobilization induced the restoration of the GAG content in most of the compartments (loaded and unloaded) (12). A decrease in indentation stiffness after 11 weeks of immobilization has also been reported by the same group. Long periods of immobilization lead to permanent alteration of biochemical and biomechanical properties of the cartilage; especially change in proteoglycan content, which jeopardizes the morphological integrity and mechanical competence (13). In addition to immobilization, the absence of load bearing during motion also causes a decrease in GAG content in the cartilage and seems to affect its integrity (14).

With regard to mechanical loading, self-reported exercise and the intensity of exercise have been found to be significantly associated with cartilage volume in children, and are thus important for growth and development (15,16). However, in adults, exercise seems to have more effect on the change in cartilage volume by increasing the surface area of the cartilage, rather than the thickness of the cartilage (17). This indicates that the capacity of cartilage to withstand load may be improved by increased surface area.

Several animal studies have revealed an increase in GAG content in the weight-bearing regions of the cartilage following exercise (18-20). Similar results have



also been reported in humans (21), in this study, using dGEMRIC, elite runners showed a higher GAG content in weight-bearing femoral knee cartilage than others with a lower level of activity.

## 1.5 Osteoarthritis

Osteoarthritis is a chronic degenerative disease of the cartilage, which is characterized by joint pain and disability. It most commonly occurs in load-bearing joints such as the knee (22,23). The prevalence of OA is 10% in people older than 55 years and 30% in people older than 65 years. Loss of GAG and subsequent disruption of the collagen network are considered early signs of OA before macroscopic changes can be detected (7,22-24). GAG loss starts in the superficial region of the cartilage before it occurs in the deeper layers (25-27). Damage to the collagen network also starts on the surface of the cartilage and then progresses into the deeper layers, leading to progressive cartilage swelling (28,29). In the later stages of OA, a progressive loss of articular cartilage is seen, finally resulting in complete loss of cartilage (28). During the progression of OA, dramatic changes take place in the subchondral bone such as: sclerosis, cyst formation and the development of osteophytes (30,31). These changes in the subchondral bone are associated with increased vascularization in the region, which invades the calcified zone of the cartilage and increases subchondral permeability (32,33).

Different methods have been developed to assess OA, such as clinical radiography (34) and arthroscopy (35), and the collection of information from patients regarding their perceptions of the disease (36,37). However, radiological features such as joint space narrowing, osteophytes and sclerosis, introduced by Kellgren and Lawrence in 1957, are still considered the gold standard for the diagnosis of OA. The most important limitation of the above methods is that physical signs and symptoms of OA do not appear until cartilage destruction is already beyond the point of repair.

Many factors are related to the onset of OA, such as age, genetics and gender, all of which are difficult to avoid, whereas others are related to lifestyle. OA usually occurs in later life, but it may also occur in children as a result of exposure to concentrated stress, for example, osteonecrosis associated with a lack of remodeling (38). The prevalence is also higher in women than in men; OA commonly developing after the menopause, which may indicate that hormones play an important role in the development of OA (39). Obesity is one of the most important lifestyle-related risk factors for OA. The incidence of OA and the progression of degenerative changes in middle-aged individuals with radiographic OA is much higher among obese than non-obese individuals (40). Other risk factors include

lifting heavy loads at work (knee bending or kneeling) and post-traumatic OA, such as meniscus injury or cruciate ligament injury, and these are associated with a high risk of OA (41).

## 1.6 The Menisci

The menisci are very important structures in the knee joint as they provide stabilization to the joints under load (42). The meniscus is semi-lunar in shape, consisting of fibrous tissue, and is situated between the femoral condyles and tibial plateau of the medial and lateral compartment of the knee. The matrix of the meniscus consists of about 70% water; the remainder being the extracellular matrix and cells (43). The extracellular matrix consists mainly of collagen (75%) and GAG (17%) (36,44).

The meniscus is highly vascular at birth, but this decreases during maturity. In the adult, the meniscus contains only 10–25% blood vessels and nerves, and these are only found in the peripheral part of the tissue, which is called the red-red area; the remaining part of the tissue is avascular aneural tissue, and is called white-white area. These two areas are separated by a transitional zone called the red-white area (45). These areas have different healing capacities directly related to the degree of vascularisation, meaning that the lesions in the white area have poor reparative response (46).

The main function of the meniscus is to withstand many different kinds of forces such as shear, tension and compression. It also plays an important role in load transmission and shock absorption, as well as lubrication and nutrition of the articular cartilage (47,48). The C-shaped medial meniscus covers 50% of the medial tibial plateau. Its anterior horn is narrower than the posterior horn, and both are firmly attached to the tibia. The outer border of the medial meniscus merges into the knee joint capsule. The lateral meniscus is more circular in shape, and covers about 70% of the lateral tibial plateau. It is more mobile as it is not attached to the lateral collateral ligaments, which makes it less susceptible to injury than the immobile medial meniscus (49,50) (Figure 1.2).

### 1.6.1 Meniscus Tear

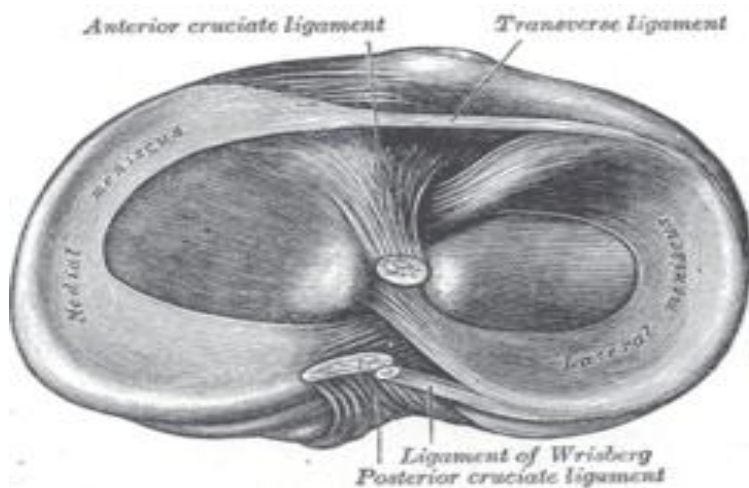
A tear in the meniscus is one of the most common intra-articular knee injuries in the United States, and a common cause of orthopaedic surgical procedures (51,52). Rupture can occur at any age, but the aetiological factors are highly related to the age of the patient, for example, the peak incidence occurs between the ages of 20 and 29 (52-54). Among the younger age group meniscus rupture is mostly

traumatic and sport related, while in older age groups, it is usually degenerative, or the result of a minor trauma or OA (55). The male to female incidence ranges between 2.5:1 and 4:1 (53,54,56).

Different systems are used to classify meniscus tears, for example, one of the common systems is based on the tear pattern: vertical = longitudinal (including bucket handle), flap = oblique, radial = transverse, and horizontal = complex (57-59). Degenerative meniscus tears are usually of the horizontal or flap type, have a very poor prognosis, and are associated with a high risk of cartilage degeneration and OA compared to traumatic tears (60).

### 1.6.2 Meniscectomy

In the past, the choice of treatment for meniscus tear was total or subtotal meniscectomy, performed as open surgery (61). However, meniscus loss has been shown to be associated with instability and a change in the biomechanics of the joint, leading to increased load on the articular cartilage and eventually more severe cartilage degeneration (37,57). The current recommendation is partial meniscectomy in the case of tears that are not repairable, in order to preserve as much normal meniscus as possible (50). Meniscus tear and meniscectomy are well-recognized risk factors for OA of the knee with a relative risk of 14, after 21 years (62).



