Quantification of the influence of the absence of normal joint loading and movement on the articular cartilage in the joints of spinal cord injured patients

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Publication Date: 2003

Permanent Link: https://doi.org/10.3929/ethz-a-004626064

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Quantification of the Influence of the Absence of Normal Joint Loading and Movement on the Articular Cartilage in the Joints of Spinal Cord Injured Patients

A dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH

For the degree of
Doctor of Sciences

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2003
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Summary

Mechanical loading influences the development, maintenance, and aging of skeletal tissues including articular cartilage. Animal studies have demonstrated that joint immobilization and stress deprivation lead to functional adaptation of articular cartilage, these changes concerning morphological, biochemical and biomechanical characteristics of the cartilage matrix. An understanding of the relationships between joint use/disuse and joint degeneration represents a critical step towards developing strategies to prevent and treat joint diseases such as osteoarthritis. Due to a lack of accurate noninvasive imaging methods, however, there has to date been no report on morphological changes of cartilage in humans following immobilization. This knowledge is important to anticipate cartilage changes in patients who require immobilization after surgical procedure, accident, or after spinal cord injury.

Recently, 3D magnetic resonance imaging (MRI), combined with state-of-the-art post-processing, has been shown to provide accurate and highly reproducible data on cartilage morphology in vivo. Therefore, MRI was used in this research as a method to identify cartilage changes in human articular cartilage. Available MRI methods identify only global morphological characteristics of the cartilage, while evidence indicates that joint degeneration is a focal problem. As a consequence, these methods are rather insensitive in detecting early signs of degeneration. We therefore developed a technique to measure and evaluate local changes in articular cartilage (chapter 3). Animal studies have shown that immobilization results in several major changes of articular cartilage (chapter 2). Mechanical, biochemical, and morphological characteristics are altered and do not always totally recover upon remobilization of the joint. However, these data cannot be transferred directly to human subjects, which makes it necessary to study cartilage reaction to restricted motion and loading in human subjects.

We could detect a difference in mean cartilage thickness between healthy volunteers and spinal cord injured (SCI) patients 6 months post injury for the patella and medial tibia. The differences became larger and significant for all joint compartments when comparing healthy volunteers to SCI subjects 12 and 24 months post injury (chapter 4). In the longitudinal study we found thinning of the articular cartilage over a period of one-year post injury. In patella, in medial and lateral tibia thinning of respectively
9%, 11% and 11% was measured one year after the injury. In the same group of patients, no changes in the humeral head articular cartilage were found (chapter 5). The reproducibility of the MRI cartilage measurements is established for the knee and other joints with thin cartilage but not for the shoulder joint, yet. Consequently an analysis of the reproducibility of the shoulder cartilage measurement from MRI has be performed. Our data showed, for the first time, that articular cartilage mean thickness of the humeral head can be quantified with a precision of 4.5% in vivo. In the last chapter, we addressed the problem of measuring mechanical properties of the cartilage in vivo. We developed a MRI compatible loading device and measured systematically the cartilage deformation during 80 minutes of static loading (400N). A deformation of the mean thickness of 7% was measured after 80 minutes.
Zusammenfassung


Wir konnten einen Unterschied in der mittleren Knorpeldicke der Patella und des medialen Bereichs der Tibia zwischen gesunden Probanden und Querschnittsgelähmten, 6 Monaten nach deren Unfall, messen. Der Unterschied wurde noch deutlicher und auch für die laterale Tibia signifikant, wenn man die
Chapter 1
Introduction
Joint pain and loss of mobility are among the most common causes of impairment in middle-aged and elderly people. In many instances, articular cartilage degeneration and concomitant alterations in other joint tissues, cause pain and a decreased range of motion. An understanding of the degeneration process in articular cartilage, and the potential for restoring its properties depend to a large extent on an appreciation of the biological behavior and the responsiveness of articular cartilage to injury and immobilization. In this dissertation, the effects of restricted movement and loading on articular cartilage are investigated in humans. At the starting point of this Ph.D., data on the effect of immobilization on the biochemical, biomechanical and morphological characteristics were only gathered using large laboratory animals. However, no human data were available. After a thorough review, two major problems in studying the effect of immobilization on articular cartilage in human subjects emerged. One is a lack of non-invasive measurement techniques, which are accurate and reproducible enough to detect small changes in articular cartilage. The other problem is related to the ethical and medical issues of immobilizing healthy volunteers during a longer period of time for research purposes. During the last few years, major efforts have been made in the field of imaging articular cartilage. Magnetic Resonance Imaging (MRI) makes it possible to visualize soft tissue in a non-invasive and direct manner. This technique can be used to overcome the first problem. The lower extremities of spinal cord injured patients cannot be actively moved because of neuromuscular transmission and can therefore be considered immobilized and unloaded. Consequently, this subject group is particularly suited to study the effects of restricted loading and movement on human articular cartilage.

1.1 Articular Cartilage

The articulating bone ends of diarthrodial joints, such as knee and shoulder, are covered by a thin (1mm to 5mm), dense white connective tissue called hyaline articular cartilage. In the knee joint, articular cartilage covers the patella, femur and tibia (Figure 1-1). The patellar cartilage is the thickest (2.8 mm on average) and the tibial is the thinnest (1.65 mm on average). The
Articular cartilage in the shoulder joint covers the humeral head and the glenoid (Figure 1-1) and is much thinner (1.3mm on average) as compared to the knee joint. The textbook: *Basic biomechanics of the musculoskeletal system* (Nordin and Frankel, 1989) can be used for a more detailed description of these two joints.

![Figure 1-1: The two joint studied in this dissertation: the knee (left) and the shoulder joint (right).](image)

Articular cartilage is a highly specialized tissue precisely suited for its functions. These functions consist of distributing the joint load over a wider area and allowing movement of the opposing joint surfaces with minimal friction and wear.

Articular cartilage is a viscoelastic material with two distinct phases: a solid phase (the organic solid extracellular matrix) and a movable fluid phase (the interstitial water with the inorganic salts dissolved in it) (Mow et al., 1984). The extracellular matrix is composed of a dense network of collagen fibers (10-30% of wet weight), and large aggregating and non-aggregating proteoglycans (3-10% by wet weight). The remaining 60 to 80% is water, inorganic salts, and small amounts of other matrix proteins, glycoproteins and lipids. Collagen fibrils and proteoglycans are the structural components of hyaline cartilage supporting the internal mechanical stresses that result from loads applied to the articular surface. Distributed within the matrix is a sparse population of...
cells (with a density less than 10% of the tissue’s volume), the chondrocytes, which are responsible for the synthesis and the maintenance of the matrix components. Articular cartilage can be considered as a fluid-filled, porous-permeable medium. Biomechanically, articular cartilage distributes load and provides a smooth, lubricated surface that facilitates movements with little friction between the articulating surfaces. The compressive viscoelastic behavior of articular cartilage is caused primarily by the flow of the interstitial fluid and the intrinsic viscoelasticity of the matrix. Articular cartilage also exhibits viscoelastic behavior in tension, which is attributable to both the internal friction associated with polymer motion and the flow of the interstitial fluid. Morphological, biochemical and biomechanical properties of articular cartilage show distinct topographical variations in human and animal joints (Jurvelin et al., 2000, Kiviranta et al., 1987, Arokoski et al., 1999).

Mechanical loading influences the development, maintenance, and aging of skeletal tissues including articular cartilage. There is also an influence of gender on cartilage morphology. Women show smaller cartilage volume, which is originates from smaller joint surface area. These two parameters are still smaller compared to men if normalized for body weight and height (Cicuttini et al., 1999, Faber et al., 2001). The effect of age on the cartilage morphology has been studied in 82 cadavers by Meachim et al (Meachim G et al., 1977). They found a progressive thinning with increasing age in women more than 50 years. In men, this thinning was much less severe.

### 1.2 Spinal Cord Injury

Spinal Cord Injury (SCI) involves the transection of the spinal cord, which results in a complete or incomplete loss of function such as the ability to muscle contraction or sensation. Frequent causes of damage are trauma (car accident, gunshot, falls, etc.) or disease (polio, spina bifida, Friedreich's Ataxia, etc.). The consequences of SCI depend on the type of injury and the level of the lesion. SCI can be divided into two categories of injury - complete SCI and incomplete SCI. A complete injury results in a total loss of functionality below the level of the injury, no sensation and no voluntary movement. Both sides of
the body are equally affected. An *incomplete* injury means that functions below the primary level of the injury are not completely absent. A person with an incomplete injury may be able to move one limb more than another, may be able to feel parts of the body that cannot be moved, or may have more functioning on one side of the body than the other. With the advances in the acute treatment of SCI, incomplete injuries are becoming more common. The resulting paralysis is generally classified in two categories: *paraplegia* and *tetraplegia*, depending on where the lesion occurred. Tetraplegia refers to injuries of the cervical region of the spinal cord. Paraplegia refers to injuries, which occur in the thoracic, lumbar, or sacral segments. The attention of this thesis is mainly drawn to the effects of complete traumatic spinal cord injury resulting in para- as well as in tetraplegia.

1.2.1 Statistics
At least 330,000 people with SCI (paraplegia and tetraplegia) are estimated to live in the member states of the Council of Europe, with about 11,000 new cases every year. The majority of the SCI subjects are males (82%) between the ages of 16-30. These injuries result from motor vehicle accidents (36%), violence (28.9%), or falls (21.2%). No exact data are available about the incidence and prevalence of SCI in Switzerland. But data from the Swiss Paraplegic Center of Nottwil, one of the largest rehabilitation centers in Switzerland, showed that in 2002 199 new spinal cord injured patients were treated. The major causes of the traumatic injury were road accidents for male and falls for female. The majority (72%) of the patients were male. The approximately hospital stay was 147 days. (Source: Jahresbericht 2002, SPZ Nottwil)

1.2.2 Secondary problems
Spinal cord injury has major physical, social and emotional implications for the patients and their family. In recent years medical care has improved considerably, resulting in a higher life expectancy of those who suffer from SCI (Whiteneck et al., 1992). However, persons with SCI still have an increased risk of secondary medical complications, such as urinary tract complications, pressure ulcers, respiratory infections, musculo-skeletal injuries, obesitas, and coronary heart disease (Whiteneck et al., 1992, Kocina, 1997, Haassard,
Some of the consequences of the SCI such as loss in muscle mass, bone density and cardiovascular fitness have been documented in a quantitative way. But although some studies suggested that SCI patients suffer from joint effusion (Betz et al., 1996, Buschbacher et al., 1991, Levi et al., 1995), joint stiffness (Levi et al., 1995), fibrofatty connective tissue growing (Enneking and Horowitz, 1972) and heterotopic ossification (Haassard, 1975), no quantitative data about the effects of SCI on the articular cartilage are available. Two older studies investigated the influence of paralysis on the articular cartilage of lower extremity joints with radiography. Pool (Pool, 1974) reviewed 200 cases of flaccid paralysis: in 25 hip joints the radiologic joint space was found to be narrowed by at least 50%. Richardson (Richardson et al., 1984) evaluated the skeletal changes in neuromuscular disorders and concluded that apparent overgrowth of the epiphyses, periarticular osteoporosis and joint-space narrowing are the most common findings. In these studies, X-ray films were used but these cannot visualize cartilage directly and suffer from projectional artifacts (Buckland-Wright et al., 1995). Moreover, they cannot differentiate between femoral and tibial cartilage loss (femoro-tibial joint) or femoral and patellar cartilage loss (femoro patellar joint) and cannot visualize the pattern of cartilage loss throughout the joint surface.

In a later study, changes in the lower extremity joints of spinal cord injured patients were evaluated by the means of magnetic resonance imaging (MRI) (Betz et al., 1996). MRI’s were obtained in the sagittal, coronal and axial planes on each subject. Betz and associates found that functional electrically stimulated (FES) -driven cycling training has no negative effects on the lower extremity joints and in some patients even has a positive effect (less joint problems). In the latter study, the MRI’s were evaluated from a clinical viewpoint only and no attempt was made to quantify their contents. To develop a fundamental understanding of the changes that occur in articular cartilage after SCI, it is necessary to quantify these changes. New developments in the MRI research field allow such quantification. With newly developed methods, articular cartilage volume and thickness can be measured in an accurate and reproducible way (Cohen et al., 1999, Peterfy et al., 1994, Stammberger et al., 1999b).
1.3 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is an imaging technique used primarily in the medical setting to produce high quality images of the human body. In this setting, a 1-1.5 Tesla MRI machine is used (Figure 1-2). MRI started out as a tomographic imaging technique, producing an image of the nuclear magnetic resonance (NMR) of protons in a thin slice through the human body. The technique has advanced beyond a tomographic imaging technique to a volume imaging technique. MRI is based on the principles of NMR from the hydrogen atoms in the object to be analyzed. In medical MRI, radiologists are most interested in looking at the NMR signal from water and fat, which are the major hydrogen containing components of the human body.

![Figure 1-2: 1.5 Tesla Magnetom MRI machine (Siemens)](image)

The principle behind all MRI is the resonance equation, which shows that the resonance frequency $\nu$ of a spin (the property exhibited by atomic nuclei that contain either an odd number of protons or neutrons) is proportional to the magnitude of the magnetic field, $B_0$, it is experiencing.

$$\nu = \gamma \cdot B_0$$

$\gamma$ is the gyromagnetic ratio.
In practice, the subject is placed in a very strong and homogeneous magnetic field. This is the polarizing field denoted as $B_0$. At thermal equilibrium and 1.5 Tesla, a few parts per million of the spins, typically protons, in the subject align preferentially in the direction of the field. The MRI machine applies a radio frequency pulse that is specific only to hydrogen. The system directs the pulse toward the area of the body we want to examine. In our study, this is the knee or the shoulder. The pulse causes the protons in that area to absorb the energy required to make them spin, or precess, in a different direction. This is the "resonance" part of MRI. The RF pulse forces them (only the one or two extra unmatched protons per million) to spin at a particular frequency, in a particular direction. This specific frequency of resonance is called the Larmor frequency and is calculated based on the particular tissue being imaged and the strength of the main magnetic field.

These RF pulses are usually applied through a coil. MRI machines come with many different coils designed for different parts of the body such as knees (Figure 1-3), shoulders (Figure 1-3), wrists, heads, necks and so on. These coils usually conform to the contour of the body part being imaged, or at least reside very close to it during the examination. At approximately the same time, the three gradient magnets co-act. They are arranged in such a manner inside the main magnet that when they are turned on and off very rapidly in a specific manner, they alter the main magnetic field on a very local level. What this means is that the system can pick exactly the specific area of which a picture has to be taken. In MRI this is called "slices." Any part of the body can be sliced in any direction, which gives a huge advantage over any other imaging modality. When the RF pulse is turned off, the hydrogen protons begin to relatively slowly return to their natural alignment within the magnetic field and release their excess stored energy. When they do this, they give off a signal that the coil now picks up and sends to the computer system. As a result the system receives mathematical data which are converted, through the use of a Fourier transform, into a picture that be put on film. This process is the "imaging" part of MRI.
To visualize hyaline cartilage, a good signal-to-noise ratio (the ability to obtain a clear image), contrast-to-noise (the ability to distinguish hyaline articular cartilage from neighboring structures and synovial fluid) and a maximal spatial resolution is needed. During this research project two MRI protocol are used to fulfill these requirements: fat suppressing gradient echo sequence and a spoiled 3D gradient echo sequence with selective water excitation.

A fat suppressing gradient echo sequence was used to get an optimal contrast between cartilage and bone. This process utilizes specific parameters to remove the deleterious effects of fat from the resulting images. There are two ways to do fat suppression: fat saturation and water excitation. The first method takes advantage of the difference in resonant frequencies between water and fat. A $90^\circ$ RF pulse, tuned to the resonant frequency of fat is applied and flips the bulk magnetic vector from fat into the transverse plane. Spoiler gradients are then applied to destroy the phase coherence of the signal. This saturation routine is followed immediately by the imaging sequence and the images will only show signal from the remaining water nuclei. Fat based nuclei will not produce a signal until there has been time for significant T1 based recovery.
The second method selectively excites the water-bounded protons by a binominal excitation scheme. A 1:2:1 pulse amplitude ratio was used. This technique takes advantage of the difference in Larmor frequency (145 Hz/T) between protons by combining a series of short excitation pulses with an appropriate time interval between the separate pulses. This process results in selective excitation of the water bound protons whereas the resultant excitation of the fat bound protons is zero and thus they do not contribute to the MR signal.

1.4 Segmentation of MR images

Segmentation is the process by which appropriate image points are assigned to a specific anatomic structure, such as a cartilage plate. For the clinical studies described in this thesis, a segmentation software package developed in collaboration between the Musculoskeletal Group (Ludwig Maximilians University München), the Institute of Medical Informatics (GSF National Research Center, Oberschleissheim), and the Institute for Diagnostic Radiology (Klinikum Grosshadern, München) was used. This segmentation technique is based on a slice-by-slice segmentation using B-splines. The user initializes the procedure by drawing a roughly approximating initial line around the cartilage. This initial contour is parameterized as B-spline curve. For more detailed information on B-spline curves, the textbook of Mortenson (Mortenson, 1985) can be consulted. The procedure uses three energy terms that guide the matching of the deformable contour to the image boundaries: the internal deformation energy, the image forces and the coupling forces. The internal deformation energy characterizes the elastic properties of a flexible contour and controls therefore its rigidity. The image forces attract the contour to the boundaries of the cartilage that are given by the gradient of the image intensity $I(x,y)$. The strength of the gradient is estimated by convolving the image data with Gaussian derivative filters integrated along the B-spline curve. The coupling force obliges the contour to be in accordance with the segmented cartilage boundaries of the previous sections. This is important in order to guide the contour in image regions with no significant edge information.
A more detailed description of the image forces and this segmentation procedure can be found in the following article: Stammberger et al (Stammberger et al., 1999b).

The accuracy of this method was determined in comparison to other methods. Knee joint cartilage volume measurements (obtained with T1-weighted, fat suppressed gradient echo sequence) have been shown to deviate not more than 5 to 10% on average from water displacement of surgically retrieved tissue (Dupuy et al., 1996, Peterfy et al., 1994, Cicuttini et al., 1999), anatomical sectioning (Eckstein et al., 1996, Sittek et al., 1996) and with CT arthrography (Eckstein et al., 1998a, Eckstein et al., 2000b). Comparison with CT arthrography gives an underestimation of the cartilage volume of 10 to 15% in MRI (Eckstein et al., 1998a). Regional distribution patterns of cartilage thickness were also found to be consistent with those derived from sectioning (Eckstein et al., 1996, Kladny et al., 1996, Sittek et al., 1996), A-mode ultrasound (Eckstein et al., 1997) and stereophotogrammetry (Cohen et al., 1999).

A critical issue when determining cartilage tissue loss longitudinally is the technical precision (reproducibility) of the measurement. Reproducibility of the method has been established in healthy volunteers as well as in osteoarthritic (OA) patients, by repetitive measurements after joint repositioning and reshimming of the magnet. The standard deviation of repeated measurements in healthy volunteers has been shown to be around 1.5% (coefficient of variation, CV%) for patellar cartilage volume (Eckstein et al., 1998c), and around 4% in the tibia (Eckstein et al., 1998c) in sagittal image data of normal cartilage. Similar values were obtained for computations of the mean 3D cartilage thickness (Stammberger et al., 1999a). This precision could be improved by using a transverse section orientation in the patella (< 1%; (Eckstein et al., 2000b)), and a coronal section orientation in the tibia (approx. 2.5%; (Hyhlik-Durr et al., 2000)). In patients with severe OA (imaged prior to total knee replacement), the coefficients of variation for volume were found to be somewhat higher (around 5% in the tibia), but the standard deviations were similar (around 50 mm³). Given an estimated cartilage loss of > 1000 mm³ in OA patients (Burgkart et al., 2000, Hyhlik-Durr et al., 2000), it appears feasible
to follow the tissue breakdown with high precision, using quantitative cartilage MR imaging. In this thesis, the transversal section orientation for the patella and the coronal section orientation for tibial and femoral cartilage were used.

As suggested by Eckstein et al (Eckstein et al., 2002), longitudinal data were segmented in one session, as resegmentation errors at separate sessions have proven to be somewhat higher (ranging from 4.5% to 2.5%). In OA patients, the CV was higher, but the standard deviation of repeated measurements was similar to those in healthy volunteers (Burgkart et al., 2001). Other joints in the human body display thinner cartilage than in the knee. However they can also be measured in an accurate and reproducible way. The quantitative imaging reproducibility was satisfactory for the elbow (Graichen et al., 2000), hindfoot (Al-Ali et al., 2002) and metacarpophalangeal articulation (Peterfy et al., 1994).

For quantitative magnetic resonance imaging 5 parameters are used: maximal and mean thickness, volume and joint surface area, and cartilage joint area. The thickness values are calculated using an algorithm based on 3D Euclidean distance transformation. It allows the accurate and efficient calculation of the 3D thickness distribution between two extracted 3D surfaces avoiding at the same time the difficulties in defining and computing normal vectors on the discrete surfaces (Stammberger et al., 1999a). The volume is computed by numerical integration, and triangulation is used to calculate the size of the surface areas.

