Loading and unloading in the development and treatment of osteoarthritis

Femke Intema

The research described in this thesis was financially supported by the Dutch Arthritis Association (Reumafonds), the Anna fund (Annafonds), and the department of Rheumatology, University Medical Center Utrecht.
Printing of this thesis was enabled by financial support of the Dutch Arthritis Association and the Anna fund.

Thesis, Utrecht University, the Netherlands

ISBN: 978-90-393-5397-4
Printed by Gildeprint Drukkerijen, Enschede

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Belasting en ontlasting in de ontwikkeling en behandeling van artrose
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. J.C. Stoof, ingevolge het
besluit van het college voor promoties in het openbaar te verdedigen
op donderdag 7 oktober 2010 des middags te 12.45 uur

door

Femke Intema

geboren op 10 oktober 1980 te Niedorp

Promotoren:	Prof. dr. F.P.J.G. Lafeber
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Introduction Chapter 1

Osteoarthritis

Osteoarthritis (OA) generally is a slowly developing joint disorder, characterized by progressive cartilage damage and loss, changes in bone and peri-articular tissues, and commonly also secondary joint inflammation[1]. These changes in tissue structure are associated with pain, stiffness, and functional disabilities[2, 3]. OA is the most common joint disorder and responsible for a large group of disabled[4].

Risk factors

Although the exact pathogenesis is still unclear, it is generally appreciated that the etiology is multi-factorial, with genetic, biochemical, and mechanical factors playing a role[5]. Besides (known or unknown) trauma to the joint or adjacent areas or structures, aging and overloading are the main risk factors[6]. Previous trauma to the joint (meniscal tear, ligament insufficiency, cartilage defect, intra-articular fracture) inevitably results in a certain degree of joint degeneration[7]. A change in mechanical (e.g. malalignment, muscle weakness) or biochemical (e.g. inflammation) peri-articular environment may lead to crossing of a point of no-return in tissue degeneration (a disturbed balance between tissue synthesis and breakdown) and with that inevitable initiation of OA[2, 6]. Also congenital joint deformities (e.g. hip dysplasia) may predispose and eventually lead to osteoarthritic changes[8]. In all cases, a combination of biochemical and biomechanical factors are expected to play a role in development and progression of the disease. Despite its multiple etiologies, the ultimate joint changes in OA are relatively uniform.

Prevalence & Impact

Osteoarthritis can affect all synovial joints, with knee OA having the largest impact on society. Symptomatic knee OA affects roughly 6-10% of the adult population and is the most common form of OA, with a huge socio-economic and health care burden[4]. Radiographic knee OA affects 30% of females over the age of 45 and 25% of males[9]. The disease occurs in all ethnic groups and in all geographic locations. Interestingly, the risk of disability attributable to knee OA, is as great as that attributable to cardiovascular disease, and greater than that caused by any other medical condition in the elderly[10]. As the population is aging, with older people still demanding an active lifestyle, and the incidence of obesity is growing, the prevalence of knee OA is projected to increase even more, clearly with huge public health consequences[11].

Clinical symptoms

Clinical symptoms of OA include pain, stiffness, and a diminished range of motion; eventually leading to joint dysfunction. Pain is described as relatively continuous and nagging, but is typically most severe at the initiation of activities and has commonly been present for years. Joint stiffening occurs especially in the morning or after other long periods of immobilization[12]. Eventually, range of motion of the joint decreases due to pain or mechanical blocking. In more advanced stages, joint effusions can occur and joint shape alters by adaptive changes in the surrounding bone[1]. Flexion is accompanied by crepitus

and there is muscle atrophy due to disuse. Diagnosis of OA in hip or knee is based either solely on clinical findings or on joint pain in combination with radiographic joint damage[13].

Joint tissues involved

Joints are part of the bony skeleton and allow movement within the rigid pillars of our body. The range of movement of joints depends on their specific shape. Joint surfaces are covered with hyaline cartilage. The majority of joints with hyaline cartilage are synovial joints characterized by the presence of a capsule with synovial lining on the inside and a thin layer of synovial fluid within the joint space. Joints that are susceptible to OA are synovial joints, involving primarily the hip and knee joints as well as joints of the hands, feet and spine[1].

Cartilage

Articular cartilage is an avascular, aneural connective tissue important in resistance against compressive force and shear stresses during joint use. It is capable to absorb stresses by deforming under mechanical load and thereby minimizing peak stresses on subchondral bone[14]. Cartilage has great durability since in most people it provides normal joint function for a lifetime, despite wear and tear due to aging[15]. Articular cartilage consists of an abundant extracellular matrix that is deposited and maintained by a (in humans and larger animal species) relatively small number of highly specialized cells, the chondrocytes[16]. The extracellular cartilage matrix consists for 70% out of water and has two main other components: collagen type II forming a network, and proteoglycans embedded in this network[17]. The collagen network defines the form and tensile strength of articular cartilage while the highly hydrophilic proteoglycans are responsible for the resilience of cartilage[18]. Proteoglycans are proteins with one or more glycosaminoglycans covalently attached. The predominant proteoglycan in articular cartilage is aggrecan. Monomers of primarily chondroitin sulphate chains connected to core protein, are non-covalently associated with hyaluronic acid, the bonding stabilized by a link protein[19]. The aggrecan molecules are immobilized in the collagen network providing a large negative charge within the cartilage matrix. Cations are drawn into the tissue to balance the negative charge, creating a large osmotic potential. As a consequence, water is absorbed by the tissue. In the unloaded condition, swelling of the tissue is constrained by tensile stiffness of the collagen network[20]. On compressive loading of the joint, water is squeezed out of the cartilage and absorbed again in unloaded condition. The turnover of proteoglycans is much higher than that of collagens, both being relatively low at the tissue level and maintained by chondrocyte activity[14].

In healthy individuals, cartilage-matrix turnover has a balance in synthesis and degradation. Chondrocytes respond to changes in extra-cellular matrix, such as the presence of fragmented matrix molecules (breakdown products), the presence of cytokines, growth factors, and small molecules with catabolic and anabolic effects, and the frequency and intensity of joint loading[21]. As such, to maintain the balance in synthesis and breakdown of extracellular matrix, intermittent loading of the cartilage is required to stimulate chondrocytes

mechanically and biochemically[22]. Note that the presence of nutrients and soluble stimuli depends on diffusion of these components through the matrix as there is no vascularization of the matrix.

In the process of OA, the cartilage surface gradually becomes damaged. The first sign is surface fibrillation, followed by the appearance of fissures, which increases till pieces of cartilage are worn off and eventually areas of bear bone are exposed to direct loading and synovial fluid[23]. In this process, the collagen network is ruptured, leading to swelling of the cartilage, followed by loss of proteoglycans from the matrix[24]. As such, the unique biomechanical properties (tensile strength and resilience) are lost, making the cartilage prone to further mechanical damage. The progressive loss of articular cartilage matrix is accompanied by an attempt to repair the cartilage[25]. Chondrocytes respond to the changed environment with increased cell division and increased matrix synthesis which results in a temporarily stable situation or even repair. When eventually a point of no return is reached, there is progressive loss of cartilage, as well as a decline of chondrocyte anabolic and proliferative respons[2]. Finally, cells dedifferentiate and numbers decrease[26]. In end-stage OA, dispersed loss of cartilage areas leads to bone to bone contact during loading, with loss of joint congruity and increased friction with increased peak loads on the remaining cartilage and underlying bone[27].

Calcified cartilage,

Between articular cartilage and bone there is a layer of calcified cartilage separated from the articular cartilage by a tidemark. This zone of calcified cartilage is a highly modified mineralized region of articular cartilage that provides the attachment of cartilage to bone. Calcified cartilage gradually submerges into the subchondral bone, provides transmission of forces across the joint, and limits diffusion from and to the subchondral bone[28]. Calcified cartilage functions as an intermediate stiffness layer between the articular cartilage and the subchondral bone[29]. There is extensive vascular and neural intrusion from the subchondral bone plate into the calcified cartilage[30]. In case of OA we see disruption of the tidemark, with formation of multiple tide marks as a sign of increased turnover, and thinning of the calcified cartilage layer. This all might be related to micro injuries to the subchondral region[31]. Also ingrowth of blood capillaries and nerve endings from the subchondral bone area into the damaged cartilage has been described[32]. The tight border between cartilage and bone, as the tide mark is considered to be in a healthy state, is lost and soluble mediators may be exchanged between both tissues[33].

Subchondral bone

Subchondral bone consists of a cortical bone plate which is supported by the cancellous bone, a network of bone trabeculae which architecture is responsible for its strength and capacity to withstand loading forces[34]. Subchondral bone changes are a distinctive feature in OA development, and they include sclerosis, cyst formation or bone marrow lesions (evidenced by MRI or CT), bone attrition, and osteophytes[12].

One of the most remarkable and consistent features of joints affected by OA, whether naturally occurring or experimentally induced, is the development of prominent osteochondral nodules at the joint edges known as osteophytes [13]. It seems likely that both mechanical and biochemical factors are involved in stimulating the formation of osteophytes. Osteophytes are an example of cartilage and bone regeneration in OA joints. They arise from tissue associated with the chondro-synovial junction or from progenitor cells residing in the perichondrium[35-37] indicating that there is a population of pluripotential cells that is responsive to the mechanical and biochemical sequels of joint injury. Although the exact functional significance of osteophyte growth remains unclear, osteophytes might help to stabilize joints affected by OA, however this is disputed as well[38].

Subchondral bone changes beneath the weight-bearing joint surface are commonly referred to as subchondral sclerosis. On radiographic imaging this generally reflects an increase in apparent bone density. Despite the fact that the material density of the bone is significantly lower than in healthy conditions, the apparent density of the subchondral bone in this area seems to have increased, probably by the increased number and reduced separation of trabeculae[32]. The reduction in mineral density can be explained by the increased turnover rate in osteoarthritic bone, which results in younger, less mineralized bone. It is commonly accepted that the trabecular bone and the subchondral plate each respond differently and should be approached as separate structures[39]. MRI studies have shown that increased bone density (sclerosis) coinciding with excessive loading, is associated with bone marrow lesions[40]. These MRI-apparent lesions are marked by bone marrow necrosis, fibrosis, and trabecular abnormalities[41]. Bone marrow lesions may play a role in the pathogenesis of subchondral cysts, as cysts have been observed to arise within regions of marrow edema-like signal[42]. Subchondral cysts can frankly communicate with the joint space or not, and are lined with fibrous connective tissue containing adipocytes and osteoblasts[43].
The role of subchondral bone in OA appears to be complex. Subchondral bone is thought to be important in absorbing loads in the joint. In the progression of cartilage damage, a sharp gradient of stiffness of the underlying bone may play a role. It is suggested that the increased stiffness of sclerotic bone in OA might play a role in the progression of cartilage degeneration[44]. Whether these subchondral bone changes precede, occur simultaneously, or are the end result of cartilage degeneration, is still subject of discussion[45]. The role of the variety and sequence of subchondral bone changes in the development of OA is not yet clear but inevitably the mechanical integrity of the joint surface is disrupted and cartilage responds.

Recent literature has emphasized the relationship between subchondral bone changes and clinical outcome in OA. None of the pathological changes in other damaged tissues show such strong correlations with clinical symptoms[46]. MRI studies have emphasized the importance of large bone marrow lesions[47] and the combination of bone marrow lesions and bone surface attrition[48] and their relationship with clinical features in knee OA. Subchondral cysts in the knee joint have been associated with an increased risk of knee replacement[49].

Consequently, subchondral bone has been identified as an attractive target for treatment in OA.

Synovial tissue

The synovial tissue is lining the joint cavity and consists of one or two cell layers of synovial cells. The synovial lining cells play a role in removal of waste products from the joint cavity and in immunological defense. The cause of synovial inflammation and its role in the pathogenesis of OA remains unclear. Although OA is commonly described as a non-inflammatory disease, secondary inflammation will contribute to the cartilage damage by production of catabolic cytokines, such as TNFα and IL-1 which in turn induce release of matrix metalloproteinases (MMPs)[16]. These catabolic mediators are also produced by chondrocytes and are involved in normal matrix turnover as well. However, they are produced in elevated amounts in OA, contributing to joint pathology[50]. The role of synovial changes in cartilage damage in OA remains minor compared to that in rheumatoid arthritis, where aggressive invasion of synovial tissue in cartilage and bone significantly contributes to joint damage. The exact role of synovial tissue and synovial inflammation in the process of OA might even be considered less explored than the role of peri-articular bone[51, 52]. As such, its role in the clinical perspective of the disease, its contribution to the intrinsic degenerative process of cartilage, as well as its relation to bone changes needs attention in future studies[53].

Studying OA

Finding targets for treatment of OA requires knowledge of disease pathology. This knowledge is significantly hampered by the lack of sensitive and specific markers describing the disease process. Such markers are needed for diagnosis, to follow the progression of the disease, and to evaluate intervention strategies. Current outcome parameters can be divided in clinical (the burden of disease as experienced by the patient), and structural parameters (actually representing tissue changes).

Clinical parameters

Clinical symptoms in knee OA are commonly measured by The Western Ontario and McMasters Universities Osteoarthritis Index (WOMAC) being the most used condition specific questionnaire, including pain, physical assessment, and functional restrictions[54]. Comparable questionnaires have been developed for other joints, like hip and hand OA[55]. Visual analogue scales for joint specific pain are applied as well. Additionally, more general questionnaires of patients wellbeing, such as SF36, can add to joint specific ones[56]. In general, outcome of these questionnaires and scales depend on the moment (of the day, before or after exercise, etc) and momentary condition of a patient. As such, they have large variability and might not be very suitable to draw conclusions for the individual patient.

Biomarkers

Biochemical markers are molecules that are released into biological fluids during matrix

turnover of articular cartilage, subchondral bone, and synovial tissue. These molecules could serve as markers reflecting the development of OA[57]. Since the rationale behind biomarker application seems so logical, expectations of the applicability of these biomarkers for describing the disease process and/or predicting progression of OA were high initially[58]. Unfortunately, advances in the OA biomarker field are limited thus far. Although some markers may be helpful, at present, none of the current (combinations of) biomarkers is sufficiently discriminating to aid to diagnosis and prognosis of OA in individual patients, or performs so consistently that it could function as an end point parameter in clinical trials[59]. Quality standards for future design of studies should be developed and significantly more research is needed to investigate biomarker performance adequately. On the other hand, sensitive biomarkers might provide the first objective measures to demonstrate preclinical tissue degeneration in OA, or structure repair as a result of treatment, expectedly much earlier than can be detected by imaging techniques.

Imaging

Radiographs provide a two dimensional overlay image of X-ray absorbing tissue, predominantly calcified tissue. By taking X-rays during joint loading, the distance between the two cartilage-bone interfaces provides a surrogate measure for cartilage thickness[60]. Bony changes such as osteophytes, surface attrition, and subchondral sclerosis can also be visualized. However, the two dimensional aspect of the images has significant limitations. Standardization of acquisition of images is a major issue and conventional radiographs are notoriously insensitive. Irrespectively, X-rays are still the gold standard, and demanded by the American food and drug administration (FDA) and the European Medicines Agency (EMEA) to demonstrate structure modification[60, 61]. The presence and severity of OA in the knee is classified using the Kellgren and Lawrence grading system, an ordinal grading system using a 5 point scale that severely depends on the presence of osteophytes followed by joint space narrowing[62]. At present, several improvements using continuous measures for joint space width but also osteophytes have been described. Semi-objective computer programs to quantify different parameters as continuous variables on plain X-rays such as joint space width (JSW), osteophyte area and subchondral bone density, have been designed for different joints[63, 64]

Computed tomography (CT), implies a series of X-rays from different angles from which a three dimensional view with little loss of pixel resolution can be created. This improved imaging quality comes at the cost of significant radiation exposure and higher costs. But also CT poorly visualizes soft tissue structures like cartilage[65]. Intra-articular or intra-venous contrast can be used to improve visualization of other tissues than bone[66], but for analysis of cartilage morphology, as well as other soft tissues, other imaging techniques are preferred. The major applicability of CT lies in the field of visualizing subchondral bone. Unfortunately, clinically applied CT is not yet able to reach resolutions high enough to define bone structure to the level of separating cortical plate from trabecular bone. Visualization of trabecular architecture is possible by use of micro-CT, which enables scanning at high resolution[67].

Unfortunately, only small objects can be visualized at present, but also these techniques are evolving. Another challenge of CT might be that different calcified tissues, like subchondral bone and calcified cartilage, are difficult to separate. When the cortical plate is analyzed with micro-CT, this plate actually is a combination of subchondral cortical bone and overlying calcified cartilage.

A shift from X-ray to magnetic resonance imaging (MRI) in visualizing cartilage and the subchondral region (as well as peri-articular soft tissues) has emerged. MRI is able to quantify cartilage structure as well as bone changes[68]. A lot of effort is put in development of reliable tools to describe morphological changes, cartilage volume and even cartilage composition by use of MRI. In addition, several MR acquisition techniques are explored including delayed contrast-enhanced MRI of cartilage (dGEMRIC) or T1rho imaging to provide information on cartilage quality[69]. However, all these techniques need further study to proof their added (and specifically, cost effective) value.

Tissue analysis

The ultimate analysis of tissue structures in OA is biochemical and histochemical evaluation of the tissues itself, in addition to the biochemical and imaging surrogate markers. However, taking biopsies of synovial tissue, bone and cartilage has major restrictions. Biopsies only represent the specific location where the biopsy is taken from, rather than an overall view of the joint. In general only *post mortem* and in major surgery, such as joint replacement procedures, sufficient amounts of tissue become available for these analyses. With respect to the latter, it normally implies that only end stage disease can be studied. When joint tissue is available, preferable full-thickness samples of cartilage on bone, multiple biochemical, molecular and histochemical techniques can provide in-depth knowledge on the tissues obtained. As mentioned, this is largely restricted to end stage disease or *post mortem* material. Therefore, this technique in addition to the biomarkers and imaging techniques need to be complemented with studies on in vivo models of OA.

Animal models

Early pathology in OA and early treatment strategies can be studied in animal models of OA. Actual biochemical, molecular, and histochemical analyses of tissues can be combined with longitudinal imaging and biomarker evaluation. Many animal models for the purpose of studying OA have been developed, most of them in rodents and canines but also in goat and sheep. Joint degeneration occurs spontaneous in certain breeds or can be induced chemically or mechanically. Depending on the model, the disease develops fast or slowly, in a localized or more generalized manner, with differences in relative involvement of synovium, cartilage or bone[70, 71]. The most frequently used model is the canine anterior cruciate ligament model (ACLT)[72]. This model has also been applied in smaller animals like rats and other rodents. OA is induced by creating instability and thus disruption of normal joint biomechanics due to transection of the anterior cruciate ligament. In addition to the ACLT model, the canine Groove model of OA has been developed. By surgically applying

grooves in the cartilage of the femoral condyles, a biomechanical change within the joint and the cartilage itself has been induced leading to overall joint degeneration. The advantage of the Groove model over the ACLT model is the lack of instability and consequently permanent disruption of joint biomechanics, potentially allowing the joint to regenerate[73]. Advantages of experimentally induced models include the ability to define precisely the type of injury, the severity of injury, as well as the time of onset and progression (rate) of injury and to relate these events to markers of disease activity and even severity (pain/loading by force plate analyses). Moreover, appropriate controls are available like the contralateral joint, assuming contralateral overloading does not play a role, or sham surgery groups with identical genetic background.

Management

As yet, OA cannot be cured in the late stage of the disease. Current treatment aims at reducing symptoms, until replacement of the affected joint is inevitable. Cure might be possible when OA is diagnosed and treated in the early stages of the disease. However, early features of OA are not yet properly defined, and the proper targets for treatment still remain to be found.

Non-surgical treatment

Conservative treatment methods aim at pain reduction and improving (or preserving) joint function. Physical therapy might have a positive influence on symptoms by keeping the surrounding tissues like muscle, tendons, and ligaments in good condition. Pain medication might be able to reduce the burden of OA to a bearable level, and might be helpful to maintain normal joint use/loading important for normal tissue physiology. On the other hand it might induce overload and with that progression of tissue damage. Braces and insoles might play a role in symptom reduction in a selected group of patients, by changing/shifting the load pattern. Also anti-inflammatory treatment might be of use in case synovial involvement is significant, as long as the medication has no significant adverse direct effects on cartilage, as has been suggested of several non-steroidal anti-inflammatory drugs (NSAIDs). Disease modifying osteoarthritic drugs are an important field of research[74]. Despite severe claims of disease modifying properties of glucosamine sulphate, chondroitin sulphate[75], and hyaluonic acid[76], no undisputable evidence for either of them can be given yet. Surgical options for intermediate disease severity are in general not indicated (with the exception of re-alignment osteotomy in case of joint malalignment). As such the disease will progress and finally joint replacement surgery is indicated.

Surgical treatment

Bone marrow stimulation

In more severe stages of OA, effective treatment methods aiming at joint preservation and joint regeneration are scarce[77]. Bone marrow stimulation was the first and still most commonly used method to stimulate articular resurfacing. Either drilling holes or microfracturing of the bone plate are applied with more or less clinical benefit. When the

highly vascularized bone is penetrated, a fibrin clot is formed over the eburnated bony surface, from which in some circumstances a fibrocartilaginous tissue is formed. The formation of this new articular tissue does not always lead to fewer symptoms[78]. This might be because the source of pain does not necessarily originate from cartilage damage. Alternatively, the newly formed tissue may not have the characteristics that functional cartilage need.

Tissue transplantation

In the last decade, tissue engineering approaches for the repair of joint cartilage and bone defects have reached the clinic[79, 80]. At present, tissue engineering is applied mostly for the clinical treatment of traumatic cartilage defects and uses autologous chondrocytes or *in situ* recruited bone marrow stem cells (MSCs)[81]. Beyond that, clinical applications for the treatment of OA, based on chondrocytes or MSCs combined with absorbable transplants (allowing a stable fixation in defects without peri-focal solid cartilage shoulder) are on the way, but might not succeed solitarily and require a combination with a mechanical treatment approach[82].

Osteotomy

In case of OA that may be related with an unfavorable mechanical loading of the joint due to clear deviation of the leg axis, osteotomy might be a treatment of choice. In general, an osteotomy is applied to correct the joint (mal)alignment and decrease the load on the affected location. Symptoms might decrease as a result of an osteotomy and articular restoration of cartilage thickness has also been observed[83]. The decrease in pain could be due to less load on the affected side, a decrease in intra-osseous pressure, changes in subchondral bone integrity, or to the formation of new articular tissue. Unfortunately, clinical improvement deteriorates over time as does the regained radiographic joint space width[84].

Endoprosthesis

Finally, in end-stage disease, partial or total replacement of the joint is indicated. Replacing the destroyed joint with an endoprosthesis is the currently accepted treatment option for end-stage knee OA. Consequently, the number of total knee prosthesis (TKP) is exponentially increasing and represents a major economic burden[11]. Over 40% of all knee replacements and up to 44% of all total knee revisions are performed in patients aged under 65[85]. The increase of failure risk in younger patients, due to the still high demands of their joints is a major concern. As such there is still an increasing need for joints preserving therapies.

Joint distraction

Joint distraction is a surgical procedure in which the two bony ends of a joint are gradually separated to a certain extent for a certain period of time. Initially, joint distraction was used in the treatment of joint mal-alignment and joint contracture[86]. An external fixation frame was used to actively reposition the joint and to increase range of motion. Distraction was

performed to prevent damage (compression) of the joint cartilage during the forced repositioning. In some of these patients, OA was present in the treated joint and an unexpected clinical improvement of the OA was observed. These clinical observations led us to a proof-of-concept study examining the benefits of joint distraction, by treating young patients suffering from severe ankle OA. Two thirds of the patients treated for 3 months with joint distraction experienced significant clinical benefits[87] for a period of up to ten years[88]. Based on preliminary radiographic outcome in a limited number of patients, it was suggested that joint distraction may lead to tissue structure modification as well. But, although with good success, thus far this method has only been studied extensively for severe ankle OA, which is not the most common form of OA, with only limited socio-economic impact.

Outline of thesis

Loading a joint is thought to be essential for keeping the joint in good shape with healthy cartilage and healthy underlying bone. In case of established OA, focal overloading might play a major role in development and adds to progression of the disease. Interventions that address the mechanical exposure of (certain areas of) the affected joint (conservatively by braces or insoles or surgically by osteotomy or joint distraction) might beneficially influence the disease. More insight in the influences of loading and unloading on joint tissues like bone and cartilage early in the disease is needed to understand the pathogenic interplay between these tissues and to provide leads to, and explanations of, possible treatments.

Therefore, after this general introduction (Ch 1), in the first part of this thesis there is a focus on the development of OA as represented by animal models; how they relate to clinical OA (Ch 2) and how bone changes relate to cartilage changes over time (Ch 3), between models (Ch 5) and within a joint (Ch 6). For proper evaluation, a new bilateral animal model had to be developed (Ch 4). From these studies it appeared that (un)loading in addition to the intrinsic degenerative process is involved in OA related bone changes. As such, in the second part of this thesis there is a focus on mechanical unloading in treatment of OA. How can unloading be obtained (Ch 7), whether unloading leads to structural repair in an animal model (Ch 8) and in human OA, with a focus on bone (Ch 9) and cartilage (Ch 10) tissue repair. Finally, the results are summarized and put into a general perspective (Ch 11).

A larger animal in vivo model with characteristics representing human OA might be of great value as it provides the opportunity to study OA during early development and allow analysis of the involved tissues in more detail. Therefore, in **chapter 2** for the first time the question was addressed: *Is cartilage damage in the canine Groove model representative of cartilage degeneration observed in human clinical OA?*

The sequence of cartilage and bone changes is subject of debate, specifically early in the disease. In **chapter 3** a first pilot study addressed the question: *What is the sequence of subchondral bone changes in the process of osteoarthritis and do these changes differ between the unilateral ACLT and Groove model?*

Changes in the different joint tissues in an experimental model of OA might not only be intrinsic to the degenerative process in the affected joint, but might also be influenced by

unloading due to surgery. Additionally, overloading might influence the contralateral control joint, which is used as a reference. In bilateral models this bias is absent. The bilateral ACLT model has been described[89]. To make a comparison with the bilateral Groove model, this model had to be developed. As such **chapter 4** addresses the question: *Are cartilage changes in the bilateral Groove model of OA comparable to those found in the unilateral model?*

The differences in induction of joint degeneration in the developed bilateral Groove model and the existing bilateral ACLT model provided the opportunity to study the relationship between cartilage changes and bone changes early in the disease under different mechanical conditions. As such **chapter 5** deals with the question: *What are the similarities and discrepancies in subchondral bone structure in two differently induced canine models of OA?*

This inter joint/model comparison has its restrictions. Therefore, subsequently in **chapter 6**, intra-joint variability, with regard to cartilage degradation and local mechanical characteristics was studied, with the question: *Is in the canine ACLT-meniscectomy model, thinning of the subchondral bone directly related to cartilage damage?*

From these studies it appeared that (un)loading in addition to the intrinsic degenerative process is involved in OA related bone changes. As such in the second part of my thesis there is a focus on unloading in treatment of OA. In **chapter 7**, different approaches of unloading in the treatment of OA were reviewed with the query: *Is unloading of joints, including joint distraction, useful in treatment of osteoarthritis?*

In the following chapters a focus on the potential structure modifying capacity of joint distraction was made. First in **chapter 8** the question was addressed: *Has joint distraction structure modifying properties in addition to clinical benefit when studied in canine experimentally induced OA?*

Subsequently, in **chapter 9**, the potential structural benefit of temporarily unloading joints by joint distraction was studied in clinical trials. First, bone changes were subject of study, by addressing the question: *Can subchondral bone density changes explain the clinical benefit of ankle distraction?*

Finally, cartilage repair in end stage disease by use of transient unloading of the joint was the main topic of study. As such in **chapter 10**, the ultimate question was addressed: *Does joint distraction results in cartilage regeneration in osteoarthritic knees?*

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Chapter 2

Cartilage integrity and proteoglycan turnover are comparable in canine experimentally induced and human clinical joint degeneration

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Rheumatology Reports; conditionally accepted

ABSTRACT

Background. The value of experimental models of osteoarthritis (OA) largely depends on the ability to translate observations to human OA. A suitable model is expected to show quick and lasting development of joint degeneration with features comparable to human OA. It does not have to be an exact copy of the disease. Surprisingly, direct comparison of characteristics of human and experimental OA is scarce. In the present study, cartilage integrity and matrix turnover in a canine model of joint degeneration were compared to human clinical OA.