**Figure 1.2** The meniscus and attached ligaments, seen from above (Gray H, 1918).

## 1.7 Magnetic Resonance Imaging

Magnetic resonance imaging has the unique ability to non-invasively display high-resolution images of all the structures in the knee: cartilage, menisci, ligaments, muscle and subchondral bone. It is also capable of detecting changes resulting from OA such as bone marrow lesions, synovial changes, capsule thickening, and meniscus maceration and extrusion (63). Measurements of cartilage thickness and volume have been used as a means of monitoring progression of the disease in patients with knee OA (64,65).

Conventional MRI methods show morphological changes in the cartilage at the stage when OA has already progressed. The early stage of OA is characterized by biochemical and structural changes in the extracellular matrix, which lead to changes in the biomechanical properties of the tissue. Thus, conventional MRI cannot be used for the detailed assessment of early cartilage pathology (66). New modalities, such as dGEMRIC, are needed to detect OA in the early stages.



# 2 The Physics of Magnetic Resonance Imaging

## 2.1 Introduction

The best way of understanding MRI, is through the physics of proton nuclear magnetic resonance, which is based on the interaction of a magnetic nucleus and its spin with an external magnetic field,  $B_0$ . All atomic nuclei consist of nucleons: protons (positively charged) and neutrons (uncharged), both of which possess a characteristic spin. For most atomic nuclei, the spins of the nucleons cancel each other out; however, for some atoms with an odd number of protons (e.g.  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{19}\text{F}$ ,  $^{31}\text{P}$  and  $^{23}\text{Na}$ ), their spin results in a magnetic moment. MRI is based on the spin of the nucleus of the hydrogen atom in water.

## 2.2 Nuclear Spin of the Hydrogen Atom

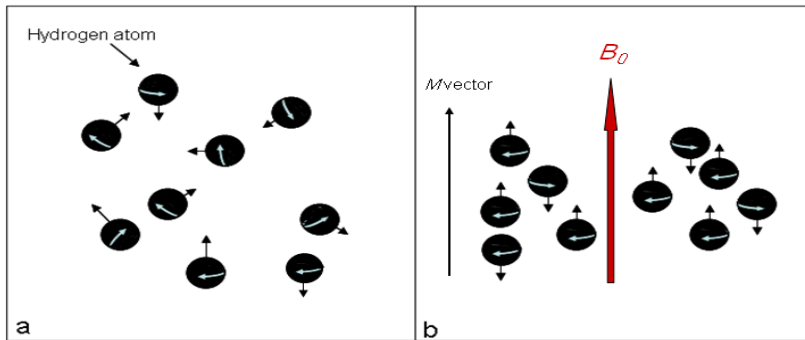
Hydrogen nuclei consist of a single positively charged proton. The spin of this positively charged proton is equivalent to a rotating current. This generates a small magnetic field along the axis of rotation. Hence, each nucleus has its own intrinsic magnetic vector, similar to a small compass needle. When there are no external influences, the spin vectors of the hydrogen nuclei (protons) are randomly distributed (Figure 2.1a), but when they are subjected to an external magnetic field, they will align in the direction of the applied magnetic field (z-direction) (Figure 2.1b).

However, these vectors are not stationary, but precess around the magnetic field direction with an angular frequency called the Larmor frequency, which is dependent on the strength of the external magnetic field:

$$\omega = \gamma B_0 \tag{2.1}$$

where  $\omega$  is the angular frequency,  $\gamma$  is the gyromagnetic ratio of the nucleus, and  $B_0$  is the magnetic field strength. The magnetic field strength commonly used for clinical purposes is 1.5 to 3 Tesla.

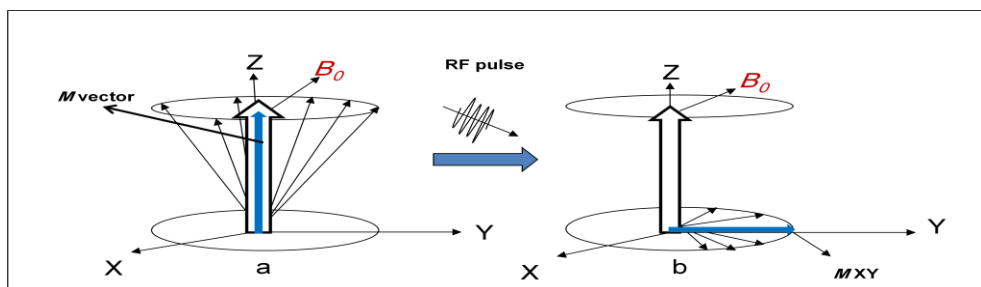
In any tissue sample, there will be millions of hydrogen nuclei. The precession of their intrinsic magnetic vectors under the force of an external magnetic field forms a bulk magnetization vector ( $M$ ) parallel to the direction of the magnetic field.



**Figure 2.1** Illustration of the magnetic vectors of hydrogen nuclei (protons): (a) without an external magnetic field and (b) in the presence of an external magnetic field of strength  $B_0$ .

## 2.3 Transverse Magnetization and Signal Detection

In order to detect a nuclear magnetic resonance signal, it is necessary to excite the nucleus of the hydrogen atoms in the tissue, in order to tilt the bulk magnetization vector from  $M$  into the XY plane (perpendicular to  $B_0$ ) (Fig. 2.2). This can be done by applying a radio frequency (RF) pulse. Such pulses are generated by an external coil, which oscillates at the Larmor frequency.



**Figure 2.2** Hydrogen spin under the influence of an external magnetic field: (a) before the radio frequency (RF) pulse and (b) after the RF pulse.

The precessing proton will pick up some energy from the RF pulse, resulting in the formation of transverse magnetization, which will also precess at the Larmor frequency. This time-varying transverse magnetization can induce an electric current in an external receiver coil (the magnetic resonance signal), as shown in Figure 2.3.

The strength of the signal depends on the amplitude of the transverse magnetization. When the RF pulse is turned off the protons return to their equilibrium state ( $M$ ).

## 2.4 Signal Localization and Image Formation

In order to localize the signal in the body, three more magnetic fields must be superimposed on the main magnetic field along the X, Y, and Z axes. This can be achieved by using gradient coils. The strength of each magnetic field varies with location, hence these fields are called “gradients”.

### 2.4.1 Slice Selection Gradients

The slice selection gradient gradually increases the magnetic field strength from one end of the investigated volume to the other. Hence, the nuclei at different positions in the investigated volume will precess with different Larmor frequencies. Thus, they will be sensitive to different RF pulse frequencies. The slice thickness is determined by the bandwidth of the RF pulse and the strength of the applied magnetic field. To separate the information from different parts of the selected slice, two more gradients, the phase encoding gradient and the frequency encoding gradient, are applied perpendicular to each other, and perpendicular to the slice selection gradient.

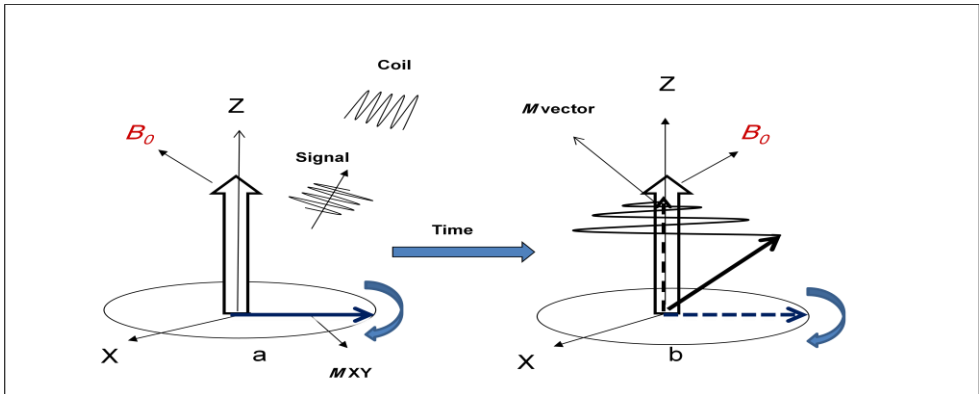
### 2.4.2 Phase Encoding Gradients

This gradient is turned on for a short period immediately after the RF pulse. This creates a difference in signal phase from hydrogen nuclei at different positions. Signal acquisition must be repeated several times, using phase encoding gradients with different strengths. The number of times the signal is measured depends on the desired resolution of the image; e.g. 256 times for a 256 x 256 matrix.