1.5 Aim of the thesis

Due to the lack of accurate noninvasive methods, no report on morphological changes of cartilage in human subjects following immobilization was available. This knowledge is important to anticipate and, if possible treat cartilage changes in patients who are immobilized after surgical procedures or accidents, or after spinal cord injury. The goal of this research was to determine to what extent articular cartilage morphology is altered and modulated by joint disuse in spinal cord injured patients. It was postulated that the reduction in cartilage thickness is following a decrease in mechanical
stimulation, an altered nutrition associated with the lack of load and a decrease in production of synovial fluid altogether reducing chondrocytes metabolism and matrix production.

The second aim of this thesis was to develop a device to measure articular cartilage mechanical properties in vivo. Until now mechanical properties of cartilage are estimated using confined or unconfined compression or indentation test on explants. However, the situation in the joints is much more completed compared to the experiments. Using MR gives us the opportunity to measure systematically deformation of articular cartilage. We wanted to build a MR compatible pressure device, which can put load on the patello-femoral joint during one hour and measure the deformation by means of MRI. Later on, we want to use these deformational data as a basis for our finite element simulation. By matching the experimental data with the numerical results, it should become feasible to analyze the mechanical parameters of articular cartilage.

1.6 Outline of the thesis

Chapter 1 provides the motivation for the research carried out in this thesis. The second chapter describes the improvements done to the segmentation procedure and the post-processing of cartilage volume. Using a global parameter per joint surface does sometimes ignore local changes in the cartilage. We tried to identify to what extent local changes in articular cartilage can be detected. The third chapter provides a critical review on the research addressing the question of the effect of immobilization on articular cartilage. The results of the first clinical investigation are discussed in chapter four. This describes a cross-sectional study with the goal assessing the magnitude and the time course of cartilage changes in SCI. The outcome of this study helped to determine the design of the longitudinal study, the results of which are described and discussed in chapter five. In contrast to the lower extremities of SCI subjects, which are unloaded and restricted in movement, the upper extremities, especially the shoulder joints, are loaded more frequently and with higher forces. Therefore, the effects of this new loading situation on shoulder
joint cartilage was analyzed using the same MRI protocol and study design as in chapter 5. Chapter six discusses the outcome of these measurements. Chapter seven describes our first attempt to quantitate cartilage deformation in human subjects during one-hour static compression test of the patello-femoral cartilage.
Chapter 2
Quantitative Analysis of Local Changes in the Patellar Cartilage
Background
The following chapter describes the work done on the MRI segmentation technique and on the characterization of the cartilage. Available segmentation techniques are time consuming and require human interaction and knowledge. Human interaction causes errors and makes the procedure less reproducible. Therefore new methods are needed that reduce human interaction to a minimum, and improve inter- and intra observers’ reproducibility. Time needed for the segmentation becomes a critical issue if this procedure is used in clinical practice.

Most MRI investigators studying cartilage morphology identify global parameters such as volume and thickness (mean and maximal) for each joint surface. This means that, using these global parameters, a lot of critical information is lost. As most joint diseases start with small local problems, they cannot be detected by these global parameters. The idea behind this research was to find a reasonable way to detect local changes in the articular cartilage thickness. Therefore, a good balance between precision of the measurement and the dimension of the local regions has to be determined.

In 2001, collaboration was initialized between the Laboratory for Biomechanics and the Computer Vision Laboratory, ETHZ. The goal of this collaboration was first to make the segmentation procedure automatic and secondly to characterize articular cartilage in a more local manner. The future dissertation of Cristian Pirnog deals with the automatization part. My contribution was in the characterization of local changes in articular cartilage, and was accomplished in close collaboration with Prof. G. Székély and Dr. Martin Maechler.

Own scientific contributions
- Select a suitable MRI protocol and collect MR images.
- Evaluate and give feedback on the semi-automatic segmentation, and the registration procedures.
- Design and implement the programs to detect morphological properties of articular cartilage
- Design and implement the algorithms to divide cartilage on an anatomical basis.
- Design and implement the thickness maps and the model to evaluate local cartilage changes.
- Determination of the reproducibility of the method.
ABSTRACT

OBJECTIVE: There is evidence that cartilage changes in joint degeneration are rather a local effect. However, most studies investigating articular cartilage changes only use global parameters to identify cartilage morphology of one joint surface. Consequently, these methods are rather insensible in detecting disease progression. Therefore, the aim of this study was to develop and validate a computational method for detecting local changes in patellar articular cartilage thickness.

METHODS: The knee of 3 healthy volunteers was imaged 4 times using a spoiled 3D gradient echo sequence with selective water excitation. Additionally, the knee of one spinal cord injured subject was measured 3, 6 and 12 months post injury. Morphologic parameters for the patellar cartilage (mean and maximum thickness) were computed. These parameters were also calculated for the medial and lateral facet of the patella. Three-dimensional thickness maps were generated and orthogonal projected. The mean value, the standard deviation (SD) and the coefficient of variation (CV%) from the four replicated data sets for the different morphological parameters and for the thickness projection maps were determined.

RESULTS: A CV of 0.7% was found for the mean thickness of the whole patellar cartilage. For the mean cartilage thickness of the medial and lateral patella, the CV was respectively 1.4 and 0.7%. The CV of maximal thickness was respectively 2.03%, 2.79% and 4.43% for patella, medial and lateral patella. The global standard deviation of the thickness maps was 0.147. Correlation between the SD maps could be found neither with the thickness maps nor with the difference maps. P values for every point of the thickness maps were generated and a clear significant thinning in certain region was detected.

CONCLUSION: The computational method, which combines rigid registration of the patellar bone with semi-automatic cartilage segmentation, is highly reproducible. It gives us the opportunities to quantify the differences in articular cartilage thickness of not only medial and lateral patellar cartilages but also within smaller regions.
2.1 Introduction

Changes in articular cartilage morphology can cause major joint problems such as osteoarthritis (OA). The ability to determine changes in articular cartilage is not only important for the accurate diagnosis of OA but can also be crucial for the evaluation of therapies, such as osteochondral autographs, autologous chondrocyte implants, and artificial tissue repair. Other study, such as the analysis of long-term functional adaptation to specific mechanical demands (Mühlbauer et al., 2000), the instant reaction of cartilage to physiological exercises (Eckstein et al., 2000a) and systematically measurement of articular cartilage deformation during static compression (Chapter 7) also use quantitative Magnetic Resonance Imaging (MRI) measurements of cartilage morphology. The in vivo quantification of cartilage thickness (Recht et al., 1993) has evolved quickly during the recent years. While joint narrowing, often a sign of advances joint degeneration, can be determined on X-rays, MRI is better suited for the identification of cartilage lesions non-invasively and without radiation. Using a high resolution, three-dimensional gradient echo sequence with fat suppression provides a good spatial resolution, good contrast-to-noise ratio, and good signal-to-noise ratio. The accuracy and reproducibility of cartilage thickness and volume measurement using this technique has been established (Cohen et al., 1999, Peterfy et al., 1994, Eckstein et al., 1998a, Eckstein et al., 1998c). These studies identified a global mean thickness and volume per joint compartment as morphological parameter. Although animal studies showed that there exist a large topological variation in morphological, biochemical and biomechanical characteristics and also in their response to immobilization and remobilization (Jurvelin et al., 1986), this information is ignored in the MRI studies. There is also evidence to believe that cartilage problems are rather a local effect and consequently difficult to detect using global parameters (Waterton et al., 2000). Using a global parameter, all thicknesses are averaged over the surface area, which means that important information about local changes is lost e.g. if a large thinning happens in only a very small region it won’t have a major effect on the global mean thickness; or thinning in certain region can be compensated by a swelling in another region of the joint compartment, resulting in a constant
mean thickness. No specific technique to evaluate local rather than global changes is available for quantifying changes in longitudinal studies.

Some attempts have been made to register cartilage and compared specific areas (Stammberger et al., 2000, Waterton et al., 2000). Waterton et al (Waterton et al., 2000) measured cartilage thickness changes in the femoropatellar joint during a day of standing. They detected changes in thickness only if they divided the joint surface in smaller areas. Stammberger et al. used a combination of rigid and elastic registration to identify corresponding points of bone cartilage interface (Stammberger et al., 2000). They reported a positive maximal difference of 0.48mm and a maximal negative difference of –0.47mm. However, these methods used elastic registration, which has the disadvantage that there is no guarantee that anatomically equivalent regions of cartilage are corresponded, even in normal subject, and the correspondences become unpredictable when the cartilage shape changes during disease.

We propose to use the patellar bone as an anatomical reference for corresponding cartilage thickness maps of one subject over time. As the patellar bone shape is not affected by disease over time, this eliminates the effect of cartilage changes over time on the registration. So the patellar bone can be segmented out of the baseline image (source) and can be used to register the MRI data set (target image).

The aim of this study was:

1) to determine the reproducibility of our registration and segmentation method. This reproducibility was established for the whole patellar cartilage surface as well as for the medial and lateral facet of the patella.

2) The second aim was to examine the use of this technique for defining small localised regions of interest in which changes in cartilage thickness could be measured from serial scans.
2.2 Imaging

2.2.1 Healthy volunteers
All three subjects were examined with a 1.5 T MRI scanner (Gyroscan Intera, Philips), and a C4 flexible coil. Magnetic resonance images were acquired in the transversal plane with a spoiled 3D gradient echo sequence with selective water excitation (TR: 17 ms, TE: 6.6 ms, Flip angle: 20°, acquisition time 2.48 min). A 256x256 matrix was used with a field of view of 80 mm and a slice thickness of 1.4 mm. Four repeated scans were taken from three healthy male volunteers. Between measurements, the subjects moved their legs and were repositioned in the MR coil. The magnet was reshimmed between repeated acquisitions.

2.2.2 Spinal Cord Injured patients
The same imaging protocol was used to measure patellar cartilage changes over time in SCI patients. Measurements took place as soon as possible (3 months) after the SCI, 6 months and 12 months after SCI. No repeated measurements were done on the patients.

2.3 Segmentation of the bone
On a slice-by-slice base, one 3D data-set of 34 MRI’s is loaded. The observer marks a rectangular region including the patellar bone. A histogram of the image intensity of the pixels lying within the region is calculated and the threshold value is set on 17% of the pixel value. This threshold can be manually adapted when necessary. A binary threshold is applied to each of the 34 images (Figure 2-1). Additionally, a few morphological operators such as dilation and erosion can be applied, if necessary. Erosion operators shrink the objects, smoothes the objects boundaries and removes fingers and small objects. Dilation expands the size of 1-value objects, smoothes the objects boundaries and closes holes and gaps. A 3D reconstruction of the patellar bone helps to visually control this procedure (Figure 2-2).
Figure 2-1: First step in the segmentation procedure is the segmentation of the patellar bone. This bone will be used for the registration and for the initialization of the patellar cartilage.

Figure 2-2: Visual control for the segmentation of the patellar bone. A 3D mesh of the segmented patellar bone.
2.4 Registration and segmentation of the cartilage

The segmented bone is then used to determine a region of interest (ROI). In this ROI the automatic ‘Multimodality Image Registration using Information Theory’ (MIRIT) registration algorithm is applied (Maes et al., 1997). This technique is used to calculate the 3D transformation (including 3 translations and 3 rotations), which matched the follow up scans to the coordinate system of the baseline scans. The registration method is based on maximization of the mutual information between corresponding voxel intensities in the two images to be registered. The mutual information of the images at a current point is evaluated from the joint histogram of the image intensities of the overlapping volume of the images at this position. The result of this registration algorithm can be visually checked using Rview which overlays the red colored source image and the green colored target image (Figure 2-3). The registered images which are in the analyze format are then converted to dicom using Xmedcon software.

Figure 2-3: Visual control of the results of the registration algorithm. The source image is colored red, the target image green, which colors the perfectly overlapping bone a yellow color.
The registered images are then segmented using the software developed by Cristian Pirnog. The MR images are interpolated until a slice thickness of 0.31 mm is reached. This semi-automatic segmentation procedure is based on medial representation model (M-REP), modified to incorporate additional information (Pirnog C et al., 2003). The initialization of the cartilage is accomplished by projecting the M-REP model on the lower part of the segmented bone (the bone-cartilage interface) and is adjusted by performing a local search in directions normal to the medial grid. The result is the lowest resolution medial grid, containing between 6 and 8 slices, evenly distributed to cover the cartilage (around 80 slices, in an isotropically interpolated dataset) (Figure 2-4). The operator has to correct possible inaccuracies. The advantage of this approach is that, at the first step, the operator has to check only about 10% of all slices and to adjust only small number of points (around 15-20). Resulting in a much shorter initialization time for the segmentation procedure.
Figure 2-4: Initialization of the patellar articular cartilage segmentation on slice-by-slice basis. This first coarse level in the segmentation procedure puts point on the cartilage boundaries in about 7 slices and approximately 22 points per slices.

The next step is the 3D refinement of the medial grid followed by an automatic adjustment of the refined points. The adjustment, similar to the one during the initialization stage, optimizes the position of the interpolated points. The last two steps are performed in an alternating fashion until the desired resolution has been achieved (usually 2 to 3 iterations are necessary). The operator can evaluate the quality of the segmentation both on the image slices and the 3D view of the segmented cartilage (continuously updated throughout the process), and perform corrections if necessary (Figure 2-5). The segmented cartilage can be exported in a binary raw data format.
2.5 Morphological parameters

2.5.1 Volume measurement
Volume was calculated by numerical integration, which is the voxels size multiplied by the number of pixels wholly inside the segmentation contour together with those on the contour.

2.5.2 3D Euclidean Thickness
The 3D thickness distribution of the cartilage was obtained by an automatic method developed by the author. The program reads in the 3D binary raw data-set of the voxelized cartilage volume. On a slice-by-slice manner, the contour is extracted out of the cartilage volume. Then using the 2 corners and searching clockwise start with the northern neighboring pixels, the bone cartilage interface and the cartilage joint interface are separated (Figure 2-6).
Figure 2-6: Steps to extract the bone cartilage contours out of the voxelized cartilage slices.

For each voxel of the cartilage bone interface the 3D Euclidean distance was calculated to the cartilage joint interface. This thickness value is then projected on the voxel. Mean and maximal thickness, and volume are calculated as morphological parameters. The 3D thickness maps are reconstructed using the isosurface module of the AVS software. These 3D thickness maps are orthogonal projected to result in a 2D thickness plot.

The projected thickness maps were used because they cause much less problems and errors with registration of cartilage thickness maps at different time points. It was checked several pixels were projected on the same point (if they had the same x coordinate). If this was the case it was first checked if it was a neighboring point, if yes than an average of their thickness value was taken and the corresponding pixels became the averaged thickness value. If no, this was counted as a failure. No failure should happen.

2.5.3 Anatomical defined regional thickness

From the first set of images a grid on the bone cartilage surface was generated which will be used to define local zones in the cartilage. The patellar surface was first subdivided in a medial and a lateral facet. This is done by looking for the point with the largest y coordinate in the first and the last image, when more than 1 point has the same y coordinate the middle point in x direction is taken. A straight line is drawn between these points. This division was used for all serial scans.
2.5.4 Statistics

Healthy volunteers data
Standard Deviation (SD) for each point of the four projection maps is calculated, resulting in a SD map. Only when in each of the four projection maps, a thickness value was found the SD was calculated. These SD maps are averaged using different window size. We tried to create a model for SD to predict local variety in SD and to transfer this to the thickness differences over time in the SCI patients. Therefore, we needed to detect if differences in standard deviation could be correlated to the morphological characteristic of the thickness maps. As morphological characteristics spatial variation, location and the thickness map itself were defined. Variation maps characterized the spatial variation in thickness. They were calculated with the following formula:

\[ S = \sqrt{0.8 \cdot (X_i - \frac{1}{4}(X_{ie} + X_{iw} + X_{is} + X_{in}))^2} \]

\( X_i \): point on the thickness map and \( X_{ie, iw, is, in} \) : the 4 neighbors (east, west, south, and north) of \( X_i \)

This formula was used because the formula for standard deviation requires a normal distribution of the thickness values. Using the 4 neighboring thickness values a normal distribution is not guaranteed. Therefore a real SD can not be calculated and a difference with the average of the 4 neighboring thickness values has to be taken as a value for the variation in local thickness values. These variation maps are averaged using different window sizes (3, 5, 11, 15 and 31). We looked for a correlation between these maps.

The \( \sigma \) for the standard deviation map was calculated by averaging the means of all 3 SD map.

Spinal Cord Injured patient data
Local difference in mean thickness between two measurement, respectively between 3 and 6 months after the SCI, and 3 and 12 months post-injury were calculated for each point in the projection maps. Quantile-quantile plots are generated of these difference maps to check if there exists a normal
distribution. If normal distribution is observed, z-values are generated by dividing the differences by the global $\sqrt{2}\sigma$. P values are then calculated using the \texttt{pnorm} function of the R program.

### 2.6 Results

#### 2.6.1 Reproducibility of the anatomical defined regional thickness

Using our segmentation technique human interaction per scan is reduced and segmentation needs approximately 10 minutes. The mean value, root mean square (RMS) of SD’s and coefficient of variation (CV%) of the mean thickness for the repeated measurement of the 3 subjects are shown in Table 2-1. The RMS of SD for the whole surface is 0.021 mm and for the lateral and medial facet of the patella, respectively 0.021 and 0.04 mm.

<table>
<thead>
<tr>
<th></th>
<th>Patella</th>
<th>Lateral Facet</th>
<th>Medial Facet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mm)</td>
<td>2.946</td>
<td>3.037</td>
<td>2.831</td>
</tr>
<tr>
<td>RMS SD (mm)</td>
<td>0.021</td>
<td>0.021</td>
<td>0.040</td>
</tr>
<tr>
<td>CV (%)</td>
<td>0.71</td>
<td>0.708</td>
<td>1.40</td>
</tr>
</tbody>
</table>

Table 2-1: Mean, root mean square (RMS) of SD and coefficient of variation (CV) for measurement of patellar cartilage mean thickness (mm) within the whole joint, the medial and lateral facet of the patella.

<table>
<thead>
<tr>
<th></th>
<th>Patella</th>
<th>Lateral Facet</th>
<th>Medial Facet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mm)</td>
<td>5.350</td>
<td>4.906</td>
<td>5.253</td>
</tr>
<tr>
<td>RMS SD (mm)</td>
<td>0.123</td>
<td>0.108</td>
<td>0.133</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.30</td>
<td>2.20</td>
<td>2.53</td>
</tr>
</tbody>
</table>

Table 2-2: Mean, root mean square (RMS) of SD and coefficient of variation (CV) for measurement of patellar cartilage maximal thickness (mm) within the whole joint, the medial and lateral facet of the patella.
2.6.2 Projection of the 3D thickness map
An visual representation of a 3D thickness map with its corresponding orthogonal projected is presented in Figure 2-7.

The orthogonal projection of the 3D thickness maps did not cause problems. All pixels who were projected on the same point were neighbors. On average 2 to 4 pixels were projected on the same point and this happened about … times per map.

![Figure 2-7: 3D thickness map and the corresponding 2D projection. Color code is linear from blue to red between 0 and 5.0 mm.](image)

2.6.3 Reproducibility of the projection maps
The result of one subject, which is representative for the other subjects, is presented in detail in this paragraph. The standard deviation maps of the four replicated measurement and averaged over a different window are represented in Figure 2-8. Averaging the standard deviation map did not influence the results significantly. Figure 2-9 shows a projection map together with variation map (averaged with a window of 14x14) and the standard deviation map (averaged with a window of 15x15). No correlation was detectable between the thickness distribution, variation maps and the standard deviation distributions (Figure 2-9). In Figure 2-10, standard deviation maps of the 3 subjects are represented, showing no clear pattern.
Figure 2-8: Standard Deviation maps of the four repeated scans with no averaging (A), with averaging over window size of 3 (B), 5 (C), 11 (D), 15 (E), and 31 (F) pixels. Color legend: blue: 0mm, red: 0.312 mm

Figure 2-9: Standard deviation of the 4 repeated measurements of subject1 (dark blue:0; dark red:0.312); projection map of the thickness (dark blue:0; dark red: 5mm); variation maps averaged over a window of 15x15pixels(dark blue:0; dark red:0.1mm)
Therefore we decided to use one global sigma for the reproducibility of the thickness maps. The global $\sqrt{2}\sigma$ is 0.21.

### 2.6.4 Results spinal cord injured individual

The values for mean thickness of the patella, medial and lateral patella during the first year following the accident are represented in Table 2-3.