Methods. In 23 Beagle dogs, joint degeneration was induced in one knee, the contra-lateral knee served as a control. For comparison, human osteoarthritic and healthy knee cartilage were obtained at arthroplasty (n=14) and post-mortem (n=13). Cartilage was analyzed by histology and biochemistry.

Results. Control values for cartilage integrity and proteoglycan (PG) synthesis showed species specific differences; GAG content was 2-fold higher in canine cartilage and PG synthesis even 8-fold. However, the relative decrease in PG content was not statistically different in humans compared to canines (17% vs. 15%, respectively), as was the histological damage of the cartilage (+7.0 vs. +6.1, respectively) and the increase of PG synthesis (100% vs. 70%, respectively). The percentage release of total and of newly formed PGs in human and canine controls was similar, as was the increase due to degeneration (+65% vs. 81% and +91% vs. 52%, respectively).

Conclusion. Despite differences in control conditions, the observed changes in characteristics of cartilage integrity and matrix turnover are similar in a canine model of joint degeneration and human clinical OA. Canine experimental OA does not represent human OA in all its aspects. Nevertheless, this model shows that its characteristics reflect those of human OA which makes the model appropriate for studying human OA.

Osteoarthritis (OA) is a highly prevalent degenerative joint disorder. Structural changes primarily comprise degeneration of articular cartilage, accompanied by changes in peri-articular bone and synovial inflammation. The disorder develops over years and age and obesity are the main risk factors[1, 2]. Currently, OA cannot be cured and treatment primarily aims at reducing symptoms (pain) and restoring function[1, 2]. Ideally, disease modifying therapies to prevent progression or to induce repair of joint damage should be applied early in the course of OA. For this reason, studying early changes in the process of OA is a necessity, as well as studying treatment strategies applied early in the disease. Unfortunately, clinical *in vivo* studies of early disease have their limitations. Analysis of early (small) cartilage changes (e.g. cartilage matrix integrity and turnover) cannot yet be performed easily by common imaging techniques such as radiographic analysis or MRI[3, 4]. Other non-invasive techniques, like urine and serum biomarker analysis, are still not applicable to monitor changes at an individual level[5]. Therefore, *in vivo* animal studies in addition to human *in vitro* studies are of great value to analyze cartilage at an individual and detailed biochemical level, early in the course of disease.
Animal models have the advantage that the disease can be synchronized and they are relatively quickly progressive. The potential difference in characteristics with human OA is the main disadvantage. Because the etiology of OA is poorly understood, it is very hard to measure how well any animal model mimics the disease in humans, even if it may display some features of OA. The importance of inflammation in OA is uncertain, as well as the importance of other etiologic factors, such as increase in bone density, and inherited defects in the structure of cartilage collagen. Also major differences among species exist with respect to the relative contribution to pathological changes of various mediators, receptors or enzymes. Nonetheless, models have an important role to play in basic research.

To study the changes associated with cartilage degeneration in OA *in vivo*, several animal models of OA have been developed. Models in horses, goats, sheep, dogs, guinea pigs, rats and mice have been applied (bio)chemically, mechanically, surgically, genetically, etc[6, 7]. Most of these models have been validated in such a way that degenerative changes in cartilage matrix occur. Sometimes they have demonstrated to be progressive over time, to be accompanied by intra-articular bone changes, and to have more or less synovial inflammation.
One of the most frequently used joints for a model of OA is the canine knee joint. Arguments in favour of the use of canine (stifle) knee joints are the presence of spontaneously occurring OA in dogs with a pathology and pathogenesis comparable to human OA, a relative good matching anatomy with the human knee joint, and not unimportantly, size allowing a broad spectrum of analysis and (surgical) interventions.
Recently, in succession to the anterior cruciate ligament transaction (ACLT[8, 9] and meniscectomy model[10], the canine Groove model of joint degeneration was developed[11-13]. Features of OA were induced by surgically applied grooves in the weight bearing cartilage of both condyles without damaging the subchondral bone. The contra-lateral knee was validated to be useful as an internal control[11]. A bilateral model with a bilateral sham surgery group was

validated as well[14].The model showed very consistent changes in cartilage bio- and histochemistry in all animals, very similar to those seen in the ACLT model[11].
While the main goal of experimental models is extrapolation of results to the human pathology, hardly any study has focused on directly comparing cartilage integrity and in particular matrix turnover to human cartilage. When studying treatment strategies, repair capacity of the tissue is expected to be closely related to matrix turnover among other important features such as normalization of intra-articular stress and movement of the joint. Therefore, in the present study we directly compared parameters of cartilage integrity and matrix turnover as observed in the canine Groove model to those observed in clinical human OA.

MATERIALS AND METHODS

Canine experimentally induced joint degeneration

Twenty-three female Beagle dogs, skeletally mature, aged 2.6 ± 0.4 years, weighing 11.9 ± 0.2 kg, were obtained from the animal laboratory of the Utrecht University, the Netherlands. They were housed in groups of 2-3 dogs per pen, and were let out on a patio in large groups for at least 2 hours daily. They were fed a standard diet and had water *ad libitum.* For this study, ethical approval was given by the Utrecht University Ethical Committee for animal studies.
Induction of OA was performed as described previously[11, 12]. In short, surgery was carried out in the right knee through a 2-3 cm medial incision while care was taken to prevent bleeding and soft tissue damage as much as possible. The cartilage of both femoral condyles was damaged by making approximately 10 diagonal grooves to a depth of maximum 0.5 mm on the weight-bearing area preventing subchondral bone damage[15]. The latter was checked by histology at the end of the experiment. There was no absolute visual control over the procedure, but macroscopic evaluation after termination of the animals showed similar patterns in all knees. After surgery, synovium, fasciae and skin were sutured. The contra-lateral left unoperated knee served as a control. Starting 2 days after surgery, the dogs were let out on the patio to exercise, 5 days a week for 4 hours a day. The dogs were forced to load the affected joint by fixing the contra-lateral limb to the trunk, 3 days a week for approximately 4 hours a day for 10 weeks.
At 20 weeks post-surgery the dogs were killed with an intravenous injection of Euthesate. Control and degenerated joints were amputated and within 2 hours full thickness cartilage slices were collected from the weight-bearing area of both condyles of both knees and processed under laminar flow conditions.

Human joint degeneration

Human degenerated cartilage was obtained from the weight bearing area of the femoral condyles during joint replacement surgery from 14 patients with clinically defined OA (mean age 67±3 years; male-female 6:8). It should be kept in mind that only the cartilage that could be cut from the joint surfaces after replacement surgery was used. Similar, from 13 donors,

intact healthy femoral knee cartilage was obtained from the weight-bearing area post-mortem (mean age 66±5 years; male-female 5:8). Macroscopically, the cartilage had a smooth glossy appearance without any signs of degeneration, which is been confirmed by histology [16]. Full thickness cartilage slices of both condyles were kept in phosphate buffered saline and were processed under laminar flow conditions within 2 hours after collection.

Cartilage analysis.

From each joint, full thickness cartilage slices were cut into square full-thickness pieces and weighed (5-15 mg; accuracy 0.1 mg).

For histology, four samples randomly taken from the femoral condyles were fixed in 4% phosphate-buffered formalin containing 2% sucrose (pH 7.0). Cartilage degeneration was evaluated in safranin-O fast-green iron hematoxylin stained sections by light microscopy according to the OARSI scale for canine experimental models of OA, a new scoring method for cartilage and bone, initiated by the Osteoarthritis Research Society International (OARSI) and developed by experts in the field (personal communication with T. Aigner, manuscript under revision). The scale is based on three categories (similar to the Mankin scale), cartilage surface, cellular characteristics and reduction of safranin-O staining (indicating loss of proteoglycans). Each category has a maximum score of twelve dependent on the severity and extent of the features, bringing the overall maximum score to thirty-six. Specimens were graded in random order by an observer unaware of the source of the cartilage. The mean score of the four samples was used for statistical evaluation.

For biochemical analysis the cartilage samples (at least 6 for each dog joint and 10 for each human joint) were cultured individually according to standard procedures as described previously[17] and cartilage proteoglycan (PG) content, PG release, PG synthesis, and release of newly formed PG were determined.

Proteoglycan (PG) content and turnover have been determined as described before[11, 17]. In short, cartilage samples were exposed to radioactive sulphate for 4 hours, thoroughly rinsed and cultured in medium without sulphate label for 3 days. Thereafter, medium was collected and the cartilage tissue samples were digested with papain. GAGs in the papain digest and in the 3-day culture medium were precipitated and stained with Alcian Blue dye solution. The amount of $^{35}SO_4^{2-}$-labelled GAGs in the papain digest and medium was measured by liquid scintillation analysis and normalized to labelling time and wet weight of the explants. Total PG synthesis is the sum of the labelled GAGS from the papain digest and medium and was expressed as the amount of sulphate incorporated per hour per gram wet weight of the cartilage (nmol/h.g), expressed as nmol. Release of newly formed PGs (in the medium in 3 days) was expressed as percentage of the originally synthesized (labelled) PGs. Blue staining of the digested cartilage and the 3-day medium was quantified photometrically with chondroitin sulphate (Sigma C4384) as a reference. The GAG content is the sum of the content in papain digest and medium, corrected for wet weight of the cartilage tissue (mg/g). The amount of GAGs released to the medium from the matrix is expressed as percentage of total GAG content (percentage release).

Calculations and statistical analysis. Mean values of different animals or donors (n=23 degenerated and 23 (contra-lateral) control animal joints, n=13 human healthy cartilage donors and n=14 human OA cartilage donors) ± SEM are presented. Unpaired non-parametric statistical evaluation was used (Mann-Whitney U test) for optimal comparisons for both animal and human cartilage. Additionally, the percentage or absolute (histology) difference between the degenerated cartilage samples was compared to the mean values of control canine and human cartilage. As such the change between control and degenerated cartilage could be compared between canines and humans by use of Mann-Whitney U test.

RESULTS

Characteristics of control cartilage

Histological grading according to the newly developed OARSI scale showed an average score (±SEM) of 7.2 ±1.3 for human control cartilage and 2.4 ±0.5 for canine control cartilage. This difference between human and canine controls was statistically significant ($p<0.05$). Also control values of PG-content and PG synthesis rate were different for both species (20.9±2.1 vs. 45.1±2.0, $p<0.01$ and 1.1±0.2 vs. 8.2±0,6, $p<0.05$, for human vs. canine control cartilage, respectively). On the other hand, percentage release of total PGs from the matrix during 3 days of culture was similar for human and canine control cartilage (7.4±0.6 and 6.2±0.5, respectively, ns). Also percentage release of newly synthesized PGs during 3 days is similar for human and canine control cartilage (8.4±0.4 and 8.8±0.4, respectively, ns).

Changes in characteristics of degenerated compared to control cartilage

Representative micrographs are depicted in figure 1. The degenerated cartilage of the experimental canine joints was on average graded 8.5 ±0.9, which is indicative of mild damage (figure 1E), statistically significantly higher than that of the control cartilage. Histological damage in human degenerated cartilage as compared to human control cartilage was also observed with an average grade of 14.2 ±1.0, representing moderate cartilage damage. It should be kept in mind that only the cartilage that could be cut from the joint surfaces after replacement surgery was used. Thus in fact the entire joint had a worse appearance than represented by the score of the cartilage used. The mean change in OARSI grade in the canine degenerated cartilage (+6.1) was not statistically significantly different compared to human osteoarthritic cartilage (+7.0). In addition, only minor differences were observed in the contribution of each of the categories to the total score. Cellular characteristics had exactly the same contribution (37% in both species), while cartilage surface damage (37% in human OA vs. 32% in canine degeneration) and loss of staining (26% vs. 31%, respectively) differed insignificantly.

The microscopically observed loss of Safranin–O staining was supported by the biochemical analysis of the cartilage. In both human OA and canine experimental cartilage a statistically significant lower PG content was observed (figure 2A). Despite the fact that the PG content

of canine control cartilage was twice as high as that of healthy human cartilage, the relative difference of PG content in both human OA cartilage and the experimental canine cartilage was comparable (-17% vs. -15%, respectively).

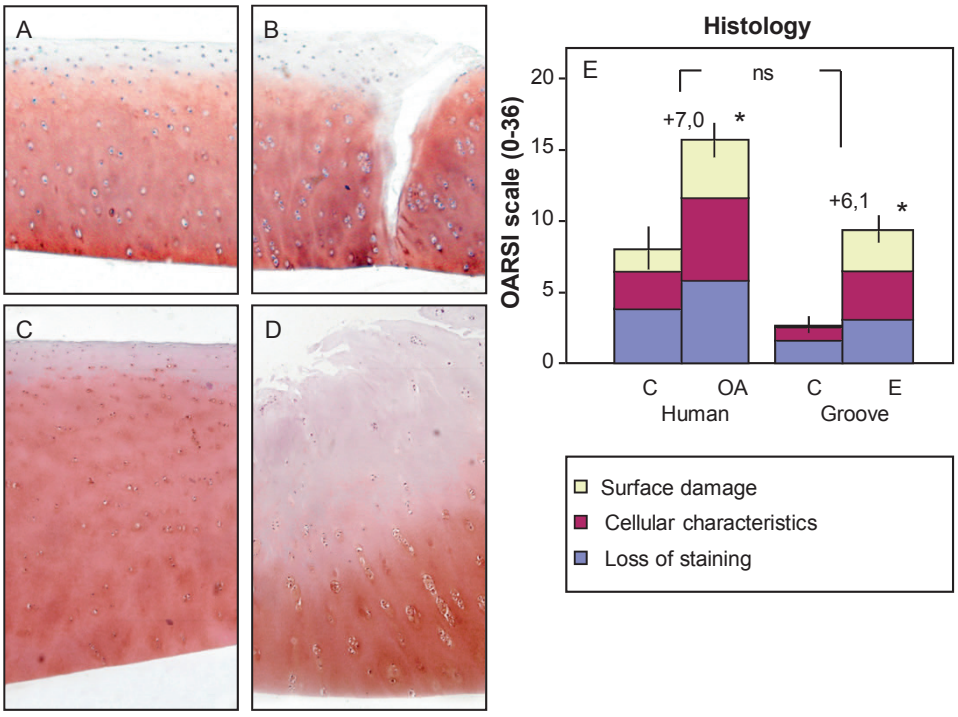

Figure 1. Representative photographs of the control and experimental cartilage of the canine Groove model (A/B) as well as human healthy and OA cartilage (C/D) are given, safranin-O fast-green iron hematoxylin staining. Figure 1E represents histological grading of osteoarthritis (OA) according to the OARSI scale for experimental models (0-36). An asterisk indicates statistical significant differences compared to control cartilage, $p<0.05$. The changes (control vs. OA/degenerated) between human and experimental OA cartilage were not statistically significantly different (ns).

The lower PG content is the result of an increased loss of PGs from the cartilage matrix. When measuring PGs released from the matrix over a period of three days of both human OA and canine degenerated cartilage, there was a statistically significantly higher release (Figure 2B). The percentage change was comparable for both species.

To compensate for this increased release there is an increased synthesis of proteoglycans. Synthesis rate of PGs (depicted in figure 2C) was on average almost twice as high in the experimental joints of the canine model compared to the contra-lateral control joints, very

similar to the doubling of PG synthesis rate as seen in the OA compared to healthy human cartilage. This higher synthesis of PGs is largely ineffective since the percentage release of these newly formed PGs is also higher in the osteoarthritic human knee joints compared to human healthy control joints (figure 2D). Again, this enhanced release of newly formed PGs is also seen in the canine experimental joints, not statistically different between both species. There was no statistical significant change in cartilage DNA content due to induction of OA (OA: 0.14 ±0.04 mg/g *vs.* control: 0.18 ±0.07 mg/g and 0.15 ±0.04 mg/g *vs.* 0.17 ±0.04 mg/g, for femoral cartilage of canines and humans respectively, data not shown).

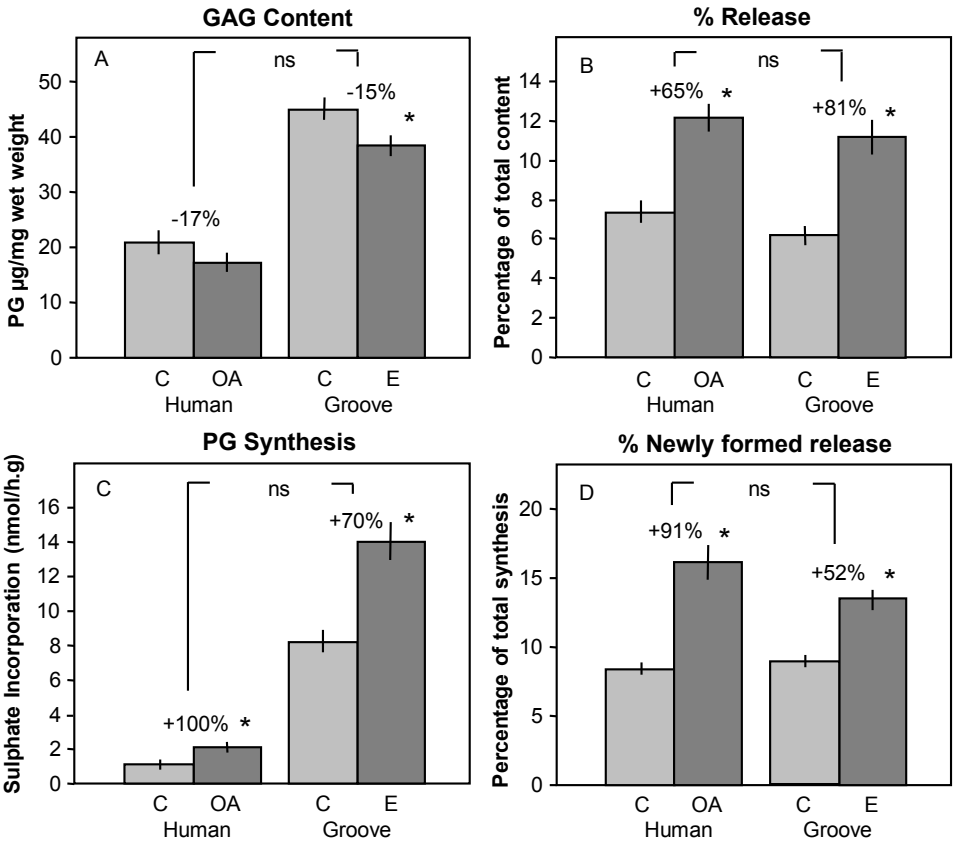

Figure 2. A. PG content (mg/g wet weight cartilage), Percentage release of total PG content (B), PG synthesis rate (C) and percentage release of total newly formed PGs are shown. The absolute differences between control (C) and OA or experimental (E) cartilage are given for human OA and the canine groove model, respectively. An asterisk indicates statistical significant differences compared to control cartilage, $p<0.05$. The changes (control vs. OA/degenerated) between human and experimental OA cartilage were not statistically significantly different (ns).

DISCUSSION

Generally appreciated, but for the first time demonstrated by direct comparison, canine and human cartilage differ significantly with respect to parameters of proteoglycan content and turnover. Irrespectively, changes due to experimentally induced joint degeneration or osteoarthritis in the canine and human cartilage, respectively, are very similar.

In most animal *in vivo* studies on osteoarthritis, species differences in cartilage structure and chondrocyte activity are either accepted or ignored. However, we hardly have any knowledge on how these differences are of influence on development of osteoarthritis in a quantitative and qualitative way, and even less on how efficacy of treatment modalities is influenced by these differences. Actually, we even have limited knowledge of the exact differences in characteristics of healthy cartilage.

When we look at cartilage structure, thickness of the hyaline cartilage layer in both humans and canines seem to depend on species size, approximately 2.3 mm for human femoral condyles and 0.8 mm for canine condyles[15, 18]. As for chondrocyte density, there is an opposite relationship between cartilage thickness and cell density, resulting in a fourfold increase in cell density in canines compared to humans[18, 19]. The specific activity of chondrocytes from different species can also be variable[20]. In our study it appeared that proteoglycan synthesis as measured by $^{35}SO_4$ incorporation in Alcian Blue precipitated GAGs upon papain digestion is eight fold higher in canines compared to humans. It is tempting to suggest that taking the four fold chondrocyte number into account the canine cells have a 2 fold higher proteoglycan synthesis rate. However, it should be kept in mind that matrix composition (characteristics) could be of influence on digestion and precipitation procedures and with that outcome in absolute numbers.

In our study the PG content as measured by Alcian Blue staining and precipitation in healthy canines is higher than in humans. The higher PG content is expressed per wet weight. As such, the difference might be explained by a lesser capacity to absorb/retain water in the matrix. A species specific artefact due to swelling as a result of collagen damage at the cutting edges might be responsible. The thinner canine cartilage of comparable weight as the thicker human cartilage has a different surface/volume ratio, due to the different shape of samples. In addition, qualitative differences in proteoglycan composition between both species can be of influence. Such differences might result in differences in precipitation and staining abilities.

Separated analyses of cartilage sampled from area with lesions versus non-lesion areas might give more information regarding differences between degenerated and healthy cartilage in both species. However, there is a rather large variation in the lesions occurring, especially in the human OA samples.

Also the age of the animals differ from the human sample population. Articular cartilage aging changes that may lead to articular cartilage degeneration include fraying and softening of the articular surface, decreased size and aggregation of proteoglycan aggrecans and loss of matrix tensile strength and stiffness. These changes most likely are the result of an age related decrease in the ability of chondrocytes to maintain and repair the tissue[21].

However, irrespective of all these potential differences, the evaluated characteristics are in a comparable order of magnitude in both species. This is a major advantage over smaller animals used for OA models. In rats and mice the cellularity increases a 26- and 33-fold, respectively[18]. Cartilage thickness is only 0.06-0.07 mm[18]. For these smaller species, to our knowledge there are no data of biochemically determined proteoglycan content or synthesis rate, expressed in such a way that they can be compared to human data.

Osteoarthritis is a disease which not solely affects cartilage. Indeed other tissues are involved as well, such as subchondral bone, synovial tissue among others. Especially the role of subchondral bone in OA has gained a lot of interest the last years. A lot of new knowledge has come forward, but whether subchondral bone changes occur first, later, or at the same time is still subject of discussion. However, in the present study there was a focus on proteoglycan turnover of cartilage. Also with respect to cartilage itself many other parameters could have been analyzed. We primarily want to show that evaluation of proteoglycan turnover in the canine model of OA might be of relevance to proteoglycan turnover in human OA. For other parameters of cartilage and for other tissues such a comparison would be of value also.

The pathogenesis of human OA is not completely understood. Osteoarthritis is a group of overlapping presumably distinct diseases, with different aetiologies, but with general similar biologic, morphologic and clinical outcome[22]. Indeed, irrespective of the (unknown) mechanism(s) responsible for OA development, in all of the 14 cases of OA in this study, cartilage changes with respect to the parameters evaluated were in the same order. In the present study we have chosen for a comparison of characteristics of human OA cartilage with presumably different aetiology with those of degenerated cartilage as observed in the canine Groove model OA with a single aetiology. Although the model has its specific characteristics with respect to induction (aetiology) of cartilage degeneration, it has been described that the degenerative changes are very similar to those seen in other models like the ACLT model of OA[11]. This fits the idea that also in the canine knee joint different aetiologies e.g. cartilage damage as in the Groove model and joint instability as in the ACLT model, lead to similar biologic and morphologic outcome. An identical similarity of human OA cartilage with canine degenerated cartilage due to joint instability would have been found.

The comparable changes due to joint degeneration in both species with respect to cartilage integrity and turnover, show us that despite basic differences, an experimental model for a complex disorder like OA might be suitable to study OA in many aspects.

However, we still have to guess about the effect of the observed basic differences in cartilage structure and turnover on attempts to remodel or even repair the tissue. Although the degenerative changes with respect to proteoglycan turnover are comparable for canine degenerated and human OA cartilage, differences may not be ignored when this and other models are used to study treatment modalities. It may well be that the turnover rate (under control conditions 8 fold higher) in canine cartilage may demonstrate treatment effects that will take a much longer time span to lead to actual changes in matrix integrity in humans. In general, this may be a major flaw of animal models representing a chronic very slowly

developing disease as OA. The smaller the species used, the larger the potential difference to humans since slow development is expected to be related to slow cure, assuming cure is possible. Good results of treatment modalities in smaller models might need much longer treatment periods in larger models or may never be found.

In conclusion, animal models are an essential part of research on OA, aiming at better understanding the pathophysiology of OA, especially in its early phases, and to study effects and mechanisms of treatment. Despite species-specific differences in cartilage characteristics in human OA and canine joint degeneration, similarities in changes due to the degenerative process prevail.

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Chapter 3

A role for subchondral bone changes in the process of osteoarthritis; a micro-CT study of two canine models

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BMC Musculoskelet Disord 2008; 12(9):20

ABSTRACT

Background. This study evaluates changes in peri-articular bone in two canine models for osteoarthritis: the Groove model and the anterior cruciate ligament transection (ACLT) model.

Methods. Evaluation was performed at 10 and 20 weeks post-surgery and in addition a 3-weeks time point was studied for the Groove model. Cartilage was analyzed, and architecture of the subchondral plate and trabecular bone of epiphyses was quantified using micro-CT.

Results. At 10 and 20 weeks cartilage histology and biochemistry demonstrated characteristic features of osteoarthritis in both models (very mild changes at 3 weeks). The Groove model presented osteophytes only at 20 weeks, whereas the ACLT model showed osteophytes already at 10 weeks. Trabecular bone changes in the Groove model were small and not consistent. This contrasts the ACLT model in which bone volume fraction was clearly reduced at 10 and 20 weeks (15–20%). However, changes in metaphyseal bone indicate unloading in the ACLT model, not in the Groove model. For both models the subchondral plate thickness was strongly reduced (25–40%) and plate porosity was strongly increased (25–85%) at all time points studied.

Conclusion. These findings show differential regulation of subchondral trabecular bone in the Groove and ACLT model, with mild changes in the Groove model and more severe changes in the ACLT model. In the ACLT model, part of these changes may be explained by unloading of the treated leg. In contrast, subchondral plate thinning and increased porosity were very consistent in both models, independent of loading conditions, indicating that this thinning is an early response in the osteoarthritis process.