### 2.4.3 Frequency Encoding Gradient

This gradient is perpendicular to the slice selection gradient and the phase encoding gradient, and it is applied at the time as signal detection. This causes the frequency of the nuclei along the axis of the gradient to be different. Thus, each column of pixels (picture elements) will contain signals with a different frequency. By analysing the phase and frequency of each emitted signal, information can be obtained on the nuclei in different parts of the investigated volume. An image is formed by reconstruction of the acquired data, using a two-dimensional Fourier transform.



**Figure 2.3** Magnetic resonance signal detection by an external receiver coil.

## 2.5 $T_1$ Relaxation Time

The  $T_1$  relaxation time is the time taken for the longitudinal magnetization to recover after the RF pulse is switched off.  $T_1$  is a time constant corresponding to the time taken for the longitudinal magnetization to return to 63% of its original value.

$T_1$  depends on the composition and structure of the tissue, and the surrounding matter (lattice). When the fluctuations of the magnetic fields in the lattice occur at the Larmor frequency, the transfer of thermal energy between protons and the lattice is facilitated and faster. Thus, protons have a shorter  $T_1$  under such conditions.  $T_1$  can be quantitatively measured, and this has been done in many areas, such as perfusion studies, contrast agent dynamics, the diagnosis of neurological disorders and dGEMRIC.

## 2.6 $T_2$ Relaxation Time

The  $T_2$  relaxation time is the time taken for the transverse magnetization to disappear.  $T_2$  is also similar to  $T_1$  in that it is a time constant. It is the time required for the transverse magnetization to decay to 37% of its initial value.

$T_2$  depends on inhomogeneities in the external magnetic field and inhomogeneities in the local magnetic field within tissues. Water molecules move very fast, which means there will be no difference in magnetic field inhomogeneity within the tissue. Thus, water has a long  $T_2$ . In contrast, liquids with larger molecules, such as fat, have a shorter  $T_2$ , because larger molecules move more slowly than water molecules, and are more affected by magnetic field inhomogeneities.

Other factors affecting both  $T_1$  and  $T_2$  are the magnetic field strength, the temperature and the presence of paramagnetic ions. Paramagnetic substances, such as gadolinium, have small magnetic fields, which cause shortening of the relaxation time of the surrounding protons. Gadolinium is toxic and in order to reduce its toxicity sufficiently for use in clinical studies it is bound to a chelating substance such as  $\text{GD-DTPA}^{2-}$ , which has a high affinity for metal ions.



# 3 delayed Gadolinium-Enhanced MRI of Cartilage (dGEMRIC)

dGEMRIC is one of the new MRI methods developed to assess the GAG content in cartilage, in order to obtain information on the early stages of cartilage pathology, before radiographic changes appear (67). It has been shown that the dGEMRIC index adequately describes cartilage quality in studies on subjects with OA, rheumatoid arthritis and hip dysplasia (68-70).

In dGEMRIC, the negatively charged contrast agent Gd-DTPA<sup>2-</sup> is injected intravenously. It is then distributed throughout the cartilage inversely to the negatively charged GAG content. High uptake of the contrast agent by cartilage indicates a low GAG content, and vice versa (67). Common doses of Gd-DTPA<sup>2-</sup> are: 0.1 (single), 0.2 (double), and 0.3 (triple) mmol/kg body weight. Triple dose showed more sensitive to GAG changes than the lower doses (71,72). However, there are more concerns in radiology community in using double and triple dose of gadolinium-based contrast agents because of its toxicity (73-75). Gadolinium (Gd) has a paramagnetic effect that can shorten the longitudinal relaxation time (T<sub>1</sub>), thereby making it possible to measure the concentration of the contrast agent:

$$[\text{Gd}] = (1/T_{1\text{Gd}} - 1/T_{1\text{pre}})/r_1, \quad (3.1)$$

where T<sub>1Gd</sub> is the value of T<sub>1</sub> at a certain point in time after injection of the contrast agent, T<sub>1pre</sub> is the value of T<sub>1</sub> before Gd-DTPA<sup>2-</sup> injection, and r<sub>1</sub> is the relaxivity of Gd-DTPA<sup>2-</sup>, (r<sub>1</sub> = 4.1 s<sup>-1</sup>mM<sup>-1</sup> in human plasma at 37°C (76). Thus, a higher value of T<sub>1Gd</sub> indicates a low Gd concentration (high GAG content), and vice versa.

The measurement of pre-contrast T<sub>1</sub> has been considered unnecessary, as it has been assumed that the T<sub>1</sub> of native cartilage is relatively constant (77,78). This suggests that it is sufficient to measure T<sub>1Gd</sub> to obtain the Gd concentration, which simplifies the procedure significantly (78). It has been shown that the relaxivity varies between the different cartilage layers due to their different contents of macromolecules, thereby introducing some uncertainty into the calculation of Gd-DTPA<sup>2-</sup> concentration (79,80).

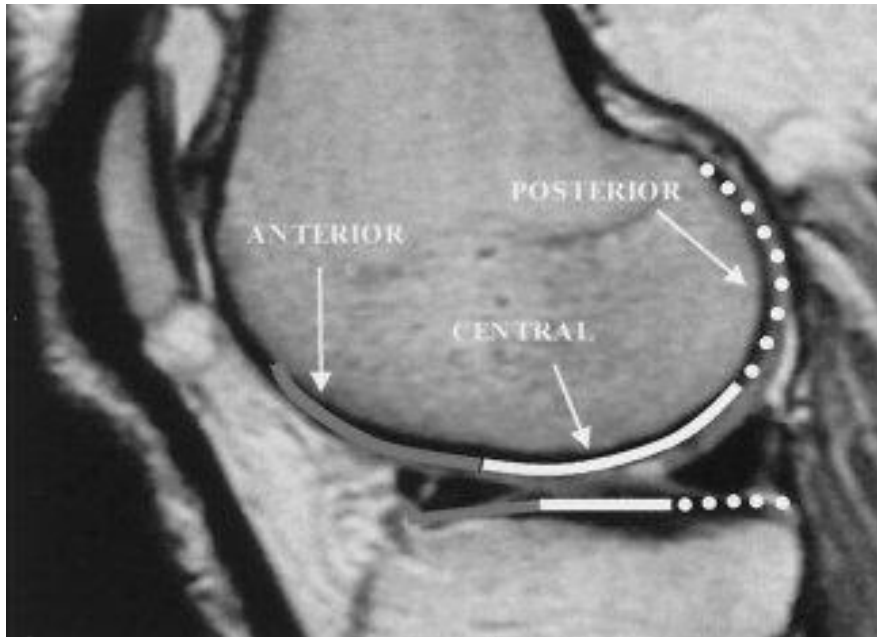
The maximum recommended time point for image acquisition of the knee joint is 90-120 minutes after injection of the contrast agent (72,77). In addition, moderate exercise is usually performed following contrast agent administration before the MRI scan, to optimize the uptake of the contrast agent by the articular cartilage (77).

In a study of patellar cartilage comparing intravenous and intra-articular injection of Gd-DTPA<sup>2-</sup>, the saturation of cartilage was faster after intravenous injection, indicating that contrast agent penetrated the cartilage from both the synovial fluid and the subchondral bone (71,81). However, it was also concluded that the optimal time for dGEMRIC measurements depends on the thickness of the cartilage.