**Table 2-3: Mean thickness (mm) of the patella, medial and lateral patella of 1 spinal cord injured subject measured 0 months, 6 and 12 months after the accident**

<table>
<thead>
<tr>
<th>Joint Surface</th>
<th>0 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patella (mm)</td>
<td>2.764</td>
<td>2.671</td>
<td>2.510</td>
</tr>
<tr>
<td>Lateral Patella (mm)</td>
<td>2.915</td>
<td>2.909</td>
<td>2.922</td>
</tr>
<tr>
<td>Medial Patella (mm)</td>
<td>2.572</td>
<td>2.293</td>
<td>1.948</td>
</tr>
</tbody>
</table>

During the first 6 months the mean patellar thickness decreased with 3.4%. The thinning was most visible in the medial facet of the patella (-11%) and no changes could be detected in the lateral facet of the patella.

The mean thickness of the patella was decreased with 9.5% one year after the accident. The thinning reached a maximum of 27% in the lateral facet while in the medial facet no changes were observed.

Difference maps for the cartilage thickness of the SCI patients’ patella were generated (Figure 2-12). Quantile-quantile plot was calculated and a normal distribution of the differences could be concluded (Figure 2-11). This $\sqrt{2}\sigma$ parameter is used to generate first the Z-score and secondly a p value.
map, which will give an idea of the significance of differences of patients in
time (Figure 2-13).

Figure 2-11: Quantile-quantile plot from the thickness differences from one
spinal cord injured patient measured as soon as possible and 6 months after
the accident.

Figure 2-12: Maps of the local difference in thickness of one patient 3 months
and 6 months post-injury (left image) and 3 - 12 months post-injury (right
image).(dark blue:0mm; dark red:2mm)

Figure 2-13: Map of the -log10(p-values) for the differences in local thickness
between 3 months and 6 months post-injury (left image) and between 3 months
and 12 months post injury (right image). (white color: >3)
2.7 Discussion

The objective of this study was to develop and validate a computational method detecting local changes in patellar articular cartilage thickness. The method combines the registration of the serial scans using the patellar bone as reference frame and a semi-automatic segmentation based on a M-REP model. The reproducibility for the mean and maximal thickness for the whole patella, and for the medial and the lateral facet of the patella, and for the thickness map was computed. The possibility to predict areas of the patellar surface, which have higher and lower reproducibility, using the thickness maps, geometry or spatial variation maps was examined.

A coefficient of variation of 0.71% and 2.3% was computed for respectively patellar mean and maximal thickness. The reproducibility for the mean cartilage thickness is higher than those reported in previous studies (Eckstein et al., 2000b, Eckstein et al., 1998c, Peterfy et al., 1994). However, reproducibility is comparable with results of studies combining registration with semi-automatic segmentation techniques (Lynch et al., 2001). Registration of the MR images seems to have a positive effect on the reproducibility of the segmentation. The bone provides a stable reference frame for examining surfaces built from segmentations of cartilage scans taken at different time points. It makes it possible to determine corresponding slices and consequently influence the reproducibility positively. Dividing the patellar surface in a lateral and medial facet still gives reasonable precision, with a CV between 0.7 and 1.4%. This mean thickness is averaged over a smaller area, which results normally in a smaller reproducibility. Lateral and medial patella are subjected to different loads and therefore react very differently to immobilization or joint disuse/overuse. This technique makes it possible to detect smaller changes or defects and to differentiate between changes in the medial and lateral facet of the patella.

The standard deviation of the maximal thickness is larger compared to those of the mean thickness. This is consistent with other investigations (Hyhlik-Durr et al., 2000, Stammberger et al., 1999b).
The possibility to find a model for the standard deviation was examined. The model should let us predict the significance of thickness changes in small region of cartilage surface. Comparing the standard deviation maps with the spatial variation maps and the projection maps, showed no clear relationship between them. It seems that segmentation failure are randomly distributed over the patellar cartilage surface and no local distribution of the SD can be predicted. Therefore a global reproducibility error (\(\sigma\)) was computed and used to calculate Z-scores. Before Z-scores can be calculated, it should be checked if the thickness changes at the two time points (0 months compared to 6 months and 0 months compared to 12 months) are normally distributed. The normal distribution was verified using quantile-quantile plots of the thickness changes. Evaluation of these plots confirmed a normal distribution. The Z-scores are computed by dividing the local mean thickness differences by 0.21 (\(\sqrt{2\sigma}\)). P values or the significance of the difference can now be calculated and visualized. Studying the P-maps, regions where the cartilage did changed significantly could be identified. All regions with a value more than 3.0 show a significant cartilage decrease.

In the spinal cord injured subject, a thinning was clearly observed if the changes in mean thickness of medial and lateral facet of patella are calculated, after 6 months and 12 months. The difference maps showed however, even more clearly very significant change in the medial facet of the patellar surface after 6 months. The evolution of this region can be followed over the following 6 months. The maps show that the region is growing bigger and even new regions are developing during the following 6 months. This example shows the potential of the novel methods to detect small local changes in articular cartilage thickness and to follow development of these local changes. With this method we developed a tool to quantify local changes in patellar cartilage thickness.
Chapter 3
The Effects of Immobilization on the Characteristics of Articular Cartilage

- CURRENT CONCEPTS AND FUTURE DIRECTIONS –

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Background

This paper is a review of the current knowledge concerning the changes that occur in articular cartilage during joint immobilization and remobilization. A lot of previous studies investigated the effects of immobilization on articular cartilage. However, they all used different methods, looked at different joints, had different periods of immobilization or used different animals. The results of these studies are organized into structural, biochemical, and biomechanical changes of articular cartilage. Tables of comparative findings among different laboratories are specified. The introductory section of each paragraph discusses briefly the anatomy of articular cartilage from respectively the structural, biochemical and biomechanical point of view. A final section discusses novel tools for quantitative analysis of articular cartilage in human subjects.

Own scientific contributions:

- Collection and review of the relevant literature
- Evaluation of the selected literature
- Classification of the literature
ABSTRACT

OBJECTIVE: The purpose of this paper is to review current data and concepts concerning the effect of immobilization on articular cartilage in animal models. We also evaluate the methods to measure articular cartilage changes in humans.

METHODS: Studies looking at the effects of immobilization on morphological, biochemical, and biomechanical characteristics of articular cartilage are reviewed.

RESULTS: Articular cartilage changes in immobilized animals include altered proteoglycan synthesis, as well as thinning and softening of the tissue. The overall thickness of articular cartilage in the knee decreases up to 9% after 11 weeks of immobilization and the deformation rate under test load increases up to 42%. Quantitative data about changes in human articular cartilage following immobilization are not available. This is mainly due to the lack of an accurate, reproducible, and non-invasive method to characterize articular cartilage.

DISCUSSION: An understanding of the alterations in articular cartilage following short and long term immobilization in humans is essential for the optimization of rehabilitation programs. Refined imaging techniques combined with state-of-the-art visualization tools could allow the systematical monitoring of articular cartilage morphology changes in immobilized humans.
3.1 Introduction

Joint pain and loss of mobility are among the most common causes of impairment in middle-age and elderly people. In many instances, articular cartilage degeneration and concomitant alterations in other joint tissues, cause pain and a decreased range of motion. An understanding of the degeneration process in articular cartilage, and the potential for restoring its properties depend to a large extent on an appreciation of the biological behavior and the responsiveness of articular cartilage to injury and immobilization.

Mechanical loading influences the development, maintenance, and aging of skeletal tissues including articular cartilage. Specifically, intermittent hydrostatic pressure is thought to maintain cartilage. Shear stresses, prolonged static loading or absence of loading encourage cartilage destruction and ossification (Buckwalter, 1995). An understanding of the relationships between joint use/disuse and joint degeneration represents a critical step in the process of developing strategies to prevent and treat joint diseases such as osteoarthritis.

In this review, we focus our attention to the problems resulting from joint immobilization and discuss the ability of articular cartilage to recover following remobilization. In section 2, we review the effects of reduced joint motion on articular cartilage morphology. Section 3 focuses on experiments aimed at quantifying biochemical changes in articular cartilage after immobilization. The effects of reduced joint motion on the mechanical behavior of articular cartilage are discussed in section 4. Finally, in section 5 we present novel techniques that can be applied to monitor early changes in articular cartilage in humans non-invasively.

3.2 Morphological changes

3.2.1 Structural characteristics of articular cartilage

The structure of articular cartilage changes with depth from the joint surface (Clark, 1971, Buckwalter et al., 1988). Although these variations are continuous, articular cartilage has been divided into four distinct zones or
layers referred to as the superficial tangential zone, the middle or transitional zone, the deep or radial zone, and the calcified zone (Figure 3-1) (Mow et al., 1974). The superficial zone is thin and exhibits the largest amount of collagen and the lowest amount of proteoglycans (a more detailed description is given in section 3). The collagen fibers in the superficial zone are oriented parallel to the joint surface and the chondrocytes appear flattened. The middle zone is oriented in a cross pattern with a transition from horizontal to vertical cell and collagen orientation. The collagen fibers in the deep zone are oriented vertically. The fibrils emerge from the underlying calcified cartilage where they are anchored. The calcified cartilage represents a transition zone between the articular cartilage and the underlying subchondral bone (Figure 3-1) (Mankin et al., 1994).

Figure 3-1: Representation of collagen fibril ultrastructure throughout the depth of the cartilage depicting the distinct idealized zones (Reproduced with permission from Mow et al, 1974) (Mow et al., 1974).

Collagen fibrils and proteoglycans are the structural components of hyaline cartilage supporting the internal mechanical stresses that result from loads applied to the articular surface. The general orientation of the surface collagen fibrils was first shown by pricking the surface, which resulted in a split-line pattern (Figure 3-2) (Hultkrantz, 1898, Benninghoff, 1925). The orientation of fibrils in the superficial collagen matrix was found to be approximately coincident with the sliding direction of the joint. Based on X-ray diffraction studies (Aspden and Hukins, 1981), polarized light microscopy (Speer and Dahners, 1979), and electron microscopy studies (Speer and Dahners, 1979),
the fibers seem to be aligned to the split line pattern. Benninghoff however, postulated that the fibrils would originate at the osteochondral junction and run radially towards the surface. There, these fibrils bend over tangential to the surface and, finally continue to the articular margin (Benninghoff, 1925). These observations were later supported by investigators using scanning electron microscopy (Jeffery et al., 1991, Clark, 1985) and a multiple-plan freeze fracture technique (Clark, 1990) but are still object of controversy. Studies by Broom (Broom, 1984) suggest that the fibrils are not continuous over their entire length but only continue for a short distance along the articular surface. The superficial tangential layer, which is near the articular surface, consists of sheets of tightly woven collagen fibrils (Mow et al., 1984). This region accounts for the highest concentration of collagen. The fibers in the middle zone on the other hand appear randomly oriented and homogenously dispersed. In the deep zone, the fibers come together to form larger, radially oriented fiber bundles. These bundles enter the calcified zone, crossing the tidemark, to form an interlocking network that anchors the tissue to the bony substrate (Figure 3-1) (Askew and Mow, 1978, Buckwalter et al., 1988).

![Figure 3-2: Representation of collagen fibril ultrastructure throughout the depth of the cartilage depicting the distinct idealized zones (Reproduced with permission from Mow et al, 1974) (Mow et al., 1974).](image)

The morphological development of articular cartilage is influenced by its adaptation to functional demands of absorbing and redistributing compressive forces. The tidemark represents the interface between the hyaline cartilage
and the calcified cartilage (Figure 3-1). Macro-morphological parameters, such as tissue volume, thickness, and joint surface areas can be used to characterize the differentiation and functional adaptation of the cartilaginous tissue to the mechanical stresses. Detailed data on the quantitative morphology of articular cartilage are important input parameters for computer models of diarthrodial joints. These models allow the prediction of load transmission in joints (Blankevoort et al., 1991, Heegard et al., 1995), and the simulation of adaptational processes in the tissue (Burgkart et al., 2000). In orthopedics and skeletal radiology, reliable determination of cartilage thickness is useful for the staging of joint disease and for the evaluation of pharmacological or surgical chondroprotective treatments.

### 3.2.2 Morphological changes due to immobilization

Most investigations examining the consequences of joint immobilization were performed using large laboratory animals (dogs). The studied animals were immobilized by means of a cast (non-rigid) or an external fixator (rigid) for a given time period. The control animals were free to move within their cages. Both groups were sacrificed at the end of the prescribed study period and the joints were evaluated (Behrens et al., 1989, Haapala et al., 1999, Haapala et al., 2000, Jurvelin et al., 1986, Setton et al., 1997). On gross examination, the articular cartilage appeared smooth and continuous with no signs of fibrillation. Jurvelin et al. measured articular cartilage at 20 different locations across the femoral and the tibial joint surfaces (Jurvelin et al., 1986). Large thickness variations were demonstrated in control dogs. In these animals, the thickest cartilage was located at the central areas of the medial condyle of the femur and tibia. The thinnest cartilage was found in the posterior part of the lateral condyle of the femur. After 11 weeks of splinting the right leg, the overall thickness of the articular cartilage in femur, tibia, and patella was decreased by 9% (Table3-1)(Jurvelin et al., 1986). The immobilization significantly decreased the thickness of the hyaline cartilage at the summit of the medial condyle (20%) and at the patellar surface (19%) of the femur (Haapala et al., 1999). After a remobilization period of 50 weeks, no significant difference was seen in comparison to age-matched controls. No changes were seen in the medial condyle of the tibia or in the patella. The cartilage thickness of the summit of
The femoral lateral condyle or the central point of the intermediate part of the tibial lateral condyle showed no changes after immobilization (Table 3-1) (Haapala et al., 2000).

Leroux et al. immobilized 10 skeletal mature dogs with a cast during 4 weeks (Leroux et al., 2001). The thickness of articular cartilage, calcified cartilage, and subchondral bone was measured from scanned and digitized images of stained sections as the average of 5, 10 and 10 equidistant measurements. They did not find changes in the cartilage, calcified cartilage, or subchondral bone thickness on the medial tibia. These results are consistent with previous findings from Haapala et al., at this part of the knee joint (Haapala et al., 1999).
Table 3-1: Morphological changes due to immobilization.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Time</th>
<th>Parameter</th>
<th>Measurement Points</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haapala et al (1999)</td>
<td>11 weeks, rigid</td>
<td>Mean thickness</td>
<td>Med Femur</td>
<td>Decrease of 20%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Summit</td>
<td></td>
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<tr>
<td></td>
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</tr>
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<td>Femur Post</td>
<td>Decrease</td>
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<td>Femur Ant</td>
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<td></td>
<td>Subchondral bone thickness</td>
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Legend: Lat=lateral, Med=medial Post=posterior, Ant=anterior.
3.3 Biochemical changes

3.3.1 Composition of normal articular cartilage

Articular cartilage is a specialized connective tissue with a large amount of extracellular matrix. This matrix is composed of a dense network of collagen fibers (10 to 30% by wet weight), large aggregating and non-aggregating proteoglycans (3 to 10% by wet weight). The remaining 60 to 80% is water, inorganic salts, and small amounts of other matrix proteins, glycoproteins and lipids (Sandell and Hering, 2001).

The collagen fiber network, mainly consisting of type II collagen (with small amounts of collagen type V, VI, IX, and XI), provides the shape and serves as confinement to the entrapped proteoglycans, which cause the tissue to swell because of the large osmotic pressure they generate (Sandell and Hering, 2001). This enables articular cartilage to withstand the compressive stresses associated with load bearing. Cartilage proteoglycans are large protein-polysaccharide molecules that exist either as monomers or as aggregates (Rosenberg, 1975). The monomers form consists of an approximately 200 nm-long protein core to which about 150 glycosaminoglycan (GAG) chains and both O-linked and N-linked oligosaccharides are covalently attached (Heinegard and Paulsson, 1984). The glycosaminoglycans are relatively short chains of repeating disaccharide units of sulphated hexosamines. In cartilage, the most important proteoglycan molecule is aggrecan, which consists of numerous PG monomers non-covalently bond to hyaluronic acid to form aggregates with a molecular weight of up to 2 X 10^8 Dalton and a length of approximately 2 µm. Besides aggrecan, several small non-aggregating proteoglycans, such as decorin and biglycan, are also present. Unlike collagen, the percentage of proteoglycans is lowest near the articular surface and increases with depth. Finally, other glycoproteins such as fibronectin, anchorin, and cartilage oligomeric matrix protein (COMP) are far less abundant, but also play a role in cartilage biology (Sandell and Hering, 2001).

Distributed within the matrix is a sparse population of cells (with a density of less than 10% of the tissue’s volume), the chondrocytes, which are responsible for the synthesis and the maintenance of the matrix components. The turnover
The Effects of Immobilization on the Characteristics of Articular cartilage of the cartilage matrix is regulated by the chondrocytes, which are capable of synthesizing a variety of proteolytic enzymes, such as matrix metalloproteases (MMPs) (Arner and Tortorella, 1995, Bluteau et al., 2001, Freemont et al., 1997, Tetlow et al., 2001). It is well known that in osteoarthritis both aggrecan and collagen are degraded (Arner et al., 1998, Billinghurst et al., 1997, Hollander et al., 1994, Lohmander et al., 1993, Lark et al., 1997, Vankemmelbeke et al., 1998). The proteolytic cascade involves collagenases (interstitial collagenase or MMP-1, neutrophil collagenase or MMP-8, and collagenase-3 or MMP-13), gelatinases (MMP-2 and MMP-9), and stromelysins (in particular stromelysin-1 or MMP-3) (Sandell and Hering, 2001). Tissue inhibitors of metalloproteases (TIMPs) inhibit the catabolic effects of MMPs. It is believed that the ratio MMP-to-TIMP is tightly regulated by the chondrocytes themselves to warrant tissue homeostasis.

3.3.2 Effects of immobilization on the biochemical composition of articular cartilage

The effects of 6 weeks of immobilization on the biochemical characteristics of articular cartilage were thoroughly investigated for the first time by Behrens et al (Behrens et al., 1989). Adult dogs (2-3 years old) were immobilized by rigid external fixation or by casting, allowing limited knee motion but normal transarticular forces, as generated by the muscles. Water content increased at 20 different locations across the patella, the femur, and the tibia (Table 3-2) (Behrens et al., 1989). These findings are partly in agreement with the results of Setton and coworkers (Setton et al., 1997), where an increase in hydration of articular cartilage at the posterior site of the tibial plateau after 8 weeks of joint disuse (non-rigid immobilization) was found (Table 3-2). However, Setton’s group did not report changes in the femur. Müller et al. also did not found changes in water content in femur after 4 and 8 weeks non-rigid immobilization (Table 3-2) (Muller et al., 1994). In this study, the right limb of 16 skeletal mature female dogs was bounded and strapped to the trunk in 90 degrees flexion. Only after 8 weeks, an increase in water content was observed, but confined to the tibial plateau. Leroux et al. did not find any changes in water content after 4 weeks of cast-immobilization in the medial
tibia (Leroux et al., 2001). This result is consistent with the findings of Müller et al., where changes appear only after 8 weeks.

Behrens' group also reported a 6.4% decrease in the total solid component after 6 weeks of casting. In the externally fixed joints, the difference was almost 30% (Table 3-2) (Behrens et al., 1989). Looking closer to this solid component, a decrease of the proteoglycan content at almost all joint locations was observed in the casted and in the external fixator group (Table 3-2) (Behrens et al., 1989). The proteoglycan content in the other joint locations showed a clear decreasing trend. When no joint movement was allowed the loss of hexuronic acid was more dramatic than in the case of limited knee motion (casting) (Behrens et al., 1989). The values returned to normal after 1 week of cast removal, but in some areas of the external fixator joints, hexuronic acid levels were still significantly depressed after 1 week of recovery. Allowing a small movement during the immobilization was already sufficient to permit a faster recovery. These findings were confirmed by Haapala et al. who found a decrease in total uronic acid concentration of up to 32% in patella, tibia and femur after 11 weeks of rigid immobilization (Table3-2) (Haapala et al., 1996). The same investigators observed that the glycosaminoglycan concentration of the articular cartilage was slightly decreased, but not significantly reduced, at the lateral condyle of femur and tibia (Haapala et al., 2000). In this study, 29 weeks old female dogs were immobilized for 11 weeks in 90 degrees flexion with a fiberglass cast, tied to the body to prevent loading. Earlier microspectrophotometric and polarized light microscopy results from the same experiment showed, however, that a significant decrease in the GAG concentration took place in the medial femoral and tibial condyle (20 to 23%) but the recovery in these sites after 50 weeks of remobilization was not complete (Haapala et al., 1999). These results are in contrast to Behrens et al., who found a decrease in the medial as well as in the lateral condyle of the tibia and femur after a shorter immobilization period and a full recovery (Behrens et al., 1989).

After a short (4 weeks) period of immobilization, no changes in sulfated GAG concentration at the medial tibia were found by Leroux and associates (Leroux et al., 2001). Unfortunately, a direct comparison of these studies is not
possible because the authors used animals of different age and of different breed. Additionally, the immobilization time was different.