Osteoarthritis (OA) is a degenerative joint disease, which causes pain and disability and is characterized by progressive damage of articular cartilage, changes in the underlying (subchondral) bone, and occasional mild synovial inflammation. Increasing evidence suggests that subchondral bone plays an important role in the etiology of OA[1, 2], but studies thus far do not provide a consistent view on this subject. Subchondral bone changes have been studied in both humans with OA and in animal models of OA. In human studies, an increase in trabecular bone volume fraction and trabecular thickness was found[3, 4], as well as an increase in cortical subchondral plate thickness[4]. However, other studies found a lower bone volume fraction and trabecular thickness in patients with OA[5, 6] or a decrease in stiffness[7, 8]. Even within one patient, areas with high and low bone volume fraction have been reported, depending on the condition of the overlying cartilage[9]. A problem of the human studies is that mostly established (severe) OA is studied, and longitudinal data showing the changes from onset until full clinical osteoarthritic signs do not exist. A problem is that there are no objective criteria that indicate early OA with mild pre-clinical signs and therefore the design of longitudinal studies is difficult. Several animal models have been developed to study osteoarthritis and changes in the subchondral bone. Some animal studies reported a decrease in bone volume fraction and trabecular thickness[10-13], whereas in other studies these parameters increased[14, 15]. These differences may be explained by the type of model used and the time at which the measurements were performed. Some bone parameters may occur in two phases: an initial decrease followed by an increase[16]. A frequently used animal model of OA is anterior cruciate ligament transection (ACLT) in dogs. ACLT results in permanent instability of the knee joint, which is followed by osteoarthritic features[17]. The ACLT model has been used for in-vivo evaluation of several treatment strategies[18-21]. However, the instability remains present, and may counteract the possible beneficial effects of treatment. For this reason, the canine Groove model has been developed. In this canine model, surgically applied damage to the articular cartilage of the weight-bearing areas of the femoral condyles, not damaging the subchondral bone, is the trigger for development of OA features[22]. The model is distinctive in that the osteoarthritic trigger is not permanent and the degenerative changes are progressive and not just the expression of surgically applied chondral damage, while synovial inflammation diminishes over time[22-24]. In the current study, we report changes in the subchondral bone of the canine Groove model and compare these with changes in the ACLT model. Because the cartilage damage induced in the Groove model appeared less drastic than in the ACLT model, the Groove model could be very useful to investigate the subtle relationship between bone and cartilage during the development of OA. Therefore, we studied the Groove model also at a very early time point. Specifically we used micro-CT analyses to quantify subchondral trabecular bone volume and architecture, the subchondral plate thickness and porosity, and osteophytosis and related this to the changes in cartilage integrity.

METHODS

OA was induced according to the ACLT model[25] or the Groove model[22]. For the ACLT model, knee joints were available from 10 and 20 weeks post-surgery (both n = 5). For the Groove model, knees were available from 3, 10 and 20 weeks post-surgery (all n = 4). In short, the following procedures were followed:

Animals

22 female beagle dogs, aged 1.5–3 years and weighing 10–15 kg, were obtained from the animal laboratory at the Utrecht University, the Netherlands. They were housed in pairs in pens, and were let out for at least 2 hours daily on a patio in large groups. They were fed a standard diet and had water ad libitum. Ethical approval was given by the Utrecht University Medical Ethical Committee for animal studies.

Anaesthesia, surgery, and post-surgical treatment

Procedures were carried out as described before[22-24]. Surgery was carried out through a 2–2.5 cm medial incision close to the ligamentum patellae in the right knee. Care was taken to limit bleeding and soft tissue damage. After surgery, synovium, fasciae and skin were sutured. The left unoperated knee served as a control. During the first 3 days after surgery, the dogs received analgesics (Buprenorphine 0.01 mg/kg) and antibiotics (Amoxicyclin 400 mg/ kg). Daily release on the patio started 2 days after surgery. At the end of the experiment, the dogs were killed with an intravenous injection of Euthesate. Both hind limbs were amputated and within 2 hours proximal tibias and distal femurs were isolated and cartilage samples were collected.

Groove model

In 12 animals, the cartilage of the lateral and medial femoral condyles was damaged with a Kirschner-wire (1.5 mm diameter) that was bent 90° at 0.5 mm from the tip as described before[22-24]. In this way the depth of the grooves was restricted to 0.5 mm. In utmost flexion, ten longitudinal and diagonal grooves were made on the weight-bearing parts of femoral condyles without damaging the subchondral bone (Fig. 1A). The latter was checked by histology at the

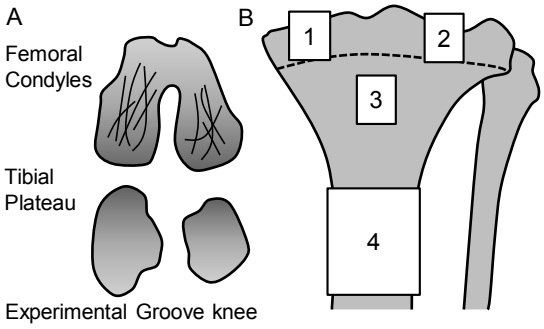

Figure 1. Schematic clarification of methods used. A. Localization of grooves made exclusively in the femoral condyles in the groove model. B. Selected regions that were analysed in the tibia using micro-CT. 1: cylinder in medial epiphysis; 2: cylinder in lateral epiphysis; 3: cylinder in metaphysis; 4: diaphysis. Cylinders 1 and 2 contain subchondral plate and trabecular bone. Cylinder 3 contains only trabecular bone. Region 4 contains only cortical bone. Dashed line indicates growth plate remnants.

end of the experiment. There was no absolute visual control over the procedure, but macroscopic evaluation after termination of the animals showed similar patterns in all knees treated. Two days after surgery, the dogs were forced to load the joint with the mechanically damaged cartilage by fixing the contralateral left limb to the trunk 3 days per week for approximately 4 h per day until the end of the experiment. The cartilage integrity and bone changes were evaluated 3, 10, and 20 weeks post-surgery (n = 4 in each group).

ACLT model

In 10 animals, anterior cruciate ligament transaction (ACLT) was carried out according to standard procedures using blunt curved scissors[25]. A positive anterior drawer sign confirmed completeness of the transection. The cartilage integrity and bone changes were evaluated 10 and 20 weeks post-surgery (n = 5 in each group).

Cartilage integrity analysis

Cartilage integrity was evaluated both histochemically and biochemically[22-24]. Cartilage samples were obtained from predetermined locations on the weight bearing areas of the femoral condyles and the tibial plateau of both experimental and control joints[22]. Cartilage was cut as thick as possible, while excluding the underlying bone (confirmed by histochemistry) and subsequently samples were cut into full-thickness cubes, weighing 3–10 mg (accuracy 0.1 mg). For histology, 4 samples from tibial plateau and 4 from femoral condyles from each knee were fixed in 4% phosphate- buffered formalin containing 2% sucrose (pH 7.0). Cartilage degeneration was evaluated in safranin-O-fastgreen iron hematoxylin-stained sections by light microscopy using the slightly modified[26] criteria of Mankin[27]. Specimens were graded in random order by two observers unaware of the source of the cartilage. For assessing the overall grade, the scores of the four specimens from each knee surface and of the two observers were averaged (a maximum of 11). This score of each joint surface was used as a representative score. For femoral condyles and tibial plateau, the amount of GAG was determined as a measure of proteoglycan (PG) content of the cartilage. Six explants were taken from the experimental joint at fixed locations, which were paired with identical locations at the contralateral control joint. All samples were handled individually. The amount of GAG was determined as described previously[28]. Alcian blue staining of the medium was quantified photometrically with chondroitin sulphate (Sigma C4384) as a reference. Values were normalized to the wet weight of the cartilage explants (mg/g). The average result of the six samples was taken as representative of that joint surface[25].

Micro-CT analysis

The proximal part of the tibias and the distal part of the femurs were scanned in a micro-CT scanner (Skyscan 1076, Skyscan, Antwerp, Belgium) with isotropic voxel size of 18 µm. The x-ray tube voltage was 60 kV and the current was 170 µA, with a 0.5 mm aluminium filter. The exposure time was 1180 ms. X-ray projections were obtained at 0.75° intervals with a scanning angular rotation of 198°. The reconstructed data set was segmented with a local

thresholding algorithm[29]. The presence or absence of osteophytes in the reconstructed dataset was scored. In both the medial and the lateral part of each femoral scan, a cylinder (diameter: 5.5 mm, height: 4.9 mm) was selected. Similarly, in the tibial scan, cylinders were selected with a diameter of 4.0 mm and a height of 3.5 mm (medial) or 3.1 mm (lateral) (Fig. 1B, regions 1 and 2). The cylinders were located in the middle of the load bearing areas using anatomical landmarks. They contained trabecular bone and subchondral plate, but did not contain growth-plate tissue. The trabecular bone and subchondral plate were separated automatically using in-house software. For the trabecular bone, bone volume fraction, which describes the ratio of bone volume over tissue volume (BV/TV), three-dimensional thickness (TbTh)[30], structure model index (SMI), a quantification of how rod-like or plate-like the bone structure is[31], and connectivity density (CD), describing the number of connections per volume[32], were calculated. For the subchondral plate, the three-dimensional thickness (PlTh)[30] and porosity (PlPor), describing the ratio of the volume of the pores in the plate over the total volume of the plate, were calculated. For these bone parameters, the data from the lateral and medial epiphyseal cylinders were averaged. The potential effect of disuse of the joints due to the treatment procedures and/or the process of OA was investigated by analyzing additional regions, further away from the joint space. A cylinder (width: 5.5 mm, height: 3.5 mm) was selected in the metaphysis of the tibia (Fig 1B, region 3), containing only trabecular bone, of which bone volume was calculated. Additionally, more distal in the tibia, a part of the diaphysis (height: 15.7 mm) was scanned at a resolution of 36 µm (Fig 1B, region 4). The diaphyseal scans were segmented with the same thresholding algorithm as the epiphyseal scans. The bone area and the corresponding moment of inertia (a parameter that reflects the distribution of the bone in each cross section) were calculated in the entire region, which contained predominantly cortical bone.

Data analysis

The data are presented as absolute values, and as percentage difference or absolute difference of the experimental joint relative to the control joint. Since the sample sizes are small, a non-parametric paired test, the Wilcoxon signed rank test, was used to compare data for experimental and control joints. The cartilage parameters have been examined in previous studies with the same models[22-24], therefore we know the direction of the effect of these parameters. Thus for cartilage parameters one-sided p values are given. Since the bone parameters have never been studied in the Groove model, we didn't know in advance in which direction the changes would evolve. Therefore two-sided p values are given for the bone parameters.

RESULTS

Changes in cartilage and in bone were similar for femoral condyles and tibial plateau. But for reasons of clarity the tibial plateau is shown as representative for both cartilage and bone parameters, since this surface was not surgically damaged when osteoarthritis was induced in the Groove model, making comparison with the ACLT model the most sound.

Groove vs. ACLT at 10 and 20 weeks post-surgery

Cartilage integrity

Histological cartilage damage was increased in the experimental tibias of all animals in both models. (Table 1 and Fig 2A). This cartilage degradation was supported by biochemical analysis. A decrease in GAG content, representing impaired cartilage integrity, was found in the tibial plateau cartilage of both models. The GAG content was decreased with 10–25% in the experimental knee compared to the control knee (Table 1 and Fig 2B).

Osteophytes

In the Groove model at 10 weeks post-surgery no osteophytes were found whereas at 20 weeks post-surgery they were clearly seen at the micro-CT images of the experimental tibial plateau in all four animals (Fig 3).

In the ACLT model already at 10 weeks and also at 20 weeks post-surgery osteophytes were found at the experimental joint in all animals. For both models, the osteophytes were located predominantly at the medial site, below the rim of the tibia plateau. The osteophytes in the Groove model were smaller than in the ACLT model. In none of the control joints osteophytes were observed.

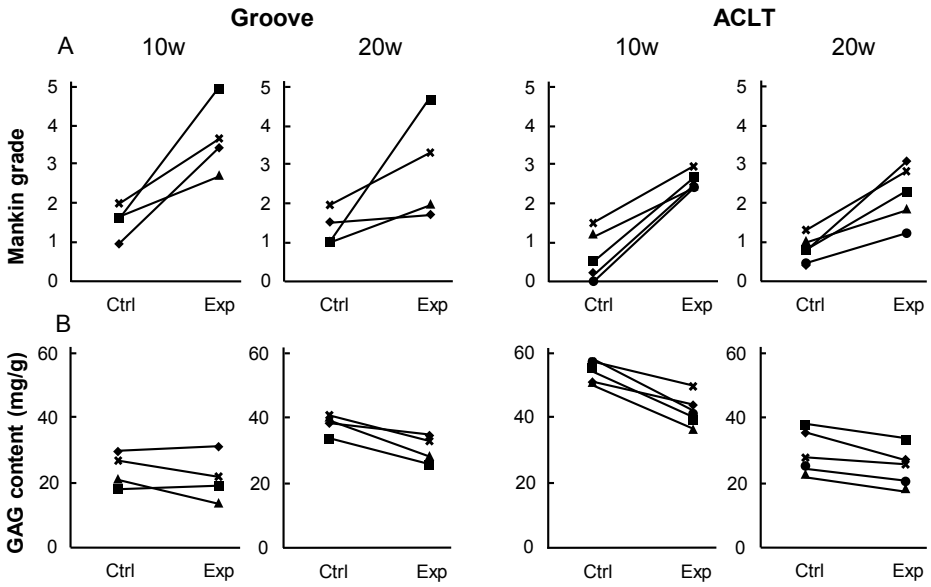

Figure 2. Cartilage integrity markers for individual animals. Data are shown for the tibial plateau of the groove and ACLT model at 10 and 20 weeks post-surgery. A. Mankin grade. B. GAG content.

Bone changes

Subchondral trabecular bone

Overall, the trabecular bone changes in the epiphysis of the experimental groove knee compared to its contralateral control were small. At 10 weeks there was a small increase in the trabecular bone volume fraction in the Groove model. Also trabecular thickness was slightly elevated at 10 weeks. At 20 weeks the bone volume fraction and the trabecular thickness were slightly decreased (Table 1 and Fig. 4A, B). The subchondral trabecular bone in the ACLT model showed a decrease in bone volume fraction (BV/TV) and trabecular bone thickness (TbTh) in all animals, at 10 and at 20 weeks. This was also reflected in the increase of the Structure Model Index (SMI) and Connectivity Density (CD) that indicate a more rod-like structure by the generation of more pores in the original structure, see table 1.

Metaphyseal trabecular bone

In the metaphyseal region (region 3 in Fig 1B), which contained only trabecular bone, the differences in bone volume between control and experimental knee in the Groove model were small. In the experimental ACLT knee, the bone volume was decreased up to 28% at 10 weeks (Table 1).

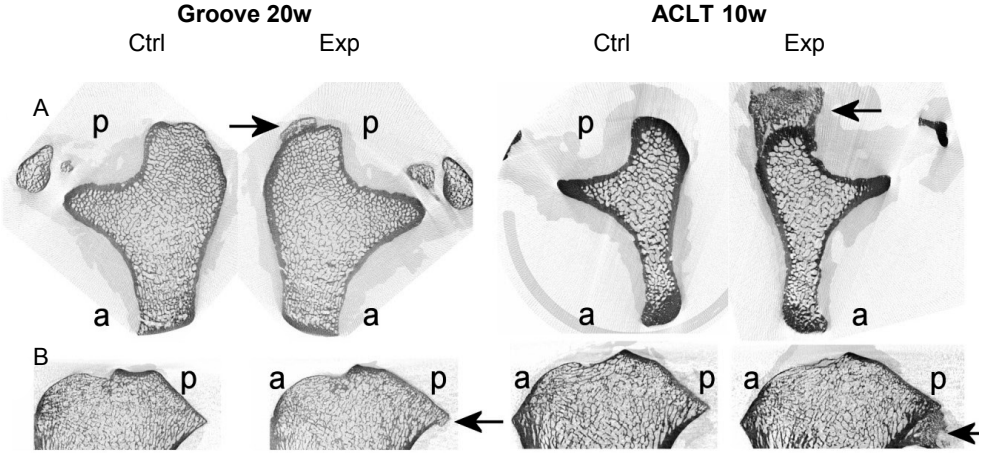

Figure 3. Osteophytes. A. Cross-sections of control and experimental tibia of groove at 20 weeks and ACLT at 10 weeks. Arrows indicate osteophytes; a = anterior, p = posterior. B. Longitudinal sections of tibias in A. Arrows indicate osteophytes; a = anterior, p = posterior.

Subchondral plate

In contrast to the trabecular bone parameters, the changes in the subchondral plate were similar in both models. The thickness of the subchondral plate in the cylinders decreased in all animals with about 25 to 40% in both the Groove and ACLT model at both time points.

The porosity of the subchondral plate increased severely in both ACLT and Groove model, at all time points in all animals (Table 1 and Fig 4C, D).

Diaphyseal cortical bone

In the diaphyseal part of the tibias, more distal from the joint, there were no differences in bone area and moment of inertia between the control knee and the experimental knee (data not shown) for both models.

	Cartilage				Epiphyseal trabecular bone								Metaphysis		Subchondral plate			
	GAG		Mankin		BV/TV		ThTh		SMI		CD		BV		PlTh		PlPor	
	ƍ(%)	p	ƍ(-)	p	ƍ(%)	p	ƍ(%)	p	ƍ(-)	p	ƍ(%)	p	ƍ(%)	p	ƍ(%)	p	ƍ(%)	p
Groove																		
3w	-4.5	0.137	+0.17	0.055	+3.9	0.465	0.0	1.000	-0.05	0.465	+3.8	0.068	-0.9	0.715	-40.7	0.068	+84.8	0.068
10w	-11.1	0.233	+2.15	0.034	+6.0	0.068	+4.2	0.144	-0.3	0.068	+0.3	1.00	-3.1	0.068	-28.6	0.068	+47.7	0.068
20w	-20.9	0.034	+1.56	0.034	-3.5	0.144	-4.2	0.144	+0.28	0.068	+15.3	0.068	-12.5	0.144	-35.7	0.068	+72.2	0.068
ACLT																		
10w	-22.3	0.022	+1.95	0.021	-16.6	0.043	-12.2	0.043	+0.67	0.043	+20.9	0.225	-28.1	0.042	-28.7	0.043	+37.5	0.043
20w	-16.5	0.022	+1.45	0.021	-17.2	0.043	-13.6	0.043	+0.77	0.043	+19.5	0.043	-16.0	0.043	-30.9	0.043	+26.2	0.043

Table 1. Cartilage and bone parameters of the tibial epiphysis. Data are displayed as mean percentage difference (ƍ) or absolute difference (for Mankin grade and SMI) of the experimental OA joint relative to the contralateral control joint, for the groove model and ACLT model, at 3, 10, and 20 weeks post-surgery.

Groove model at 3 weeks post-surgery

The development of OA appeared less advanced in the Groove model than in the ACLT model. Therefore we used the Groove model to gain further insight in the subtle relationship between cartilage and bone in the process of OA development. Thus, an additional time point was studied, at 3 weeks post-surgery.

Cartilage integrity

The histological cartilage damage in the experimental tibia was minimal at 3 weeks, while at 10 and 20 weeks, more cartilage damage was present and in all animals. The GAG content was minimally reduced at 3 weeks and gradually decreased over time (Table 1 and Fig 5A).

Bone changes and osteophytes

Subchondral trabecular bone

At 3 weeks, there were no consistent changes in trabecular bone. Also in the metaphyseal area, no changes in trabecular bone were observed between experimental and control tibia.

Subchondral plate

In contrast to the trabecular bone, there were already clear changes in the subchondral plate at 3 weeks in the Groove model. In all animals the subchondral plate thickness was decreased, on average with 40%. The plate porosity was increased in all animals, with on average 85%, which is even larger than at the later time points (Table 1 and Fig 5B). No

diaphyseal cortical bone changes or any osteophytes were found at 3 weeks post-surgery in the Groove model.

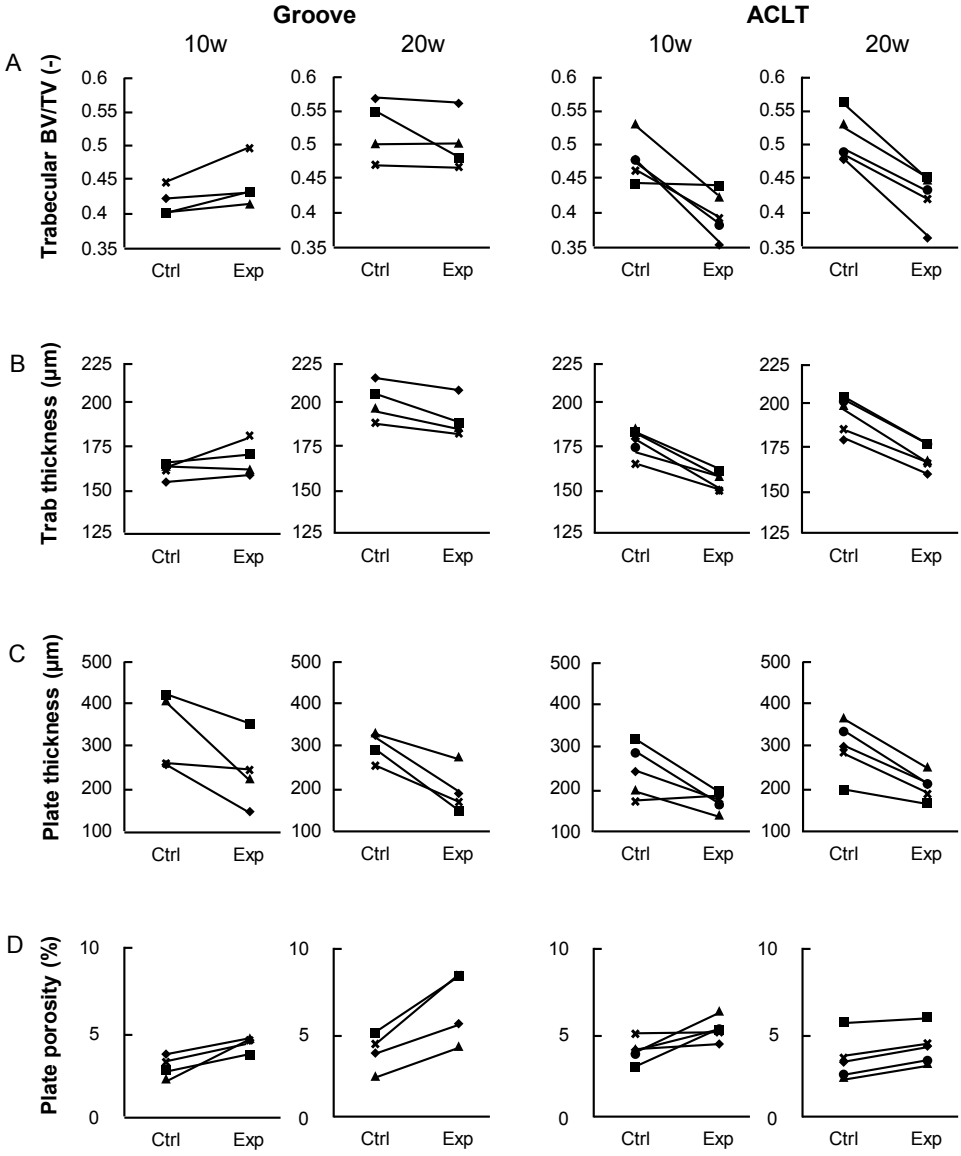

Figure 4. Bone parameters for individual animals. Data are shown for the tibial epiphysis of the groove and ACLT model at 10 and 20 weeks post-surgery. A. Trabecular bone volume fraction. B. Trabecular thickness. C. Subchondral plate thickness. D. Subchondral plate porosity.

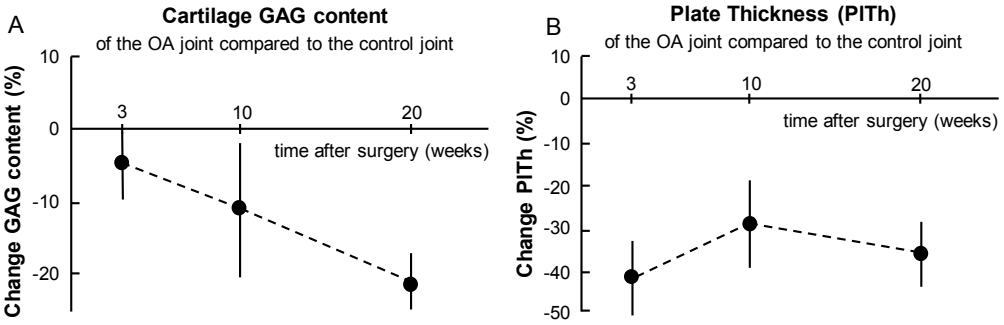

Figure 5. Relative change of experimental joints compared to control joints. Data are shown for the tibial epiphysis of the groove model at 3, 10, and 20 weeks post-surgery. A. Cartilage GAG content. B. Subchondral plate thickness. Error bars represent SEM.

DISCUSSION

The thickness of the subchondral plate decreased very consistently in two different canine models of osteoarthritis: the Groove model and the ACLT model. In contrast, the changes in the trabecular bone at the tibial epiphysis in the Groove model were relatively small and not consistent over time whereas these changes in the ACLT model were larger, with up to 20% loss in bone volume fraction with significant changes in the corresponding architectural parameters. Due to the low number of animals in the Groove model, the bone parameters could not reach statistical significance in this model. Although the trabecular parameters were not consistent, the changes in the subchondral plate were very consistent in the Groove model, with a clear and early reduction of the plate thickness and an increase in plate porosity.

Although the grooves in the Groove model were made in the femur only, the changes in subchondral bone were found in both the femur and in the tibia. This is in concurrence with the cartilage changes found in the Groove model which also showed changes in both femur and tibia[22]. Since the subchondral bone changes in the tibia cannot be caused directly by the grooves, we believe that these changes are part of the osteoarthritic process. This suggests an interaction between the bone and the cartilage through diffusive molecules that originate from the degenerated cartilage or the synovial fluid.

The cartilage changes in both models were similar to the changes previously described for larger groups of animals[22-24] and thus the data concerns a representative set of these earlier studies. The Groove model showed only very mild changes in cartilage integrity at 3 weeks, which progressed at 10 and 20 weeks. In the ACLT model the changes were comparable to those in the Groove model, but slightly more progressive.