Body mass index (BMI), has been found to affect the dosage in dGEMRIC, and a correction factor has been proposed (82). A high BMI is associated with low dGEMRIC index, and vice versa. The dGEMRIC index can be corrected for BMI dosing bias using the equation:

$$T_{1\text{Gd correct}} = T_{1\text{Gd}} (\text{measured}) + 3 (\text{BMI} - 20) \quad (3.2)$$

The delineation of the region of interest (ROI) in femoral cartilage imaging differs from study to study, and depends mainly on the area of cartilage that is of interest in the particular study (80,81). ROIs in the femoral cartilage have also been subdivided according to the loading, into anterior (non-weight-bearing), central (weight-bearing) and posterior (non-weight-bearing) (Fig. 3.1) (83). The intra-observer variation in ROI delineation is generally low (84).



**Figure 3.1** Subdivision of the region of interest according to the loading (Sandip, 2002).

### 3.1 Previous dGEMRIC Findings

In previous dGEMRIC studies of patients with early OA, signs of GAG loss (a low dGEMRIC index) have been found in both the knee and the hip (23,85). Compartmental dGEMRIC analysis has shown a subtle difference between the dGEMRIC index in the medial and lateral femoral cartilage, with a higher dGEMRIC index laterally (21). This indicates a higher GAG content in lateral than in medial femoral cartilage, although other explanations have not been ruled out. dGEMRIC studies at our department, and at others, suggest that human femoral weight-bearing cartilage has adaptive capacity, and that physical exercise increases the GAG content in the weight-bearing area in both normal and injured cartilage (86,87).



# 4 Aims of this Work

## 4.1 General Aims

In most previous *in vivo* dGEMRIC studies, only the bulk dGEMRIC index in the weight-bearing regions of cartilage has been analysed and compared. The aim of the work described in this thesis was to evaluate the GAG content of articular cartilage using sub-regional analysis in both weight-bearing and non-weight-bearing femoral knee cartilage.

## 4.2 The Specific Aims

The specific aims were:

- I. to investigate the transport of Gd-DTPA<sup>2-</sup> into human knee cartilage *in vivo* by depth-wise dGEMRIC analysis.
- II. to investigate the significance of cartilage thickness, and to compare the ability of the dGEMRIC index with gadolinium concentration in differentiating the quality of articular cartilage in individuals with different physical activity levels *in vivo*.
- III. to evaluate the ability of *in vivo* sub-regional dGEMRIC analysis to provide additional information, compared with the bulk value, in identifying the effects of changes in physical activity level on differently loaded regions of cartilage, in patients at high risk of developing OA.





# 5 Methods

## 5.1 Subjects and Patients

The aim of the first study (Paper I) was to investigate the temporal and spatial dynamics of Gd-DTPA<sup>2-</sup> contrast agent in femoral knee cartilage. Twenty-three healthy volunteers, 12 male and 11 female, aged between 19 and 45 (mean age 25.4 years; SD 6.35) were recruited. The exclusion criteria were as follows: (i) history of trauma or pain in either knee, (ii) regular medication with the exception of oral contraceptives and vitamins, (iii) contraindications for MRI (i.e., metal prosthesis, claustrophobia or serious allergy), (iv) receiving or scheduled to receive another contrast agent within 1 week before, or 2 weeks after the proposed examination, (v) abnormality at physical examination of the knee, and (vi) different positioning of the knee between investigations, as shown by changes in the thickness of the cartilage (number of pixels).

Study II was carried out to investigate the significance of cartilage thickness, and to compare the ability of the dGEMRIC index with Gd concentration in differentiating between articular cartilage quality in individuals with different physical activity levels *in vivo*. Seventeen healthy volunteers were included. The study was approved by the local ethics committee. The subjects were divided into two groups according to their level of physical activity: Group I (the sedentary group) consisting of 9 volunteers who did not participate in any kind of physical activity and Group II (the exercise group), which consisted of 8 male elite athletes running an average of 90 km per week. The characteristics of the two groups are given in Table 5.1.

**Table 5.1** Gender, age, height, weight and BMI in the sedentary group (Group I) and the exercise group (Group II). Mean (SD, and ranges).

Study II	Group I (n=9)	Group II (n=8)
Males / Females	7/2	8 males
Age (y)	23.8 (2.8;21–30)	25.2 (2.3; 23–29)

Height (cm)	173 (10; 159–187)	182 (3.9; 175–188)
Weight (kg)	69 (15.2; 50–91)	71 (2.1; 69–76)
BMI (kg/m <sup>2</sup> )	23 (3; 17.3–26.9)	21.4 (0.8; 20.4–23.2)

The aim of Study III was to evaluate the ability of *in vivo* sub-regional dGEMRIC analysis to provide additional information, compared with bulk value, in identifying the effect of changes in physical activity level on different loading cartilage, in patients at high risk of OA. Thirty patients were included, 20 male and 10 female, aged 38.4–49.4, mean age 45.7 years, mean BMI 26.6. They were followed up by dGEMRIC measurements. The subjects were middle-aged patients of both sexes who had been treated with partial medial meniscus resection, and who were deemed to be at high risk of developing knee OA. The inclusion criteria were as follows: (i) partial medial meniscectomy 3–5 years previously, (ii) current age between 35 and 50 years, (iii) willingness to participate in the study, (iv) and provision of signed informed consent. The exclusion criteria were: (i) no meniscectomy, (ii) history of cruciate ligament injury, (iii) arthroscopic OA changes (defined as deep clefts or visible bone in the arthroscopy report), (iv) too high a physical activity level, (v) too low a physical activity level (indoor walking), (vi) a self-reported limiting condition, and (vii) not being in the geographical area during the whole study period. The study was approved by the local ethics committee. The subjects were divided into three groups according to self-reported changes in level of physical activity: Group I (increased level of physical activity), 11 subjects who had increased their level of physical activity over the 4-month period of study, Group II (no change in level of physical activity) 13 subjects reporting no change in physical activity over the 4-month period of the study, and Group III (reduced level of physical activity), which included 6 subjects who had reduced their daily physical activity over the 4-month period of the study. The characteristics of the three groups are given in Table 5.2.

**Table 5.2** Gender, age, height, weight and BMI in the three groups in Study III. Mean (SD, range).

Study III	Group I (n=11)	Group II (n=13)	Group III (n=6)
Males / Females	8/3	10/3	2/6
Age (y)	45.4 (3.1; 38.9–49.6)	45.3 (3.5; 38.4–49)	47.3 (1.6; 44.4–49.6)

Height (cm)	1.78 (0.08; 1.63–1.93)	1.74 (0.08; 1.55–1.86)	1.73 (0.09; 1.6–1.89)
Weight (kg)	85 (10; 66–102)	80 (7.9; 69–90)	79 (12.9; 61–96)
BMI (kg/m <sup>2</sup> )	26.9 (3.3; 22.8–34.2)	26.6 (2.9; 22.8–31.6)	26.1 (2.7; 21.3–30.1)

## 5.2 MRI

MRI examinations were performed using a 1.5 T MRI system (Siemens Magnetom Vision) with a dedicated knee coil. A triple dose of Gd (0.3 mmol/kg of Gd-DTPA<sup>2-</sup>, Magnevist, Bayer Healthcare Pharmaceuticals, Germany) was used in all studies, injected slowly, over a period of 2–3 minutes, intravenously. To optimize the contrast uptake, volunteer's walked up and down stairs or cycled, corresponding to seven minutes of exercise, immediately after injection of the contrast agent.