Haapala et al. found a decrease in the hyaluronan content after 11 weeks rigid immobilization at the tibial condyle and the patellar surface of the femur (Table 3-2) (Haapala et al., 1996). The proportion of hyaluronan to total uronic acid remained unchanged because of a concurrent decrease in aggrecan. In contrast to these results, Müller and associates showed that the hyaluronan content was maintained at control values after 8 weeks of joint disuse (Müller et al., 1994). The results of the centrifugation studies of the nondissociatively extracted proteoglycans indicated a decrease in the amount of aggregates. However, the loss of aggregates is primarily associated with a decrease of the slow-sedimenting ones.
Table 3-2: Results of several studies on the effect of immobilization on the biochemical characteristics of articular cartilage. (PG=proteoglycan, GAG=glycosaminoglycan, MMP=matrix metalloproteases, TIMP=tissue inhibitors of metalloproteases, Prox=proximal, Post=posterior, Ant=anterior, Lat=lateral, mol=molecular, *=full thickness, **=surface zone).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Time</th>
<th>Parameter</th>
<th>Measurement Points</th>
<th>Results</th>
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<td>Med Tibia</td>
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<td>Overall</td>
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<td>Overall</td>
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<td>Distal Femur</td>
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<td></td>
<td></td>
<td>PG content</td>
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</table>
Some authors studied the metabolism of cartilage following immobilization and measured the rate of PG synthesis in explant cultures. Behrens et al. showed that the proteoglycan synthesis decreased by 48% in the external fixation group and by 34% in the casted group as compared to controls (Table 3-2) (Behrens et al., 1989). Only in the external fixation group a significant difference was measured. These results agree with the results of Palmoski, (Palmoski et al., 1980) who observed a 41% reduction in synthesis (on a wet-weight basis) in pooled femoral condyle and patellar cartilage from a single 6 weeks casted dog. The external fixator group also showed an increase in a low-molecular weight proteoglycan (as measured by chromatography) that was present only in very small amounts in normal cartilage and in only slightly higher quantities in the casted animals (Tabel 3-2) (Behrens et al., 1989). The rate of proteoglycan synthesis and aggregation is very sensitive to the physico-chemical environment (i.e., hydration, pH, osmotic pressure, etc.) of the chondrocytes (Gray et al., 1988, Hall et al., 1996, Urban and Bayliss, 1989, Urban, 1994). Consequently, it seems reasonable to postulate that chondrocytes may respond to changes induced by unloading and disuse, and remodel their surrounding extracellular matrix. Markers of cartilage and synovium metabolism (MMPs and TIMPs) were measured by Grumbles and collaborators (Grumbles et al., 1995) and by Haapala and co-workers (Haapala et al., 2001) in an attempt to detect proteolytic events associated with joint immobilization. Grumbles et al. found an elevation of neutral metalloprotease (MMP-2) after 4 weeks of non-rigid immobilization in adult mongrel dogs. Concurrently, a striking fall in TIMP levels was displayed (Grumbles et al., 1995). A return of TIMP and MMP-2 towards control values was observed two weeks after removal of the sling. Haapala et al. monitored the concentration of markers of cartilage and synovium metabolism in the knee joint synovial fluid of young beagles subjected to 11 weeks of rigid immobilization (Haapala et al., 2001). The joint lavage fluid levels of interleukin 1α, TIMP-1 and the concentration of chondroitin sulphate were decreased. In contrast to Grumbles’ data, MMP-3 was not affected by the immobilization period. Joint remobilization during 50 weeks restored the decreased concentrations of markers to control levels.
The other solid part component of articular cartilage, namely the collagen, appeared to be very resistant to reduced joint loading, as shown in several studies (Haapala et al., 1999, Haapala et al., 2000, Setton et al., 1997). No changes in concentration could be detected (Table 3-2). However, immobilization reduced the amount of collagen crosslinks after an 11 weeks period of rigid immobilization in the femur of dogs. Remobilization restored crosslink levels to normal (Haapala et al., 1999). The ability of articular cartilage to sustain repeatedly applied compressive stresses over a lifetime of normal joint function is attributed to the tightly woven collagen network and the high concentration of proteoglycans (Weightman and Kempson, 1979). It has been suggested that once the integrity of this network is lost, the cells might be exposed to abnormally high stresses (Kiviranta et al., 1997). The observation of a decreased number of collagen crosslinks might therefore result in altered biomechanical properties and, ultimately, lead to an abnormal biological response of the residing chondrocytes.

### 3.4 Biomechanical changes

#### 3.4.1 Deformation of normal articular cartilage upon application of load

Articular cartilage is a viscoelastic material, with two distinct phases: a solid phase (the organic solid extracellular matrix) and a movable fluid phase (the interstitial water with the inorganic salts dissolved in it) (Mow et al., 1984). It can be considered as a fluid-filled, porous-permeable medium. Biomechanically, articular cartilage absorbs and distributes load and provides a smooth, lubricated surface that facilitates movements with little friction between the articulating surfaces. Biomechanical properties of articular cartilage show distinct topographical variations in human and animal joints (Arokoski et al., 1999).
Figure 3-3: Setup of an unconfined indentation method and the typical biphasic behavior of articular cartilage. When compressive stress ($\sigma_0$) is applied to the tissue (A), fluid exudes, resulting in compaction of the solid matrix (B). At equilibrium (C), there is no fluid flow; the equilibrium compressive strain of the solid matrix is given by $\varepsilon_0$. (adapted from Armstrong et al, 1980 with permission) (Armstrong and Mow, 1980)

The compressive viscoelastic behavior of articular cartilage is due primarily to the flow of the interstitial fluid and to the intrinsic viscoelasticity of the matrix (Mak, 1986). Articular cartilage also exhibits viscoelastic behavior in tension (Woo et al., 1987), which is attributable to both the internal friction associated with polymer motion and the flow of the interstitial fluid. In tension, the tissue is strongly anisotropic (being stiffer and stronger for specimens harvested in the direction parallel to the split line pattern than for those harvested perpendicular to this pattern) (Woo et al., 1976). Also, cartilage is inhomogeneous (Woo et al., 1976). These anisotropic and inhomogeneous characteristics are believed to be due to the varying collagen and proteoglycan structural organizations of the joint surface and the layering structural arrangements found within the tissue (Mow et al., 1989)

3.4.2 Effect of immobilization on biomechanical parameters

The effect of 11 weeks immobilization on the stiffness of articular cartilage in the canine knee was investigated by Jurvelin and associates (Jurvelin et al., 1986). They characterized the mechanical properties in 20 different locations using the in situ indentation method (Figure 3-3). In an indentation test a punch with a certain radius ($a$) is pressed under constant load ($P$) normally to the articular surface and the deformation ($\omega$) is measured at specific time points during the test. (Armstrong and Mow, 1980)

From the deformation at a given time point ($t_0$), the elastic modulus ($E(t_0)$) is calculated as follows:
\[ E(t_0) = \frac{P(1 - \nu^2)}{2a \omega(t_0) k} \]

\( K \) is a geometrical scaling function (using the value of thickness at the measuring point) and \( \nu \) the Poisson’s ratio. In cartilage, the instant elastic deformation after application of the constant load is followed by a time-dependent creep deformation. From the obtained load-displacement curves, they calculated the instantaneous elastic modulus \( E(t_0=0s) \), and the modulus 15 second after load application \( E(t_0=15s) \). The retardation time spectrum, which characterizes the time-dependent deformation in viscoelastic material, was calculated using the 2 s and 15 s shear compliance (shear compliance = 1/shear modulus), according to the method described by Parsons and Black (Parsons and Black, 1977). The retardation spectrum is then used to assess the rate of fluid flow.

Splinting of the knee joint caused an overall softening of the articular cartilage (Jurvelin et al., 1986). The value of the instantaneous elastic modulus decreased by 17%, and the 15-second elastic modulus was reduced by 25% compared to the control dogs (Table 3-3). After 11 weeks of immobilization, the changes in \( E(t_0=0s) \) were found at 4 indentation points, 3 on the femur and 1 on the tibia. Six indentation points on the femur and three on the tibia showed significant changes in the 15-second elastic modulus. The rate of fluid flow under compression, used as a measure for the tissue permeability, increased in the cartilage of the immobilized leg. As reported in earlier studies all biomechanical parameters showed large variability depending on their anatomical location (Jurvelin et al., 1987, Arokoski et al., 1999). After splinting, softening was more remarkable in non-contact areas of the joints studied (Jurvelin et al., 1986). This classification in contact versus non-contact areas was estimated radiographically and is purely indicative.

In contrast to these results, the instant shear modulus \( G(t_0=0s) \) of cartilage on compressive loading measured by means of the in-situ indentation test did not change after a rigid immobilization during 11 weeks in female Beagle dogs (Table 3-3) (Haapala et al., 2000). In this study only the summit of the lateral condyle of the femur and the central point of the intermediate part of the lateral
condyle of the tibia were mechanically tested. The instant modulus $E(t_0=0s)$ and instant shear modulus

$$G(t_0 = 0s) = \frac{E(t_0 = 0s)}{2(1+\nu)} = \frac{P(1-\nu)}{4\alpha \omega(t_0 = 0s)k}$$

were calculated to indicate the tissue stiffness according to Hayes et al (Hayes et al., 1972). These results are similar to data gathered at approximately the same indentation points in a study previously published by the same investigators (Table 3-3) (Jurvelin et al., 1990). Another remarkable point is that in this previous study, two points very close to each other (i.e. central and marginal intermediate points on the lateral tibia (TLic and TLIm)), can largely differ in terms of $E(t_0=0s)$. In the control dogs this parameter is already much larger at TLIm then at TLic. The effect of immobilization on the $E(t_0=0s)$ as well as on the $E(t_0=15s)$ is much more pronounced in TLic than TLIm. Because of this important site-dependency, it is extremely difficult to compare results.

The equilibrium shear modulus measured 1 hour after load application i.e. $G(t_0=3600s)$ decreased in the lateral femur by 28% and in the lateral tibia by 25% (Table 3-3) (Haapala et al., 2000). In this study, the instantaneous modulus did not change but the equilibrium modulus was reduced, i.e., the articular cartilage matrix alone was softer. After 11 weeks, the dogs were allowed cage activity for 50 weeks. The equilibrium shear modulus recovered to control levels in the tibia but was still reduced by 15% in the femur. These observations show that the effects of immobilization are not totally reversible.
Table 3-3: Results of investigations looking at the effect of immobilization on the biomechanical properties of articular cartilage.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Time</th>
<th>Parameter</th>
<th>Measurement Points</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jurvelin et al</td>
<td>11 weeks, rigid</td>
<td>Instant EM</td>
<td>Femur, Tibia and Patella</td>
<td>Decrease of 17%</td>
</tr>
<tr>
<td>(1986)</td>
<td></td>
<td>15-second EM</td>
<td></td>
<td>Decrease of 25%</td>
</tr>
<tr>
<td>Haapala et al</td>
<td>11 weeks, rigid</td>
<td>ESM</td>
<td>Summit Femur Lat,</td>
<td>Decrease of 30%</td>
</tr>
<tr>
<td>(2000)</td>
<td></td>
<td></td>
<td>Summit Tibial Lat</td>
<td>Decrease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ISM</td>
<td>Summit Femur Lat,</td>
<td>No changes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Summit Tibial Lat</td>
<td>No changes</td>
</tr>
<tr>
<td>Setton et al</td>
<td>4 weeks, non-rigid</td>
<td>Compressive EM</td>
<td>Medial Tibia Post,</td>
<td>No changes</td>
</tr>
<tr>
<td>(1997)</td>
<td></td>
<td></td>
<td>Medial Tibia Ant</td>
<td>No changes</td>
</tr>
<tr>
<td></td>
<td>8 weeks, non-rigid</td>
<td>Compressive EM</td>
<td>Medial Tibia Post,</td>
<td>No changes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Medial Tibia Ant</td>
<td>No changes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tensile EM</td>
<td>Lat Femur Ant</td>
<td>Tendency to increase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lat Femur Post</td>
<td>Tendency to increase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Distal Groove Femur</td>
<td>Tendency to increase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prox Groove Femur</td>
<td>Tendency to increase</td>
</tr>
<tr>
<td>Leroux et al</td>
<td>4 weeks, non-rigid</td>
<td>EM</td>
<td>Medial tibia</td>
<td>Trend to decrease</td>
</tr>
<tr>
<td>(2001)</td>
<td></td>
<td></td>
<td></td>
<td>Decrease of 75%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ESM</td>
<td>Medial tibia</td>
<td>Decrease of 71%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dynamic SM</td>
<td>Medial tibia</td>
<td></td>
</tr>
</tbody>
</table>

EM=elastic modulus, ESM= equilibrium shear modulus, ISM= instant shear modulus, Prox=proximal, Post=posterior, Ant=anterior, Lat=lateral.

Leroux and coworkers calculated the equilibrium shear modulus from linear regression of the equilibrium shear stress as a function of the applied shear strains over the four step-wise applied strain increments ($\gamma_0=0.005, 0.01, 0.02,$ and 0.03) (Leroux et al., 2001). The resulting torque was monitored for 1200 s, followed by a 600 s recovery period upon removal of the strains. They observed even a bigger reduction of approximately 70% for the equilibrium shear modulus in the medial tibia following 4 weeks of joint immobilization.
The Effects of Immobilization on the Characteristics of Articular cartilage

(151, 749) They found an equal decrease for the dynamic shear modulus, which was calculated by applying an oscillatory displacement, corresponding a shear strain with an amplitude of 0.01 and an angular frequency from 0.1 rad/sec to 100 rad/sec. Calculations assume a linear viscoelastic behavior. In contrast, the equilibrium elastic modulus did not change.

Setton et al. reported no significant changes in compressive properties of the articular cartilage after 4 or 8 weeks of joint disuse (Table 3-3) (Setton et al., 1997). The non-rigid immobilization was used as a model for joint disuse, eliminating weight-bearing forces across the joint, while allowing for some limited limb motion. The indentation tests were performed at two sites on the medial tibial plateau, corresponding to the anterior and posterior sites of contact to the femoral condyle. The in situ indentation protocol and biphasic theoretical analysis were used to numerically determine the permeability of the articular cartilage, and the elastic properties of the matrix (aggregate modulus corresponding to the equilibrium modulus and Poisson’s ratio). In contrast to Jurvelin et al (Jurvelin et al., 1986) no changes were found. However, looking at the equivalent positions (tibia medial anterior and posterior) in this study, no changes were observed in this latter study. The period during which the animals were immobilized was also shorter in this study. Setton et al. used female dogs of older age and the immobilization method was different (Setton et al., 1997). The same group studied the effect of non-rigid immobilization on the tensile elastic modulus of the femur (Setton et al., 1997). Test strips of articular cartilage were placed in the jaws of the testing apparatus and straightened under a tare load of 0.02 N. Series of successive tensile stress-relaxation experiments was then performed as described (Setton et al., 1997). A significant increase of the elastic modulus after 8 weeks of immobilization was found in the distal groove of the femur, where the femur is in contact with the patella (Table 3-3). There was already a clear tendency to this result after 4 weeks in all measured points. A period of 3 weeks of remobilization after 4 or 8 weeks of immobilization did not restore the value for the tensile modulus measured in control cartilage.

Indeed, there is a lot of discrepancy between results from different studies. This discrepancy may be related to differences in the protocol used for
biomechanical testing, the type and duration of immobilization, and finally the age and breed of the animals used. Inherent to the indentation method, used in most biomechanical investigations, is the site-dependency, which renders a direct comparison between results from different studies very difficult. Also, the technique is invasive since it requires the opening of the joint to expose the articular surfaces. Additionally, in the animal studies discussed, the gathered data were fitted to different models. Jurvelin et al. assumed that articular cartilage can be modeled as an elastic layer with finite thickness bonded to a rigid half-space (Jurvelin et al., 1989). In contrast, Setton et al. applied the biphasic theory and calculated the permeability, aggregate modulus, shear modulus, and Poisson’s ratio (Setton et al., 1997). One of the reason of discrepancy between Leroux’s results and the previous results (Haapala et al., 2000, Setton et al., 1997) may be that torsional shear, used in this study, directly measures the average response of the full-thickness sample, while compressive indentation testing is strongly influenced by the properties of the superficial articular cartilage and the Poisson’s ratio needed for the theoretical solution.

Although it is an invasive technique, indentation has been recently used to assess biomechanical properties of articular cartilage in humans (Lyyra et al., 1995, Lyyra et al., 1999). Other non-invasive methods to asses human articular cartilage, albeit not from a biomechanical point of view, will be presented in the next section.

3.5 Monitoring cartilage changes in humans

Animal studies showed that immobilization result in several major changes of articular cartilage. Mechanical, biochemical, and morphological characteristics are altered and do not always totally recover upon remobilization of the joint. Due to the lack of an accurate, reproducible, and non-invasive method to characterize articular cartilage, quantitative data about changes in human articular cartilage after a given period of immobilization are not yet available. However, novel techniques will allow the measurement of morphological parameters in the near future.
Conventional radiography can provide indirect information about cartilage destruction from signs such as “joint space” narrowing, subchondral sclerosis or cysts, or the presence of osteophytes (Pool, 1974, Richardson et al., 1984). However, it is a two-dimensional technique that is sensitive to artifacts resulting from malpositioning (Buckland-Wright et al., 1995). Another disadvantage of radiography is the inability to differentiate between femoral and tibial cartilage loss. Radiography is less sensitive to local than to general cartilage lesions, and cannot demonstrate the pattern of cartilage destruction throughout the joint surface. Due to these limitations, measurements of the joint space width in radiographs are not ideal for reliably evaluating cartilage thickness and surface alterations. An attempt to use ultrasound for determination of articular cartilage thickness has been made by McCune et al, Aisen et al, Castriota-Scanderbeg et al, and Martino et al. (Aisen et al., 1984, McCune et al., 1990, Martino et al., 1993, Castriota-Scanderbeg et al., 1996). The technique is fast, cheap, and non-invasive. The problems of ultrasound are the lack of reproducibility and its insufficient accuracy (Castriota-Scanderbeg et al., 1996, Martino et al., 1993). Additionally, this method is only applicable to superficially located joint surfaces, and the position of the images relative to the joint surface is hard to define. Computer tomography (CT) arthrography is invasive, requiring a contrast agent to be injected into the joint cavity (Ihara, 1985, Boven et al., 1982). Its routine use in clinical medicine is therefore problematic and the technique cannot be employed in healthy volunteers. Diagnostic arthroscopy is considered the gold standard for evaluating surface alterations. The examination can provide information on cartilage mechanical properties if an arthroscopic indenter instrument is used (Lyyra et al., 1995, Lyyra et al., 1999). Again, this method is invasive, which restricts its routine clinical use. Additionally, it is very difficult to define the measuring location in an accurate and reproducible way, which is essential for cross-sectional as well as longitudinal studies.

Magnetic Resonance Imaging (MRI) is presently the most accurate imaging modality to evaluate the articular cartilage, due to the high soft tissue contrast and the opportunity to evaluate the total joint surface (Figure 3-4). MRI uses a strong static and high frequency spatially inhomogeneous magnetic fields to
obtain sectional images. In MRI, the tissue contrast can be substantially modulated by choosing different types of pulse sequences, and by changing the specific parameters of the sequences (repetition time, echo time, flip angles, etc). For the analysis of cartilage macro-morphology, the bone-cartilage interface and the articular surface need to be delineated accurately (Peterfy et al., 1994, Eckstein et al., 1996). In particular, the spatial resolution must be sufficient to permit quantitative measurements. Therefore, a high resolution fat suppression gradient sequence is used to visualize the articular cartilage with a high contrast to the surrounding tissues (Figure 3-4) (Eckstein et al., 1997, Losch et al., 1997, Haubner et al., 1997). Fat-suppression is achieved by applying a pre-pulse, preventing the fat-bound protons from creating a signal during the subsequent data acquisition (Recht et al., 1993). However, sequences with selective excitation of only the water-bound protons have been introduced recently, in which fat-signal elimination can be obtained with much shorter acquisition times (Hardy et al., 1998, Hyhlik-Durr et al., 2000, Eckstein et al., 1998c).