Osteophytosis, visible on the CT-images, occurred in all the experimental ACLT knees at 10 and 20 weeks. This contrasts the Groove model in which osteophytes only were detected at 20 weeks and not at 3 and 10 weeks. This corroborates the less progressive development of OA in the Groove model compared to the ACLT model. However, a cartilaginous pre-form of the osteophytes may develop earlier, but is not detectable on the micro-CT images. In both models the osteophytes start below the rim of the medial tibia plateau and extend to more distant regions. This location is in line with osteophyte location in a rabbit ACLT model[16]. In human osteoarthritis, osteophytes are found close to the joint surface; it has been suggested that the load bearing area increases as to compensate for instability[33]. However, in our study, the osteophytes were also found in the Groove model, in which the joint does not become unstable arguing against their role in joint stabilization. An explanation for the different location in comparison to humans may be that, in dogs, the ligaments are attached to the bone at a different location than in humans, thereby causing high stresses on the bone in a different location. In addition to this, cytokines such as TGFβ, which is elevated after OA induction[34, 35], stimulate osteophyte formation[36]. Since the synovial capsule in dogs extends more to the proximal and distal part of the joint than in humans, the interface between synovial capsule and bone is more distant from the joint space. Assuming synovial tissue derived cytokines to play an important role in osteophyte formation[37], this may explain their location in dogs compared to humans.

The changes in the trabecular bone were not very pronounced in the Groove model. However, in the ACLT model, the bone volume fraction and trabecular thickness were clearly reduced. This corroborates the difference in rate of development of cartilage changes in both models. The changes in the ACLT model fit with previous studies in this model in dogs as well as cats[10-13]. The fact that other studies find an increase in bone volume fraction and trabecular thickness[14, 15] may be explained by the use of a different type of model, evaluated at a longer time period. Irrespective of the different changes in trabecular bone, similar changes in cartilage and subchondral plate were found in both models. Thus, it seems that the trabecular bone changes are not directly related to the changes in subchondral plate and cartilage. Since the subchondral plate changes consistently follow the cartilage changes, and the trabecular bone changes do not, the subchondral plate may play a more important role in the OA process than the trabecular bone changes.

The subchondral plate thickness decreased in both models at all time points in all experimental knees. This is in line with findings from previous studies concerning various animal models for OA, where subchondral plate thinning was documented in the early stage of the disease[12, 13, 38, 39]. In some of these studies, this early phase of thinning was followed by a later phase of plate thickening[12, 39]. This also explains the discrepancy with the sclerosis seen in most human studies[3, 4, 9], since such studies often concern patients with late osteoarthritis, whereas our present study examined only relatively early time points.

In order to justify the use of the contralateral knee as control, we calculated bone parameters in the diaphyseal and metaphyseal tibia, distal from the joint, containing cortical and trabecular bone, respectively. The bone volume of the metaphyseal tibia was significantly decreased in the experimental ACLT tibias, indicating disuse of the experimental ACLT knee. Thus, the trabecular bone loss in the epiphysis in the ACLT model may be explained by disuse. However, the tibias of the Groove model showed hardly any changes in the diaphyseal and metaphyseal bone parameters. Hence, we have no signs of disuse in this model. Both the ACLT and Groove model show similar subchondral plate thinning and increased porosity. Since the diaphyseal cortical bone showed no differences between control and experimental knee, we assume that in both models these subchondral plate changes are not caused by disuse of the treated leg.

The consistent decrease in subchondral plate thickness occurred already at 3 weeks post-surgery in the Groove model, whereas the cartilage changes were only very mild at this early time point (Fig 5, table 1). This suggests that the subchondral plate changes occur fast. Taken together with the fact that this cannot be explained by disuse, this indicates (at least in the Groove model) an interaction between cartilage and subchondral plate that induces bone resorption as a consequence of initiation of cartilage damage induced by the grooves. The thinning and drastically increased porosity of the subchondral plate may facilitate vascular invasion of the cartilage and diffusion of molecules from the damaged cartilage through the subchondral plate and vice versa, thereby enhancing the biochemical communication between bone and cartilage[40]. It is not clear if this bone cartilage communication interacts with an intrinsic repair activity of cartilage[41] or plays a role in the progression of the disease process[42].

We see differences in subchondral trabecular bone changes and osteophyte formation between the Groove model and the ACLT model, with the Groove model clearly showing a slower development of these changes. However, the severe loss of thickness and increased porosity in the subchondral plate are the same in both models. This quick and extensive loss of the subchondral plate thickness and increase in plate porosity cannot be explained by unloading and strongly suggests that cartilage- bone interplay is part of the etiology of osteoarthritis.

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Chapter 4

The canine bilateral Groove model of osteoarthritis

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J Orthop Res 2008; 26(11):1471-1477

ABSTRACT

Background. In studies aiming at local treatment of experimental osteoarthritis (OA) it is optimal to have an internal (untreated) OA control. Such an approach excludes inter-animal variation and allows paired statistical evaluation of treatment efficacy. For this purpose, we developed and characterized a bilateral version of the canine Groove model. We hypothesized that the bilateral version of the canine Groove model would show consistent and clear development of features of OA similar to those found in the unilateral version.

Methods. In 6 Beagle dogs, grooves were surgically made in the articular cartilage of the femoral condyles of both knee joints. Six additional dogs underwent bilateral sham surgery. The degree of OA was quantified 20 weeks after surgery and was compared in retrospect to 23 animals that underwent the same procedure in a single knee joint with the contra-lateral knee serving as a non-OA control.

Results. Bilateral groove surgery resulted in OA. This was based on the observed ineffective repair response in which an increase in proteoglycan synthesis, a diminished retention of these newly formed proteoglycans, and an enhanced loss of resident proteoglycans resulted in decreased cartilage proteoglycan content. These biochemical effects were corroborated by clear histological features of OA. All these effects were found in femur as well as in (surgically untouched) tibia. Interestingly, features of OA were slightly more severe in the bilateral model than in the unilateral variant.

Conclusion. The bilateral canine Groove model showed consistent and clear development of features of OA, comparable to the unilateral model.

Osteoarthritis (OA) is a degenerative joint disease, characterized by focal cartilage damage associated with changes in subchondral and marginal bone and synovial tissue. OA presents with pain and can be accompanied by stiffness, crepitus and swelling of the joint. Eventually this results in loss of joint function. Although age is the main risk factor for developing OA, this degenerative joint disease is not simply the result of lifelong use. Articular changes due to aging differ from those due to OA[1]. Age can be seen as one of many predisposing factors that make the joint more prone to develop the disease. A secondary (mechanical) trigger is likely to be involved in the actual set off of irreversible joint damage and by that induction of OA[2], specifically in larger weight-bearing joints such as the knee[3]. A mechanical trigger can be of high impact, as in trauma, or moderate but structural overload, as in obesity or malaligned joints[4].

One of the major problems of OA is that presently available treatment options are limited and aim at relieving symptoms instead of curing the disease[5]. An additional problem is that diagnosis cannot be made until advanced degenerative characteristics cause radiographic changes, since clinical symptoms are not specific enough for diagnosis to be based upon[6]. This leads to delayed diagnosis and with that delayed treatment. Therefore, more knowledge on early OA characteristics is necessary for early diagnosis, as well as unraveling the pathogenesis of OA to provide therapeutic targets for possible cure or attenuation early in the disease process.

In the clinical situation, a limited number of diagnostic and research parameters are available. Analysis of the quantity and quality of cartilage changes by X-ray and MRI is thus far not sufficiently sensitive. The use of diagnostic laboratory parameters such as biomarkers is also still in a developmental phase[7]. Research in animal models can help to reveal early symptoms, contribute to unraveling the pathogenesis of OA, and help to optimize treatment strategies by means of detailed biochemical, histological and macroscopical measurements[8].

Several animal models of OA have been developed over the years. Features of OA can be induced mechanically, surgically and chemically, and there are spontaneous models as well. All these models have their advantages and disadvantages[8, 9]. In surgical models, OA is usually induced by meniscectomy, meniscial tear or cruciate ligament transection (e.g. ACLT model)[9, 10]. The disadvantage of all these models is that the trigger for OA is permanent. When testing treatment strategies, except for surgical interventions, it is unexpected that a ligament or meniscus can be cured. Studies aiming at curing OA or slowing down the disease process are hampered by this permanent trigger which will interfere with treatment efficacy, assuming cartilage (and bone) repair is possible. Moreover, a permanent trigger does not provide the opportunity to test prolonged follow-up after transient treatment strategies.

To overcome these disadvantages, the unilateral canine Groove model was developed[11]. In this model, the articular cartilage of the weight-bearing areas of the femoral condyles is damaged to initiate development of OA. In this model progressive cartilage damage appeared intrinsic to the tissue itself and is not primarily depending on synovial inflammation[11].

In the Groove model care is taken not to damage subchondral bone in order to exclude possible repair activities by bone marrow derived precursor cells[12]. Also surgically-induced synovial damage and intra-articular bleeding are kept to a minimum to prevent inflammatory activity. Loading of the experimental joint is thought to be of influence in the development of osteoarthritis. To ensure loading, in the unilateral Groove model the contra-lateral control joint is fixed to the trunk of the animal intermittently (3 times weekly) for short periods (2-3 hours). In 10 to 20 weeks this leads to experimental OA demonstrated by the characteristic macroscopic, histological, and biochemical features. Progression of these degenerative features is observed between 20 and 40 weeks after induction[13]. The model is not simply the expression of the surgically applied grooves because shortly after surgery (3 weeks) repair activity, without features of OA, is demonstrated[14]. Overall, the model resembles an early stage of (secondary) OA, specifically suitable to test disease-modifying osteoarthritis drug (DMOAD) activity, aiming at curing the disease.

OA treatment can be applied locally or systemically. When testing OA treatment, efficacy is measured by comparing the treated joint with an untreated joint. With local treatment, a bilateral OA model has the advantage of providing an internal animal OA-control, enabling paired statistical evaluation of treatment efficacy and excluding inter-animal variations. This results without loss of statistical power in a reduction of the number of animals needed or with the same number of animals in the advantage that smaller differences can be detected.

Surprisingly few studies have been published on bilateral animal models for OA. Marshall was one of few evaluating the ACLT model bilaterally[15]. Symmetrical canine knee OA was induced and the model was thought to be a potent model for investigating fundamental OA mechanisms and therapeutic approaches. Unfortunately, this model still has the disadvantage of permanent joint instability. Therefore, in the present study the bilateral variant of the Groove model is evaluated. We hypothesized that the bilateral version of the canine Groove model would show consistent and clear development of features of OA similar to those found in the unilateral version.

MATERIALS AND METHODS

Animals

Beagle dogs (n=12 females, mean age 1.9 ± 0.02 years, weighing 11.5 ± 0.3 kg), were obtained from the animal laboratory of Utrecht University, The Netherlands. They were housed in groups of two dogs in indoor-outdoor pens and were let out on a large patio in groups for at least two hours a day. The feeding consisted of a standard diet and water *ad libitum*. The dogs were divided randomly in two groups of six animals each. The ethical committee for animal studies of Utrecht University gave its approval for this study.

Anesthesia, general surgery and post-surgical treatment

After induction with Nesdonal, the dogs were anaesthetized with halothane in a mixture of oxygen and nitrous oxide. Surgery (see below for details) was performed through a 2-2,5 cm medial incision close to the *ligamentum patellae* in both knees. Bleeding and soft tissue

damage was prevented as much as possible. After surgery, synovium, fasciae and skin were sutured separately. The animals received analgesics (Buprenorphine 0.01 mg/kg) and antibiotics (Amoxicillin 400 mg/kg) during the first three days after surgery. Starting two days after surgery, the dogs were allowed on a daily basis on the patio again. Twenty weeks after surgery the dogs were killed by an intravenous injection of Euthesate (sodium pentobarbital). Both hind limbs were amputated.

Surgical procedures

In 6 animals the cartilage of the lateral and medial condyles of both knees was damaged using a Kirschner-wire (1.5 mm diameter), which was bent at a 90 degree angle at 0.5 mm from the tip. This ensures that the depth of the grooves was restricted to 0.5 mm. The thickness of articular cartilage of the condyles in a canine knee joint is 0.73 ± 0.03 mm as measured by Frisbie[16]. In utmost flexion, approximately ten longitudinal and diagonal grooves were made on the weight-bearing parts of the femoral condyles without damaging the subchondral bone. The latter was checked by histology at the end of the experiment. There was no absolute visual control over the procedure, but macroscopic evaluation at the end of the experiment showed similar patterns in all affected knees. The other six animals received a sham treatment. Identical procedures were followed except for the actual grooving of the cartilage. Groove and sham dogs and right and left knees were alternately operated to prevent surgical differences between the sham and groove dogs and right and left joints.

Cartilage and synovial tissue

Within four hours after the hind legs were amputated, the following procedures were performed under laminar airflow conditions. From each animal the left (n=3) or right (n=3) knee joint was evaluated (the other knee joints were used for alternative purposes).

After opening the knee joint, synovial tissue and cartilage samples were taken of predefined positions. All samples were weighed (accuracy 0.1 mg) and placed immediately in 200 µl culture medium (Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 0.085 mM ascorbic acid, 2 mM glutamine (Gibco), 100 IU/ml penicillin, 100 mg/ml streptomycin and 10% heat-inactivated Beagle serum).

Synovial tissue analysis

Three retro-patellar synovial tissue samples per joint (medial, middle and lateral) were fixed in 4% phosphate-buffered formalin (pH 7.0) and embedded in paraffin. Deparaffined sections were stained with haematoxylin-eosin. The histological sections were examined separately in random order and independently by two observers who were not aware of the source of the tissue. Each specimen was analyzed to determine the degree of inflammation, using slightly modified[17] criteria described by Goldenberg and Cohen[18]. For assessing the overall grade the three specimens from each knee were considered as a unit and scores of the two observers were averaged.

Cartilage analysis

Cartilage samples for histological and biochemical analyses were obtained from predefined locations on the weight-bearing areas of the femoral condyles and the tibial plateau[11]. Cartilage was cut as thick as possible, while excluding the underlying bone.

For histology, four samples from the tibial plateaus and four from the femur condyles were taken. All samples were fixed in 4% phosphate-buffered formalin containing 2% sucrose (pH 7.0). Cartilage degeneration was evaluated in safranin-O-fast-green iron haematoxylin-stained sections by light microscopy according to the slightly modified[19] criteria of Mankin[20]. Specimens were graded in random order by two observers unaware of the source of the cartilage. The average scores of the four different cartilage samples were used for statistical evaluation.

For biochemical analysis, the cartilage samples were cultured individually in 96-well culture plates (NUNCLON®, Denmark) in 200 µl culture medium. Cartilage explants were cultured according to standard procedures as described previously[19]. For femur condyles and tibial plateau, cartilage proteoglycan (PG)-synthesis, retention of newly formed PGs, PG-release and PG-content were determined and averaged for eight explants per parameter per joint surface.

PG-synthesis. As a measure of PG-synthesis, the rate of sulphate incorporation was determined *ex vivo*. After one hour of pre-culture, 370 kBq $Na_2{}^{35}SO_4$ (Dupont, NEX-041-H, carrier free) in 10 µl DMEM was added to each sample. After four hours of labeling, the cartilage samples were washed three times with medium for 45 minutes. Subsequently samples were cultured for three days without label. Cultures were stopped by washing two times with cold phosphate buffered saline (PBS) and freezing of the samples.

Glycosaminoglycans (GAGs) in a papain digest of cartilage samples were precipitated with Alcian Blue and $^{35}SO_4{}^{2-}$–labeled GAGs were measured by liquid scintillation counting. The total sulphate incorporation rate of each cartilage sample was calculated using the specific activity of the medium and was normalized for labeling time and wet weight of the explants.

Synthetic activity (nmol/h.g) is calculated by the sum of the total sulphate incorporation in the explant and the release of newly formed PGs in the medium during the successive three day culture period.

Retention of newly formed proteoglycans. To determine the release of the newly synthesized PGs as a measure of the retention of these PGs, the release of $^{35}SO_4{}^{2-}$-labeled GAGs in the three day culture medium was determined. GAGs were precipitated from the medium with Alcian Blue, as described[19]. The $^{35}SO_4{}^{2-}$–labeled GAGs were measured by liquid scintillation counting and the release was calculated using the specific activity of the medium normalized to the wet weight of the explants. The release of newly formed PGs is corrected for the synthesis rate and expressed as percentage release of newly formed PGs in three days (% new PG release).

PG loss. For the total release (loss) of PGs, GAGs in the culture medium were precipitated and stained with Alcian Blue and quantified photometrically by the change in absorbance at 620 nm with chondroitin sulphate (Sigma C4384) as a reference. The total amount of GAGs released (blue staining) is expressed as a percentage of the PG-content (% GAG release).

PG-content. To measure the PG-content of the cartilage samples, the amount of tissue GAG was determined. The GAGs in the papain digest of cartilage samples were precipitated, stained with Alcian Blue, and quantified as described above. Values were expressed per wet weight of the cartilage tissue (mg/g).

The unilateral canine Groove model of OA

Retrospective data from twenty-three female Beagle dogs, previously the OA controls from four different studies were used (mean age 2.6 ± 0.4 years, weighing 11.9 ± 0.2 kg; both not statistically significantly different from the presently used group of animals). In these animals OA was induced unilaterally, according to identical procedures as described above and evaluated twenty weeks after induction of OA. Animals were housed, fed and treated identically as described above. In these studies the contra-lateral control joint was fixed to the trunk of the animals for at least two hours a day to assure loading of the affected joint[11, 13, 14]. Contra-lateral unoperated knee joints served as controls.

Calculations and statistics

Absolute mean values ± SEM of six bilateral shams, six bilateral OAs, and twenty-three unilateral OAs with the twenty-three contra-lateral control joints are presented. To analyze differences between bilateral OA and bilateral sham animals the unpaired non-parametric Mann-Whitney U test was used. For the evaluation of the OA joints and their contra-lateral controls in the unilateral model the non-parametric paired Wilcoxon test was used. To compare the differences between the bilateral sham and unilateral controls and bilateral OA and unilateral OA joints unpaired Mann-Whitney U test was used as well. Two-sided *p*-values are presented and when less than 0.05 they were considered to indicate statistically significant differences.

RESULTS

In general no differences were observed between the control joints of both models (i.e. between the joints with sham surgery in the bilateral model and the unoperated contra-lateral in the unilateral model). When occasional small differences reached statistically significance, they were considered of limited biological significance since these differences were much smaller than the differences between controls and OA joints in each of the models (see figures 1-3, where all p values are shown).

Cartilage integrity

Microscopical evaluation of cartilage in the bilateral Groove model showed for both femoral condyles and tibial plateau (representative micrographs shown in figure 1A/B) features of OA. These features included loss of safranin-O, fibrillation of the articular surface, and chondrocyte clustering as represented by the modified Mankin grade (figure 1C) and corroborated the decrease in GAG-content (figure 1D) observed in both femoral and tibial cartilage. With respect to cartilage integrity, the changes in femoral and tibial cartilage were

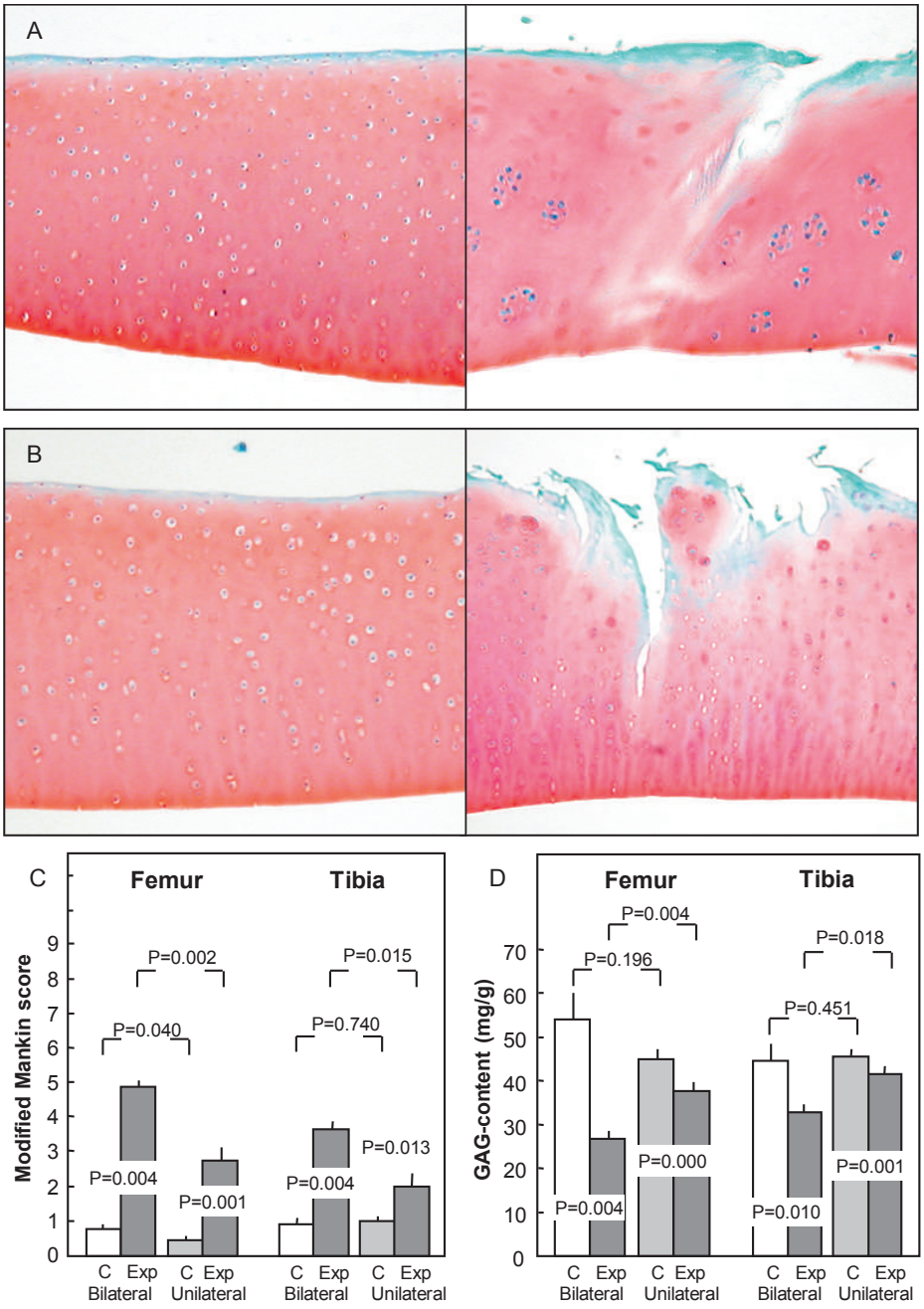

Figure 1. Cartilage Integrity. Representative photographs of control (sham operated; right) and grooved (left) femur condyle (A) and tibial plateau (B) cartilage of the bilateral model. Average histological scores (Modified Mankin grade) for both the bilateral (C = sham operated joints, Exp = grooved OA joints) and unilateral (C = contra-lateral control joints, Exp = grooved OA joints) model are shown in panel C. GAG-content (biochemically determined) as a parameter representing cartilage integrity is shown in panel D. P-values are given.

comparable in the bilateral Groove model, as well as in the unilateral model. Notwithstanding, GAG content and microscopic cartilage damage were more severe in the bilateral model than in the unilateral variant of the model, reaching statistical significance for both parameters in femur and tibia.

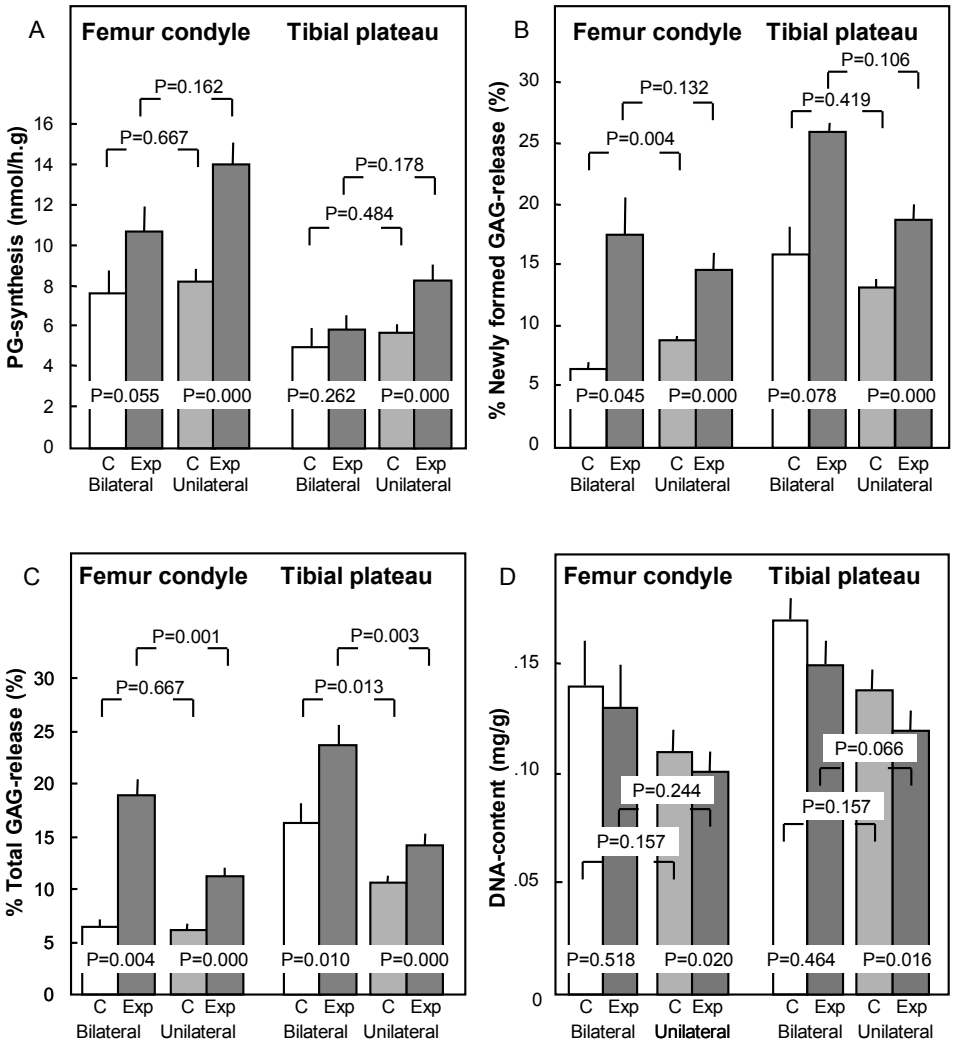

Figure 2. Chondrocyte activity as measured by proteoglycan synthesis rate (panel A), release of newly formed proteoglycans normalised to synthesis rate, expressed in percentage (B), total proteoglycan release normalised to proteoglycan content, expressed in percentage (C) and DNA-content as a measure of cartilage cellularity (panel D) is shown. Average results for both the bilateral (C = sham operated joints, Exp = grooved OA joints) and unilateral (C = contra-lateral control joints, Exp = grooved OA joints) model are shown. P-values are given.

Chondrocyte activity

The synthesis of proteoglycans (figure 2A) was increased in the experimental joints compared to the control joints in the bilateral model although not statistically significant for either femur or tibia.

Retention of these newly formed proteoglycans was diminished in the bilateral Groove model; percentage loss of newly formed proteoglycans was enhanced (figure 2B). Loss of proteoglycans as measured by relative total GAG release (figure 2C) was enhanced in femoral condyles and the surgically untouched tibial plateau, statistically significant for both. The DNA-content was slightly decreased in the OA cartilage but not statistically significant between shams and grooved joints in the bilateral model (figure 2D).

All osteoarthritic changes that were previously observed in the unilateral model were also observed in the bilateral model. However, the proteoglycan metabolism was more severely disturbed in the bilateral model. Synthesis rate was less enhanced, retention was more impaired, and loss of proteoglycans was higher in the bilateral model compared to the unilateral model, although only statistical significant for the latter. No statistical significant differences were found for DNA content between both models.