In Study I, both pre- and post-contrast measurements were performed. Consequently, post-contrast T<sub>1</sub> was analysed a total of eight times (from 12 minutes to 4 hours p.i.). The weight-bearing central cartilage was analysed before contrast agent administration and at eight points in time after the intravenous injection of Gd-DTPA<sup>2-</sup>: 12–60 minutes (4 volunteers) and 1–4 hours (19 volunteers). Pre- and post-contrast measurements were also performed in Study II. Post-contrast T<sub>1</sub> measurements were performed 2 hours after injection of the contrast agent. In Study III, only post-contrast T<sub>1</sub> measurements were performed, 2 hours after contrast agent injection.

### 5.2.1 Quantitative T<sub>1</sub> Analysis

T<sub>1</sub> calculations and segmentation of the femoral cartilage regions were performed using an MRI scanner (1.5 Tesla) before (T<sub>1pre</sub>) and after (T<sub>1Gd</sub>) injection of the contrast agent, using the computational software MATLAB<sup>®</sup> (Mathworks Inc., Natick, MA, USA). All dGEMRIC indices were corrected for BMI dosing bias according to Eq. (3.2).

In Study II, dGEMRIC was corrected for the thickness of the cartilage according to:

$$[\text{dGEMRIC}_{\text{corr}}] = \text{dGEMRIC} + (\text{thickness} - 1.5) K \quad (5.1)$$

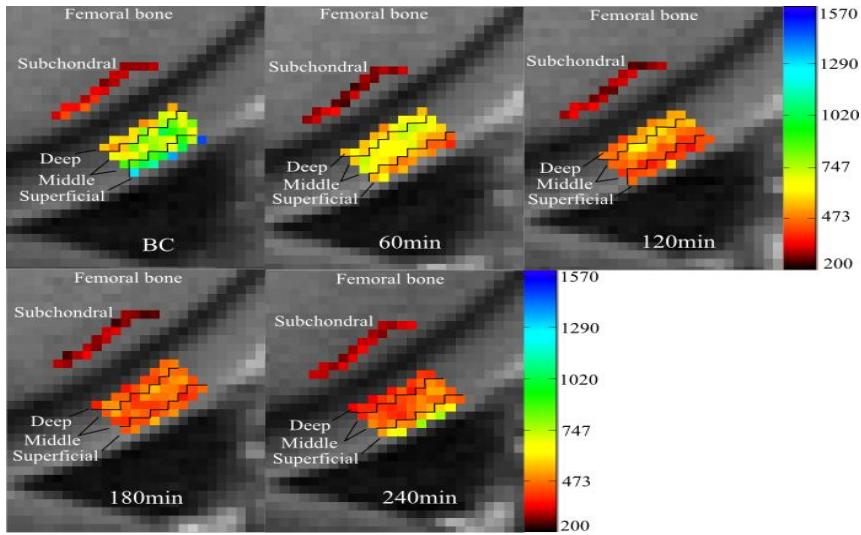
where dGEMRIC has been corrected for BMI, and  $\text{dGEMRIC}_{\text{corr}}$  denotes the thickness-corrected dGEMRIC. K is a factor, which describes how much  $T_1$  changes with respect to cartilage thickness (59.8 ms/mm for bulk cartilage and 148.2 ms/mm for deep cartilage).

In Studies I and II, the gadolinium concentrations for all regions of interest were calculated according to Eq. (3.1).

## 5.2.2 Cartilage Regions of Interest

Sagittal slices covering the central parts of the lateral and medial femoral condyle were localized using the MRI scanner. In Study I, the central parts of the medial and lateral femoral weight-bearing cartilage covered by the posterior horn of the meniscus were divided into segments manually for  $T_1$  analysis. Care was taken by the MRI technician to ensure that the locations of the two sagittal slices, (one in the medial and one in the lateral femoral compartment) were exactly the same in all scans of each patient. In addition, the thickness of the cartilage within each ROI did not change between repeat scans (Figure 5.1). The width of the ROI in the sagittal plane was on average 12 pixels (range 10–14). The thickness of the cartilage within the ROI was uniform in each individual, although it varied between individuals. The cartilage in the ROI was manually segmented into layers with increasing depth. To avoid potential partial volume artefacts, pixels that appeared to include signal originating from both the synovial fluid and the superficial cartilage were excluded.

In Studies II and III, also using slices in the sagittal plane, the central parts of both the medial and lateral femoral condyles were divided into two segments (weight-bearing and non-weight-bearing). The deep and the superficial layers of cartilage were analysed as separate ROIs, and the whole as a full-thickness ROI (bulk value). Cartilage thickness was measured using the Image J program (88). The mean value of three measurements in each segment of femoral cartilage was calculated. As in the first study, pixels that included signal originating from the cartilage and synovial fluid were excluded, as were pixels that included signal from both cartilage and the subchondral bone.



**Figure 5.1** T<sub>1</sub> maps of the analysed regions of interest in lateral femoral cartilage, divided into deep, middle and superficial layers, and subchondral bone, before contrast agent (BC) and 60, 120, 180 and 240 minutes after injection. The thickness of the cartilage within each ROI did not change between repeat scans.

### 5.3 Statistical Analysis

Non-parametric test was used in all the studies. The Wilcoxon signed rank test and the Mann-Whitney rank sum test were used for statistical analysis. To analyse correlations between cartilage thickness and the dGEMRIC index or Gd-DTPA<sup>2-</sup> concentration, Spearman's rank correlation coefficient was calculated. In Study II, Spearman's rank correlation coefficient was also used to compare bulk dGEMRIC index and Gd concentration in the superficial layer.

To evaluate the reproducibility of manual segmentation, segmentation was performed on three consecutive days by the author, and the coefficient of variation (CV) calculated (Paper I).



# 6 Summary of the Results

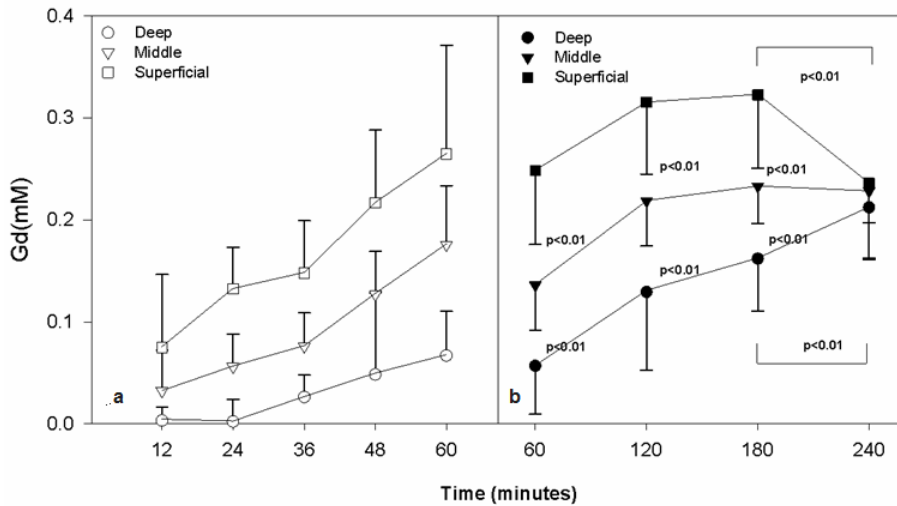
## 6.1 Paper I In vivo Transport of Gd-DTPA<sup>2-</sup> in Human Knee Cartilage Assessed by Depth-wise dGEMRIC Analysis

Measurements of  $T_1$  were performed in the femoral knee cartilage of 23 healthy volunteers. Values of  $T_1$  in the weight-bearing central cartilage were measured before the intravenous injection of Gd-DTPA<sup>2-</sup> and at eight times p.i. Measurements were made on four volunteers 12 to 60 minutes p.i. and on 19 volunteers 1 to 4h p.i. The deep, middle and superficial layers of cartilage were studied separately.