![Figure 3-4: Methods for quantitative 3D analysis of cartilage morphology from MR imaging. Panel A: Coronal MR image of the human knee obtained with a fat-suppressed gradient echo sequence; segmentation of medial tibial cartilage with B-spline Snake algorithm. Panel B: 3D volume reconstruction of the medial and lateral tibial cartilage.](image)

Quantitative evaluation requires some image-processing steps including the segmentation of the cartilage, 3D analysis (3D reconstruction and 3D thickness computations), and potentially 3D registration.
Knee-joint cartilage volume measurements did deviate not more than 5 to 10% on average from data collected from anatomical sections (Eckstein et al., 1996), and from CT arthrography (Eckstein et al., 2000b, Eckstein et al., 1998b, Graichen et al., 2000). The thickness distribution calculated out of the MRI showed no differences compared to histological sections (Kladny et al., 1996) A mode ultrasound, (Graichen et al., 2000, Eckstein et al., 1997) and stereophotogrammetry (Cohen et al., 1999). The reproducibility has been studied in healthy volunteers and patients, by repeating measurements after joint repositioning and reshimming of the magnet (Eckstein et al., 1998b, Cicuttini et al., 1999, Stammberger et al., 1999b). The reproducibility of cartilage volume and thickness measurement are relatively high, ranging between 1.5% and 3.8% (Eckstein et al., 1998b). The cartilage volume and thickness results agreed within 4% between the fat-suppression and the water excitation sequence with slightly higher values for the water excitation (Hyhlik-Durr et al., 2000). Another potentially valuable image sequence for quantitative cartilage imaging is driven equilibrium Fourier transform imaging (DEFT) (Hargreaves et al., 1999). It generates contrast between cartilage and joint fluid by enhancing the signal from the joint fluid, rather than by suppressing the cartilage. This is a promising technique but the accuracy and precision of quantitative measurement remains to be established. Biochemical information of the cartilage can be obtained by using the negatively charged contrast agent gadolinium diethylene triamine pentaacetic acid (Gd(DTPA)\textsuperscript{2-}). This contrast agent distributes in cartilage in inverse relation to the negatively charged GAG concentration. Using this premise, the spatial distribution of GAG can be easily observed in T1-weighted and T1-calculated MRI studies in intact human knee joints (Bashir et al., 1999), with good histological correlation. Furthermore, in vivo clinical images of T1 in presence of (Gd(DTPA)\textsuperscript{2-}) correlated well with the validated ex vivo results after total knee replacement surgery (Bashir et al., 1999). It could be demonstrated that degenerated cartilage had a higher Gd(DTPA)\textsuperscript{2-} uptake than normal cartilage (Tiderius et al., 2000). Consequently Gd(DTPA)\textsuperscript{2-} enhanced MRI gives us the opportunity to get biochemical and structural information of articular cartilage. However, it necessitates the injection of contrast agents, which is invasive and risky.
Several studies showed the power of MRI to detect early degenerative changes in articular cartilage (Tiderius et al., 2000, Thut et al., 2001, Calvo et al., 2001, Ghosh et al., 2001). Thut et al. used six different image sequences and a self-developed score system which takes into account the signal abnormality, cartilage thickness change, and surface irregularity (Thut et al., 2001). Early degenerative changes were distinguished in thicker cartilage regions of rabbit knees, whereas some changes were still undetectable at locations where the cartilage is very thin. This demonstrates not only the power of MRI to detect early degenerative cartilage changes but also the importance of image resolution. Calvo et al. showed that focal increases in cartilage thickness is one of the earliest measurable changes in osteoarthritis and proceeds subchondral bone remodeling (Calvo et al., 2001). Four weeks after partial medial meniscectomy in the left knee of rabbits, the femoral articular cartilage was already significantly increased. Tibial cartilage showed a significant increase after 6 weeks, while no changes were detected in the microradiographs of the subchondral bone even 10 weeks after inducing osteoarthritis. Measuring the cartilage thickness variations with MRI can be used to follow the course of OA and to evaluate potential beneficial effects of novel therapies. Another parameter derived from MR images is the T2 map (Ghosh et al., 2001). This map is generated, on a pixel by pixel basis, by extrapolating from the signals obtained at two different echo times. Significant T2 differences are observed between healthy volunteers and osteoarthritic patients. The MRI technique for T2 mapping complements the high-resolution cartilage thickness and volume measurement technique. Vanwanseele et al. showed recently that cartilage thickness decreases after spinal cord injury (Vanwanseele et al., 2001). They used MRI to monitor systematically morphological changes in articular cartilage. Preliminary results showed that after 6 months of immobilization, there is already a clear tendency to thinning in medial tibia. Twelve months after the spinal cord injury the decrease in cartilage thickness was even bigger.
3.6 Summary

Articular cartilage alterations following immobilization have been thoroughly studied in laboratory animals. These investigations demonstrated that stress deprivation alters the morphological, biochemical and biomechanical characteristics of articular cartilage. Data about changes in human articular cartilage after immobilization are not yet available. In healthy volunteers and in patients suffering from severe osteoarthritis, cartilage thickness and volume could be measured by MRI using a special pulse sequences combined with the state-of-the-art visualization tools. This technique allowed also first assessments of how physical activity could influence articular cartilage morphology. Measuring parameters such as tissue volume and mean or maximal cartilage thickness give some insight into the influence of unloading and/or restricted motion on the articular cartilage in patients immobilized for a certain period. Such data can provide important information on the effects of immobilization on human articular cartilage and on the time course of these changes. This knowledge will help physicians and therapists in the planning and optimization of rehabilitation programs after surgical procedures or prolonged motion restriction or immobilization.

Acknowledgments:
Support for this project was provided by the Swiss Paraplegic Foundation, Basle, Switzerland.
Chapter 4
Knee Cartilage of Spinal Cord Injured Patients Displays Progressive Thinning in the Absence of Normal Joint Loading and Movement

Reprinted from:
Vanwanseele, B; Eckstein, F; Knecht, H; Stüssi, E; Spaepen, A. Knee Cartilage of Spinal Cord Injured Patients Displays Progressive Thinning in the Absence of Normal Joint Loading and Movement. *Arthritis and Rheumatism* 46(8), pp 2073–2078
Background

The following paper is the first published study that measured differences between the knee articular cartilage thickness of healthy volunteers and spinal cord injured patients. Until now investigations studying the effects of immobilization on articular cartilage were done using large laboratory animals. As previous chapter described, thinning of the cartilage as a reaction to the immobilization of the leg was observed in those studies. However, it is difficult to transfer animal data to human beings. The magnitude and the time frame of the reaction of human cartilage on immobilization are unclear. With a cross-sectional study design, an idea of the magnitude and the time frame in which changes in human knee cartilage happen, was collected fast. With this approach, differences between healthy volunteers and spinal cord injured patients 6 months after the injury, 12 months and 24 months post-injury were measured. The outcome of this study gave for the first time an idea of cartilage changes as a reaction to joint immobilization and provided the necessary knowledge to define the measurement protocol for a new longitudinal study design.

Own scientific contribution

- Selection of the best suitable MRI protocol and the research design
- Segmentation of the cartilage
- Statistical analysis and interpretation of the data
ABSTRACT

OBJECTIVE: Alterations of cartilage morphology, biochemical and mechanical properties occur after unloading and immobilization in animals. However, findings have been inconsistent, and it is unclear whether such changes also take place in humans. In this in vivo study, we tested the hypothesis that progressive thinning of knee joint cartilage is observed after spinal cord injury.

METHODS: We evaluated knee cartilage of patients with complete, traumatic spinal cord injury at 6 (n = 9), 12 (n = 11), and 24 months (n = 6) post-injury. Morphologic parameters for the knee cartilage (mean and maximum thickness, surface area) were computed from magnetic resonance imaging data, and results were compared with those of young healthy volunteers (n = 9).

RESULTS: After 6 months of injury, the mean articular cartilage was significantly less in the patella and medial tibia (-10 and -16%, respectively, p < 0.05), but not in the lateral tibia (-10%) compared with healthy volunteers. After 12 and 24 months, the differences amounted to -21%/-23% for the patella, -24%/-25% for the medial tibia, and -16%/-19% for the lateral tibia. The changes were significant in all three surfaces (p < 0.05 to 0.01).

CONCLUSION: Our data show, for the first time, that progressive thinning (atrophy) of human cartilage occurs in the absence of normal joint loading and movement. This may have important implications for patient management, in particular for spinal cord injured patient and patients immobilized after surgery.
4.1 Introduction

Loading and movement of the joint are of major importance for the maintenance of the morphological and functional integrity of articular cartilage. Animal studies have demonstrated that joint immobilization and stress deprivation lead to functional adaptation of articular cartilage, these changes concerning morphological, biochemical and biomechanical characteristics of the cartilage matrix (Jurvelin et al., 1986, Grumbles et al., 1995, O'Connor, 1997, Behrens et al., 1989, Haapala et al., 1996, Haapala et al., 2001, Haapala et al., 1999, Haapala et al., 2000, Muller et al., 1994, Setton et al., 1997).

Jurvelin et al. (Jurvelin et al., 1986) observed an average decrease in cartilage thickness of 9% in the canine knee after 11 weeks of rigid immobilization, and Haapala et al. (Haapala et al., 1999) a decrease of approximately 20% in the dogs’ medial femur. In contrast, Leroux et al. (Leroux et al., 2001) found no significant changes in cartilage thickness after 4 weeks of non-rigid immobilization in tibial cartilage. Other authors have reported altered proteoglycan synthesis and content, fibrofatty proliferation at the articular surface, and softening of the cartilage during joint immobilization in animals (Jurvelin et al., 1986, Grumbles et al., 1995, O'Connor, 1997, Behrens et al., 1989, Haapala et al., 1996, Haapala et al., 2001, Haapala et al., 1999, Haapala et al., 2000, Muller et al., 1994, Setton et al., 1997).

Due to a lack of accurate noninvasive imaging methods, there has, however, so far been no report on morphological changes of cartilage in humans following immobilization. This knowledge is important to anticipate cartilage changes in patients that are immobilized after surgical procedures or accidents, or after spinal cord injury.

In patients that suffer from a traumatic spinal cord injury (SCI) the lower extremity joints are unloaded and restricted in movement for long periods of time. These patients consequently encounter secondary complications, such as a decrease in muscle mass, cardiovascular fitness, and bone density (Whiteneck et al., 1992, Kocina, 1997). Although there have been suggestions that these patients suffer from joint effusion (Buschbacher et al., 1991, Levi et
al., 1995, Betz et al., 1996), stiffness (Levi et al., 1995), fibrofatty connective tissue growing (Enneking and Horowitz, 1972, Betz et al., 1996) and heterotopic ossification (Haassard, 1975), only two prior studies have attempted to assess the influence of paralysis on the articular cartilage of lower extremity joints (Pool, 1974, Richardson et al., 1984). In these studies, a narrowing of the radiographic hip joint space by more than 50% was observed in 25 of 200 patients with flaccid paralysis (Pool, 1974). Richardson et al. (Richardson et al., 1984) reported an apparent overgrowth of the epiphyses, periarticular osteoporosis and joint space narrowing in patients with neuromuscular disorders. However, radiography cannot directly visualize cartilage, nor discriminate between different cartilage plates in the knee. In particular, accurate and reproducible measurements require a semiflexed, weight-bearing configuration of the knee (Buckland-Wright et al., 1995), which cannot be achieved in these patients.

Recently, however, 3D magnetic resonance imaging (MRI), combined with state-of-the-art post-processing, has been shown to provide accurate and highly reproducible data on cartilage morphology in vivo (Burgkart et al., 2001, Cohen et al., 1999, Eckstein et al., 1998c, Peterfy et al., 1994, Stammberger et al., 1999b). The objective of this study was therefore to assess, for the first time, morphological alterations in human articular cartilage during long-term unloading and immobilization in patients with spinal cord injury. To determine the time course of potential change, we examined patients at various time periods after injury in a cross-sectional study.

4.2 Patients and Methods
The right knees of 26 male patients with traumatic, and complete spinal cord injury (age 37 ± 13.7 years, range 19-58) were investigated. Nine individuals were 6 months post injury (age 45.5 ± 17 years), 11 were 12 months (age 32.4 ± 9.6 years), and 6 were 24 months post injury (age 31.8 ± 7.5 years). In the 6 months group, 7 patients were paraplegic (range L1-Th5) and 2 were tetraplegic (C2, C5). Seven patients in the 12 months group were paraplegic (range L1 – Th4) and 4 were tetraplegic (range C5 – C7). In the 24 months
group, one patient was paraplegic (Th6) and 5 were tetraplegic (range C5 – C7). Patients with a history of knee pain, trauma, or surgery were excluded from the study. All participants signed a statement of informed consent as approved from the Ethical Committee Lucerne, after receiving oral and written information.

Figure 4-1: Transverse MR image (top) and coronal image of the knee joint at 6 months post spinal cord injury. Fat suppressed FLASH sequence (TR/TE 53/10 msec, flip angle 30 deg) with a resolution of 0.31 mm x 0.31 mm x 1.5 mm (matrix = 512 x 512 pixels, field of view = 160 cm).

The patients were examined with a 1.5 T scanner (Magnetom Symphony, Siemens, Erlangen, Germany), and a circular polarized (CP) transmit-receive extremity coil. A previously validated fat-suppressed gradient echo sequence (FLASH = fast low angle shot; repetition time = 53 msec, echo time = 10.3 msec, flip angle = 30 degrees) (Glaser et al., 1997) was used to acquire one transverse data-set of the patellar cartilage, and one coronal data-set of the tibial cartilages(Figure 4-1). Images were obtained at an in plane resolution of 0.31 mm x 0.31 mm and a slice thickness of 1.5 mm (matrix = 512 x 512 pixels, field of view = 160 cm). These orientations were chosen because they involve smaller precision errors than a sagital protocol. In the patella, a precision error of only 1.0 % (RMS average CV%) has been reported for cartilage thickness measurements in healthy volunteers (Eckstein et al., 2000a), and precision errors of 2.6% and 2.5% for the medial and lateral tibia, respectively (Hyhlik-Durr et al., 2000).
Segmentation was performed by one single person (B.V.) on a graphics computer (Octane Duo, Silicon Graphics Inc., CA) on a section-by-section basis, using a B-spline Snake algorithm (Stammberger et al., 1999b). Patellar, medial and lateral tibial cartilage plates were reconstructed three-dimensionally, and the volume determined by numerical integration of the segmented voxels. The size of the joint surface area and the cartilage-bone interface area were computed after triangulation of these surfaces (34), and the mean and maximal cartilage thickness values were calculated independent of the original section orientation by applying three-dimensional Euclidean distance transformation algorithm (Stammberger et al., 1999a). The thickness values in the patients were compared with a control group, consisting of 9 male healthy volunteers (age 28 ± 7 years, range 25 to 36), with no history of knee pain, trauma, or surgery, and no abnormalities of the cartilage in the MR images. The data in the control group were segmented by the same observer (B.V.), and were consistent with data reported in 49 young healthy men (age 25.8 ± 3.2 years, range 20 to 30) (Eckstein et al., 2001b) (Figure 4-2).

The statistical significance of the differences between patients and the control group was first assessed by a Kruskal-Wallis test. In a next step, each patient group (6 months, 12 months, 24 months post injury) was compared with the control group using a Mann-Whitney U test.

### 4.3 Results

There was a significantly ($p < 0.01$, Kruskal Wallis test) lower mean cartilage thickness in the patella, medial and lateral tibia of the spinal cord injured patients vs. the control group of young healthy volunteers (Figure 4-2: Mean cartilage thickness of the patella, medial tibia, and lateral tibia of 49 young, healthy men (database), 9 healthy control subjects, and spinal cord-injured subjects (n=9), 12 months (n=11), and 24 months (n=&) after injury. Bars show the mean ± SD. * = $p < 0.05$ and ** = $p < 0.01$ compared with controls, by Mann-Whitney U test.). The maximal cartilage thickness in the medial tibia was also significantly decreased, but changes in the patella and lateral tibia failed to reach statistical significance at the 5% error level.
Knee Cartilage of SCI Patients Displays Progressive Thinning

* $p < 0.05$ (compared to control; Mann-Whitney-U test)

** $p < 0.01$ (compared to control; Mann-Whitney-U test)

![Figure 4-2: Mean cartilage thickness of the patella, medial tibia, and lateral tibia of 49 young, healthy men (database), 9 healthy control subjects, and spinal cord-injured subjects ($n=9$), 12 months ($n=11$), and 24 months ($n=8$) after injury. Bars show the mean ± SD. * = $p < 0.05$ and ** = $p < 0.01$ compared with controls, by Mann-Whitney U test.]

In the patella, the differences of mean (maximal) cartilage thickness in the patients vs. volunteers amounted to 10% (8%) at 6 months, 21% (15%) at 12 months, and 23% (16%) at 24 months post injury (Table 4-1). Differences in mean thickness were significant for all groups of patients ($p < 0.01$; Mann Whitney U test).
Table 4-1: Cartilage morphology in the patella of healthy young men (control) and spinal cord injured subjects 6, 12, 24 months post-injury. Average values and Standard deviation for mean and maximal thickness, joint surface area and, cartilage volume.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>6 months (n = 9)</th>
<th>12 months (n = 11)</th>
<th>24 months (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean thickness (mm)</td>
<td>2.79 ± 0.19(*)</td>
<td>2.48 ± 0.20(**)</td>
<td>2.17 ± 0.30(**)</td>
<td>2.12 ± 0.47(**)</td>
</tr>
<tr>
<td>Maximal thickness (mm)</td>
<td>5.55 ± 0.53(*)</td>
<td>5.13 ± 0.58(*)</td>
<td>4.70 ± 0.82(*)</td>
<td>4.69 ± 1.40(*)</td>
</tr>
<tr>
<td>Joint Surface Area (cm²)</td>
<td>13.8 ± 1.8(*)</td>
<td>14.5 ± 1.7(*)</td>
<td>12.9 ± 1.9(*)</td>
<td>13.6 ± 1.8(*)</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>4.43 ± 0.67(*)</td>
<td>4.27 ± 0.71(*)</td>
<td>3.39 ± 0.55(**)</td>
<td>3.49 ± 0.95(*)</td>
</tr>
</tbody>
</table>

* p < 0.05 vs. control group with Mann-Whitney-U test
** p < 0.01 vs. control group with Mann-Whitney-U test

In the medial tibia, the differences were higher than in the patella: Changes in mean (maximal) thickness amounted to 16% (16%) at 6 months, 24% (19%) at 12 months, and 25% (14%) at 24 months (Table 4-2). Differences in mean and maximal thickness were significant for all groups (p < 0.05).

Table 4-2: Cartilage morphology in the medial tibia of healthy young men (control) and spinal cord injured subjects 6, 12, 24 months post-injury. Average values and Standard deviation for mean and maximal thickness, joint surface area and, cartilage volume.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>6 months (n = 9)</th>
<th>12 months (n = 11)</th>
<th>24 months (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean thickness (mm)</td>
<td>1.65 ± 0.17(*)</td>
<td>1.38 ± 0.17(*)</td>
<td>1.26 ± 0.20(**)</td>
<td>1.24 ± 0.22(**)</td>
</tr>
<tr>
<td>Maximal thickness (mm)</td>
<td>3.77 ± 0.39(*)</td>
<td>3.16 ± 0.44(*)</td>
<td>3.05 ± 0.49(**)</td>
<td>3.22 ± 0.39(**)</td>
</tr>
<tr>
<td>Joint Surface Area (cm²)</td>
<td>12.0 ± 1.7(*)</td>
<td>12.3 ± 2.0(*)</td>
<td>11.8 ± 1.2(*)</td>
<td>1.11 ± 0.8(*)</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>2.34 ± 0.46(*)</td>
<td>1.89 ± 0.27(*)</td>
<td>1.81 ± 0.34(*)</td>
<td>1.63 ± 0.26(*)</td>
</tr>
</tbody>
</table>

* p < 0.05 vs. control group with Mann-Whitney-U test
** p < 0.01 vs. control group with Mann-Whitney-U test
In the lateral tibia, the percentage differences were lower than in the medial tibia: They amounted to 10% (10%) at 6 months, 16% (16%) at 12 months, and 19% (7%) at 24 months post injury (Table 4-3). The mean cartilage thickness was significantly lower at 12 and 24 months (p < 0.05), but not at 6 months. The maximal cartilage thickness did only display significant differences 12 months post injury (p < 0.05).

Table 4-3: Cartilage morphology in the lateral tibia of healthy young men (control) and spinal cord injured subjects 6, 12, 24 months post-injury. Average values and Standard deviation for mean and maximal thickness, joint surface area and, cartilage volume.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>6 months (n = 9)</th>
<th>12 months (n = 11)</th>
<th>24 months (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean thickness (mm)</td>
<td>2.08 ± 0.20</td>
<td>1.86 ± 0.30</td>
<td>1.73 ± 0.29</td>
<td>1.67 ± 0.27</td>
</tr>
<tr>
<td>Maximal thickness (mm)</td>
<td>4.56 ± 0.71</td>
<td>4.12 ± 0.54</td>
<td>3.85 ± 0.69</td>
<td>4.24 ± 0.55</td>
</tr>
<tr>
<td>Joint Surface Area (cm²)</td>
<td>11.6 ± 1.7</td>
<td>11.4 ± 1.4</td>
<td>10.9 ± 1.1</td>
<td>11.0 ± 1.1</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>2.53 ± 0.44</td>
<td>2.52 ± 0.61</td>
<td>2.25 ± 0.36</td>
<td>2.17 ± 0.40</td>
</tr>
</tbody>
</table>

* p < 0.05 vs. control group with Mann-Whitney-U test
** p < 0.01 vs. control group with Mann-Whitney-U test

The size of the bone-cartilage interface or articular surface showed no significant differences between groups. Changes in cartilage volume in patients were thus very similar to those in mean cartilage thickness (Table 4-1 to Table 4-3).