Synovial inflammation

The microscopic score of the synovial tissue of the OA joints compared to the sham operated joints (representative micrographs given in figure 3A and 3B), showed mild inflammation with occasional villi formation and minor thickening of the synovial lining. On average, synovial inflammation was of higher statistical significance in OA joints than in sham joints.

Mild inflammation was also seen in the unilateral variant of the model. Based on the microscopical score the degree of inflammation in the bilateral model was not different from the unilateral model. The control joints in the unilateral model demonstrated slightly more synovial triggering then the controls (shams) in the bilateral model (figure 3C).

DISCUSSION

The present study evaluates the bilateral version of the canine Groove model of knee osteoarthritis (OA), in which OA is induced by surgically applied chondral damage. The bilateral version shows consistent and clear development of features of OA, slightly more severe than those found in the unilateral version.

A bilateral model provides an internal animal OA-control, enabling paired statistical evaluation of treatment efficacy in case of local treatment, excluding inter-animal variations. The Groove model is distinctive in that the canine experimental OA seems intrinsic to the cartilage which allows evaluation of efficacy of therapies directed at cartilage metabolism. Combining these two aspects, the present description of the bilateral version of the Groove model is innovative and may add to further understanding of pathogenesis and treatment efficacy of OA.

When evaluating the bilateral canine Groove model, all parameters showed (statistical) significant changes pointing towards OA-related joint degeneration. Although the groups consisted of only six animals, this small number was sufficient to demonstrate the changes with unpaired statistical analysis compared to sham operated animals. This underscores that OA changes in this model are very consistent between animals. Chondrocyte activity revealed an increased PG-synthesis rate, in an attempt to repair cartilage, a mechanism characteristic for mild to moderate OA. This enhanced synthesis rate was ineffective as retention of these newly formed PGs was poor, demonstrated by an enhanced release of these newly formed proteoglycans. Also the total loss of proteoglycans was enhanced demonstrating a net loss of one of the major cartilage matrix components. Cartilage integrity, as a result, was clearly impaired as demonstrated by a decreased GAG-content, corroboration histology of the cartilage and macroscopic evaluation of the cartilage surfaces.
All parameters for degenerative changes as seen in the surgically harmed femoral cartilage are also observed in the surgically untouched tibial cartilage. Incongruity of the cartilage surfaces, as well as the release of soluble mediators such as proteases[21, 22] by the cartilage, are the mechanical and biological triggers for the spreading of degenerative changes.
In all these aspects, the bilateral Groove model shows clear characteristics of degenerative cartilage damage as seen in human OA[23] and other canine models[11]. Although several other OA related parameters have been determined, only parameters were described that were available in retrospect from the unilateral Groove model. In this way, outcome could be compared to the unilateral version of the model.
Care was taken to prevent a left-right bias (e.g. during surgery or processing of materials) but no comparison between left and right joints could be made because it was chosen to use only one joint of each animal (three left and three right joints) for the evaluation of the parameters as described. The contra lateral joints were used for other purposes. A comparison between these three left and right joints did not reveal a difference other than the variation between animals. Therefore, it is expected that the joint degeneration in this bilateral model develops with the same pace and to the same extend in both knee joints.

Results demonstrate that the bilateral version of the Groove model, at 20 weeks post-surgery, reveals slightly more severe OA than the unilateral version. This difference could be the result of a comparison of prospective data with retrospectively obtained data. However, surgery and all additional handling of the animals as well as all histological and biochemical analyses were for both the unilateral and the presently described bilateral model, performed by the same personnel, using the same techniques, under the same conditions. Moreover, parameters for bilateral sham operated animals were not different from those of the control joints in the unilateral model. This corroborates previous statements that the contra-lateral knee can be used as an internal control[13]. Whether the minor differences in severity between the bilateral and unilateral version are more or less apparent at other time points needs further study.

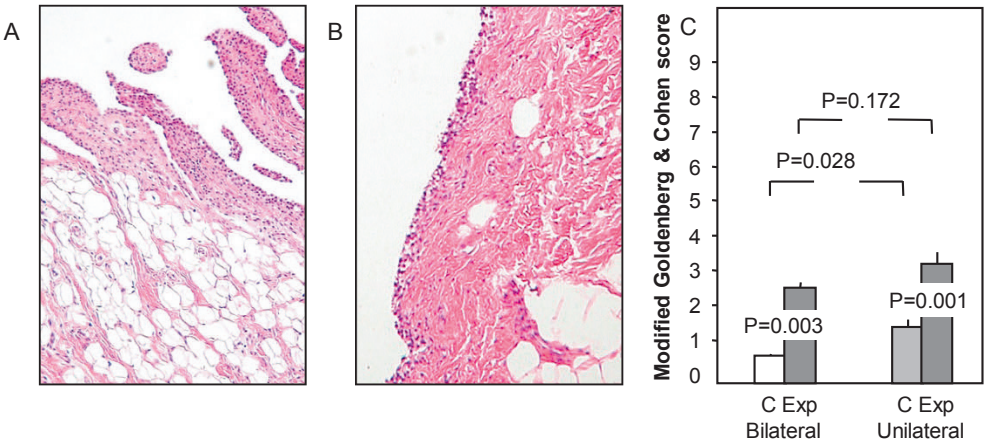

Figure 3. Representative photographs of synovial tissue obtained from grooved (A) and control (sham operated; B) joints of the bilateral model. Averaged histological score (Modified Goldenberg & Cohen score) of the bilateral (C = sham operated joints, Exp = grooved OA joints) and unilateral model (C = contra-lateral control joints, Exp = grooved OA joints) are shown in panel C. P-values are given.

A difference in actual loading of the affected joints might account for the difference in severity of OA between both versions of the Groove model. In the unilateral Groove model, forced loading of the experimental joint is applied because the animals are quadruped and can easily unload the affected joint. Although never tested, this is, based on literature[24-26], expected and presently under investigation a prerequisite to develop OA in this model. Assuming that loading of the experimental joint is of major importance in the development OA in the Groove model, it might be concluded that in case of the bilateral model experimental joints receive more loading when compared to the unilateral Groove model. This might indeed be the case because fixating the contra-lateral leg to the trunk of the animal is inconvenient for the animals, and although it forces to load the experimental joint, this joint will only be loaded when the animals are moving around. The bilateral operated animals are not hampered by this inconvenience and because they do not have instable joints (as in case of ACLT induced OA), and the damaged cartilage itself is not painful (cartilage is not innervated[1]), the animals may have loaded their joints better than in case of unilateral induced OA model. Subjectively evaluated this appeared indeed the case. This might explain the slightly more severe cartilage damage in the bilateral model compared to the unilateral Groove model.

Inflammation in the Groove model in both versions is mild[11]. This corroborates with primary OA in humans, where inflammation is secondary and mostly mild.

Despite its possibilities and advantages, the use of bilateral animal models for OA is uncommon. The present study demonstrated that a bilateral canine model of OA provides

reliable macroscopic, histological, and biochemical OA-related degenerative changes in cartilage accompanied by mild synovial inflammation in a period of 20 weeks post surgery. For systemic treatment the unilateral version has the advantage that an internal non-OA control is present, limiting the number of animals needed to design a proper study. The bilateral model has the advantage that it allows evaluation of local (intra-articular) treatment with the advantage of an internal OA animal control. This results, without loss of statistical power, in a reduction of the number of animals needed, or with the same number of animals, in the advantage that smaller differences can be detected. In this respect the bilateral canine Groove model might be the OA model of choice in case local chondroprotective treatment has to be evaluated.

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Chapter 5

Similarities and discrepancies in subchondral bone structure in two differently induced canine models of osteoarthritis

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J Bone Mineral Res 2010;25(7):1650-1657

ABSTRACT

Background. In osteoarthritis (OA), cartilage degradation is accompanied by subchondral bone changes. The pathogenesis and -physiology of bone changes in OA are still unclear. The changes in subchondral bone architecture and cartilage damage were compared in differently induced experimental models of OA.

Methods. Experimental OA was induced bilaterally by Anterior Cruciate Ligament Transection (ACLT) or by cartilage trauma (Groove model); bilateral sham surgery served as control. Lysylpyridinoline (LP; bone resorption) and C-telopeptide of type II collagen (CTX-II; cartilage breakdown) were measured over time. At 20 weeks post-surgery, the subchondral cortical plate and trabecular bone of the tibia were analyzed by micro-CT and cartilage degeneration was analyzed histologically and biochemically.

Results. In both models cartilage degeneration and cortical subchondral plate thinning were present. CTX-II levels were elevated over time in both models. Subchondral trabecular bone changes were only observed in the ACLT model, not in the Groove model. Correspondingly, LP levels were elevated over time in the ACLT model and not in the Groove model. Interestingly, the trabecular bone changes in the ACLT model were extended to the metaphyseal area.

Conclusion. The early decrease in plate thickness, present in both models as was cartilage damage, suggests plate thinning to be a phenomenon intrinsic to the process of OA, independent of the cause/induction of OA. On the other hand, trabecular changes in subchondral and metaphyseal bone are not part of a common pathway of OA development and may be induced biomechanically in the destabilized and less loaded ACLT joint.

Osteoarthritis (OA) often presents with pain and is accompanied by stiffness, crepitus, and swelling of the joint. Structural changes underlying these clinical features are damage of the articular cartilage, changes in the subchondral bone structure, osteophyte formation at the joint margins, and synovial inflammation. The etiology of OA is complex and includes various genetic, biochemical, and mechanical factors. In fact, the pathogenesis of OA is poorly understood[1, 2].

Radiographs of osteoarthritic joints show an increased density of the subchondral bone, defined as subchondral sclerosis. Changes in architectural structure are suggested to be responsible for this increase in density. In established OA, studies of bone structure have shown that the subchondral cortical plate has thickened[3] and the volume of the trabecular bone has increased[4, 5]. It is suggested that the increased stiffness of sclerotic bone in OA might play a role in the progression of cartilage degeneration[6]. Whether these subchondral bone changes as observed in established OA, precede, occur simultaneously, or are the result of cartilage degeneration is still subject of discussion[3, 7]. Studies in human end stage hip OA showed both cartilage deterioration without trabecular bone changes[4], and bone changes without local cartilage changes[5]. Unfortunately, in human studies (*in vivo* or *ex vivo)* mostly established (severe) OA is studied whereas longitudinal data on subchondral bone changes from onset until full blown clinical osteoarthritis are lacking. Early OA is difficult to detect clinically. Sensitivity of existing noninvasive (*in vivo*) analytical methods of subchondral bone changes is low and small changes are difficult to quantify[8].

Several studies in the rabbit and canine anterior cruciate ligament transaction (ACLT) model of OA[9] showed a decrease of trabecular bone volume[10-13] and a thinning of the subchondral plate[13-15] early in the disease process. These changes are in contrast to those found in human end stage OA[4, 5, 16]. However, changes of subchondral bone in experimental guinea pig models at a more advanced stage were found comparable to those in human end stage OA[17, 18]. This suggests that bone remodeling in preclinical animal models of OA is biphasic: an early decrease in trabecular bone volume followed by a phase in which the subchondral bone becomes denser and stiffens. Recently, the early overall decrease in bone volume was also found in early human OA[19] supporting the biphasic response theory.

In the present study we focused on the early phase of OA since early diagnosis and treatment are the challenge for the future. There are many different models of early OA with different causes. E.g. in the canine Groove model, OA is induced by surgically damaging the cartilage of the femoral condyles, leading to a progressive cartilage degeneration, also in the surgically untouched tibial plateau[20-22]. In the canine ACLT model, joint instability is the trigger for features of OA[9]. It might well be that despite a final common outcome regarding cartilage damage with plate thickening and increased subchondral trabecular bone volume the initial process with respect to bone changes may differ between various subpopulations (and models) of OA depending on the cause (or induction).

Most recently, Sniekers *et al.* published a pilot study suggesting that early in the disease process subchondral trabecular bone changes occur in the ACLT model but not in the Groove model whereas cartilage damage was comparable[13]. As such, trabecular bone changes would be unrelated to cartilage pathology. However, this study was limited in

number of animals and used a unilateral variant of both models with potential altered loading in control joints due to presence of OA in the contra-lateral joint. Therefore, the suggested uncoupling of cartilage damage and subchondral trabecular bone changes prompted us to study the structural changes of both bone and cartilage early in the process of OA in more detail in the bilateral versions of the two different models of OA[23, 24]. Biomarkers were used to study cartilage degradation and bone loss over time and as endpoint outcome, micro-CT of bone and biochemical and histological evaluation of cartilage were performed.

MATERIALS AND METHODS

Animals

Skeletally mature Beagle dogs (n=18 females, mean age 1.9 ± 0.1 years, weighing 11.8 ± 0.3 kg), were obtained from the animal laboratory of Utrecht University, the Netherlands. They were housed in groups of two dogs in indoor-outdoor pens and were let out on a large patio in groups for at least two hours a day. The feeding consisted of a standard diet and water *ad libitum*. The dogs were divided randomly in three groups of six animals each. The study was approved by the Utrecht University Medical Ethical Committee.

Surgical procedures and post-surgical treatment

Under general anesthesia, surgery was performed through a 2-2.5 cm medial incision next to the *ligamentum patellae* in both knees of the 18 animals.

In 6 animals, OA was induced bilaterally according to the ACLT model[9, 20, 24]; the anterior cruciate ligament was transected, using a pair of blunt curved scissors, making sure no other structures were damaged. A successful procedure was established by a positive anterior drawer sign.

In 6 other animals, OA was bilaterally induced according to the Groove model[23]. The cartilage of the lateral and medial femoral condyles of both knees was damaged using a Kirschner-wire (1.5 mm diameter), which was bent at a 90 degree angle at 0.5 mm from the tip. This ensures that the depth of the grooves was restricted to 0.5 mm. In utmost flexion, approximately ten longitudinal and diagonal grooves were made on the weight-bearing parts of the femoral condyles without damaging the subchondral bone. There was no absolute visual control over the procedure, but macroscopic evaluation at the end of the experiment showed a similar pattern in all affected knees.

In the remaining 6 animals sham surgery was performed. Identical procedures were followed visualizing the femoral condyles and the anterior cruciate ligament except for the actual grooving of the cartilage or transection of the anterior cruciate ligament.

ACLT, Groove, and sham dogs, as well as left and right joints were alternately operated on to prevent surgical differences between the different groups and joints. Bleeding and soft tissue damage was prevented as much as possible. After surgery, synovium, fasciae, and skin were each sutured. The animals received analgesics (Buprenorphine 0.01 mg/kg, intra-muscular injections twice daily) and antibiotics (Amoxicillin 400 mg/kg, oral administration

twice daily) during the first three days after surgery. Starting two days after surgery, the dogs were allowed on a daily basis on the patio again.

Longitudinal markers of bone and cartilage breakdown

Urine samples were collected twice before and every 2 to 3 weeks during the development of OA. As representative of bone resorption, lysylpyridinoline (LP) was measured in these samples. LP is a type I collagen crosslink residue considered specific for bone breakdown[25]. The mature collagen cross-link LP was determined in acid hydrolysates of the urine samples by HPLC as described previously[26]. In addition urine samples were assayed for CTX-II a marker considered representative of cartilage degradation[27]. CTX-II was measured by ELISA (Cartilaps; Nordic Bioscience) according to manufacturer's instructions. Both LP and CTX-II were corrected for urine dilution by urine creatinine level (Cayman Chemicals).

End-point parameters

Twenty weeks after surgery the dogs were killed by an intravenous injection of Euthesate (sodium pentobarbital). Within four hours the hind legs were amputated. From each animal alternately the left (n=3) or right (n=3) knee joint was evaluated (the other knee joints were used for alternative purposes). Because in the Groove model surgically grooving could have led to subchondral bone changes not intrinsic to the OA process, in all further analysis only the surgically untouched tibial plateaus were evaluated.

After opening the knee joint, digital high resolution photographs were taken from the tibial plateau for blinded macroscopical scoring of cartilage damage. Subsequently, cartilage samples were taken from predefined locations of the weight bearing area of the tibial plateaus as described previously[20]. All samples were weighed (accuracy 0.1 mg) and stored for further analysis.

Micro-CT analysis

The proximal part of the tibias was scanned in a micro-CT scanner (Skyscan 1076, Skyscan, Antwerpen, Belgium) with a voxel size of 18 μm. The reconstructed data set was segmented with a local thresholding algorithm[28].

In both the medial and the lateral part of each tibial scan, a cylinder with a diameter of 4.0 mm and a height of 3.5 mm (medial) or 3.1 mm (lateral) was selected. By use of anatomical landmarks the cylinders were located in the middle of the weight bearing area (figure 1A). They contained trabecular bone and subchondral plate (figure 1B).The trabecular bone and subchondral plate were separated automatically using in-house software (*Erasmus MC, Rotterdam*; for representatives see figure 3). For the trabecular bone, bone volume fraction, which describes the ratio of bone volume over tissue volume (BV/TV), three-dimensional trabecular thickness (TbTh)[29], structure model index (SMI), a quantification of how rod-like or plate-like the bone structure is[30], and connectivity density (CD), describing the number of connections per volume[31], were calculated. For the subchondral plate, the three-dimensional plate thickness (PlTh)[29] was calculated. For these bone parameters, the data from the lateral

and medial epiphyseal cylinders of each animal were averaged and used for statistical evaluation.

To analyze whether the trabecular changes were specific for the subchondral area, an additional region further away from the joint space was analyzed. In advanced stages of OA, changes of the underlying bone as shown by imaging techniques (x-ray / MRI / scintigraphy), are restricted to subchondral/periarticular area and do not extend through the metaphyseal area. If changes occur in the quickly adaptive trabecular bone of the metaphysis, they are more likely to be the result of a change in mechanical load of the whole proximal tibia. Bone changes in the metaphyseal area, distant from the cartilage, were considered not intrinsically related to cartilage degeneration as they are not likely to be directly influenced by changes in mechanical characteristics of cartilage, or by chemical factors released from cartilage and synovium during the process of joint degeneration. Therefore, a cylinder (width: 5,5 mm, height: 3,5 mm) was selected in the metaphyseal area of the tibia, distal from the growth plate remains. This cylinder contained only trabecular bone (figure 1A), of which the same trabecular bone characteristics as described above were calculated.

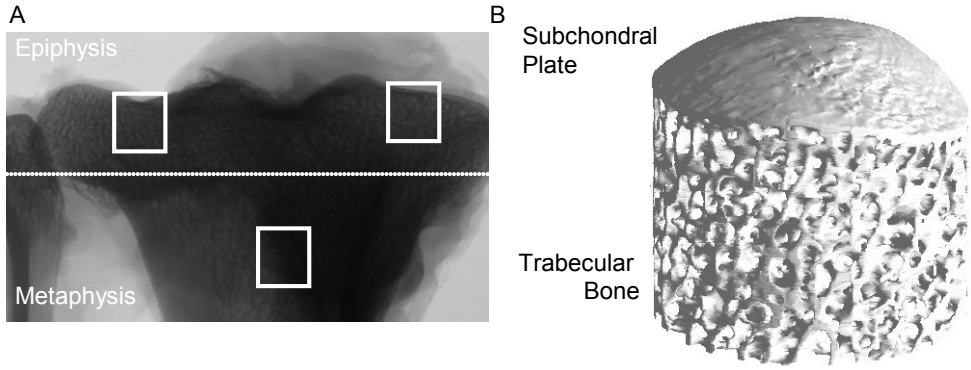

Figure 1. A. The three locations of which evaluations were performed, indicated by squares, two subchondral in the epiphysis and one in the metaphysis. B. A three dimensional representative picture of a cylinder of (diameter 4mm x height 3.1-3,5 mm) consisting of subchondral plate and underlying trabecular bone as evaluated by micro-CT (see also figure 3).

Cartilage analysis

Macroscopic cartilage degeneration was evaluated on photographs by two observers unaware of the source of the photographs. Severity of cartilage degeneration of the tibia was graded from 0 to 4: 0=smooth surface, 1=roughened, 2=slightly fibrillated, 3=fibrillated, 4=damaged[32]. Scores of the two observers were averaged (maximum of 4) and used for statistical analysis.

For histology, four samples from predefined locations of the tibial plateau (two lateral and two medial) were fixed in 4% phosphate-buffered formalin containing 2% sucrose (pH 7.0). Cartilage degeneration was evaluated in safranin-O-fast-green iron haematoxylin-stained sections by light microscopy according to the slightly modified[33] criteria of Mankin[34]. Specimens were graded in random order by two observers unaware of the source of the cartilage. The average score (a maximum of 11) of the medial and lateral tibial compartment of each animal was used for statistical evaluation.
For biochemical analysis, Proteoglycan (PG)-content, one of the main components of cartilage, was determined from 6 samples taken from predefined locations. Details of biochemical analysis, using Alcian Blue staining, have been described previously by Mastbergen *et* al[22, 32]. In addition, damage of the cartilage collagen (type II) was assessed from 4 samples from predefined locations by selective proteolysis using α-chymotrypsin, which only cleaves off damaged, denatured collagen and leaves the intact triple-helical collagen behind. The soluble fraction (denatured collagen) was quantitatively separated from the insoluble fraction (intact collagen). Hydroxyproline levels were colorimetrically determined in both fractions after acid hydrolysis. The percentage of denatured collagen was calculated as (hydroxyproline in supernatant) / (total hydroxyproline) x 100%[10].
Results for PG-content and denatured collagen were averaged for all the samples from the lateral and medial compartment.

Calculations and statistics
Absolute mean values ± SD of six sham, six Groove, and six ACLT animals are presented. To analyze differences between OA and sham and between the two OA models the unpaired non-parametric Mann-Whitney U test was used.

RESULTS

Longitudinal evaluation of bone breakdown
Urine level of lysylpyridinoline (LP) as a representative of bone resorption, was elevated from week 6 until week 20 (the end of the experiment) in the ACLT model. In the Groove model no such increase in bone resorption was observed. At all time points from week 6 on the levels were statistically significantly higher in the ACLT model compared to the sham surgery group (figure 2A; a: $p<0.05$) as well as compared to the Groove model group (all $p<0.05$). Also the area under the curve of the whole 20 weeks was statistically significantly higher in the ACLT group compared to both other groups ($p<0.05$ and $p<0.01$, for sham and Groove group, respectively).

Subchondral bone
Subchondral plate: The subchondral plate thickness was significantly reduced in the Groove model and in the ACLT model compared to the sham group (both statistically significant; figure 3). Although the decrease in thickness tended to be larger in the ACLT model, this was not statistically significantly different from the Groove model.

Subchondral trabecular bone: Subchondral trabecular bone in the ACLT model showed statistically significantly less bone volume fraction (BV/TV; figure 4A left panel) and less trabecular bone thickness (TbTh; figure 4B left panel). This was also reflected in the higher structure model index (SMI; figure 4C left panel) and higher connectivity density (CD; figure 4D left panel) that indicated a more rod-like structure and generation of more pores in the original structure, resulting in more connections per volume. Interestingly, in the Groove model, these differences compared to sham animals were only seen as a tendency, not statistically significantly different from the sham group.

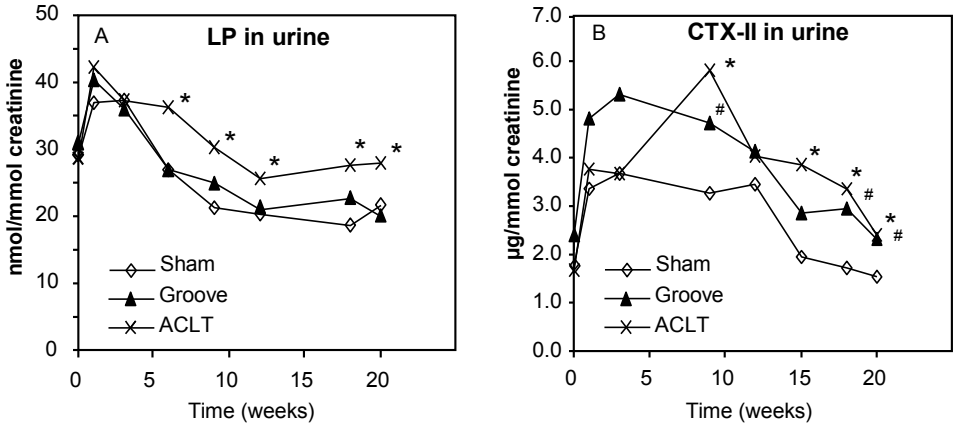

Figure 2. Levels of Lysylpyridinoline (LP) (A) and CTX-II (B) in urine normalized for urinary creatinine during the course of OA development for the three groups of animals. Mean values for each time point are given. * and # indicate statistically significant difference ($p<0.05$) from the sham group for the ACLT and Groove model, respectively.

Metaphyseal trabecular bone

Because of the significant difference in subchondral trabecular bone characteristics between both models trabecular bone changes in the metaphyseal part of the tibia were studied as well. Potential changes in this part of the bone are considered not to be intrinsically related to the cartilage degenerative process. Although absolute values of healthy bone at the two locations (subchondral epiphysis and metaphysis) were different due to differences in actual structure of the trabecular bone, it appeared that in the ACLT model bone volume fraction and trabecular thickness were lower compared to the sham group, while the structure model index was higher, similar as found in the subchondral trabecular bone (figure 4A, B, and C; right panel). In the Groove model, metaphyseal trabecular bone structure was not statistically different from the sham group, as observed for the subchondral trabecular bone. Again a slight tendency to a change in the direction of the ACLT model was observed. Connectivity density in the metaphyseal bone was in both models not different from the sham group (figure 4D, right panel).

Longitudinal evaluation of cartilage breakdown

Urinary CTX-II levels as a representative of cartilage breakdown were elevated from week 10 until week 20 in the ACLT model, and from week 1 in the Groove model. At all time points from week 10 on (except for one) the levels were statistically significantly higher for the ACLT model and Groove model when compared to the sham surgery group (figure 2B). In addition, the area under the curve was statistically significantly higher in the ACLT and Groove model when compared to the sham group ($P<0.05$ and $P<0.01$, respectively). No difference was observed between both OA models except for an earlier (at 1 and 3 weeks) increase in CTX-II levels in the Groove model compared to the ACLT model, which was anticipated based on the way cartilage damage is induced in both models (direct cartilage damage and joint instability, respectively).