Measurements of  $T_1$  before the injection of Gd-DTPA<sup>2-</sup> showed a variation in  $T_1$  with depth in the cartilage. Values in the superficial layer were 50% higher than those in the deep layer. Measurements at various times after injection showed that the uptake of Gd-DTPA<sup>2-</sup> was not detected in the deep layer until 36 minutes after injection, and the concentration increased up to 240 minutes p.i., whereas in the superficial layer, uptake was seen after only 12 minutes, and the concentration decreased after 180 minutes ( $p < 0.01$ ) (Figure 6.1). A difference was seen in the bulk concentration of DTPA<sup>2-</sup> in the medial and lateral compartments, but not between the concentration in the bulk and the superficial layer. The bulk Gd concentration was negatively related to cartilage thickness ( $r_s = -0.68$ ;  $p < 0.01$ ).

From the above results it could be concluded that the variation in Gd-DTPA<sup>2-</sup> concentration with depth in the cartilage affects the interpretation of bulk dGEMRIC measurements *in vivo*. In thick cartilage, incomplete penetration of Gd-DTPA<sup>2-</sup> will yield a falsely too long value of  $T_1$ .





**Figure 6.1** Estimated Gd-DTPA<sup>2-</sup> concentration (mM, mean  $\pm$  SD) in the lateral femoral cartilage with a thickness of 3–5 pixels. (a) Shows the results from subjects who were recruited to study the early phase after contrast injection (n=4 compartments) while (b) shows the results from subjects studied over a longer period (n = 16 compartments). No Gd-DTPA<sup>2-</sup> was detected in the deep layer until 36 min p.i., whereas an increase was seen in the superficial layer after only 12 min. The maximum concentrations in the different layers were observed at different times p.i.

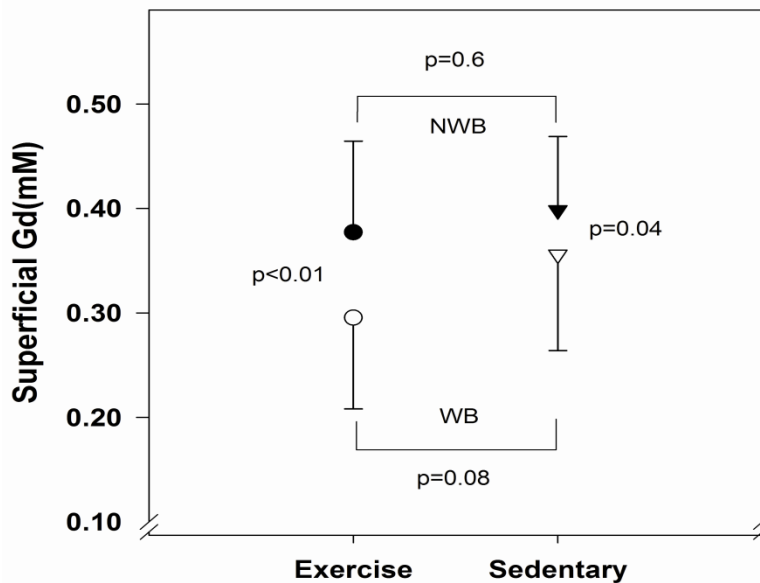
## 6.2 Paper II Can Cartilage Adaptive Capacity Studies Be Improved by Sub-regional dGEMRIC Analysis?

Seventeen healthy volunteers, were divided into two groups: one containing 9 individuals regarded as sedentary and the other containing 8 elite runners. The  $T_1$  relaxation time was measured in the central part of the femoral condyles before, and two hours after, intravenous administration of Gd-DTPA<sup>2-</sup>. Femoral cartilage was divided into two segments: weight-bearing and non-weight-bearing, and each segment was further divided into a superficial and deep layers/ROI. Cartilage thickness was measured in all segments.

Pre-contrast values of  $T_1$  were higher in the superficial layer of cartilage than in the deep layer in both groups, as in the first study ( $p < 0.01$ ). The Gd concentration

in the superficial layer was found to be independent of cartilage thickness. The Gd concentration in weight-bearing compartment in the superficial layer were lower than in the non-weight-bearing compartment in both the exercising and the sedentary group ( $p < 0.01$  and  $p < 0.04$ ) (Fig. 6.2). A correlation was found between bulk dGEMRIC index and gadolinium concentration in the superficial layer ( $r = -0.48$ ,  $p < 0.01$ ).

The present results indicate smaller differences in cartilage structure due to exercise than previously reported. Different cartilage thickness in different compartments and individuals is a source of error in dGEMRIC that should be considered when determining bulk values. However, the dGEMRIC index remains a relevant indicator of cartilage quality.



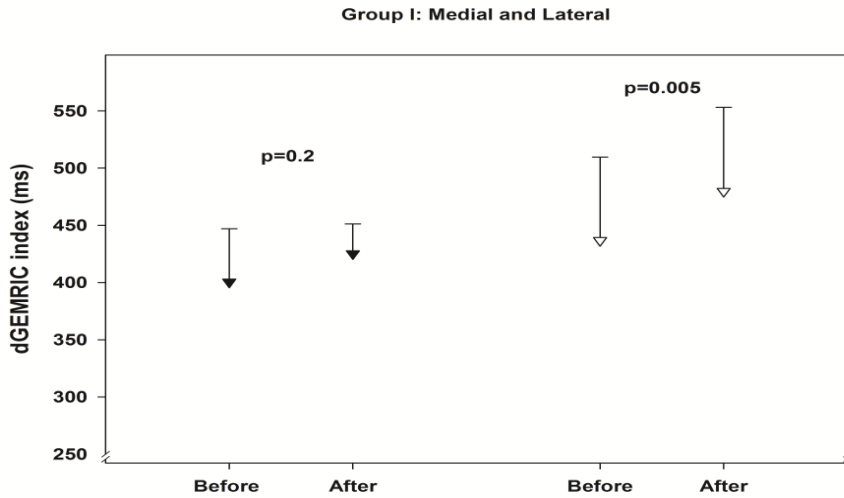
**Figure 6.2** Gd concentration in the superficial layer in two groups of healthy individuals: elite runners taking regular exercise and a sedentary group, in non-weight-bearing (NWB) and weight-bearing (WB) compartment. The difference in Gd concentration between the WB and NWB compartments, was statistically significant in both groups. The Gd concentration in the WB cartilage of the sedentary group showed a tendency to be higher than in the exercise group.

### 6.3 Paper III Sub-regional dGEMRIC Analysis in Patients at Risk of OA Provides Additional Information on Activity-Related Changes in Cartilage Structure

Thirty patients (aged: 38–50 years) having previously undergone medial meniscus resection were divided into three groups according to self-reported changes in level of physical activity after participating in a 4-month randomized trial comparing exercise with no exercise. Group I (n=11, all from the group taking regular physical exercise) consisted of those reporting increased level of physical activity, Group II (n=13, including 2 from the exercise group) consisted of those reporting no change in physical activity, and Group III (n=6, 3 from the exercise group), those reporting reduced level of physical activity. The dGEMRIC index was analysed at inclusion and after 4 months. The ROIs analysed were the superficial and deep layers of weight-bearing and non-weight-bearing cartilage of the medial and lateral femoral cartilage.

Group I showed a significant increase in dGEMRIC index in the weight-bearing cartilage ( $p=0.004$ ). The increase was greater in the lateral ( $p=0.005$ ) than in the medial compartment (Figure 6.3), in both the superficial and deep cartilage layers. The dGEMRIC index did not change in Group II. In patients reporting decreased activity (Group III), the dGEMRIC index decreased in the medial weight-bearing cartilage ( $p=0.03$ ).