There were no statistically significant differences in cartilage thickness, joint surface area, or cartilage volume between the reference group used for the comparison with the patients, and the larger database of 49 healthy volunteers.
4.4 Discussion

In this study, we investigated whether human articular cartilage shows morphological changes in spinal cord injured patients. Our data show, for the first time, that progressive thinning of human cartilage occurs in the absence of normal joint loading and movement.

So far, in vivo analysis of human articular cartilage has been hampered, because no validated noninvasive measurement method has been available. Only recently, 3D magnetic resonance imaging (MRI) combined with state-of-the-art post-processing has been shown to provide accurate and highly reproducible data on cartilage morphology in the living (Burgkart et al., 2001, Cohen et al., 1999, Eckstein et al., 1998c, Peterfy et al., 1994, Stammberger et al., 1999b). Precision errors for the given imaging sequence and resolution have been shown to range from only 1 to 3% (Eckstein et al., 2000a, Hyhlik-Durr et al., 2000), whereas we observed differences between groups of up to 20% in this study. The technology available therefore allows us to characterize the time-dependent changes of human articular cartilage following spinal cord injury with high reliability.

At six months post injury, the largest change in mean and maximal thickness was found in the medial tibia. In the lateral tibia, no significant changes occurred, but a trend was also apparent. These results are consistent with findings reported in some animal studies (Jurvelin et al., 1986, Haapala et al., 1999): In dogs, the cartilage thickness of the medial tibia showed more substantial changes than the patellar or lateral tibial cartilage after a rigid immobilization of 11 weeks (Jurvelin et al., 1986). Haapala et al. (Haapala et al., 1999) did not find significant changes in the lateral tibia after 11 weeks of rigid immobilization in dogs, while Jurvelin found only a small degree of thinning of the cartilage of about 4%. In contrast to these results, a recent study of Leroux et al. (Leroux et al., 2001) reported no changes in cartilage thickness in the medial tibia after 4 weeks of non-rigid immobilization in dogs. Thickness measurements in these studies were derived from histological sections at only a few points. In contrast, the technique used here allows us to analyze the cartilage thickness throughout the whole joint, taking into account
out-of-plane deviations of the minimal distance between the articular surface and bone cartilage interface.

There are numerous clinical implications of the current findings. Thinning of the cartilage may render the joint unstable, and these joints may encounter abnormal stresses during passive standing training in spinal cord injured patients. Adjunct therapies, such as functional electro stimulated cycling, may, however, be able to prevent cartilage thinning following spinal cord injury (Betz et al., 1996). Other forms of exercise, such as continuous passive motion (Salter et al., 1980), may be used to prevent cartilage changes during postoperative immobilization. This is of particular importance, as some animal studies have indicated that changes of the cartilage during immobilization are not fully reversible during remobilization (Haapala et al., 1999, Haapala et al., 2000).

O’Connor made a distinction between unloading alone, and unloading combined with non-rigid immobilization (O’Connor, 1997). Unloading alone caused thinning of the uncalcified cartilage in the medial tibia, but did not change the total thickness of the cartilage. Unloading combined with restricted movement did not cause changes in the uncalcified cartilage thickness of the medial tibia, but the uncalcified cartilage in the medial anterior tibia was decreased by 10%. Further studies will therefore have to identify, whether thinning of human cartilage is caused by a decrease in weight bearing or by a decrease in joint movement. Moreover, it will be necessary to investigate to what extent thinning of human cartilage is associated with biochemical, structural, and mechanical changes, whether changes proceed after 24 months, and to what extent the changes can be reversed during remobilization. Such data will be important to anticipate cartilage changes in patients that are immobilized after surgical procedures. The current methodology will also permit to evaluate the effectiveness of potential countermeasures, designed to preserve the morphological and functional competence of articular cartilage.
Acknowledgment:
The author thanks all subjects for participating in this study and acknowledge the contribution of Dr. Phil Jungen, Dr. Hans Hawighorst (Swiss Paraplegic Centre, Notwill, Switzerland) in data collection. We thank Dr. Christian Glaser and Prof. Dr. Maximilian Reiser (Institute of Clinical Radiology, LMU München, Germany), and PD Dr. Karl-Hans Englmeier (Medis Institute, GSF National Research Center, Neuherberg, Germany) for technical advice, and for allowing us to use the software for quantitative cartilage analysis. This work was supported by the Swiss Paraplegic Foundation, Switzerland.
Chapter 5

Longitudinal Analysis of Cartilage Atrophy in the Knees of Spinal Cord Injured Patients

Reprinted from:
Background

This paper reports for the first time changes in articular cartilage in the knee joints of spinal cord injured (SCI) patients. In the previous chapter, we detected differences between knee cartilage thickness of healthy volunteers and spinal cord injured patients. Significant difference in the patella and the medial tibia were found between the healthy volunteers and the SCI patients 6 months post-injury. These results were very interesting however with a longitudinal study changes over time within one patient can be measured and the reaction of cartilage between different joint compartments can be compared. This can be done with a much higher statistical significance. Therefore, a longitudinal design was selected and spinal cord injured subjects were measured at the beginning of their injury, 6 months and 12 months post injury. This study is important for the understanding of the response of articular cartilage to restricted motion and loading. Results are crucial for rehabilitation programs used in SCI patients but also for individuals who are immobilized after surgical procedures, and long-term illness.

Own scientific contribution:

- Selection of the best appropriate MRI protocol and research design
- Segmentation of the cartilage
- Statistical analysis and interpretation of the data
ABSTRACT

OBJECTIVE: A previous cross sectional study has indicated that patellar and tibial cartilage morphology are subject to change after spinal cord injury (SCI). Here we report, for the first time, a longitudinal analysis of cartilage atrophy in all knee compartments, including the femoral condyles, in SCI patients over 12 months.

METHODS: The right knees of 9 patients with complete, traumatic SCI were examined shortly after the injury (9 ± 4 weeks), at 6, and at 12 months post-injury. Three-dimensional morphology of the patellar, tibial, and femoral cartilage (mean and maximum thickness, volume, and surface area) was determined from coronal and transversal magnetic resonance images (fat-suppressed gradient echo) using validated post processing techniques.

RESULTS: The mean thickness of knee joint cartilages significantly decreased during the first six months after injury (range 5% - 7%, p< 0.05). Changes at 12 months amounted to 9 %, in the patella, 11 %, in the medial tibia, 11 % in the medial femoral condyle, 13 % in the lateral tibia and 10 % in the lateral femoral condyle (p < 0.05 for all compartments).

CONCLUSION: These data show that human cartilage atrophies in the absence of normal joint loading and movement after SCI, with a rate of change that is higher than that observed in osteoarthritis (OA). As a potential clinical implication, cartilage thinning after SCI may affect the stress distribution in the joint and render it vulnerable to OA. Future studies will have to show whether cartilage thinning can be prevented using specific exercise protocols and rehabilitation programs.
5.1 Introduction

The morphological and mechanical integrity of articular cartilage is a prerequisite for appropriate function of diarthrodial joints. As suggested by various animal studies, a certain amount of mechanical loading and movement of the joint is required, to maintain normal cartilage morphology, biochemical composition and biomechanical properties (Vanwanseele et al., 2002b). However, results have been highly variable and inconsistent between studies. Jurvelin et al (Jurvelin et al., 1986), for instance, observed a decrease in cartilage thickness of 9% in the canine knee after 11 weeks of rigid immobilization. Haapala et al (Haapala et al., 1999) reported a high decrease (approximately 20%) in the dogs’ medial femur, but did not find any changes in the lateral tibia and lateral femur (Haapala et al., 2000). Leroux et al. (Leroux et al., 2001), in contrast, found that a shorter period of non-rigid immobilization (4 weeks) did not change the knee cartilage thickness in dogs.

Spinal cord injury (SCI) causes unloading and restricted movement of the lower limb joints for substantial periods of time. SCI patients consequently encounter secondary complications, such as a decrease in muscle mass, cardiovascular fitness, and bone density (Kocina, 1997, Whiteneck et al., 1992). One study reported radiographic hip joint space narrowing by more than 50% in 25 of 200 patients with flaccid paralysis (Pool, 1974) and another study (Richardson et al., 1984) observed overgrowth of the epiphyses, periarticular osteoporosis and joint space narrowing in patients with neuromuscular disorders. Recently, it has been shown that 3D magnetic resonance imaging (MRI), combined with state-of-the-art post-processing, can provide accurate and highly reproducible data on cartilage morphology in health and disease in vivo (Stammberger et al., 1999b, Burgkart et al., 2001).

In a recent cross-sectional study, we have shown that there exist significant differences in patellar and tibial cartilage thickness of SCI patients compared with age matched healthy volunteers (Vanwanseele et al., 2002a).

The objective of the current study was to longitudinally assess the magnitude and rate of morphological cartilage changes in all knee compartments, including the femoral condyles, during unloading and immobilization after SCI.
5.2 Materials and Methods

5.2.1 Subjects
We examined the right knees of 9 male patients with traumatic, and complete SCI (mean ± SD, age 47 ± 18 years, range 17 –65 years). The first measurement was obtained as soon as possible after the injury (9 ± 4 weeks). The patients were measured again at 6 and 12 months after injury. Seven patients were paraplegic (range Th12-Th4) and 2 were tetraplegic (range C4-C6). Patients with a history of knee pain, knee trauma, or knee surgery prior to SCI were excluded from the study. One patient developed clear signs of osteoarthritis, as observed on the MR images, during the study and was therefore excluded from the statistical analysis. During the 12 months, all patients received a standardized therapy program at the Swiss Paraplegic Center, including passive range of motion exercises, aqua therapy, and cardiovascular training (arm crank ergometer training). This program did not differ considerably between patients. All participants signed a statement of informed consent as approved by the Ethical Committee of the canton Lucerne, Switzerland, after receiving oral and written information.

5.2.2 Imaging and imaging processing
The patients were examined with a 1.5 T MRI scanner (Magnetom Symphony; Siemens, Erlangen, Germany), and a circular polarized (CP) transmit-receive extremity coil. Transverse and coronal images were obtained using a validated fat-suppressed gradient echo sequence (fast low angle shot; repetition time = 53 ms, echo time = 10.3 ms, flip angle = 30 deg, spatial resolution 0.31 x 0.31 x 1.5mm, matrix 512x512) as described previously (Vanwanseele et al., 2002b). These orientations were chosen because they involve the smaller precision errors than sagittal scans (Burgkart et al., 2001). In the patella, precision errors of only 1.0% (root mean square coefficient of variation) have been reported with this technique, whereas precision errors in the tibia and femoral condyles range from 2.5 to 3.2 %. Semi-automatic segmentation was performed by a single person (B.V.) on a graphics computer (Octane Duo, Silicon Graphics Inc., CA) on a section-by-section basis. After manual initialization of a contour around the cartilage, a B-spline Snake algorithm was
employed to attract the contour to the cartilage boundaries. The performance of the algorithm was then checked visually by the observer (B.V.) and corrected manually, if required. Finally, the cartilage volume, mean and maximal thickness, and size of the joint and the cartilage-bone interface area were computed as described previously (Burgkart et al., 2001, Stammberger et al., 1999b).

Figure 5-1: Transverse MR image (top) and coronal MR image of the knee joint of a spinal cord injured patient. Fat suppressed FLASH sequence (TR/TE 53/10 msec, flip angle 30 deg) with a resolution of 0.31 mm x 0.31 mm x 1.5 mm (matrix = 512 x 512 pixels, field of view = 160 cm).

5.2.3 Statistical analysis

A Wilcoxon Paired-Samples Test was used to assess statistical significance of the differences between baseline and follow up measurements in the patients. A significance level of 0.05 was chosen unless stated otherwise. The same test was used to compare the rate of change, as a percentage of the initial value between joint surfaces, at 12 months.

5.3 Results

The total knee cartilage volume (patella, medial and lateral tibia, and medial and lateral femur) was reduced by 7% at six months post injury and by 10 % at twelve months post injury compared to the baseline measurements.
The magnitude (and rate) of change in mean and maximal cartilage thickness for the patella, medial tibia, lateral tibia, medial femoral condyle, and lateral femoral condyle are displayed in Table 5-1 and Figure 5-2. At 6 months post SCI, changes in mean cartilage thickness ranged from 5% in the patella to 7% in the medial and lateral tibia. The change was significant in all compartments, but there was no significant difference in the rate of change between the five cartilage plates.

At 12 months post injury, changes in mean cartilage thickness ranged from 9% in the patella to 13% in the lateral tibia. Again, the changes were significant in all compartments (p < 0.05). As for 6 months, the relative rate of thinning in the 5 compartments did not differ significantly (Figure 5-2). When comparing the relationship between the age of the patients and the magnitude (rate) of change at 12 months in the various cartilage plates, no significant association was observed.

The size of the bone-cartilage interface or the articular surface showed no significant differences between baseline and follow up. The changes in
cartilage volume thus followed a similar pattern to those of the mean cartilage thickness (Table 5-1).

Table 5-1: Cartilage morphology in the patella, medial and lateral tibia, medial and lateral femur condyles of spinal cord injured subjects (n=8) as soon as possible after injury, 6 and 12 months post-injury. Data are presented as mean ± SD; thickness (mm), volume (ml) and joint surface area (cm²).

<table>
<thead>
<tr>
<th></th>
<th>Max Thickness</th>
<th>Mean Thick</th>
<th>Volume</th>
<th>Surface Area</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patella</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 months</td>
<td>5.04 ± 0.70</td>
<td>2.53 ± 0.32</td>
<td>4.11 ± 0.73</td>
<td>13.43 ± 1.43</td>
</tr>
<tr>
<td>6 months</td>
<td>4.76 ± 0.67 (*)</td>
<td>2.40 ± 0.37 (*)</td>
<td>3.87 ± 0.68 (*)</td>
<td>13.50 ± 1.37 (*)</td>
</tr>
<tr>
<td>12 months</td>
<td>4.72 ± 0.78 (*)</td>
<td>2.30 ± 0.32 (*)</td>
<td>3.68 ± 0.64 (*)</td>
<td>13.26 ± 1.45 (*)</td>
</tr>
<tr>
<td><strong>Medial Tibia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 months</td>
<td>3.33 ± 0.44</td>
<td>1.61 ± 0.22</td>
<td>2.25 ± 0.68</td>
<td>11.58 ± 2.67</td>
</tr>
<tr>
<td>6 months</td>
<td>3.16 ± 0.46 (*)</td>
<td>1.50 ± 0.25 (*)</td>
<td>2.06 ± 0.59 (*)</td>
<td>11.40 ± 2.61 (*)</td>
</tr>
<tr>
<td>12 months</td>
<td>3.27 ± 0.54 (*)</td>
<td>1.44 ± 0.23 (*)</td>
<td>2.06 ± 0.60 (*)</td>
<td>11.62 ± 2.76 (*)</td>
</tr>
<tr>
<td><strong>Lateral Tibia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 months</td>
<td>4.48 ± 0.56</td>
<td>2.28 ± 0.27</td>
<td>2.74 ± 0.63</td>
<td>10.55 ± 1.67</td>
</tr>
<tr>
<td>6 months</td>
<td>4.26 ± 0.58</td>
<td>2.11 ± 0.25 (*)</td>
<td>2.45 ± 0.78 (*)</td>
<td>10.39 ± 1.79 (*)</td>
</tr>
<tr>
<td>12 months</td>
<td>4.35 ± 0.57 (*)</td>
<td>1.98 ± 0.28 (*)</td>
<td>2.40 ± 0.63 (*)</td>
<td>10.40 ± 1.83 (*)</td>
</tr>
<tr>
<td><strong>Medial Femur</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 months</td>
<td>3.14 ± 0.39</td>
<td>1.80 ± 0.29</td>
<td>0.99 ± 0.20</td>
<td>3.98 ± 0.68</td>
</tr>
<tr>
<td>6 months</td>
<td>2.97 ± 0.47</td>
<td>1.68 ± 0.30</td>
<td>0.94 ± 0.20</td>
<td>4.06 ± 0.80</td>
</tr>
<tr>
<td>12 months</td>
<td>2.81 ± 0.48 (*)</td>
<td>1.59 ± 0.25 (*)</td>
<td>0.87 ± 0.16 (*)</td>
<td>3.94 ± 0.69 (*)</td>
</tr>
<tr>
<td><strong>Lateral Femur</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 months</td>
<td>2.70 ± 0.29</td>
<td>1.60 ± 0.15</td>
<td>1.04 ± 0.24</td>
<td>4.81 ± 1.04</td>
</tr>
<tr>
<td>6 months</td>
<td>2.55 ± 0.27</td>
<td>1.50 ± 0.17 (*)</td>
<td>0.96 ± 0.20 (*)</td>
<td>4.71 ± 1.07 (*)</td>
</tr>
<tr>
<td>12 months</td>
<td>2.46 ± 0.33 (*)</td>
<td>1.41 ± 0.15 (*)</td>
<td>0.91 ± 0.18 (*)</td>
<td>4.66 ± 0.98 (*)</td>
</tr>
</tbody>
</table>

* p < 0.05 vs. 0 months with Wilcoxon Paired-Samples Test.
5.4 Discussion

In this study we have analyzed the magnitude and rate of cartilage thinning during restricted motion and unloading of the knee, using a longitudinal study design. Although we have previously reported significant differences in cartilage thickness in patient groups at different time intervals after SCI (Vanwanseele et al., 2002b), this cross sectional study did not allow us to reliably determine the magnitude and rate of changes of cartilage morphology, due to the high intersubject variability in morphological cartilage properties (Eckstein et al., 2001c, Hudelmaier et al., 2001). Moreover, the current study also explores changes in the femoral condyles, which have not been previously studied (Vanwanseele et al., 2002b), although animal studies have indicated that changes during immobilization may differ between different cartilage plates and compartments of the knee (Jurvelin et al., 1986, Haapala et al., 1999, Haapala et al., 2000, Leroux et al., 2001).

The changes reported here amounted to 5 - 7% after six months and to 9 - 13% after twelve months of immobilization. These annual changes exceed those during normal aging (Hudelmaier et al., 2001), which amount to approx 0.4% in most knee joint cartilage plates. The changes observed after SCI thus exceed changes during normal aging by a factor of > 20:1. The changes after SCI also exceed those reported during osteoarthritis in groups of patients with Kellgren-Lawrence grades 1 to 3 by a factor of approx. 2:1 (approx. 5% per annum (Hudelmaier et al., 2001)). It is interesting to note that restriction in movement and loading appears to have more severe effects on the maintenance of cartilage volume and thickness of previously healthy cartilage than those observed during progression of advanced OA. In this context it is also of relevance that all patients received a standardized therapy program, including passive range of motion exercise, aqua therapy, and cardiovascular training. It is possible that atrophic cartilage changes may have even been higher, without these therapeutic efforts.

We found relatively uniform changes in all knee compartments, with a trend towards lower rates in the patella, and towards higher rates in the lateral tibia, but no significant differences between cartilage plates. Whether this represents
a random finding due to the small sample size or is, for instance, due to a more flexed position of the knee after SCI is currently unclear. At any rate, this finding is in contrast with the observations of animal studies by Haapala et al, who showed only changes in medial tibia and medial femur (Haapala et al., 1999) and no changes in cartilage thickness in the lateral tibia and femur (Haapala et al., 2000) after rigid immobilization for 11 weeks. In these animal studies, however, thickness measurements were derived from histological sections at only a few points. In contrast, the technique applied here permits one to analyze the cartilage thickness throughout the entire joint surfaces, taking into account out-of-plane deviations of the minimal distance between the articular surface and bone cartilage interface (Stammberger et al., 1999b, Burgkart et al., 2001). The changes after SCI, as observed in this study, also clearly exceed the precision errors associated with the technique applied, which range from 1% (patella) to approx 3% in femorotibial cartilage plates.

Given the relatively large change in cartilage morphology during the first year after SCI, it is likely that substantial biochemical, structural, and mechanical changes are associated with these changes, but these are currently more difficult to reliably measure in vivo. Other open questions are, whether morphological changes continue at the same rate after one year, and to what extent the changes can be slowed down or stopped by therapeutic action, such as mobilization and physical rehabilitation programs.

The current findings also have some clinical implications. If the patients are immobilized for substantial periods of time, thinning of the cartilage may cause an alteration of the congruity of the femorotibial joint surfaces and thus cause changes in the stress distribution throughout the joint. This may render the joint vulnerable to osteoarthritic degeneration. Therefore, it may be important to initiate therapeutic programs as soon as possible, so that cartilage atrophy is prevented at the earliest possible time point. Several types of remobilization programs are available after SCI, such as functional electro-stimulated cycling or treadmill training using robotic orthosis (Colombo et al., 2000). It will thus be a challenging issue for future studies, to objectively evaluate which specific programs are most beneficial for maintaining normal morphological cartilage properties. Such exercise programs will also be important in the context of
postoperative immobilization and long term space flight, in which equal measures may have to be taken to avoid cartilage atrophy, loss of mechanical competence, and associated joint changes.