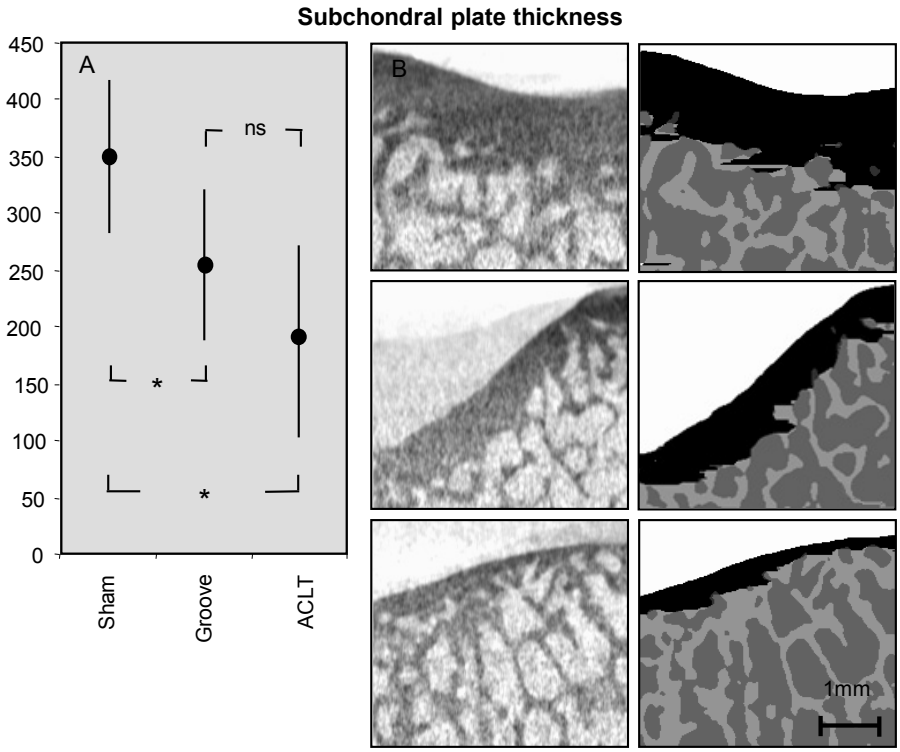

Figure 3. A. Micro-CT analysis of the tibial subchondral plate. Mean ± SD of the Plate Thickness (PlTh) is given; * indicating $p<0.05$, ns indicating not statistically significant. B. Representative single slide images of the original reconstructed CT-scans and of segmented images with separation of the subchondral plate from underlying trabecular bone as used for 3D calculation of plate thickness for the three groups (sham, Groove and ACLT, respectively).

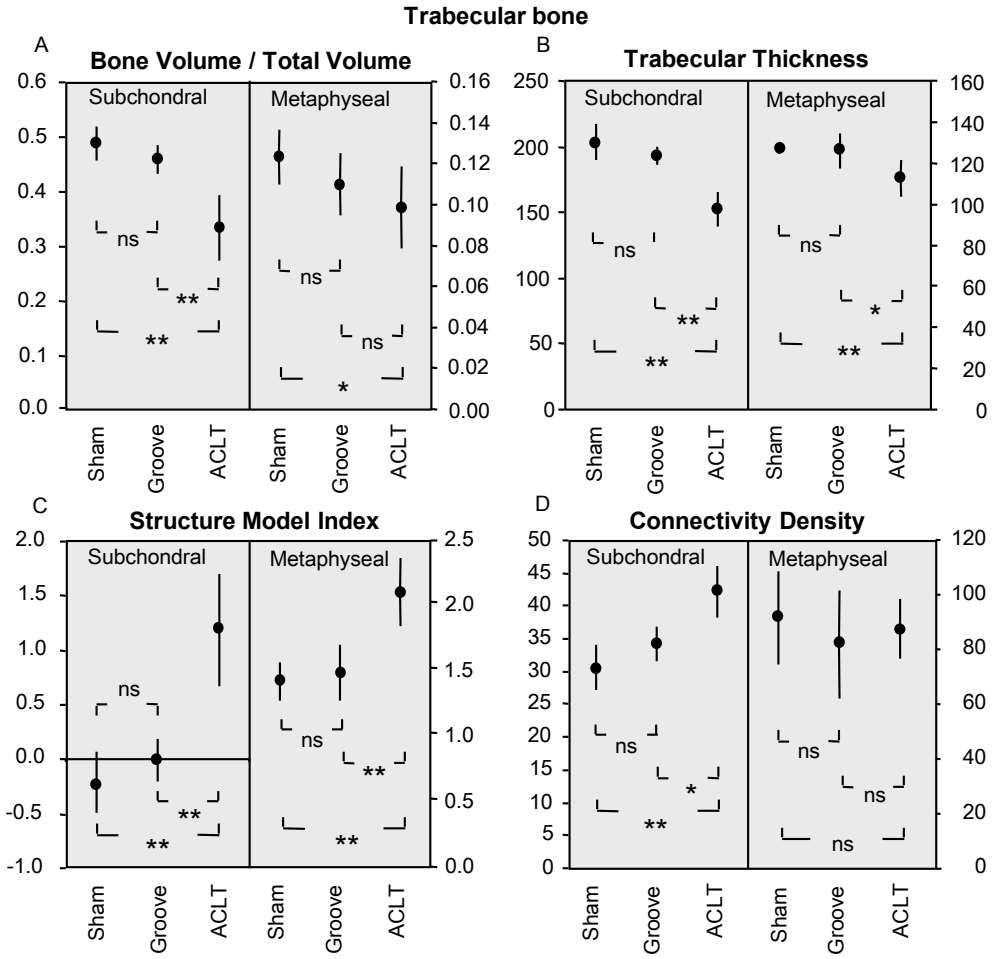

Figure 4. Micro-CT analysis of tibial trabecular bone. Mean ± SD of the Bone Volume/Total Volume (BV/TV) (A), Trabecular thickness (TbTh) (B), Structure Model Index (SMI) (C) and Connectivity Density (CD) (D) are given; * indicates $p<0.05$, ** indicates $p<0.01$ (ns: not statistically significant). Left panels represent the subchondral area and right panels the metaphyseal area.

Cartilage matrix integrity

Cartilage matrix integrity was measured by several parameters; a macroscopical score, a histological score, and biochemical analysis of proteoglycan content and collagen damage.

Macroscopical evaluation of cartilage of the tibial plateau, located above the subchondral bone areas evaluated by micro CT, showed a statistically significant increase of cartilage damage when compared to the sham group, both in the Groove and ACLT model (both

p<0.05; figure 5 top left panel). Histological evaluation of cartilage in both models confirmed the macroscopical results as represented by the increased modified Mankin grade (figure 5 top right panel; both p<0.05 compared to the sham group). Representative photographs of the cartilage in all three groups have been added (figure 5B), showing the characteristic features of OA, including loss of Safranin-O staining, fibrillation of the articular surface, and chondrocyte clustering. Loss of Safranin-O staining was corroborated by the decrease in PG-content (figure 5, bottom left panel) as biochemically determined. This loss of proteoglycans was accompanied by an increase in the amount of denatured collagen (figure 5, bottom right panel) in the Groove and ACLT model when compared to the sham group. All these changes appeared very similar in both models, although histological cartilage damage was slightly more pronounced in the Groove model than in the ACLT model (p<0.05).

DISCUSSION

This study demonstrates that thinning of the subchondral plate coincides with degenerative changes in articular cartilage independent of the model used and as such is considered an intrinsic part of the OA process. This is in contrast to the subchondral trabecular bone changes that were clear in the ACLT model but hardly present in the Groove model and that coincided with similar changes in the metaphyseal bone. As such, subchondral trabecular bone changes might not be an intrinsic part of the cartilage degenerative process and are differently regulated in different models (due to different causes) of OA. It is suggested that mechanical unloading of the bone is causative in early subchondral trabecular bone changes rather than the degenerative process itself[35].

Subchondral plate thickness was significantly reduced in both OA models as were cartilage integrity parameters. The similarity in cartilage damage for both models was corroborated by the similarity in elevation of the cartilage collagen marker CTX-II during the course of the development of the cartilage degeneration. The early reduction in plate thickness is in concurrence with a previously published pilot study which demonstrated that subchondral plate thinning at three and ten weeks of OA development may even precede cartilage damage[13]. Also others have demonstrated that plate thinning is an early feature in the OA process[15, 36]. Unfortunately, in our study group size and variation in bone and cartilage parameters were too small to perform meaningful correlations between values of plate thickness and cartilage degeneration for each group.

Although speculative, there might be a direct role for biochemical factors between cartilage and bone in the process of thinning of the underlying subchondral plate[37]. The tidemark which was previously thought to be a strict barrier between cartilage and bone, allows perfusion of chemo- and cytokines released by the diseased chondrocytes and inflamed synovial tissue[38]. In line with this assumption is that if mechanics instead of chemical components would be involved, a compensatory increase in bone tissue is anticipated due to the loss of the mechanical function and with that shock absorbance of the cartilage. This would increase local stresses on the subchondral bone and result in an increase in bone tissue volume/thickness. Whereas the opposite, loss of bone tissue is observed, a role for chemo- and cytokines rather than mechanics in early plate thinning is suggested.

Thinning of the plate was hardly represented by an increase in urine LP levels. In the Groove model only a very slight, not statistically significant increase in urine LP levels over time was observed. In contrast, LP levels were significantly increased in the ACLT model. This increase is expected to depend on the significant changes in subchondral trabecular bone that were only present in the ACLT model and although a tendency was observed, not clear in the Groove model, whereas cartilage damage actually was most pronounced in the Groove model. The trabecular changes in the ACLT model were not restricted to the subchondral trabecular area but extended throughout the bone. It is hypothesized, as has been reported before, that ACL transection results in clearly diminished loading of the whole paw (braking, stance and propelling force)[39, 40] as a consequence of joint instability. Although not objectively measured, in this study a clear decrease of overall activity in the

ACLT group due to bilateral hind limb joint instability in addition to more lameness of both hind limbs was observed, probably compensated for by increased loading of the front limbs. Note that dogs can easily transfer their load to their front limbs[41]. This changed loading pattern was not observed in the sham or Groove group, corroborating the observation in the unilateral versions of both models[39-41]. The significant increase in bone resorption over time as evaluated by urine LP levels is supportive in this respect. Importantly, the difference in bone resorption marker between both OA models with comparable cartilage damage should be taken into account when evaluating such biomarkers prospectively in clinical studies. It could well be that depending on the original cause of OA, bone resorption (turn over) levels are increased or not.
In contrast to the changes in trabecular bone volume, thickness, and SMI, the connectivity density (CD) of the metaphyseal bone did not show the same changes as in the subchondral area. It is known that CD can either increase or decrease as a result of bone volume loss[42]. With loss of bone volume, trabeculae may disappear leading to a decrease in CD. However bone loss can also increase CD by an increased porosity of the trabecular structures. Therefore, changes in this parameter are less conclusive.
Several studies showed that subchondral trabecular volume decreased early in the OA process followed by an increase later in the process. All these models are based on joint instability (ACLT or collagenase induced ligament weakening) or spontaneous OA of unknown origin. As such the initial decrease in trabecular bone volume in these models could also be caused by unloading in the early phase of OA due to changed biomechanical conditions of the joint, pain or joint stiffness. Unfortunately other primarily cartilage damage induced OA models have not been evaluated for bone characteristics in such a detailed way, to be supportive for this concept in this respect.

The present study underscores that cartilage damage in these models is not secondary to more stiffened bone, as the decrease of bone volume is likely to result in more softened and fragile bone. These early changes in mechanical characteristics of subchondral plate and trabecular bone may even have a natural protective function as the cartilage might secondary be spared from excessive forces. On the other hand, the fragility of the bone can also be causative in the process of cartilage degeneration.
As such the significance of the decrease of subchondral plate thickness and trabecular bone volume is still unknown. Do these early changes normalize over time or are they the necessary trigger resulting in subchondral sclerosis? Although a short longitudinal pilot study has performed[13], it is clear that longer follow-up studies for both models are needed. Do late stage plate thickening and increase of trabecular volume, also develop after prolonged in the Groove model or is it as consequence of the early decrease in trabecular volume parameters only seen in the ACLT model? At present we chose to compare the two models early in the disease process, but the results from this comparison urge for a comparison later in the disease process as well.
Despite common degenerative features of clinical end stage OA (cartilage damage, subchondral sclerosis, joint inflammation), the pathway from a healthy joint to a destructed

joint can be diverse. In this study different causes of joint degeneration are represented, joint instability (ACLT model) and primary cartilage damage (Groove model), both common causes for OA in the human clinic. Gaining knowledge on the differences and similarities of pathogenic events in the development of OA with different etiology can bring us one step closer to a more patient specific approach in early treatment of OA. A patient with a chondral defect may not benefit from pain medication (maintaining proper joint loading) or bisphosphonate therapy (inhibiting bone resorption), in contrast to a patient with an insufficient cruciate ligament in its early phase preventing the bone changes early in the process, by maintaining proper joint loading and/or arresting bone turnover.

In conclusion, the occurrence of early trabecular bone changes depends on the cause/induction of joint degeneration and is not an indispensable factor in cartilage degeneration. There could be an important role for unloading because natural mechanics of the joint are disrupted in the ACLT model and not in the Groove model. The early decrease in plate thickness, concomitant with the cartilage damage, present in both models, suggests plate thinning to be a process intrinsic to cartilage degeneration.

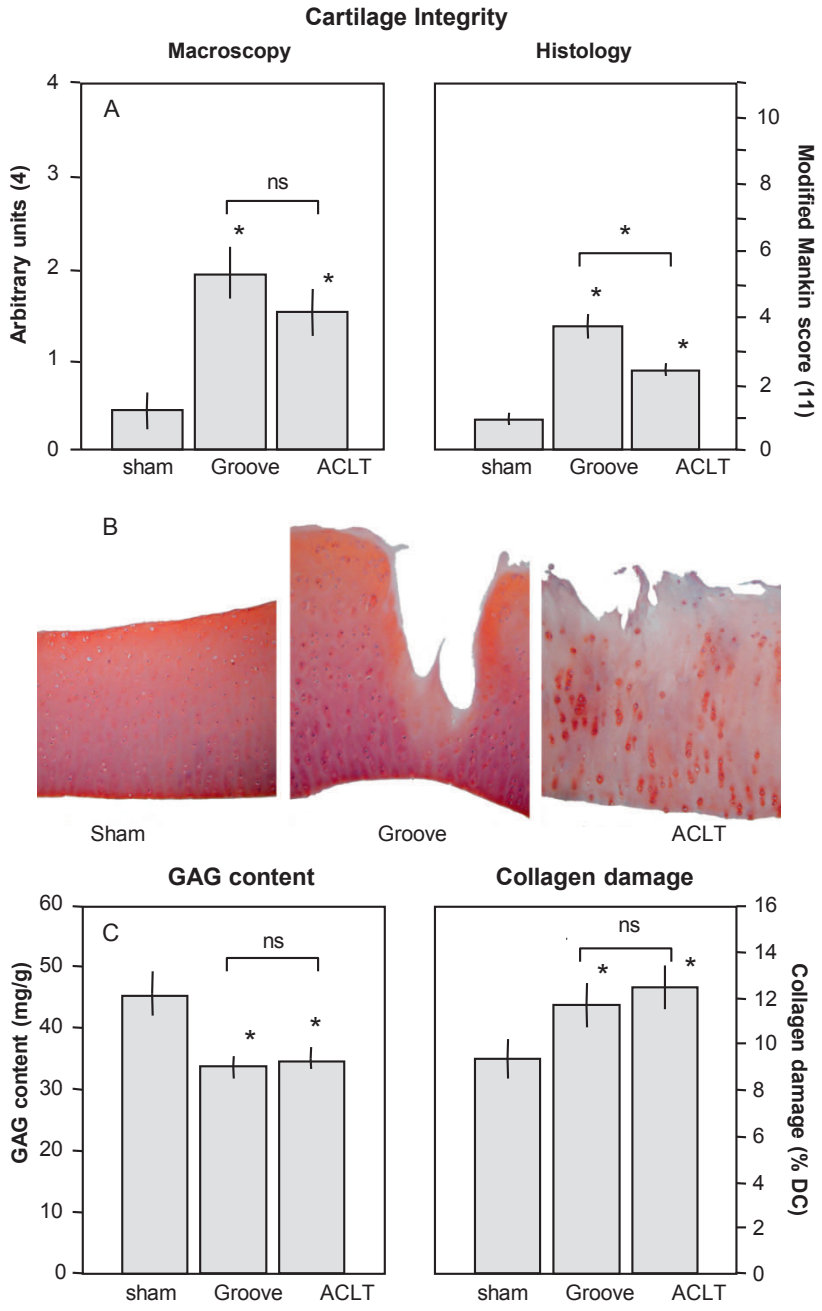

Figure 5. Cartilage integrity parameters. A. Mean ± SD of macroscopical (top left), and histological (top right; Mankin grade) cartilage damage. B. Representative micrographs of cartilage for each of the three groups, showing surface damage, cell clustering, and loss of safranin-O staining for the Groove and ACLT model. C. Biochemical (cartilage matrix GAG content (bottom left) and collagen damage (bottom right)) analysis of cartilage obtained from the tibial surface. * at the top of a bar indicates statistical ($p < 0.05$) significant difference from the sham surgery group. The differences between the Groove and the ACLT group have been indicated as well, * indicating $p<0.05$ (ns: not statistically significant).

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Chapter 6

In early OA, thinning of the subchondral plate is directly related to cartilage damage: results from a canine ACLT-meniscectomy model

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Osteoarthritis and Cartilage 2010; 18(5):691-698

ABSTRACT

Objective. The pathogenesis of osteoarthritis (OA) includes cartilage degeneration, synovial inflammation, and bone changes. Slowly, the sequence and interrelationship of these features is becoming clearer. Early models of OA suggest thinning of the subchondral plate in addition to trabecular bone changes. In the present study subchondral bone changes were studied in the canine ACLT-meniscectomy model. This model is characterized by intra-joint variability with respect to cartilage damage (predominantly medial) and loading (lateral unloading due to a shifted axis).

Methods. In 13 Labrador dogs, OA was induced by transection of the anterior cruciate ligament and removal of the medial meniscus. Twelve weeks later, cartilage integrity was evaluated histologically using the modified Mankin score (0-11), and proteoglycan content was determined by Alcian Blue assay. Bone architecture of the tibia was quantified by micro-CT.

Results .Cartilage damage was severe in the medial compartment (Mankin score +3.5, GAG content -28%) and mild in the lateral compartment (Mankin score +1.6, GAG content -15%). Thinning and porosity of the subchondral plate were only present on the medial side (-21%, +87%, respectively). Interestingly, changes in trabecular bone structure did almost not occur in the medial compartment (volume fraction -7%) but were clear in the lateral compartment (-20%).

Conclusion. Thinning of the subchondral plate is a localized phenomenon related to cartilage degeneration while trabecular bone changes are related to mechanical (un)loading. The different mechanisms responsible for bone changes in OA should be taken in account when designing and interpreting studies interfering with bone turnover in the treatment of OA.

Osteoarthritis (OA) is a slowly progressive degenerative joint disorder characterized by cartilage damage, changes of the subchondral bone, and inflammation of the synovial tissue. Clinical features include pain, joint stiffness and loss of joint function. Although the exact pathogenesis is still unclear, it is generally appreciated that the etiology is of multiple origins; with genetic, biochemical, and mechanical factors playing a role[1]. Besides (known or unknown) trauma to the joint, aging and overloading are the main risk factors[2]. Current treatment options are unable to accomplish joint regeneration and only with varying success slow down disease progression. Preferably, treatment aiming at joint preservation should be applied early in the course of the disease. Gaining more knowledge on early pathogenic events is necessary for development of early treatment methods, in addition to early diagnostic tools.
The need to clarify early pathogenic events that occur in various joint tissues at the onset and during the early progression of OA has motivated the use of animal models, which may elucidate the complex inter-relationship between different joint tissues[3].

In OA, cartilage damage is of primary concern, but total joint homeostasis relies on the biochemical and biomechanical interaction of all tissues involved, including the underlying bone and synovial tissue[4]. With respect to bone characteristics, bit by bit the role of subchondral bone changes in the development of OA becomes clearer. It is accepted that the trabecular bone and the subchondral plate each respond differently and should be approached as separate structures[5]. Thickening of the subchondral plate is evident, changes in trabecular structure such as trabecular volume are less well defined and are reported to either increase[6-8] or decrease[9, 10]. Despite its multiple etiologies, OA leads to a common final outcome with respect to bone and cartilage changes. However, it is unknown whether these changes are preceded by a common pathway with respect to the role of bone and cartilage interaction. In fact the exact changes of bone in early OA are unclear.
In experimental animal models of OA several studies described an initial decrease of volume, thickness, and number of subchondral bone trabeculae, followed by an increase when OA progresses[11-15]. These early osteopenic changes are likely to be (at least partly) the result of less or changed loading. Early thinning of the subchondral cortical plate is also demonstrated[16], even occurring in the absence of trabecular changes (personal observations) which argues a causative role of unloading. It is yet unclear what causes the decrease in plate thickness and how it is related to cartilage degeneration and mechanical loading.
Based on the knowledge thus far, it appears that stiffening of the subchondral bone, only observed in more advanced stages of OA, plays no major role in initiation of cartilage degradation in these models of OA. However, as a secondary feature, bone stiffening might add to progression of cartilage degradation later in the process. The initial decrease in cortical plate thickness and loss of underlying subchondral trabecular bone might be directly related to degeneration of cartilage, leaving open which comes first or whether cartilage and bone changes simply coincide. Analyzing the exact changes of the subchondral bone in relation to cartilage degeneration and unloading, can bring us closer to unraveling the

complex pathogenesis of the interaction between bone and cartilage in OA. Moreover, this knowledge might be of relevance in the design and interpretation of studies on bone turnover (or interfering with bone turnover) in the treatment of OA.

Our hypothesis is that thinning of the subchondral plate directly relates to cartilage degeneration while a decrease in trabecular volume is solely under influence of (un)loading. To study this hypothesis subchondral bone changes in relation to cartilage damage were evaluated in the canine Anterior Cruciate Ligament Transection (ACLT)-meniscectomy model. This model has intra-joint variables with respect to cartilage damage and loading, characterized by predominantly medial cartilage damage, obvious unloading of the lateral compartment in addition to generalized unloading of the joint.

MATERIALS AND METHODS

Animals

Thirteen skeletally mature medium-size dogs, average body weight (±SEM) of 25 ± 1 kg, were obtained from a commercial laboratory-animal breeding facility (7 males, 6 females; mean age (±SEM) of 26 ± 4 months). All dogs were without any clinical and radiological signs of orthopedic disorders. The Utrecht University Ethical Committee for Animal Care and Use approved the study. During the study, the dogs were individually housed in indoor/outdoor pens, and were fed a standard diet with water *ad libitum*. In the eight weeks prior to the surgical induction of OA, the dogs were trained to run on a treadmill.

Induction of osteoarthritis

Joint degeneration was induced in the right knee (i.e., the experimental joint) by ACLT[17] combined with medial meniscectomy (ACLT-MX)[18] under standard anesthesia. After a lateral para-patellar arthrotomy, the anterior cruciate ligament was excised, followed by careful loosening and removal of the medial meniscus. Care was taken to minimize bleeding and soft tissue damage and to avoid damage to the cartilage of the femoral condyles and tibial plateau. The joint capsule and subcutaneous tissue were closed separately and the skin was sutured. The left knee (i.e., the contralateral joint) served as an internal (unoperated) control. The animals received analgesics the first three days after surgery and antibiotics the first five days after surgery according to standard procedures. After a recuperation period of two weeks, the dogs were exercised on a treadmill for 10 minutes, five days a week at a speed of approximately 3 km/h.

Cartilage analysis

At the end of the experiment, 12 weeks after OA induction, the dogs were euthanized with an intravenous overdose of pentobarbital. Both hind limbs were removed immediately. The knee joints were opened and cartilage tissue was collected from the tibial plateau and processed within two hours after euthanasia under laminar flow conditions.

Microscopy of the cartilage degeneration was performed on two samples from predefined weight-bearing locations[19] from both the medial and lateral compartment of the tibial plateaus of all animals (see figure 1a). Samples were fixed in 4% phosphate-buffered formalin (pH 7.0) containing 2% sucrose. Sections were embedded in paraffin, stained with safranin-O-fast-green iron haematoxylin and scored blinded and in random order by two independent observers using slightly modified[20] criteria of Mankin[21] (a maximum score of 11). The scores of the two samples of the two observers were averaged for each of the two compartments for each joint and the average of all animals was used for data presentation and statistical evaluation.

Biochemically assessed cartilage matrix proteoglycan (PG) content was determined from three explants of each of the two tibial compartments from predefined locations, all handled individually[19]. The locations in the experimental OA joint were identically paired with the same location in the contralateral control joint. The average of each of the three samples was taken as a representative for each of the two tibial compartments and was used for statistical analysis (with n=13 for the number of animals). To measure the PG content of the cartilage samples, glycosaminoglycans (GAGs) were precipitated from a papain digest of cartilage samples and stained with Alcian Blue. Blue staining was quantified photometrically by the change in absorbance at 620 nm with chondroitin sulfate (Sigma C4384) as a reference value. Results were normalized to the wet weight of the cartilage explants and expressed as mg GAGs per gram wet weight of tissue[20].

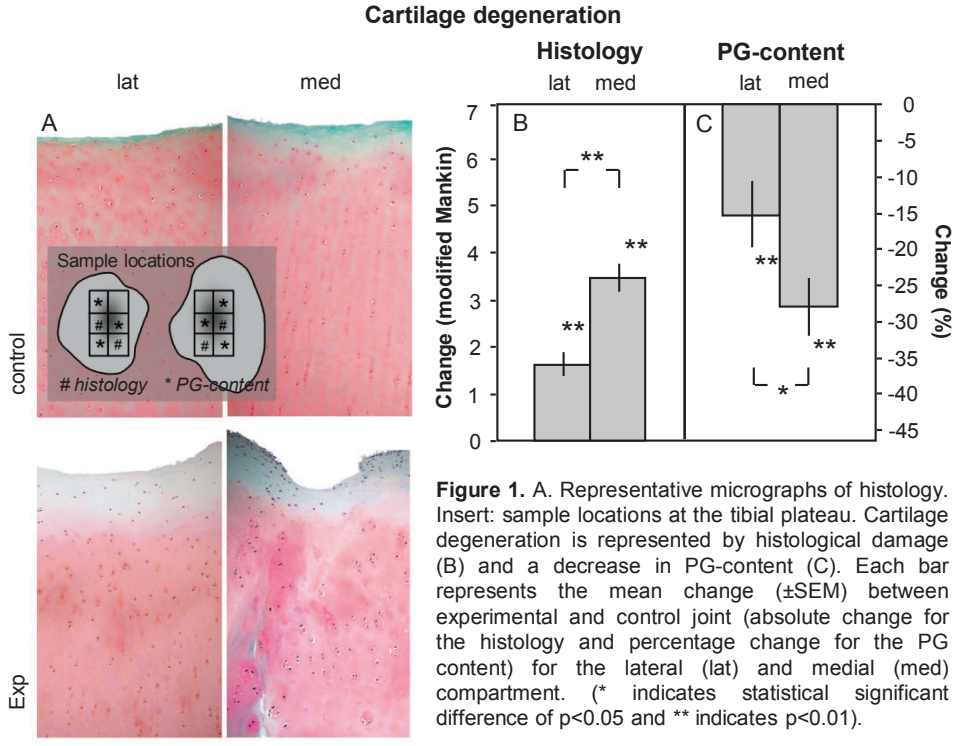

Figure 1. A. Representative micrographs of histology. Insert: sample locations at the tibial plateau. Cartilage degeneration is represented by histological damage (B) and a decrease in PG-content (C). Each bar represents the mean change (±SEM) between experimental and control joint (absolute change for the histology and percentage change for the PG content) for the lateral (lat) and medial (med) compartment. (* indicates statistical significant difference of $p<0.05$ and ** indicates $p<0.01$).