From the results of this study it can be concluded that, in patients having undergone medial meniscectomy, the beneficial effect of exercise varied between different locations within the joint; the greatest improvement being observed in lateral weight-bearing cartilage, i.e. in the compartment without a meniscus lesion. The effects of exercise did not seem to vary with depth in the cartilage. These results confirm that exercise may delay the development of OA in patients with meniscus lesions.



**Figure 6.3** dGEMRIC index in Group I (increased level of physical activity), before and after the 4-month study period, comparing medial and lateral compartments of weight-bearing femoral cartilage. The bulk dGEMRIC index increased in the lateral ( $\nabla$ ), but not in the medial ( $\blacktriangledown$ ) weight-bearing cartilage.



# 7 General Discussion

## 7.1 Pre-contrast $T_1$

In the first two studies, the precontrast  $T_1$  relaxation time was found to vary with depth in the cartilage, showing a decrease in  $T_1$  from the articular surface to the subchondral bone. It has previously been assumed that the  $T_1$  of native cartilage is relatively constant (77,78). However, more recent studies both *in vitro* and *in vivo* support the present findings (89,90). Generally, the water content of articular cartilage is considered to be more related to  $T_2$  relaxation time (91-93). However, the pre-contrast spatial differences found in this work may support findings in a recent study that suggest that  $T_1$  is also related to the cartilage water content. Measuring pre-contrast  $T_1$  in dGEMRIC has previously been considered unnecessary as it was assumed that the  $T_1$  of native cartilage is relatively constant and not related to differences in structure with changing depth (77,78). The variation in pre-contrast  $T_1$  has been considered negligible compared with the decrease in  $T_1$  observed when Gd-DTPA<sup>2-</sup> is administered (24,94). Consequently, the ability of bulk values of  $T_{1Gd}$  and  $\Delta R1(1/T_{1Gd} - 1/T_{1pre})$  to discern OA patients from healthy subjects were believed to be equivalent (69). However, the full thickness of the cartilage was included in the ROIs in those studies, thus giving bulk values. The pre-contrast results obtained in the present work indicate that the results would be different if superficial and deep cartilage layers were analysed separately, as shown by the 50% longer pre-contrast  $T_1$  in the superficial layer than in the deep layer. Furthermore, OA starts in the superficial layer before progressing deeper into the cartilage (25-27). Thus, the sensitivity of detecting disease could be increased by a depth-wise analysis of Gd-DTPA<sup>2-</sup> concentration.

## 7.2 Compartmental Differences

Previously studies have shown subtle differences between bulk cartilage  $T_{1Gd}$  in the medial and lateral femoral cartilage (21). When analysing the layer of superficial cartilage separately in normal volunteers, no difference was observed in Gd concentration between the medial and lateral compartments (Paper I). Thus, the difference in bulk Gd concentration in the two compartments probably reflects

incomplete transport of the contrast agent into the deeper regions of thicker lateral cartilage. Altogether, the results presented in Paper I suggest that the higher Gd-DTPA<sup>2-</sup> concentration in medial than in lateral femoral cartilage is due to a difference in the thickness of the cartilage, rather than a difference in GAG content, as previously suggested by our own research (21). The difference in Gd-DTPA<sup>2-</sup> concentration between medial and lateral superficial cartilage 240 min p.i. is probably due to continuing Gd-DTPA<sup>2-</sup> transport towards the deeper layers of cartilage in the thicker lateral compartment, while wash-out occurs at a similar rate in the superficial layer in both the medial and the lateral compartments.

### 7.3 Permeability of Articular Cartilage

Based on examinations of patellar cartilage in 2 healthy volunteers at 1.5 T, 50 min and 150 min after an intravenous injection of Gd-DTPA<sup>2-</sup> without a pre-contrast examination, Bashir et al. concluded that Gd-DTPA<sup>2-</sup> entered the cartilage from both the articular surface and the subchondral bone (71). They also reported that T<sub>1</sub> was shorter in both deep and superficial cartilage than in the central parts 50 min p.i.. At 150 min p.i., a general decrease in T<sub>1</sub> was seen from the superficial to deep cartilage. Similar results were found in the present work (Paper I). A consequence of not examining pre-contrast T<sub>1</sub> is that the cartilage contrast agent concentration could not be calculated. Based on the pre-contrast findings in the present work (low T<sub>1</sub> values in the deep cartilage and higher T<sub>1</sub> values near the surface), the low T<sub>1</sub> values near the sub-chondral bone at 50 min in the study by Bashir et al. are more likely to have been caused by a normal depth-wise variation in pre-contrast T<sub>1</sub>. At least in femoral cartilage, Gd-DTPA<sup>2-</sup> seems to be present only near the articular surface 50 min after injection. Several studies have demonstrated that the diffusion of small solutes from the subchondral bone to the articular cartilage is negligible compared with the diffusion from the synovial fluid (9,10,95-97). This was also demonstrated recently for different CT and MRI contrast agents, in particular Gd-DTPA<sup>2-</sup> (97). The *in vivo* results in the present work, showing no Gd-DTPA<sup>2-</sup> in the deep region until 36 min, suggest that the amount of Gd-DTPA<sup>2-</sup> that enters the cartilage from the subchondral femoral bone in clinical dGEMRIC studies is negligible.

By analysing the Gd concentration in a one-pixel-thick segment of superficial and deep cartilage, and relating the results to cartilage thickness, it was possible to show that the concentration in the superficial layer is independent of cartilage thickness, whereas the concentration in the deep layer is dependent on cartilage thickness (Paper II). Furthermore, a relationship was found between the bulk dGEMRIC index and cartilage thickness, i.e. increasing index with increasing

cartilage thickness. These findings also suggest that the contrast agent mainly enters the cartilage from the surface, and not via the subchondral bone.

## 7.4 Effect of Cartilage Thickness on Quantitative dGEMRIC Analyses

The results presented in this thesis also demonstrate the relationship between cartilage thickness and bulk Gd-DTPA<sup>2-</sup> concentration. If the contrast agent enters the cartilage mainly from the articular surface, as indicated by the present results (Paper I), thin cartilage will probably show a higher bulk contrast agent concentration than thick cartilage. In addition, the incomplete penetration of the contrast medium into deeper parts of the cartilage at the time of imaging will yield a falsely elevated dGEMRIC index for bulk regions of interest.

In the study presented in Paper II, it was confirmed that cartilage thickness is an important variable when measuring contrast agent concentration and the dGEMRIC index *in vivo*. A relationship was also found between bulk dGEMRIC index and cartilage thickness, i.e. increasing index with increasing cartilage thickness. This indicates that cartilage thickness is an important factor that affects both Gd concentration and the dGEMRIC index. Thus, cartilage thickness/volume measurements should be considered in both cross-sectional and longitudinal study protocols. In cross-sectional studies where cartilage thickness varies, comparing measurements made in the superficial cartilage layer may be sufficient. Gandy et al. found that there was no change in cartilage thickness after three years of OA (98). Thus, the results presented in this thesis suggest that a single dose of contrast agent may be adequate for comparing measurements from ROIs with similar thickness between individuals, or in longitudinal studies where cartilage thickness does not vary. It may also be possible to make dGEMRIC measurements sooner after contrast agent administration.

One limitation of *in vivo* sub-regional dGEMRIC analysis is that smaller volumes of cartilage are involved. To avoid potential partial volume artefacts and chemical shift, pixels that appeared to include signal originating from both the synovial fluid and the superficial cartilage were excluded, as were pixels including signal from both the cartilage and the subchondral bone. Thus, the use of a low magnetic field strength in MRI in clinical studies may limit its use, and larger numbers of volunteers would be needed to reach statistical significant difference between cartilage groups.