Acknowledgments:
The authors thank all subjects for participating in this study and acknowledges the contribution of Dr. Phil Jungen (Swiss Paraplegic Centre, Notwill, Switzerland) in data collection. We thank Dr. Christian Glaser and Prof. Dr. Maximilian Reiser (Institute of Clinical Radiology, LMU München, Germany), and PD Dr. Karl-Hans Englmeier (Medis Institute, GSF National Research Center, Neuherberg, Germany) for technical advice, and for allowing us to use the software for quantitative cartilage analysis. This work was supported by the Swiss Paraplegic Foundation, Switzerland.
Chapter 6

In Vivo Precision of Quantitative Shoulder Cartilage Measurements and Changes After Spinal Cord Injury

Reprinted from:
**Background**

This paper is an important study to understand the response of articular cartilage to joint disuse and overuse in a human model. In previous chapter, the response of articular cartilage to restricted motion and loading was examined. A thinning of the cartilage during a period of one year after SCI was measured. In contrast to the lower extremities of these patients, the upper extremities are loaded more frequently and with higher load. So it is possible to measure the reaction of articular cartilage to joint over/disuse in the same subjects. However measurements of the humeral head cartilage imposes higher requirements to the MRI protocol compared to the knee cartilage. The humeral head cartilage is much thinner, and has a totally different geometry compared to the knee joint. These are two factors, which influences the reproducibility of the quantitative MRI. The accuracy of quantitative shoulder MRI was recently determined but no reproducibility study has been done until now.

**Own scientific contributions**

- Adaptation and selection of MRI protocol and research design
- Segmentation the cartilage
- Statistical analysis and interpretation of the data
ABSTRACT

Recent advances in MRI have allowed for quantitative assessment of articular cartilage morphology in human joints. In this study, we test the hypothesis that the precision of quantitative shoulder cartilage measurements is sufficient to detect changes between and within patients, and that shoulder cartilage thickness in paraplegic patients increases due to increased loading. The shoulders of 7 healthy volunteers were imaged four times with a coronal 3D fat-suppressed gradient echo sequence. Humeral head cartilage of 7 paraplegic patients was evaluated early after injury, and one year post-injury. A precision of 4.5% (RMS average CV%) of shoulder cartilage thickness measurements was measured. Whereas a significant decrease of cartilage thickness (-11%, p < 0.05) was observed in the knee, there was no significant change of articular cartilage thickness in the shoulder (-1.1%). Our data show, for the first time, that articular cartilage of the humeral head can be quantified with reasonable precision in vivo. We demonstrate that, contrary to the knee, articular cartilage morphology of the humeral head is subject to little change with no significant in- or decrease after spinal cord injury.
6.1 Introduction

Mechanical loading has been shown to strongly influence the development, maintenance, and aging of muscle and bone tissue (Carter and Wong, 1988, Huiskes et al., 2000), but little is known about the functional adaptation of articular cartilage to mechanical stimuli. It has been speculated that intermittent hydrostatic pressure promotes cartilage biosynthesis and maintains its structural and functional competence, and that shear stresses, prolonged static loading or absence of loading encourage cartilage destruction and ossification (Buckwalter, 1995). However, there has been little evidence that these processes shape the morphology of articular cartilage on a macroscopic scale. An understanding of the relationships between joint use/disuse and cartilage adaptation represents a critical step in the process of developing strategies to prevent and treat cartilage disorders in patients that encounter substantial modifications of day-to-day loading, such as paraplegic patients.

Animal studies have suggested that non-strenuous physical training improves the biomechanical properties and the thickness of young canine knee articular cartilage (Jurvelin et al., 1986; Kiviranta et al., 1988). Strenuous training, in contrast, returned cartilage biomechanical properties and thickness back to the level of control animals, or even lower (Jurvelin et al., 1990). It is however currently unknown to what extent mechanical loading influences articular cartilage properties and morphology in humans, and specifically in patients with spinal cord injury (SCI). A recent study reported that there are no differences between the knee articular cartilage thickness of triathletes and physically inactive volunteers (Eckstein et al., 2001a), but we were recently able to show that cartilage thinning occurs in the knee cartilage of patients after SCI (Vanwanseele et al., 2002a, Vanwanseele et al., submitted).

In paraplegic patients, the joints of the upper extremity are loaded more heavily and more frequently during daily activities, such as body transfer and ambulation. As a consequence, the shoulder is frequently subject to overuse injury and pain (Gellman et al., 1988, Sie et al., 1992, Silfverskiold and Waters, 1991). Silfverskiold et al. found that 78% of the tetraplegic patients and 35% of the paraplegics experienced pain during the first six months after SCI.
manual wheelchair propulsion, the average compressive joint load was estimated to range from 500N to 850N. Furthermore, the peak glenohumeral contact forces during push phase can rise up to 1400N (Veeger et al., 2002) and contact forces in SCI patients can be up to 2.5 times the body weight (Anglin et al., 2000) during normal daily activities.

Recent advances in magnetic resonance imaging (MRI) have allowed direct delineation of shoulder pathology, and two studies have examined MRI abnormalities in patients with paraplegia qualitatively (Escobedo et al., 1997, Boninger et al., 2001). The authors reported a high prevalence of abnormalities in individuals with paraplegia, including rotator cuff tears and osteolysis of the distal clavicle. However, to date no specific and particularly no quantitative information is available on cartilage morphology changes in these patients, either as expression of functional adaptation or degeneration.

High resolution, fat-suppressed gradient echo sequence and advanced image post processing software have been shown to permit quantitative assessment of knee joint cartilage with high accuracy and reproducibility (Eckstein et al., 1998a, Eckstein et al., 2002, Stammberger et al., 1999a). Validation has also been performed in the human elbow (Graichen et al., 2000) and shoulder joint (Graichen et al., 2003), and a satisfactory in vivo precision has been reported in the human hind foot (Al-Ali et al., 2002). However, no studies have so far dealt with the in vivo precision (immediate test-retest reproducibility) of quantitative analysis of shoulder cartilage, which displays substantially lower cartilage thickness than the knee. Also, no previous study has investigated whether changes of cartilage morphology occur in the shoulder following SCI. The specific objectives of this study were thus:

1) to determine the precision of MR based cartilage volume, thickness, and surface area measurements of the humeral head.

2) to investigate articular cartilage changes in the humeral head after SCI.
6.2 Patients and Methods

6.2.1 Volunteers and patients
To analyze the precision of the measurements, the right shoulder of 7 healthy volunteers (age 28 ± 2.5 years, range 26 – 33) was examined. None of the volunteers reported musculoskeletal disease or internal disorders of the shoulder. We then examined the shoulder and the knee (Vanwanseele et al., submitted) of seven patients (age 45 ± 18, range 18 - 66) with traumatic and complete SCI. All individuals were paraplegics (range Th4- Th12). Patients with a history of shoulder pain, trauma, or surgery prior to the injury were excluded from the study. All participants signed a statement of informed consent that had been approved by the Ethical Committee of Lucerne, after receiving oral and written information.

6.2.2 MR imaging and processing
MR imaging was with a 1.5 T magnet (Magnetom Vision, Siemens, Erlangen, Germany) and a shoulder array coil. A previously validated (Graichen et al., 2003) fat-suppressed gradient echo sequence (FLASH = fast low angle shot; repetition time = 44 msec, echo time = 10.3 msec, flip angle = 30 degrees) was used to acquire coronal data-sets of the gleno-humeral joint (Eckstein et al., 1998a, Stammberger et al., 1999a) (Figure 6-1). Images were obtained at an in plane resolution of 0.273 mm x 0.273 mm (matrix = 512 x 512 pixels, field of view = 140 cm), and at a slice thickness of 1.5 mm. The acquisition time of the measurement was 11min 40sec.
In the volunteers, four data sets from each subject were obtained, with repositioning of the shoulder and reshimming of the magnet in between replicated acquisitions. In the patients, one measurement (baseline) was obtained as soon as possible after injury (10 ± 3 weeks) and a second measurement (follow up) was performed one year after injury.

Knee images were obtained as described previously (Vanwanseele et al., 2002a, Vanwanseele et al., submitted). Segmentation of the cartilage was performed by one person (B.V.) on a section-by-section basis, using a B-spline Snake algorithm (Stammberger et al., 1999b). Humeral head and knee cartilage plates were reconstructed three-dimensionally, and the volume determined by numerical integration of the segmented voxels (Eckstein et al., 1996). The size of the joint surface areas and the cartilage-bone interface areas were computed after triangulation (Hohe et al., 2002), and the mean and
maximal cartilage thickness by three-dimensional Euclidean distance transformation, independent of the original section orientation (Stammberger et al., 1999a).

6.2.3 Statistics

As a measurement of immediate test-retest precision, the mean value, standard deviation (SD), and coefficient of variation (CV% = SD divided by the mean value x 100) of the four replicated was determined for the morphological parameters (maximal and mean thickness, volume and surface area). As suggested by Glüer et al. (Glüer CC et al., 1995), the root mean square (RMS) SD and CV% were used to determine the average SD and CV% of the quantitative computations in all 7 subjects.

To assess the statistical significance of differences between baseline and follow up measurements in the patients, a Wilcoxon Paired-Samples Test was used. A Mann Whitney U-test was used to compare data in patients to those in healthy volunteers. The required significance level was set to p < 0.05 for both tests.

6.3 Results

The mean value, SDs and CV% of repeated maximal and mean thickness, volume and surface area for each of the 7 subjects are shown in Table 6-1. The surface area had the smallest precision error (range 1.0% to 2.3%) whereas the maximal cartilage thickness displayed the largest precision error (range 2.2% to 9.7%) amongst parameters. The RMS CV% of mean cartilage thickness was 4.5%.
<table>
<thead>
<tr>
<th>Subject</th>
<th>Mean</th>
<th>SD</th>
<th>CV(%)</th>
<th>Mean</th>
<th>SD</th>
<th>CV(%)</th>
<th>Volume (ml)</th>
<th>Surface area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject1</td>
<td>2.783</td>
<td>0.158</td>
<td>5.681</td>
<td>1.433</td>
<td>0.029</td>
<td>2.026</td>
<td>4.736</td>
<td>26.077</td>
</tr>
<tr>
<td>Subject2</td>
<td>2.464</td>
<td>0.158</td>
<td>6.403</td>
<td>1.277</td>
<td>0.060</td>
<td>4.716</td>
<td>3.845</td>
<td>22.230</td>
</tr>
<tr>
<td>Subject3</td>
<td>3.368</td>
<td>0.287</td>
<td>8.515</td>
<td>1.889</td>
<td>0.084</td>
<td>4.466</td>
<td>6.473</td>
<td>27.032</td>
</tr>
<tr>
<td>Subject4</td>
<td>2.092</td>
<td>0.126</td>
<td>6.044</td>
<td>1.165</td>
<td>0.067</td>
<td>5.758</td>
<td>3.711</td>
<td>24.557</td>
</tr>
<tr>
<td>Subject5</td>
<td>2.170</td>
<td>0.048</td>
<td>2.204</td>
<td>1.177</td>
<td>0.032</td>
<td>2.704</td>
<td>3.668</td>
<td>24.145</td>
</tr>
<tr>
<td>Subject6</td>
<td>2.486</td>
<td>0.105</td>
<td>4.230</td>
<td>1.187</td>
<td>0.058</td>
<td>4.924</td>
<td>3.930</td>
<td>25.670</td>
</tr>
<tr>
<td>Subject7</td>
<td>1.874</td>
<td>0.181</td>
<td>9.667</td>
<td>0.937</td>
<td>0.053</td>
<td>5.610</td>
<td>3.062</td>
<td>24.502</td>
</tr>
<tr>
<td>Total</td>
<td>2.463</td>
<td>0.167</td>
<td>6.530</td>
<td>1.295</td>
<td>0.058</td>
<td>4.511</td>
<td>5.337</td>
<td>24.887</td>
</tr>
</tbody>
</table>

In the volunteers, the mean cartilage thickness of the humeral head was 1.29 ± 0.30 mm, the maximal cartilage thickness 2.46 ± 0.50 mm, the cartilage volume 4.20 ± 1.12 ml, and the surface area 24.9 ± 1.55 cm² (Table 6-2). When relating the inter-subject variability (SD in the 7 healthy volunteers – Table 6-2) to the technical precision (RMS SD of repeated measurements – Table 6.1), the ratios were 6.1:1 and 4.0:1 for the cartilage volume and surface...
area, and 5.2:1 and 3.0:1 for the mean and maximal cartilage thickness, respectively.

Table 6-2: Cartilage morphology in the humeral head of spinal cord injured subjects 3 months and 12 months post-injury. Average Values and Standard Deviation for Mean and Maximal Thickness, Area of Joint Surface and Bone-Cartilage Interface.

<table>
<thead>
<tr>
<th></th>
<th>Healthy volunteers (n=7)</th>
<th>3 months post-injury (n=7)</th>
<th>12 months post injury (n=7)</th>
<th>Percent difference and level of significance between 3 and 12 months post injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean thickness (mm)</td>
<td>1.29 ± 0.30</td>
<td>1.00 ± 0.12</td>
<td>0.991 ± 0.13</td>
<td>-1% ( p = 0.3)</td>
</tr>
<tr>
<td>Maximal thickness (mm)</td>
<td>2.46 ± 0.50</td>
<td>2.02 ± 0.30</td>
<td>1.854 ± 0.25</td>
<td>- 8% ( p = 0.04)</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>4.20 ± 1.12</td>
<td>2.60 ± 0.36</td>
<td>2.45 ± 0.27</td>
<td>- 6% ( p = 0.1)</td>
</tr>
<tr>
<td>Joint Surface Area (cm²)</td>
<td>24.89 ± 1.55</td>
<td>19.23 ± 2.44</td>
<td>18.84 ± 2.44</td>
<td>- 2% ( p = 0.5)</td>
</tr>
</tbody>
</table>

In a cross-sectional comparison, the patient cohort displayed significantly lower cartilage volume, thickness, and surface areas than the healthy volunteers at baseline (Table 6-2). However, no significant differences were observed in shoulder cartilage morphology of the patients at 3 and 12 months post injury (Figure 6-2 and Table 6-2), except for the maximal cartilage thickness (-7.99%). Specifically, the change in mean cartilage thickness was only 1.1% at the humeral head, whereas the mean cartilage thickness decreased by -11% (p < 0.05) at the knee (Figure 6-3).
Figure 6-2: Shoulder cartilage morphology of healthy volunteers, spinal cord injured subjects 3 months and 12 months post injury. Average Values and Standard Deviation for Mean and Maximal Thickness and Volume.

Figure 6-3: Direct comparison of changes between 3 and 12 months of injury in the shoulder and knee joint cartilage thickness in the 7 patients with spinal cord injury; values are give as percentage of the initial (baseline) value.
6.4 Discussion

In this study we have examined the in vivo precision of quantitative cartilage measurements in the human shoulder and have analyzed changes in the articular cartilage morphology of the humeral head in SCI patients between 3 and 12 months after injury. We find that the technique displays sufficient reproducibility to quantify differences between subjects and within subjects over time. Our data show, for the first time, that, opposite to the knee, almost no changes in shoulder cartilage thickness occur during the first year after SC injury.

The in vivo precision of cartilage surface area, volume, mean and maximal thickness ranged from 1.5 to 6.5 % (CV%) and was 4.5% for the mean cartilage thickness. These precision errors are higher than those in the knee (Eckstein et al., 2002), but smaller than those in the hind foot (Al-Ali et al., 2002). The standard deviation of repeated measurements for mean cartilage thickness was 0.6 mm. This value is several times smaller than the inter-subject variability.

Quantitative imaging of thin cartilage puts high demands on the spatial resolution. On the other hand, in vivo imaging demands a short acquisition time, to avoid movement artifacts. Therefore, we have chosen a resolution of 0.273 mm x 0.273 mm x 1.5 mm, resulting in an acquisition time of 12 minutes. Because the head of the humerus is shaped like a sphere, it is difficult to circumvent the partial volume effects at the periphery. To minimize these effects, a coronal section orientation was selected, and the first and last two image slices with visible humeral head cartilage were omitted from the analysis. A recent study (Graichen et al., 2003) has shown that, in cadavers, qMRI based cartilage volume and thickness measurements of the humeral head are in close agreement to values obtained by surgical resection of cartilage (Archimedes principle) and A-mode ultrasound, and this study has thus established the accuracy of these measurements.

The somewhat lower precision of the humeral head cartilage analysis compared with the knee is not surprising, because of the thinner cartilage and the higher degree of curvature in the shoulder. Additionally, the humeral
cartilage is situated very closely to other shoulder structures with similar MR signal intensity, such as muscles and ligaments. Taking into account that the mean cartilage thickness is less than 1.5 mm, the precision is, however, highly satisfactory.

The cartilage thickness values in the healthy volunteers (1.29 and 2.46 mm for mean and maximal thickness, respectively) are very similar to the values reported in the study by Soslowsky et al. (1.44 and 2.13 mm, respectively), which was performed using stereophotogrammetry (Soslowsky et al., 1992). The size of surface areas observed in our study are substantially higher than those reported by Soslowsky et al (24.9 vs. 17 cm²), but Soslowsky et al. (Soslowsky et al., 1992) only used one paired view of digitized photographs and therefore likely did not cover the entire humeral cartilage plate with their measurements.

It has been well documented that mechanical loading of upper limb joints is altered substantially after SCI (Veeger et al., 2002, Anglin et al., 2000). Also, SCI patients display symptoms and signs of mechanical overuse, such as rotator cuff tears, osteophytes, and secondary degenerative arthritis (Bayley et al., 1987, Boninger et al., 2001, Gellman et al., 1988, Sie et al., 1992, Wylie and Chakera, 1988). Previous animal studies (Jurvelin et al., 1986, Jurvelin et al., 1990, Kiviranta et al., 1988) have shown that articular cartilage thickness increases with moderate exercises, but comparative studies in triathletes (Eckstein et al., 2001a) found no significant difference in cartilage morphology in comparison with sedentary subjects in humans. We have previously reported significant thinning of the cartilage of all knee joint compartments after SCI (Vanwanseele et al., submitted), and this was interpreted as response to the reduction in mechanical loading of this joint after the accident (Vanwanseele et al., submitted). The current study suggests that changes are much smaller (if occurring at all) in the shoulder joint, which is not deprived of mechanical usage, but, on the contrary, is used more heavily than before in paraplegic patients. However, we found no indication of an increase in cartilage thickness due to higher mechanical use, as has been indicated in some animals studies (Jurvelin et al., 1986, Kiviranta et al., 1988).
For medical and ethical reasons the patients could, unfortunately, only be examined 3 months, but not immediately after injury. Patients had to be stable in terms of cardiovascular and psychological status. We observed significant differences in cartilage thickness and volume between the group of healthy volunteers and the patients, but, given the modest sample size, this is more likely a cohort effect than an effect of the injury. Note that the patients also displayed much smaller joint surface areas than the volunteers and that differences in cartilage morphology may thus be due to differences in body constitution. Moreover, our recent longitudinal study (Vanwanseele et al., 2002a) in the knee has shown that changes within the first 6 months after injury are not larger compared to those occurring beyond 6 months after injury. We therefore believe that the conclusion of the present study is not affected by the circumstance that the first measurement could only be obtained at 3 months after (rather than immediately after) injury.

In summary, we have shown that, using a high-resolution fat-suppressed gradient echo MRI sequence and state-of-the-art post processing tools, articular cartilage of the humeral head can be quantified with reasonable precision under in vivo imaging conditions. Opposite to the knee, only little change of cartilage thickness is observed within one year post injury of paraplegic patients. This implies that, at least for the first year after injury, cartilage morphology in the shoulder remains unaltered in paraplegic patients, despite substantial changes in mechanical loading of the upper extremity.

Acknowledgment:
The author thanks all subjects for participating in this study and acknowledge the contribution of Dr. Phil Jungen (Swiss Paraplegic Centre, Notwill, Switzerland) in data collection. We thank Dr. Christian Glaser and Prof. Dr. Maximilian Reiser (Institute of Clinical Radiology, LMU München, Germany), and PD Dr. Karl-Hans Englmeier (Medis Institute, GSF National Research Center, Neuherberg, Germany) for allowing us to use the software for quantitative cartilage analysis. This work was supported by the Swiss Paraplegic Foundation, Switzerland.
Chapter 7
Quantitative Analysis of in vivo Cartilage Deformation in the Patellofemoral Joint under Static Load: Preliminary Results
Background

This paper addresses the question if it is possible to measure mechanical properties of articular cartilage in vivo in human. The idea is to measure cartilage deformation during one hour of static loading. Three-dimensional MRI gives us the opportunities to measure cartilage with a good spatial resolution, good signal-to-noise ratio and good contrast-to-noise ratio in intact joints in human. The newly developed segmentation method, which combines rigid registration of the patellar bone with semi-automatic segmentation, allows us to detect local differences in cartilage thickness. In a later project we want to use these deformation data as an input for finite-element simulations and so calculate material properties of articular cartilage.