Micro-CT analysis

For the micro-CT analysis all soft tissue was removed from the tibial bone. The proximal part of the tibia was scanned in a micro-CT scanner (Skyscan 1076, Skyscan, Antwerpen, Belgium) at a resolution of 18 µm. The reconstructed data set was segmented with a local threshold algorithm[22].

In both the medial and the lateral part of each scan, a cylinder with a diameter of 4.0 mm and a height of 3.5 mm (medial) and 3.1 mm (lateral) was selected. Cylinders were selected by two observers and results were averaged. The cylinders were located in the middle of the load-bearing areas using anatomical landmarks; middle of the line between the most proximal border and most caudal border, osteophytes not included (figure 2b). Each cylinder contained trabecular bone covered by subchondral plate. The trabecular bone and the subchondral plate were separated automatically using in-house software (ErasmusMC, Rotterdam)[22].

For the subchondral plate, the three-dimensional plate thickness (PlTh) and the plate porosity (PlPor), describing the ratio of the volume of the pores in the plate over the total volume of the plate, were calculated.

Several parameters were analyzed from the trabecular bone; bone volume fraction, which describes the ratio of bone volume over tissue volume (BV/TV), three-dimensional trabecular thickness (TbTh)[23] and structure model index (SMI), a quantification of the trabecular bone structure[24] with a rod-like structure providing an SMI value of 3, a plate has an SMI value of 0, and a structure with closed holes (Swiss cheese like) has a negative value.

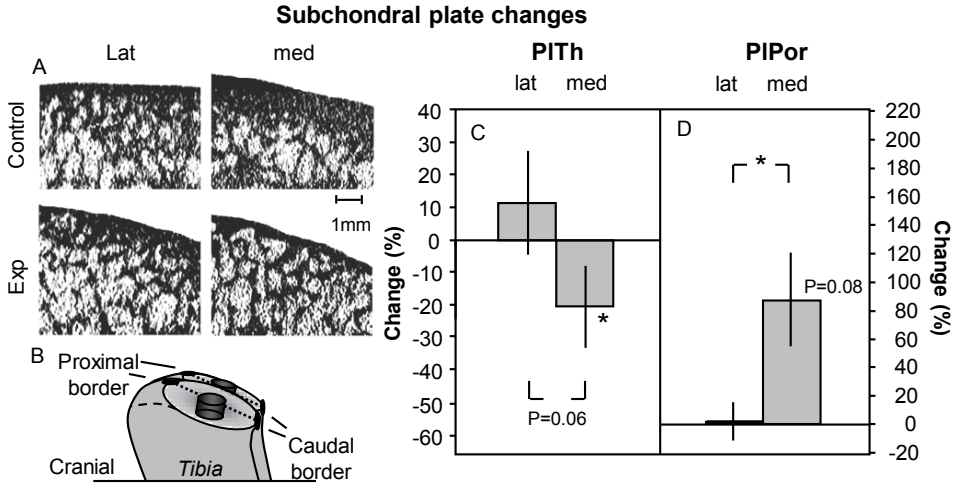

Figure 2. Subchondral plate changes. A. Representative micro-CT images of the lateral and medial subchondral plate of the control and experimental joint. B. Cylinder location; centred on the weight bearing area of the tibial plateau. C. Plate thickness (PlTh) and D. Plate porosity (PlPor), each bar represents the mean change (±SEM) between experimental and control joint (percentage change of PlTh and PlPor) for the lateral and medial compartment (* indicates statistical significance of $p<0.05$).

Statistical evaluation

For differences between the contralateral control and experimental OA joints, paired non-parametric evaluation was performed using Wilcoxon signed rank test. These changes were expressed as percentages for each animal and the average percentage change of the medial side was compared to the lateral side by paired non-parametric evaluation. Non-parametric correlation coefficients (Spearman) were calculated for comparison of the changes in bone and cartilage characteristics.

Tibia		Lateral		Medial	
		C	E	C	E
Histology (Mankin grade)	**Mean**	**1.78**	**3.40**	**1.60**	**5.06**
	SEM	0.19	0.23	0.28	0.32
PG-content (µm/mg wet weight)	**Mean**	**31.57**	**26.22**	**38.64**	**27.08**
	SEM	2.39	1.89	2.04	1.05
BV/TV	**Mean**	**0.45**	**0.37**	**0.44**	**0.41**
	SEM	0.01	0.01	0.01	0.01
SMI	**Mean**	**0.34**	**1.02**	**0.47**	**0.71**
	SEM	0.11	0.09	0.13	0.07
TbTh (µm)	**Mean**	**178.76**	**152.71**	**164.54**	**157.58**
	SEM	6.59	4.56	6.09	2.53
PlTh (µm)	**Mean**	**184.97**	**186.81**	**215.89**	**147.95**
	SEM	19.48	20.60	36.11	22.76
PlPor (% of total volume)	**Mean**	**6.32**	**6.21**	**10.25**	**15.72**
	SEM	0.99	1.24	1.98	3.49

Table 1. Mean values (±SEM) of the different parameters from the medial and lateral compartment of the contralateral control knee (C) and experimental (OA) knee.

RESULTS

Cartilage analysis

Microscopic evaluation revealed the characteristic loss of safranin-O staining, fibrillation of the articular surface, and chondrocyte clustering in the experimental knee compared to the contralateral control knee in both the lateral and medial compartment (figure 1a). Histological cartilage damage in the experimental joints was statistically more severe on the medial side when compared to the lateral side (figure 1b) while there was no significant difference in histological grading between both sides of the control joint (table 1). These degenerative microscopical features were corroborated by a decrease of *PG content*, representing loss of cartilage integrity (figure 1c). The PG content of the experimental knee was significantly lower than that of the control knee in both compartments of the tibial plateau (table 1), with statistically more severe PG-loss in the medial compared to the lateral compartment (figure 1c).

Micro-CT analysis

Subchondral plate

The subchondral plate thickness decreased drastically in the medial compartment of the experimental knee compared to the control knee (table 1). Surprisingly, no statistically significant change was present in the lateral compartment of the tibial plateau; there was even a trend (although not statistically significant) towards an increase in plate thickness (figure 2c). The difference between both compartments was almost statistically significant (p=0.06). In the control joint there was no statistical difference between the medial and lateral side (table 1), although there was a trend the plate was thicker medially.

The plate porosity did show a clear trend towards an increase on the medial side (p=0.08; figure 2d) whereas no change was observed in the lateral compartment. The change between the medial and lateral compartment was statistically significantly different.

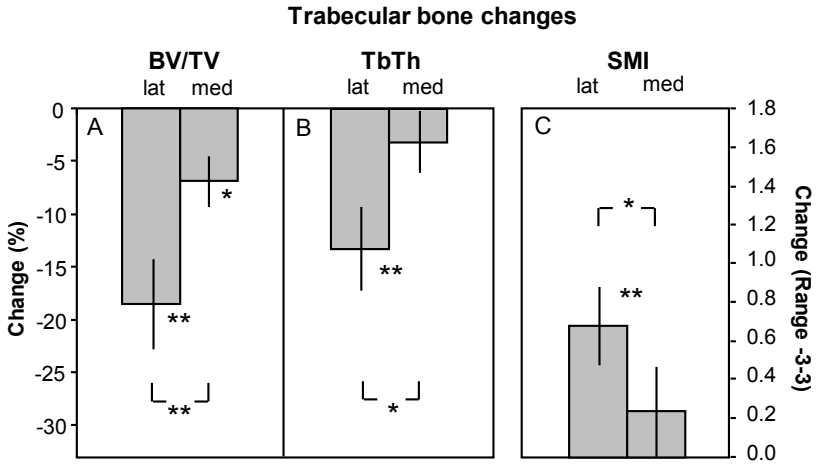

Figure 3. Trabecular bone changes. Each bar represents the mean change (±SEM) between experimental and control joint (percentage change of the fraction of (A) bone volume over tissue volume (BV/TV) and of (B) the trabecular thickness (TbTh) and (C) absolute change for the structure model index (SMI)) for the lateral and medial compartment each (* indicates statistical significant difference of $p<0.05$ and ** indicates $p<0.01$).

Trabecular bone

The subchondral trabecular bone showed a statistically significant decrease in BV/TV in both the medial and lateral compartment compared to the control joint (table 1/figure 3a). However, this decrease was much larger at the lateral side (statistically significant) which contrasts the subchondral plate and cartilage changes. In addition, trabecular thickness and SMI showed statistically significant changes on the lateral side compared to the control knee, which were absent in the medial compartment (table 1/figure 3b and 3c). The

difference between the lateral and medial compartment in the OA induced joint was statistically significant for both parameters. Whereas there was no difference between the medial and lateral side in the control joint (table 1).

Correlation between cartilage damage and bone characteristics

The individual change in histological cartilage damage and percentage change of PG-content were correlated with the percentage change of subchondral plate thickness and plate porosity. Cartilage histology showed a negative correlation (p-value: 0.085) with the plate thickness, indicating that increasing cartilage damage correlated with a decrease in

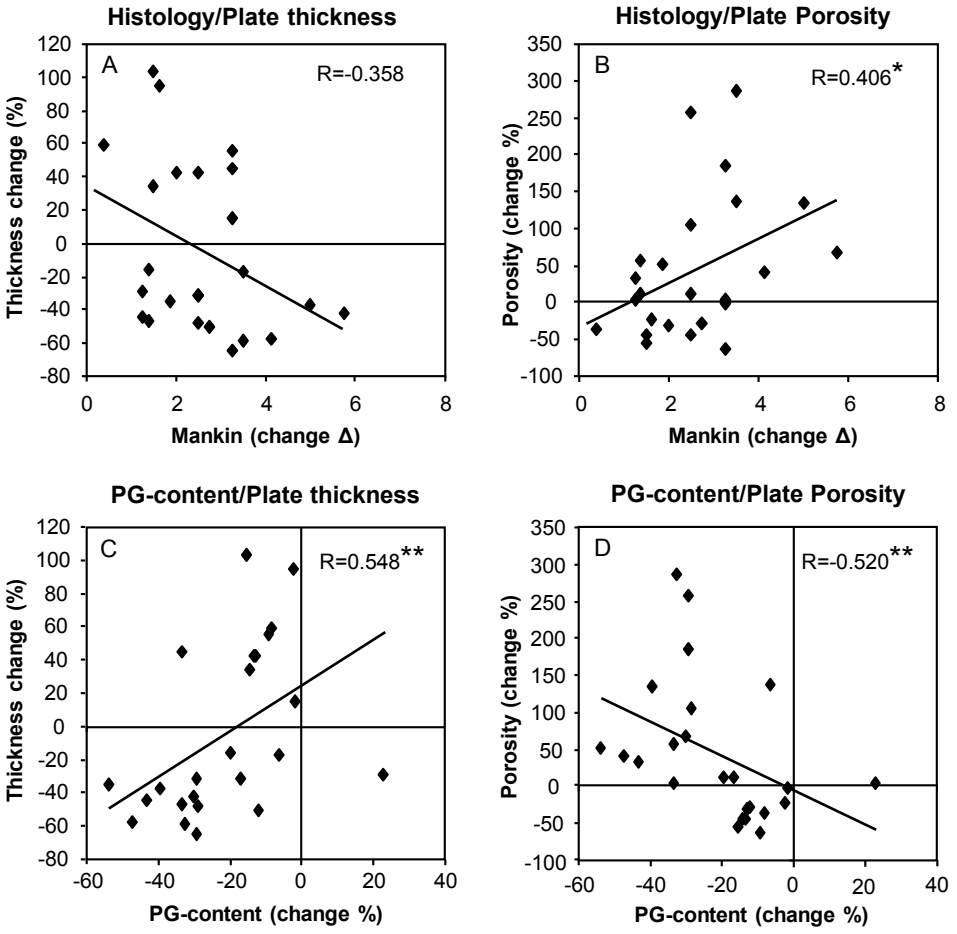

Figure 4. Correlations between individual cartilage changes and changes of the subchondral plate for the lateral and medial compartment (* indicates statistical significance of $p<0.05$ and ** indicates $p<0.01$).

plate thickness (figure 4a). Plate porosity correlated positively with histological cartilage damage indicating that increasing cartilage damage correlated with an increase in plate porosity (figure 4b). The decrease in PG-content showed similar correlations; a decrease in cartilage content was related to a thinning (figure 4c) and increasing porosity of the plate (figure 4d) both correlations being statistically significant.
The correlations for trabecular changes with cartilage changes and with plate changes showed weak and inconsistent results suggesting the absence of a clear relation with a clear direction.

DISCUSSION

This study compares cartilage degeneration with subchondral plate and trabecular bone changes in an intra-articular asymmetric ACLT-menisectomy model of OA with predominately cartilage degeneration in the meniscectomized medial compartment. The lateral compartment is relatively more unloaded, due to the created varus angle (figure 5) in addition to overall unloading of the affected limb due to the joint instability[18].
Significant changes in cartilage degeneration in the more loaded medial compartment coincided with severe thinning of the subchondral plate. There is a lack of plate thinning in the lateral compartment coinciding with only moderate cartilage degeneration. This contrasts the decrease of trabecular volume, mainly present in the unloaded lateral compartment. Despite overall limb unloading, local peak loads might counteract the decrease of trabecular volume at the medial side.

In all experimental models of OA, the natural loading pattern of the affected limb is disturbed, viz. overall diminished. This can be the result of pain, an altered loading axis, instability, or other influences on joint function. Bone is affected by the changes in loading. Especially the trabecular structure adjusts quickly to changed mechanical demands. Due to decreased weight-bearing of an extremity, the trabecular volume decreases, characterized by thinner and fewer trabeculae. In addition, the shape of the trabeculae and the direction of trabecular structure alters, adjusting to the forces it has to sustain[25]. Boyd described changed mechanical capacities of cancellous bone in the canine ACLT model due to bone architecture adaptation[26]. The subsequent change in tissue elastic modulus was confirmed by Day[7]. It was postulated that the disrupted mechanical characteristics of cartilage and bone may lead to an overshoot resulting in stiffened sclerotic trabecular bone in the end. The suggestion of an increase in trabecular volume following the primary decrease of trabecular volume was confirmed in several studies[27-29]. According to this sequence of bone changes, it can be argued whether in the present study, in the medial and lateral compartment, different stages of OA development were observed, instead of differences due to loading. Additional time points are needed to elucidate this possibility.

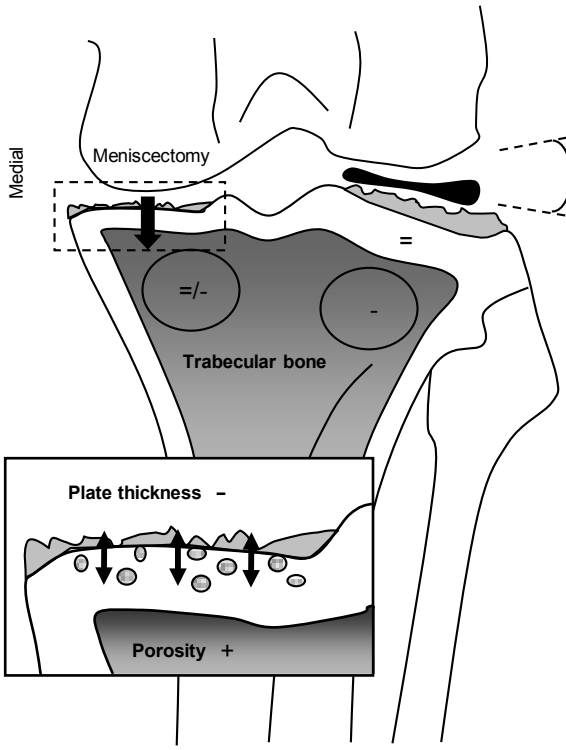

Figure 5. Schematical overview of changes in the ACLT-meniscectomy model. Cartilage damage is present in both the medial and lateral compartment, but more severe on the medial side where the meniscus is removed. Thinning and increasing porosity of the subchondral plate coincides with cartilage degeneration on the medial side (inset). Laterally, trabecular bone decreases (-), a sign of unloading, either by total paw unloading or locally due to the varus angle (arrows). These trabecular changes do not occur on the medial side (=/-) where removal of the meniscus increases local (peak) load on the bone, counteracting overall unloading.

The observed changes in trabecular structure in the lateral compartment corresponded to features resulting from less loading, overall and locally due to the functional varus angle. Lindsey described a similar phenomenon[30]. In human OA, articular cartilage thinning was associated with trabecular bone loss in the opposite compartment. In addition, it has been demonstrated that trabecular bone change does not correlate with joint space narrowing[31], suggesting that cartilage damage is not directly related to cancellous bone changes.

The absence of trabecular changes on the medial side means that locally trabular bone is less unloaded. Load distribution over the joint surface is significantly disturbed due to the absence of the meniscus resulting in increased local peak loads[32]. These peak loads probably balance the overall unloading of the joint due to the OA induction, preventing trabecular bone to change. The tibial plateau was specifically chosen for analysis because a more or less fixed area on the plateau is loaded while loading and shear stresses in the condyles are distributed over a larger, less defined area, inducing undesired variation in bone and cartilage outcome parameters. Hayami also described trabecular bone parameters in the medial compartment of the ACLT-meniscectomy model[15]. In contrast to

our results, a decrease of trabecular volume using histomorphometric analysis was observed. These conflicting results could be due to species differences. On the other hand, it might well be that the observed changes in the rat reflect mean changes of the whole tibial plateau and are not specific for the weight-bearing locations analyzed in our study.

Although unloaded, the lateral compartment demonstrates cartilage damage. It is well known that both a meniscectomy and anterior cruciate ligament transection are responsible for progressive cartilage degeneration[3]. Importantly, due to ACLT the shear stresses in both compartments increase. Additionally, there is an inflammatory (tissue destructive) component throughout the joint, adding to general cartilage damage[33]. Although absolute changes between studies remain difficult to compare, the degree of cartilage degeneration in the lateral compartment is comparable to previous studies in the ACLT model without meniscectomy[16, 19]. This suggests that even though this compartment in the present study is probably additionally unloaded due to the created varus angle, the increase in shear stresses and the inflammatory component are still responsible for cartilage degeneration in this compartment. The diminished loading on its own apparently cannot prevent cartilage degradation.

On the medial side, plate thinning coincided with severe cartilage damage. The degeneration of cartilage alters its mechanical properties. It is likely that degradation of mechanical characteristics causes an increase in load on the underlying bone, leading to a higher demand and subsequent increased thickness of the plate (and counteracting trabecular bone impairment; see above). Botter[34] and Wu[35] have already described a correlation between cartilage damage and bone plate changes. But opposed to our results they observed a correlation between cartilage degradation and increase in plate thickness, whereas in the present study plate thinning related to cartilage damage. The discrepancy could be related to differences in the models used, (ADAMTS5 knockout mice and varus osteotomy in rabbits) and to the stage of OA development (early vs. late OA).
The process of plate thinning underneath damaged cartilage suggests that mechanisms other than biomechanics are involved. Nowadays it is generally appreciated that there is a chemical interaction between bone and cartilage. It has been reported recently that fluids can flow through cartilage and bone, crossing the tidemark. The hydraulic conductance increases with progression of OA[36], allowing destructive mediators to enter bone more easily in advanced stages of cartilage damage. These mediators may initially activate bone turnover in favor of resorption[37]. Among others, RANKL, TNFα or IL-6 might be involved. They are produced in high amounts by inflamed synovial tissue and OA cartilage[38] and are known to play a role in bone resorption[37, 39].
In later phases of OA, mechanically induced bone remodeling may take over leading to the generally known sclerosis, including plate thickening and increased trabecular bone formation as seen in end stage OA.
The presence of cartilage damage at the lateral side without detectable plate thinning might be explained by a certain threshold of cartilage degeneration before plate changes can be

induced. Moreover, loading of the cartilage (stronger at the medial side) may be essential for the release and/or transport of factors to the subchondral bone. This is corroborated by the more severe plate thinning in bilateral models of OA compared to unilateral models (personal observations) where loading of the joint is maintained as unloading of both hind limbs is more difficult. In ACLT joints exposed to forced mobilization, cartilage degradation coincided with increased plate thinning and plate porosity[40]. The increased plate porosity might add to the availability of cartilage-derived bone-destructive mediators.
Assuming loading is involved in changes of subchondral bone, the influence of pain medication and physical therapy in treatment of OA might (in addition to its supposed benefits) have additional adverse effects due to increased loading. This is supported by Appleton[40] and O'Connor[41] who demonstrated increased bone changes and cartilage degeneration resulting from increased loading of the affected joint.
Additionally, from these observations we can conclude that cartilage damage can precede plate thinning (lateral side), contrasting previous reports[42]. Apparently, the sequence of events is not definite.

The use of one animal model, and especially a traumatic model like the canine ACLT-meniscectomy model, just represents one origin of OA pathogenesis. As stated in the introduction, various roads lead to a common outcome of severe end stage OA with common features. However, the path of development might not be common. In the ACLT model a specific way of OA induction in a specific joint is reflected. Another model of OA, a collegenase injected model, not only showed plate thinning in the medial compartment coinciding with cartilage degeneration but also in the lateral compartment where no cartilage damage was present[12, 13].

Involvement of bone turnover might be a target for treatment of OA. However, the present study, although limited to a single animal model, clearly demonstrates that at least in early OA this is a complex approach. In late-stage disease with subchondral sclerosis (plate thickening and increase of trabecular bone volume), approaches such as the use of bisphosphonates arresting further sclerosis have moderate results[43, 44]. Administration of bisphosphonates early in the disease did not result in less bone resorption[45]. But most important, the role of early plate thinning is unclear. Does the process of early thinning promotes further joint damage, or is it a natural protective event? However, other studies interacting with bone resorption[46, 47] by administration of calcitonin early in the disease, show that less bone resorption coincides with less cartilage damage. Chemical interactions between cartilage and bone need further study to evaluate whether specific targets are of relevance. Overall, the fact that plate thinning and loss of subchondral trabecular bone are differently regulated and are a localized phenomenon within the osteoarthritic joint should be taken in account when designing and interpreting studies interfering with bone turnover in treatment of OA.

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Unloading joints to treat osteoarthritis, including joint distraction

Chapter 7

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Current Opinion in Rheumatology 2006; 18(5):519-525

ABSTRACT

Objective. More and more, patients are interested in non-pharmacologic approaches to help manage their osteoarthritis. This review describes the recent literature on the potential beneficial effects of unloading joints in the treatment of osteoarthritis, with a focus on joint distraction.

Results. Mechanical factors are involved in the development and progression of osteoarthritis. If 'loading' is a major cause in development and progression of osteoarthritis, than 'unloading' might be able to prevent and slow down progression. There are numerous examples that unloading might indeed be effective in reducing pain and slowing down structural damage. This review describes unloading by footwear and bracing (non-surgical), unloading by osteotomy (surgical), and has a focus on unloading by joint distraction. Excellent reviews in all these three fields have been published over the past years. More recent studies do not argue the usefulness of a biomechanical approach to improve function and possibly reduce disease progression.

Conclusions. To improve patients function and possibly reduce disease progression, a biomechanical approach should be considered in the treatment plan for patients with osteoarthritis. However, further research (appropriate high quality clinical trials) and analysis (clinical as well as pre-clinical and fundamental) are still necessary to understand, validate, and refine the different approaches of unloading to treat osteoarthritis.

This review describes the potential beneficial effects of unloading of joints using non-surgical and surgical approaches with an emphasis on joint distraction in the treatment of osteoarthritis.

Osteoarthritis

Osteoarthritis is known as a degenerative joint disease, characterized by joint pain and stiffness. These clinical symptoms are the result of cartilage damage, changes in the subchondral bone, and bony outgrowth at the joint margins. As a result of these changes joint inflammation may occur. This inflammatory response, the changes in bone characteristics and additional changes in peri-articular soft tissues originate the clinical symptoms[1]. Although many predisposing factors for the development of osteoarthritis are described[2, 3], these factors on their own are not expected to cause osteoarthritis. Mechanical factors specifically in the weight bearing and larger joints are involved in the actual setoff of the joint damage.

Loading and osteoarthritis

Normal synovial joints can withstand loading during normal activities for a lifetime without developing osteoarthritis. However, a considerable number of studies support the concept that mechanical demand that exceeds the tolerance of the joint (cartilage, bone or ligaments) has a major role in the development and progression of osteoarthritis. The association between physically demanding occupations and osteoarthritis suggests that intense joint loading is associated with early onset and progression of joint degeneration[4-6]. Even so, the relationship between participation in sports and osteoarthritis indicate that those sports which subject joints to intense loading increase the risk of osteoarthritis[7]. There is the association between obesity and osteoarthritis[4, 8]. Other studies demonstrate an association between joint injury and later development of osteoarthritis; Damage to joint articular surfaces, menisci, ligaments, and capsules causes acute tissue damage and can lead to permanent joint instability or incongruity of the articular surface that cause repetitive increased contact stress on articular cartilage surfaces that can initiate or accelerate joint degeneration[7]. Normal levels of joint use may also cause articular surface injury and degeneration in unstable joints, in subluxated, dysplastic, incongruous, or malaligned joints; and in joints that do not have normal innervation[7]. The biomechanical aspect of gait and the impact of alignment have been recognized as important in the development and progression of radiographic joint space loss and deterioration in function[9]. Thus, in general, the development of symptomatic osteoarthritis (including pain) and of structural changes (including joint space loss) is depending on biomechanical processes[2].

Loading *versus* unloading

If ‘loading’ is a major cause in development and progression of osteoarthritis, then ‘unloading’ might be able to prevent and slow down progression of osteoarthritis. There are numerous examples that unloading might indeed be effective in reducing pain and slowing down structural damage. Weight loss reduces the risk of developing symptomatic

osteoarthritis[10, 11]. Measures that may decrease the intensity and frequency of impact and torsional loading of joints during sports may prevent joint injury and development and progression of osteoarthritis[7]. In addition, the symptoms of knee osteoarthritis can be improved by improving alignment and altering the dynamic forces on the involved compartment of the knee during gait[9].
Importantly, it has been demonstrated that the proper joint load (joint training) results in beneficial alterations in cartilage composition and biomechanical properties. Chondrocytes have the capacity to detect alterations in (un-)loading and change/restore the matrix accordingly. This observation supports the concept that joints can adapt to their mechanical environment, viz. loading/unloading[12]. Even in cartilage tissue engineering, in addition to cell selection, scaffold design and biological stimulation, it is recognized more and more that the proper biomechanical factors, mechanical and hydrostatic, are the challenge for the engineering of functional tissue[13, 14].

It is appreciated that new insights in the pathophysiology of osteoarthritis as well as novel therapeutics will improve the pharmacologic options in osteoarthritis[15]. However, up till now there is no medication that conclusively can be designated as having disease modifying osteoarthritic (DMOAD) activity. Moreover, patients are interested in non-pharmacologic approaches to help manage their osteoarthritis. A strategy to improve the symptoms may be to reduce the (over)load. To improve patients' function and possibly reduce disease progression, a biomechanical approach should be included in the treatment plan for patients with osteoarthritis. In addition to lifestyle changes e.g. directed at weight loss and limiting sports induced joint (over)load, strategies aiming at unloading are at hand in treatment of osteoarthritis.