## 7.5 Effect of Mechanical Load and Exercise

Experimental studies have shown chondrocytes to be sensitive to their mechanical environment; different loading in weight-bearing and non-weight-bearing articular cartilage influences the topographical variation of GAG content (4-6). Furthermore, Tiderius et al. showed that cartilage in weight-bearing regions has an adaptive capacity to exercise (21). These findings were confirmed in the present work by analysing contrast agent concentration in human superficial cartilage, showing a significant difference between weight-bearing and non-weight-bearing cartilage in both exercise and sedentary groups (Paper II). Higher Gd concentration in the superficial non-weight-bearing cartilage than in weight-bearing cartilage indicates that weight-bearing cartilage has a higher GAG content than non-weight-bearing cartilage. However, the effect of exercise was more pronounced on weight-bearing than on non-weight-bearing cartilage, since a higher concentration of Gd was only seen in the weight-bearing cartilage of the *sedentary group* rather than the *exercise group*.

Sub-regional dGEMRIC analysis revealed a depth-wise effect of exercise on articular cartilage (Paper III). In a previous study by our group (87), we found that exercise increased the GAG content in the weight-bearing medial femoral cartilage after a 4-month training period in patients who had undergone partial medial meniscectomy. These findings were confirmed in the present work, and it was also found that the changes occurred mainly in the healthy lateral compartment after exercise, in both superficial and deep weight-bearing cartilage, which indicates that moderate exercise increases the GAG content throughout the cartilage. Furthermore, no change in dGEMRIC index was seen in the medial injured compartment, which suggests that moderate exercise may prevent the loss of GAG in the injured compartment. Further evidence supporting this theory is that subjects who decreased their level of physical activity showed a decrease in cartilage GAG content.

In a previous animal study using beagles it was reported that the immobilization of an articular joint for a duration of 11 weeks reduced the GAG content in the cartilage, and that remobilization led to the restoration of the GAG content in most of the compartments (99). A decrease in indentation stiffness after 11 weeks of immobilization has been reported by the same group. A long period of immobilization will lead to permanent alteration of the biochemical and biomechanical properties of the cartilage; especially a change in GAG, which jeopardizes the morphological integrity and mechanical competence of the cartilage (13). *In vivo* results (paper III) showed a decrease in dGEMRIC index in the injured medial weight-bearing cartilage following a reduction in the level of physical activity. This indicates that, not only immobilization, but also a reduction

in physical activity will also lead to a decrease in GAG content, especially in the injured compartment.



# 8 General Conclusions

The studies presented in this thesis clearly demonstrate that the detailed information obtained from *in vivo* sub-regional dGEMRIC analysis improves our knowledge of status of the cartilage in normal and diseased conditions. The most important findings of this work are listed below.

- It is possible to follow the temporal dynamics of contrast agent permeability in cartilage using *in vivo* sub-regional dGEMRIC analysis.
- Using *in vivo* sub-regional MRI analysis revealed a depth-wise variation in pre-contrast  $T_1$  relaxation time. This may be related to the water content in the cartilage, and can be compared between individuals.
- Performing measurements on the superficial ROI, enables the effect of cartilage thickness to be eliminated as a source of error when comparing individuals.
- *In vivo* sub-regional dGEMRIC analysis provides additional information on the effect of exercise throughout the cartilage, in healthy and injured individuals.
- Sub-regional dGEMRIC analysis can also be used to identify changes in GAG concentrations in different cartilage layers, offering the possibility of identifying OA at a much earlier stage.



## 9 Future Aspects

The results presented here motivate further *in vivo* depth-wise analysis of  $T_1$  without contrast agents, especially in patients at high risk of developing OA. It may be possible to identify the early stages of OA, without using a contrast agent, which would reduce the time and cost of the examination.

Further *in vivo* sub-regional dGEMRIC studies need to be performed to confirm these findings, to follow up patients at high risk of developing OA, and to determine the progression of the disease throughout the depth of cartilage.

*In vivo* sub-regional dGEMRIC analysis deals with smaller volumes than bulk dGEMRIC analysis. Thus, the use of a higher magnetic field strength (3T) in future *in vivo* sub-regional dGEMRIC analysis may provide better information than that obtained with the lower fields commonly used today.

This work suggests that implementing *in vivo* sub-regional analysis in other MRI modalities may change the way in which OA is assessed in future clinical studies.



# Summary

The structure and composition of articular cartilage varies with depth. The water content is high in the superficial layer and decreases towards the subchondral bone. There is less glycosaminoglycan (GAG) in the superficial layer than in the deeper layers of the cartilage, while collagen fibres are highly packed in the superficial region (1). Osteoarthritis is a chronic degenerative disease of the cartilage. Early OA is characterized by the loss of GAG and the disruption of collagen molecules in the cartilage. GAG loss starts in the superficial region of the cartilage and progresses to deeper layers.

Delayed gadolinium-enhanced MRI is a technique used to detect the early stages of OA. In dGEMRIC, the distribution of the negatively charged contrast agent is distributed inversely to the amount of GAG in the cartilage. Thus, a high uptake of the contrast agent indicates a low GAG content in the cartilage, and vice versa (67). However, in most *in vivo* clinical studies, the bulk dGEMRIC value is calculated for regions of interest including the full thickness of the cartilage, referred to as the dGEMRIC index (77). In the work described in this thesis, sub-regional dGEMRIC of the femoral cartilage has been studied. ROIs were drawn manually, and layers of cartilage were analysed as separate ROIs.

In the study described in Paper I, 23 healthy volunteers were examined with MRI before and at 8 different times after the administration of the contrast agent Gd-DTPA<sup>2-</sup>. It was found that the contrast agent entered the cartilage mainly from the synovial fluid rather than from the subchondral bone. The longer T<sub>1</sub> relaxation time in the superficial region than in the deep region of the cartilage may indicate that the water content is higher in the superficial than the deep region. The results also showed that the concentration of gadolinium in the superficial layer is not dependent on cartilage thickness, whereas the bulk gadolinium concentration was negatively related to cartilage thickness. This indicates that incomplete penetration of Gd-DTPA<sup>2-</sup> in thick cartilage will yield a falsely extended T<sub>1</sub> relaxation time.

In the second study (Paper II), the gadolinium concentration in the superficial layer and the bulk value were measured using dGEMRIC in 17 healthy volunteers who were divided into two groups: 9 sedentary and 8 exercising elite runners. The gadolinium concentration in the superficial layer, which is not related to cartilage thickness, was significantly lower in weight-bearing than in non-weight-bearing cartilage in both groups. Furthermore, there was also a trend towards a lower



gadolinium concentration in the superficial region of weight-bearing cartilage in the exercise group, than in the sedentary group. These results reconfirm the adaptive capacity of cartilage to exercise. In addition, loaded compartment of cartilage had a higher GAG content than non-loaded compartment. A positive relation was also found between bulk dGEMRIC index and cartilage thickness, which indicates that differences in cartilage structure due to exercise are less pronounced than (we) previously thought. Variation in cartilage thickness is a source of error that should be considered in dGEMRIC studies. However, a negative correlation was found between the bulk dGEMRIC index and the gadolinium concentration in the superficial layer, which suggests that the bulk dGEMRIC index is still satisfactory for estimating cartilage GAG content.

In the final study (Paper III), dGEMRIC was applied to the superficial and deep region of cartilage, as well as to the bulk in 30 patients who had undergone partial medial meniscus resection. They were divided in to three groups according to self-reported changes in physical activity. The results of this study suggest that exercise increases the GAG content throughout the cartilage in the non-meniscectomized compartment. Furthermore, exercise also stops the loss of GAG in the injured compartment. The bulk dGEMRIC index showed a decrease in weight-bearing cartilage in those who had reduced their level of physical activity during the training period. This suggests that, in addition to immobility, a reduction in physical activity also leads to a decrease in the GAG content of cartilage.

In summary, the results presented in this thesis indicate that sub-regional MRI analysis can provide additional information on the status of the cartilage in normal and diseased conditions.

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