Own scientific contribution

- Test new MRI protocol to decrease the acquisition time
- Try out different coils
- Design of the loading device
- Segmenting MRI
- Analysis and interpretation of the data
ABSTRACT

OBJECTIVE: Altered mechanical behavior of articular cartilage is an important problem in joint disease. In the process of cartilage degeneration, the structural components undergo progressive changes resulting in deteriorated mechanical properties of the tissue. Mechanical properties have mostly been determined in confined or unconfined compression or indentation test. The situation in these in vitro tests is largely different compared to the conditions in the joints. Therefore it is important to analyze cartilage deformation under load in situ in living human. The aim of the study was to develop a device that statically compresses the human patello-femoral cartilage in vivo during at least one hour and concomitant determine the cartilage deformational behavior, by means of MRI.

METHOD: A custom built loading device applied mechanically an isometric load of 400N on the patella of one subject during 80 minutes. The subject’s ankle and hip were fixed to the device, the medial/lateral knee motion was restricted and the thigh and shank of the subjects were supported. Using a spoiled 3D gradient echo sequence with selective water excitation, the patellar and femoral cartilage was measured with an in-plane resolution of 0.312x0.312 mm² and 1.4 mm slice thickness. Morphological parameters (mean and maximal thickness) of the patellar and femoral cartilages were determined and thickness maps were generated.

RESULTS: We observed a mean cartilage deformation of 5% of the initial cartilage thickness. The maximal thickness was decreased with 2%. Differences in thickness maps clearly detected local differences in the contact area.

DISCUSSION: With this case study we could show that it is feasible to measure systematically articular cartilage deformation under static compression. Combined with finite-element analyses, these data may be used to compute the mechanical properties of the articular cartilage. And so give us insight in factors influencing development of joint diseases such as osteoarthritis or in effects of therapeutical programs.
7.1 Introduction

Articular cartilage distributes load over a wider area and provides a smooth, lubricated surface that facilitates movements with little friction and wear between the articulating surfaces. For this mechanical function, the structure and properties of healthy articular cartilage are optimal. Cartilage is a permeable, visco-elastic material, which consists of an organic matrix and free interstitial fluid. The organic matrix is composed by two macromolecules, collagen and proteoglycan. Collagens are proteins that form the fibrillar meshwork providing cartilage with its high tensile stiffness and strength. Glycosaminoglycans (GAG’s) attract water and repel each other due to their electronegativity, creating a swelling pressure that is restrained by the collagenous meshwork. GAG’s therefore function mainly to resist compressive forces. Using multiple linear regression, Treppo et al. (Treppo S et al., 2000) found that more than 80% of the variation in the compressive equilibrium modulus between joint surfaces could be accounted for by variations in biochemical properties such as water content, sulfated GAG/wet weight, and hydroxy-proline/wet weight.

Joint diseases are not only characterized by a change in cartilage morphology but also by alterations in mechanical properties of the articular cartilage (Buckwalter, 1995). In osteoarthritis (OA), the equilibrium compressive modulus of articular cartilage decreases and water content increases. In human OA, the compressive modulus decreases by 31 to 34% as the histological index increased to the most degenerated grade (Armstrong CG and VC, 1982, Maroudas A et al., 1985).
Figure 7-1: Three experimental set-ups to measure mechanical characteristics of articular cartilage. A) confined compression, B) unconfined compression and C) indentation.

Most investigations studying the mechanical properties of articular cartilage use explants or exposed articular cartilage surfaces. In these experiments, three different testing configurations have been used to quantify the mechanical response of articular cartilage: unconfined compression, confined compression and indentation. An unconfined compression experiment uses two platens to compress a circular plug of cartilage (Figure 7-1). The cylindrical specimen is not restrained radially, so displacement and fluid exudation occur radially as the tissue is compressed. This technique requires only simple boundary conditions to be applied when modeled analytically or numerically. Typically the upper and lower surfaces of the specimen are assumed to be in contact with frictionless, impermeable platens. A confined compression experiment uses a porous–permeable indenter to compress a cylindrical plug of articular cartilage, which is fitted inside an impermeable cylindrical sleeve to prevent radial displacement and radial exudation of fluid (Figure 7-1). Throughout the studies in the literature, by far the most commonly used experimental method to determine the in situ mechanical properties of cartilage is that of indentation. The popularity of this method is due to the relative ease of performing reproducible experiments. It does not require complex specimen preparation and allows the cartilage to be investigated while still attached to the supporting bone. The indenter is typically a plane-ended or hemispherically ended cylinder (Figure 7-1).

These experiments have provided important insights into the function of articular cartilage and have been a valuable basis for the theoretical characteristics of its mechanical behavior. In theory, using the right constitutive
law, one can calculate the mechanical characteristics of articular cartilage. Unfortunately, the use of different in vitro experiments can give different results for the same cartilage (Korhonen et al., 2002). The situation is further complicated by the fact that cartilage behavior in the joint shows considerable differences with its behavior in the in vitro experiments. Using cartilage specimens means that the anchoring of the collagen fibrils and the resistance against fluid redistribution is disrupted. The cartilage-cartilage contact can be simulated neither by porous nor by non-porous stamps. It is because of this that a large variation in mechanical parameters calculated through different experimental set-ups even using the same constitutive laws exists. Therefore, it is very interesting to look at the mechanical behavior of cartilage in living humans. This has been made possible by new developments in 3D Magnetic Resonance Imaging (MRI) technique. This imaging method combined with state-of-the-art post-processing, has been shown to provide accurate and highly reproducible data on cartilage morphology in vivo. Some studies used MRI to analyze cartilage deformation after physical activity (Eckstein et al., 2000a, Hudelmaier et al., 2001). However, they do not give a systematical analysis of cartilage deformation in vivo. Until now only one study investigated systematically, the in situ deformation in intact human cadaver joints (Herberhold et al., 1998, Herberhold et al., 1999) but no data are available in vivo.

The objective of the study was to develop a device to statically compress the human patello-femoral cartilage in vivo during one hour and determine its deformational behavior, by means of MRI.
7.2 Materials and Method

7.2.1 Subjects and Imaging
One healthy male adult (age: 28 yrs; height: 180 cm; weight: 72 kg) was measured in a 1.5T MRI scanner (Gyroscan Intera, Philips) using a C4 flexible coil. Institutional review board approval was obtained and the subject gave informed consent. Magnetic resonance images were acquired in the transversal plane with a spoiled 3D gradient echo sequence with selective water excitation (TR: 17 ms, TE: 6.6 ms, Flip angle: 20°, acquisition time 2.48 min). A 256x256 matrix was used with a field of view of 80 mm and a slice thickness of 1.4 mm (Figure 7-2).

![Figure 7-2: MR image of the femoro-patellar contact after 60 min of 400N loading.](image)

7.2.2 Loading
One subject was positioned supine in a custom built loading device (Figure 7-3). Ankle and hip were fixed to the device (Figure 7-3), the medial/lateral knee motion was restricted and the thigh and shank of the subjects were supported. Knee flexion of 30° can be individually achieved by lengthwise adjustment of the ankle fixation. An isometric load of 400 N was mechanically applied to the patella.
7.2.3 Segmentation and rigid registration
The patellar bone was automatically segmented on a slice-by-slice manner. The operator selected a region of interest (ROI) where a median filter and a binary threshold were applied. This patellar bone was then used as a region of interest for the rigid registration algorithm as described in Chapter 3. Slices with apparent patello-femoral contact completed with 4 extra slices were manually determined in the data sets after 80 min load and identified as the region of interest. The same slices were segmented at the different time points. A semi-automatic segmentation procedure was then performed on the reoriented MR images. The segmentation is based on medial representation model (M-REP), modified to incorporate additional information (Pirmog C et al., 2003). The initialization step of the cartilage is accomplished by projecting the M-REP model on the lower part of the segmented bone (the bone-cartilage interface) and is adjusted by performing a local search in directions normal to the medial grid. The operator has to correct possible inaccuracies. The patellar and femoral cartilage was only segmented in the slices in the region of interest by one single person.
7.2.4 Morphological parameters

The 3D mean cartilage thickness was computed using 3D minimal distance transformation. Cartilage volumes were determined by counting the voxels wholly inside the segmentation contour. The 3D thickness distribution of the cartilage was obtained by an automatic method developed by the author. Using the 2 corners and the segmented cartilage, bone-cartilage and the cartilage-joint contours were extracted in a slice-by-slice manner. For each voxel on the cartilage bone contours the 3D Euclidean distance was calculated to the cartilage joint contour. This thickness was then projected on the voxel. The difference maps were generated by orthogonal projection of the 3D thickness maps. The mean thickness of each point was subtracted between time points. The spatial difference between thickness distribution patterns could be directly visualized.

7.2.5 Contact area

The cartilage joint surfaces of the patella and the femur were selected and the minimal distance between both surfaces was calculated. If this distance was smaller than the voxels dimension (0.312 mm) this voxels was defined as contact.

7.3 Results

Fifty-two slices were segmented in this subject. During the compression experiment the patella was translated and rotated. After 80 min of static compression, translation in x, y and z direction was respectively −2.264, -9.461 and −1.91 mm. Rotation around the x, y and z axis was respectively −0.4039, -1.387 and 1.2922 degrees.
Figure 7-4: changes in mean thickness (mm) in patellar and femoral cartilage during 80 min static loading.

The analysis of the cartilage deformation in the selected contact slices showed a mean reduction of 5% of the initial thickness in the patella after 80 min of static loading (Figure 7-1). The patellar mean cartilage thickness decreased in an approximately exponential manner (Figure 7-5). The mean thickness in the lateral facet of the cartilage was decreased by 7%, while changes in the medial facet were only 2% (Table). The maximal articular cartilage was decreased by 10% in the lateral patella and by 6% in the medial patella (Table 7-1). In contrast to the patellar cartilage, the femoral cartilage did not show any changes in this subject (Figure 7-4). The size of the bone-cartilage surface showed no significant changes during the measurement. The changes in cartilage volume thus followed a similar pattern to those of the mean cartilage thickness.

Differences in projected thickness distributions before and during load are represented in Figure 7-6. Static loading of the patello-femoral joint deformed mainly regions of the lateral facet (Figure 7-6). These regions correspond with the contact area and not with the area of thickest cartilage.
Table 7-1: In situ thickness changes during loading with 400 N of subject 1. Mean and maximal cartilage thickness (mm) in medial and lateral patella.

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>18 min</th>
<th>31 min</th>
<th>46 min</th>
<th>60 min</th>
<th>80 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lat Patella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean thickness</td>
<td>2.91</td>
<td>2.83</td>
<td>2.75</td>
<td>2.74</td>
<td>2.73</td>
<td>2.70</td>
</tr>
<tr>
<td>Max thickness</td>
<td>4.67</td>
<td>4.44</td>
<td>4.28</td>
<td>4.31</td>
<td>4.17</td>
<td>4.17</td>
</tr>
<tr>
<td>Med Patella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean thickness</td>
<td>2.84</td>
<td>2.83</td>
<td>2.817</td>
<td>2.80</td>
<td>2.78</td>
<td>2.82</td>
</tr>
<tr>
<td>Max thickness</td>
<td>5.00</td>
<td>5.00</td>
<td>4.884</td>
<td>4.76</td>
<td>4.66</td>
<td>4.67</td>
</tr>
</tbody>
</table>

As illustrated in the Figure 7-5, the contact area did not change during the experiment.

Figure 7-5: Time dependent femoro-patellar change in one of the contact slice of the spoiled 3D gradient echo sequence with selective water excitation during 80 min of static loading.
7.4 Discussion

In this case study we investigated the feasibility of measuring systematically articular cartilage deformation \textit{in vivo} under static load. To obtain an adequate temporal resolution, we reduced the field of view to 80 mm. Using a matrix of 256x256 we obtained a resolution of 0.312x0.312 mm$^2$, giving us a reasonable spatial resolution, with high contrast-to-noise and signal-to-noise ratio. The reproducibility of this MRI protocol and the segmentation procedure developed at our lab was tested and found to be comparable to the reproducibility of the semi-automatic segmentation procedure described in the introduction (Chapter 2). We optimized the 3D MRI sequence so that the acquisition time could be reduced to 2min 45seconds. As a result of this short acquisition we could measure every 3 min, and no 2D images at the beginning of the compression were needed. Herberhold et al. showed that 7% of the final deformation
(0.069mm) occurs during the first minute; and about 10% (0.023mm) during the first 4 min. In our study, with much lower load and consequently smaller deformation, these changes during 3 minutes would not be detectable. (Herberhold et al., 1998, Herberhold et al., 1999).

The patellar bone was translated and rotated in all directions. As we are measuring living humans it is impossible to fix the patellar bone completely. One of the causes of the movement of the leg is the compression of soft tissue. However, in this study we compensated movement of the patellar bone in the image plane by a rigid registration algorithm, which allows us to identify corresponding points and therefore corresponding MRI slices.

We found a mean patellar cartilage thickness decrease of 5% after 80 min compression, which are smaller than those found by Herberhold (Herberhold et al., 1999). Differences are probably due to the fact that we applied lower load to the joint, measured in vivo and determined a 3D mean thickness. However, relating deformations with corresponding loads, our thickness changes are in the same range as those of Herberhold and coauthors. (Herberhold et al., 1999).

The observation that the patellar cartilage deformation is bigger compared to the one in the femoral cartilage is consistent with results of indentation test in animal studies (Froimson et al., 1997, Jurvelin et al., 1986) and with results of Herberhold et al (Herberhold et al., 1999). In contrast to our results Herberhold found deformation of femoral cartilage. Our applied load could be insufficient to deform significantly the femoral cartilage.

Analysis of the thickness maps demonstrates that cartilage deformations are highly location dependent and vary considerably throughout the joint. This is not surprising as the joint is highly incongruent and the contact between femur and patella occurs only over a small part of the surface.

If we compare the difference plots with contact area and projection map, it is clear that deformation mainly occurred in the contact area rather than in the region of the maximal thickness. This is in contrast with the previous results, where changes were found mainly in the area with the thickest cartilage.
(Herberhold et al., 1999). In our subject, it seems that the areas with the thickest cartilage were not in contact and consequently were not compressed. These preliminary results show the potential of this non-invasive technique to quantify systematically *in vivo* cartilage deformation under static compression. Data can be used to determine the mechanical parameters of human articular cartilage with an inverse Finite Element Method.

### 7.5 Outlook: improvement to the compression apparatus

This preliminary data, also when it is only with one subject, showed us that it is possible to measure systematically cartilage deformation under static compression in living humans. However, this study showed us also that some improvements to the compression apparatus are still necessary before starting measuring more subjects. The small deformation measured with our load, let us doubt if the effective load on the patellar bone is 400N. Due to friction, downwards movement of the patella it is possible that the load of 400N was not constantly applied to patella. Therefore, it would be a good idea to monitor during the experiment the load applied to the patella. Also a better fixation of the knee joint should be possible.
7.6 Summary and outlook

The research in this dissertation is characterized by the interplay between clinical studies, medical imaging analysis, mechanics and biology. The interaction with the physicians and patients combined with the results of the clinical studies showed us the importance of gaining new insights in this research field and the necessity of novel innovative methods to measure articular cartilage non-invasively in human. This dissertation reports for the first time quantitative data on the effects of restricted loading and motion on human articular cartilage morphology. The work also describes a novel method to detect and quantify small local changes in the articular cartilage morphology by means of magnetic resonance imaging. First attempts to measure systematically articular cartilage deformation during static loading are reported. Using this novel technique, insight in the mechanical properties of the articular cartilage and the adaptation of these properties to joint loading and unloading can be gathered.

Animal studies showed that articular cartilage characteristics change following loading situation alterations. In this dissertation, it was shown that articular cartilage morphology adapts to changes in mechanical loading, especially to a reduction in loading and movement, in human. Spinal cord injured subjects are very suitable subjects to study the influence of a restriction in loading and motion on the articular cartilage. To visualize soft tissue in a non-invasive manner, Magnetic Resonance Imaging (MRI) is an appropriate method, which allowed multiple measurements of the same patients at different time points. The reproducibility of this method for the thin cartilage of the humeral head was computed in the dissertation and was found to be 4.5 and 6.5% (CV %) for respectively, the mean and maximal thickness. A thinning of the articular cartilage during the first six months following the spinal cord injury was measured in all knee compartments (patella, medial and lateral tibia and, medial and lateral femur). This thinning gradually increased during the year following the injury. In contrast to the unloading of the lower extremities, the upper extremities are loaded more frequently and with higher stresses in spinal cord injured individuals. This new loading situation in the shoulder joint did
however have no effect on the articular cartilage in the spinal cord injured subjects.

These data are very important for the spinal cord injured subject as well as for each individual who is immobilized after surgery or during long-term sickness. It is now established that articular cartilage thins during period of restricted motion and loading, but it would be very interesting to study how and if these changes can be prevented by means of physical therapy. Several kinds of therapeutical programs are used in rehabilitation for spinal cord injured individuals but the outcome of these programs on the articular cartilage or other parts of the joint is not quantified yet. It is conceivable that some programs with high impact loads can cause major joint problems while others are beneficial for the maintenance of healthy articular cartilage.

Using the new method to characterize local changes in articular cartilage, it will be possible in the future to detect not only a global thinning in patellar cartilage but also to determine in which part of the joint the thinning occurred. It has to be shown that this method enables early detection of changes in the patellar cartilage morphology. It is known that osteoarthritis starts with local changes in the articular cartilage. Consequently this method could detect OA in an early stage and therefore make treatment easier and more efficient. The efficiency of the medical or surgical treatment can be followed using the difference maps.

In the last chapter, the potential of a novel custom made device for detection of articular cartilage deformation under load was demonstrated. With this device, the detection of changes in mechanical properties of cartilage non-invasively in human is possible. In this manner, we now systematically can measure the influence of training and/or immobilization on the mechanical as well as on the morphological parameters of articular cartilage. In the future, these data can also be used as an input for finite element analysis.
Acknowledgements

I would like to take the opportunity to thank all the people without whom this work would not have become what it is. The three years of compiling my dissertation were years of very interesting new collaborations, new scientific insights and personal development.

Everything started with the chance that my supervisor Prof. Dr. E. Stüssi gave me to be a research assistant at the Laboratory for Biomechanics. I am very grateful that he gave me this opportunity and gave me the space and the freedom to develop my own ideas.

I, as a novel in the cartilage research field, was also very fortunate to become acquainted and to collaborate with Felix Eckstein. Despite the distance between Munich and Zurich, Felix played a very crucial role in my work. He gave me the believe in the importance of my research, stimulated me at moments that I needed it and was a great help during the writing of the papers.

Since 2001, I got chance to develop my research in the medical imaging research field. I want to thank Prof. Dr. Gabor Székely for giving me this chance, for his support and for sharing his knowledge and insights.

I would like to acknowledge Prof. Dr. A. Spaepen for giving me the opportunity to start this PhD, for his interest in my work and for supporting me and believing in me from the very first start.

Das Nottwil team, Prof. Dr. Hans Knecht, Dr. Hans Hadwighorst, Mihael Abramovic und Phil Jungen möchte ich für ihren Einsatz und für die angenehme Zusammenarbeit danken. Auch eine grosse Dank an alle Patienten für die Zeit und die Mut die sie gebraucht haben um an diese Studien teil zu nehmen. Tanja Kaekebeke, merci das du mich angehört hast und mich Mut eingesprochen hast, wenn ich es gebraucht habe.

Big thanks to Roger Lüchinger for all the hours he helped me with finding the appropriate MRI protocol and with measuring subjects.

I really enjoyed working in the Laboratory for Biomechanics. Thanks to all colleagues of LfB for the positive and enjoyable working atmosphere. Special thanks goes to my officemate Eliana Lucchinetti and my Dutch language-mate
Eling de Bruin for the valuable discussions and advice they gave me. Likewise, a special thanks goes to Toru Fischbach for his friendship, listening ear and company during the long evenings at work. During my PhD time, the ‘Wagi-Wagi land’ team became a real concept and enriched my office life as well as my free time. It was a great pleasure to be part of this team on many occasions.

Dan wil ik graag nog mijn ouders en zus bedanken voor de vrijheid die ze me geven om mijn dromen waar te maken, voor de warmte en het voorbeeld die jullie mij meegaven en voor de dagdagelijkse steun die jullie voor mij zijn, zelfs vanop grote fysieke afstand.

Then there is one more person who plays the most important role in my life, who shared the joyful moments but also endured the difficult ones, who was there to listen to me but also to discuss and advice me, and the most important thing, who believed in me. Andrew, thank you for your support, encouragements and everything you did for me during our shared lifetime. Thanks for being there.

Schlieren, October 2003

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