Unloading by footwear and bracing
Varus and valgus malalignments have been related to medial and lateral knee osteoarthritis[16, 17]. The osteoarthritis in these individuals may be controlled to a certain extent by the use of footwear. Reducing the varus or valgus moment in medial compartmental osteoarthritis of the knee during gait may play a role in reducing the symptoms of knee osteoarthritis. Foot orthoses may change the mechanical forces on the knee and influence the femoro-tibial angle. A Cochrane survey by Brouwer and colleagues of the literature until end of 2002 provided a scientific basis for applying wedged insoles[18]. A recent review by Krohn of the published literature until 2004 on footwear for improving symptoms of knee osteoarthritis in an attempt to reduce pain in patients with medial compartment knee osteoarthritis upholds these findings[9]. In general both studies concluded that pain, stiffness and physical symptoms may improve, and the use of nonsteroidal anti-inflammatory drug intake may decrease.
More recent studies did not contradict the earlier findings. A small group with only six weeks follow-up of laterally wedged custom-moulded foot orthoses demonstrated reduced knee pain in subjects with mild to moderate medial compartment osteoarthritis of the knee[19]. A recent study of Toda and colleagues of which two previous studies were used for the

Cochrane survey, reported on the effects of strapped lateral wedged insole in patients with varus deformity induced osteoarthritis of the knee. The efficacy of laterally wedged insoles with subtalar strapping and a traditional shoe insert wedged insole of finally 21 patients in each group was compared at 6-months[20] and at 2 years follow-up[21]; strapping appeared superior. They also provided data on optimal duration of daily wear for the strapped insole[22] being 5-10 hours daily.
However, it should be noted that less positive effects have been reported as well: In the traditional-wedged insole group of the study of Toda, no significant effects were found at 2 years when compared to baseline[21]. This corroborates previous findings of the group of Dougados failing to demonstrate in a group of 82 *versus* 74 patients relevant symptomatic and/or structural effects of laterally wedged insoles in medial femoro-tibial osteoarthritis at 6[23] and 24[24] months.
On the other hand, laterally wedged insoles have been shown in biomechanical studies to reduce the load on the medial compartment; gait laboratory data suggest that there is a role for the use of lateral wedge orthoses for medial compartment knee osteoarthritis[9]. Most recently this was supported, although in 2 of 13 patients with osteoarthritis an adverse effect was observed[25]. This all demonstrates that insoles might be effective but that the indication and limitations of wedged insoles in general should be analysed in more detail.

Braces may be used to transfer load to the normal, or at least less diseased, compartment of the knee in order to reduce pain from the narrowed, arthritic compartment. Knee braces that stabilize the knee joint and provide a valgus stress have been shown to improve pain and function in patients with medial compartment knee osteoarthritis[9]. Radiographic images provided by fluoroscopic digital radiography during gait provided support that a proper designed osteoarthritis knee brace can change the alignment of the limb and reduce the load on the medial compartment enough to result in radiographic separation of the medial femoral-tibial joint space[26]. In several studies the use of a brace during gait gave reduction of the varus-moment by a few degrees[9, 27]. The change in joint space during gait was 1,2 mm, measured by a fluoroscopic digital radiograph[9, 28]. Significant clinical improvement was measured, compared to a control group, after 6 months of treatment with a varus-alignment brace, but also after treatment with a neoprene sleeve[29]. More recently, two types of braces have been compared, a simple hinged brace and a valgus corrective brace were evaluated in a study of 12 patients with osteoarthritis of the medial compartment. Significant improvements in pain, function and loading and propulsive forces were seen with the valgus brace. The simple brace only showed improvements in loading forces. Both braces improved confidence and function during gait[30]. In a three dimensional determination of the effectiveness of five different braces, all braces were most effective at off-loading the knee at heel strike and least effective at midstance[31]. Four of five subjects achieved at least some off-loading on the medial condyle, while one subject did not experience any benefit from the five different braces at all. It might be concluded that the use of osteoarthritic knee bracing appears to be clinically effective, although the long term effectiveness and with that the indication and limitations have to be established.

Unloading by osteotomy

Tibial osteotomy suffers as a natural experiment in which high levels of loading in a localized area of the joint are relieved by realigning the bones, distributing load. This operation often has a dramatic pain-lowering effect, proving the importance of loading in causing pain[32].

In subjects with uni-compartmental osteoarthritis, tibial osteotomy decreases joint loads and slows the progression of cartilage breakdown in the affected compartment[33]. A Cochrane survey of the literature until end of 2002 described that high tibial osteotomy can improve pain and function in osteoarthritis of the medial compartment of the knee[34]. More recent studies supported this finding. Several studies show improvement of gait and clinical measures at 2, 5 and 21 years[35-38]. When compared with uni-compartmental osteoarthroplasty no significant differences were found[36]. Also intertrochanteric varus osteotomy and chiaric pelvic osteotomy for (advanced) osteoarthritis in patients with hip dysplasia as performed by Ito and colleagues appeared successful in the long-term[39, 40]. Although seemingly beneficial it should be noted that most of these studies are in general not of high methodological quality and, most importantly, there are no studies showing that an osteotomy is preferred above other treatments.

Unloading in case of severe osteoarthritis; joint distraction

In cases of severe joint damage, with refractory (severe) pain and (significant) loss of articular cartilage, when a joint is considered for a fusion or endoprosthesis, it might seem that 'unloading' will not be sufficient anymore. However, this appears not the case. Although the aforementioned approaches might not provide adequate help in case of severe osteoarthritis, complete unloading of the joint by using an external distraction frame might (figure 1).

Despite the very promising clinical results, studies on joint distraction in treatment of osteoarthritis are limited. Slightly more groups become involved and more results on laboratory work, including *in vivo* animal studies, become available. Recently, Chiodo and McGarvey provided a good overview on this work[41].

The precise mechanism by which joint distraction works remains speculative. Joint distraction aims at the absence of mechanical stresses on the cartilage by using an external fixation frame preventing further wear and tear of the cartilage surfaces and allowing chondrocytes to initiate repair. Intermittent fluid pressure in the joint is maintained - by using hinges, thin flexible wires, or springs in the distraction frame – being expectedly important for nutrition of the chondrocytes. Distraction relieves mechanical loading of the peri-articular bone leading to temporary osteopenia within the distraction area. The turnover of bone, being a large storage of growth factors known to be involved in cartilage growth and repair[42], may provide support to the chondrocytes in a cartilage repair mode. After distraction, less dense subchondral bone will absorb greater stress, and with that lowers stress on the overlaying cartilage.

Twelve years ago the first study on joint distraction in case of severe osteoarthritis was published. A variety of hip diseases including osteoarthritis was treated with a hinged

external fixator for 6 to 10 weeks, maintaining a joint space of 4 to 8 mm. Clinically good results were found in 42 of 59 patients under the age of 45, most of these patients had osteoarthritis[43]. This work was followed by several publications on joint distraction in treatment of severe ankle osteoarthritis. Retrospectively evaluated it was described that in 11 patients (mean age 35) with post-traumatic osteoarthritis of the ankle treated with an Ilizarov distraction for 15 weeks with hinges, pain decreased in all patients, movement improved in 55% of the patients and joint space widening was seen in 50% of the patients after a follow-up period ranging from 9 to 60 months[44]. At that time this work was cited by Buckwalter[45] as being of potential importance in the treatment of osteoarthritis, especially for young, active people. Saltzman in his group started their own cohort of patients treated with severe post-traumatic osteoarthritis of the ankle and compared articulated distraction with non-articulated distraction. Although the study is not yet completed, results at 2 years of follow-up seem surprisingly good with respect to clinical symptoms as well as increase in joint space width. There appeared no difference between articulated and non-articulated distraction at that time of evaluation (personal communication with Saltzman and Buckwalter). Our own group started a prospective study: 17 patients with severe osteoarthritis of the ankle were treated with an Ilizarov external distraction frame, maintaining a joint space of 5 mm for 3 months. In a two-year follow up, 4 patients had poor results with distraction resulting in arthrodesis within one year. Of the remaining 13 patients, two thirds improved significantly as shown by physical examination, functional ability questionnaires and pain scale; effects were progressive in the second year of follow-up[46]. In the same year less encouraging results were reported in a small group of patients who underwent ankle distraction[47]. Joint distraction in treatment of ankle osteoarthritis was reviewed, stating that irrespective of underlying mechanisms, the long-term efficacy of joint distraction in the treatment of severe ankle osteoarthritis at young age validates the concept of joint distraction in the treatment of osteoarthritis, it even may be the treatment of choice[48, 49]. The work continued in 2002 in Arthritis & Rheumatism[50]: 57 patients, who were considered for arthrodesis of the ankle were treated with joint distraction in an open prospective study. Significant clinical benefit was found in three-fourths of the patients. In addition, a randomised trial was performed in 17 patients to determine whether joint distraction had a better outcome than debridement. Joint distraction showed significantly better results. Improvement increased over time and radiographic evaluation showed increased joint space width and decreased subchondral sclerosis[50]. Prolonged clinical benefit from joint distraction in the treatment of ankle osteoarthritis was demonstrated in a retrospective study of 22 patients, treated with Ilizarov distraction more than 7 years previously. In 16 patients significant improvement was observed using three different approaches at a mean follow-up of 10 years[51]. Most recently Paley and Lamm[52] described their study on ankle distraction using hinges in an Ilizarov distraction frame. Preliminary results from the first 20 joints distracted rendered good or excellent results in 18 joints with a follow-up ranging from 2 to 16 years. Presently distraction of osteoarthritic knees is evaluated in the Netherlands, and although results are preliminary (7 patients with a

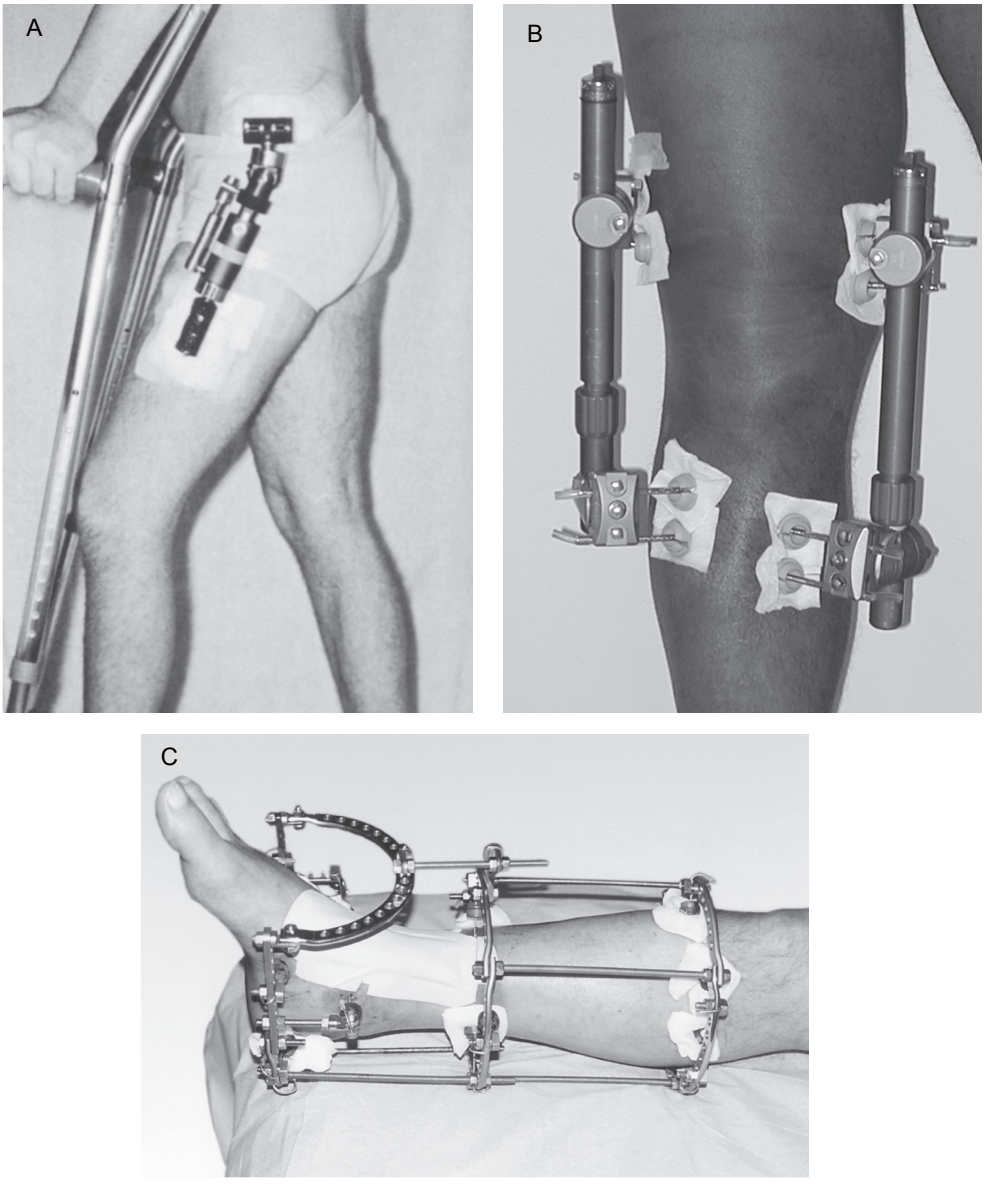

Figure 1. Different examples of joint distraction in the treatment of osteoarthritis A. Hinged, articulated hip distraction. B. Knee distraction using an Howmedica system with springs. Reproduced with permission from Aldegheri et al[43]. C. Ilizarov ankle distraction using thin wires. Reproduced with permission from van Valburg et al[44].

maximum follow up of 2 years) results again seem very promising, demonstrating reduction of clinical symptoms to a great extent.

Although clinical effects seem at least promising[41], whether structural changes expectedly underlying the clinical symptoms are induced remains less clear. In a canine study we found beneficial changes in chondrocytes activity immediately after ending the distraction, but no cartilage repair[53]. Karadam and colleagues reported 'no beneficial effects of joint distraction on early microscopical changes in osteoarthritic knees' in a rabbit study[54]. Osteoarthritis was induced by papain injection in 24 rabbits which were divided into 4 groups. One group served as control and the others were treated by simple external fixation, articulated distraction, and nonarticulated distraction. Histologically, joint distraction had no beneficial effect, whereas nonarticulated distraction worsened the results. But similarly as in our previous study, no follow-up was studied. And therefore this study is far from conclusive as was discussed[55]. More recently a study by Kajiwara et al. demonstrated that articulated distraction by an external fixator promoted repair of fresh osteochondral defects in the weight bearing area of the femoral condyls of the rabbit. Although distraction for 4 weeks was not a long enough period to repair the defect, distraction for 8 and 12 weeks resulted in good outcome[56]. At the same time a study was published by Yanai et al. on autologous culture-expanded bone-marrow-derived mesenchymal cell transplantation (ACBMT) to treat full surface and full thickness tibial plateau cartilage defects in rabbits. Articulated distraction was performed to facilitate joint repair after ACBMT, as was demonstrated by histochemistry. Interestingly, the control group treated with articulated distraction alone, also demonstrated significant cartilage repair compared to the non-distracted group[14].

Although the current literature on clinical and scientific experience with joint distraction as the ultimate form of unloading is limited, there is a steady spreading of this technique among clinicians and scientists. Further research and analysis will be necessary to understand, validate, and refine this novel approach[57].

Conclusion

Clinicians should be aware of all these non-pharmacologic treatments for some of their patients with osteoarthritis. To improve patients' function and possibly reduce disease progression, a biomechanical approach should be considered in the treatment plan for patients with osteoarthritis. However, further research (appropriate high quality clinical trials) and analyses (clinical as well as pre-clinical and fundamental) are still necessary to understand, validate, and refine the different approaches of unloading in treatment of osteoarthritis.

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Chapter 8

The effects of joint distraction in the canine Groove model of osteoarthritis

This study describes the interim analysis of the first 2 phases of a 3-phase study

ABSTRACT

Objective. Osteoarthritis is a degenerative joint disorder characterized by progressive cartilage damage, peri-articular bone changes, and often secondary joint inflammation. These tissue structure changes coincide with pain, stiffness, and functional disabilities. Few treatment options are available in end-stage knee osteoarthritis and eventually, replacement of the affected joint using an endoprosthesis is inevitable. Joint distraction has shown long term clinical benefits in the treatment of ankle OA, and tissue structure modification has been suggested. This study evaluates whether joint distraction induces tissue structure modification in a canine experimental model of osteoarthritis.

Methods. Osteoarthritis was induced in the right knee joint according to the Groove model (surgically applied cartilage damage to the femoral condyle) in 16 dogs. Ten weeks after OA induction, the right knee joint was distracted for 3-5 mm by use of a hinged external fixator for 8 weeks in 9 dogs (distraction group). Seven dogs were left untreated (osteoarthritis group). Pain/function was studied by (un)loading of the joint using force plate analysis every 5-10 weeks. Twenty-five weeks after removal of the external fixator, cartilage integrity of the osteoarthritic, surgically untouched, tibial plateau was analyzed.

Results. In the untreated osteoarthritic group, cartilage showed a decreased PG content (-18%, $p<0.01$), an increased PG release (+20%, $p<0.03$), and an increase in collagen damage (+2.5%; ns) when compared to the contralateral control joint. This was corroborated by an increased macroscopic and histological grade of cartilage damage (+1.8 and +3.3, respectively, both $p<0.05$). This loss of cartilage integrity was accompanied by decreased loading of the affected joint, especially reflected in a decreased brake and stance force (-0.35N and -0.70N, respectively, both $p<0.05$).

After a follow-up of 25 weeks, the osteoarthritic joints treated with 8 weeks distraction showed a loss of PG content of -7%, which was significantly less ($p<0.02$) then the untreated osteoarthritis group, as was the PG release (+5%; $p<0.05$), which normalized. In addition, less collagen damage was found (+0.3%) in the distracted group. Again this was reflected by both the macroscopic and histological grade of cartilage damage (+1.3 and +2.8 respectively; $p<0.05$). This relative improvement of cartilage integrity was accompanied by a normalization of joint loading (braking force and stance force; $p<0.05$).

Conclusions. Joint distraction results in less cartilage damage accompanied by less pain (based on normalization of loading of the affected knee) in a canine model of experimentally induced osteoarthritis. The actual histochemical and biochemical changes in cartilage in this animal in vivo study corroborate the observed cartilage repair activity as was suggested by imaging and biomarker analysis in clinical trials evaluating joint distraction.

Osteoarthritis (OA) is a heterogeneous condition caused by a variety of factors that result via different pathways in a common clinically characteristic pathology[1, 2]. The disease involves not only the most frequently studied articular cartilage, but the whole joint, including subchondral bone, synovial tissue, menisci (in the case of the knee), capsule, ligaments and muscles[3].

Few treatment options are available for end-stage knee OA. Eventually, when physical and pharmacological therapies do no longer provide sufficient pain relief and maintenance of function, surgical treatment of OA is indicated. Joint replacement (endoprosthesis) is a frequently used treatment in end stage OA. A relatively new approach in the treatment of severe OA is joint distraction, an extra-articular surgical method using an external fixation frame bridging the joint[4].

Initially, joint distraction was applied in different cases of joint mal-alignment and joint stiffness accompanied by joint degeneration in order to improve joint position and increase range of motion. Unexpectedly, benefit was noted with respect to clinical features of joint degeneration[4-6]. Larger proof of concept studies were performed by treating young patients suffering from severe ankle OA or knee osteoarthritis with two to three months of joint distraction, resulting in significant clinical benefits in more than two third of the patients[5] (Chapter 10). Importantly, these beneficial changes were progressive over time and lasted for at least several years, up to ten years now in the treatment of ankle OA[7].

The mechanisms responsible for these clinical benefits are unclear. Joint distraction of the ankle led to increasing joint space width (JSW) and diminished subchondral bone sclerosis as measured on radiographs in addition to clinical benefits[6, 8]. Chapter 9 of this thesis describes that subchondral sclerosis diminishes and subchondral bone cyst disappear leading to bone normalization as a results of joint distraction. Most recently, structural cartilage repair was demonstrated after joint distraction in case of severe knee osteoarthritis. X-ray, MRI as well as biomarker analyses demonstrated clearly cartilage repair activity (Chapter 10).

The mechanisms behind the prolonged clinical benefit of joint distraction and the structural changes have not yet been explained satisfactorily. Joint distraction aims at a temporary decrease of mechanical stresses on articular cartilage by preventing direct contact of articular surfaces while intra-articular intermittent fluid pressure is maintained during joint loading, important for nutrition and stimulation of chondrocytes[4]. Additionally, a significant but transient peri-articular osteopenia develops during distraction, which from a mechanical and biomechanical point of view might add to cartilage repair and might lead to clinical improvement (see Chapter 7). Although largely hypothetical, it is anticipated that the combination of these conditions is a prerequisite to obtain the benefits of joint distraction.

Unfortunately, actual repair of articular cartilage is difficult to study in the human clinical situation. Thus far only surrogate markers, such as increased joint space width on radiographs, normalisation of subchondral bone on radiographs and CT images, increased cartilage thickness and area on MRI and beneficial changes in biochemical markers of cartilage synthesis and breakdown suggested actual tissue structure repair (chapter 9 and 10). To actually demonstrate cartilage repair, macroscopical, histochemical, and biochemical

analysis of cartilage tissue integrity is needed. These analyses require tissue samples which cannot be obtained without highly invasive and possibly harmful surgical procedures. To analyse tissue structure integrity in actual tissue samples, joint distraction was performed in an animal model of OA.

In a previous animal study, joint distraction was performed after induction of OA according to the anterior cruciate ligament transaction (ACLT) model of the dog[9]. In this model 8-weeks joint distraction resulted in a decreased synovial inflammation and a normalization of the proteoglycan (PG) turnover of the articular cartilage directly after treatment. However, histological examination did not reveal cartilage repair. The lack of tissue structure modification was attributed to the short-term follow-up. It could be that normalized matrix turnover had not yet resulted in actual changes in cartilage integrity (cartilage repair). This would be in line with the clinical observations that show that clinical and structural changes are progressive over time[5-7]. Therefore, a longer follow-up was indicated to demonstrate possible cartilage repair after joint distraction in an animal model of OA.

However, when performing long-term follow-up, the ACLT-model might not be suitable. Since joint instability is still present after treatment has stopped, possible beneficial effects might be counteracted. Therefore, in the present study, a model was used without permanent joint instability: the Groove model[10-12] (Chapter 2). The Groove model shows osteoarthritic features in the canine knee joint within 10 weeks, similar to the changes found in the ACLT model[10]. These features of OA are representative of human OA (Chapter 2) and are slightly progressive over time resulting in more advanced degenerative changes at 40 weeks post-surgery[11]. Since this model uses a one-time trigger for OA (femoral cartilage damage), the Groove model allows cartilage repair after the period of distraction[12]

The purpose of the present study was to investigate whether 8 weeks joint distraction in established experimentally induced osteoarthritis in the canine Groove model at 10 weeks, results in cartilage repair at 45 weeks, i.e. 25 weeks after the joint distraction period.

MATERIALS AND METHODS

Due to the complexity and intensive logistics of the experimental set-up this experiment has been performed in 3 identical parts. Each part takes at least a year of study. So far, 2 sequences haven been completed, the 3rd one is on-going. The following description refers to an interim analysis of the first 2 parts.

Animals

Skeletally mature mixed breed canines (n=16 females, mean age 1.6 ± 0.5 years, weighing 18.0 ± 1.3 kg) were obtained from the animal laboratory of Utrecht University, the Netherlands. They were housed in groups of two dogs and were let out on a large patio in larger groups for at least two hours a day. The dogs were divided randomly in groups of at least 2 animals each. The study was approved by the Utrecht University Medical Ethical Committee for animal studies.

Surgical Procedures

OA induction

In all 16 dogs, OA was induced in the right knee joint according to the canine Groove model as described before[5]. Under general anesthesia, surgery was performed through a 2-2.5 cm medial incision close to the *ligamentum patellae*. In utmost flexion, approximately ten longitudinal and diagonal grooves were made on the weight-bearing parts of the femoral condyles using a Kirschner-wire (1.5 mm diameter), which was bent in a 90 degree angle at 0.5 mm from the tip. This ensures that the depth of the Grooves was restricted to 0.5 mm. preventing damage of the underlying subchondral bone. There was no absolute visual control over the procedure, but macroscopic evaluation at the end of the experiment showed a comparable pattern in all affected knees.

Joint distraction

Joint distraction was performed in 9 dogs. After development of OA, ten weeks after OA induction, the external fixation frame was placed under general anesthesia and pain medication. Three bone pins (self-drilling half pins, 3 mm Ø, Stryker) were drilled into the femur, two distally and one proximally on the antero-lateral side. In addition, three bone pins (self-drilling half pins, 3 mm Ø, Stryker) were drilled into the tibia, two proximally and one distally on the antero-medial side. External fixation frames were custom-made and adapted to the dog's anatomy. The design of the finally used frames was based on an extensive pre-study with several types of frames and approaches to obtain optimal distraction with flexion and loading of the joint. Two frames with three point fixation (5mm) were coupled to the pins of the femur and tibia by use of commercially available connections (Stryker). The external fixation frames of femur and tibia were connected via hinges medially and laterally of the knee joint. By use of the screw threaded connecting rods, distraction of the knee joint was carried out and visualized by X-ray, while smooth flexion of the joint during flexion and extension was maintained (figure 1).

After five days of recovery, all the dogs were let out on the patio again. Overall condition of the dogs and pin sites was controlled at least twice a week. In case of clinical signs of pin tract infection, the dog was treated with antibiotics which was necessary in one case.

After 8 weeks of distraction, distraction was eliminated by removing the hinges. One week later, the fixation frames and pins were removed under general anesthesia. This two-phase strategy was used to achieve a gradual reloading of the joint surfaces. The severity of OA was evaluated 45 weeks after induction of osteoarthritis in all animals, i.e. 25 weeks after end of treatment in the distraction group.

At the end of the experiment all the dogs were killed with an intravenous injection of Euthesate. Both hind limbs were amputated and processed within 2 hours. All further analyses include only the surgically untouched tibial plateau to prevent interference with the surgically applied damage to the femoral condyles, less representative for joint degeneration.

After opening the knee joint, digital high-resolution photographs were taken from the tibia plateau and supra-patellar synovial tissue for macroscopical scoring of cartilage damage

and synovial inflammation. Subsequently, cartilage samples were taken from predefined locations of the weight bearing area of the tibia plateaus as described previously[10]. All samples were weighed (accuracy 0.1 mg) and stored for further analysis. In addition, synovial tissue samples were collected. Procedures were carried out under laminar flow conditions.

Conditions of joint distraction

Distraction of the knee joint was checked every 2 weeks by comparing radiographs of both the distracted joint and the contralateral control joint in identical loaded position, and if necessary adjusted (a representative radiograph presented in figure 2A and B). Two observers measured joint space width on the radiographs and the mean value was used for statistical evaluation.

Intra-Articular Fluid Pressure

Besides absence of mechanical stresses, intra-articular intermittent fluid pressure changes, as observed during clinical treatment[4], were measured. Upon flexion and extension, changes in intra-articular fluid pressures were measured in the first 5 dogs treated with joint distraction by means of a pressure transducer connected to an intra-articular positioned canule. During measurement dogs were mildly sedated with preservation of muscular tension. Pressure recordings during flexion and extension of the joint were made and maximum pressure interval represented the intermittent intra-articular pressure. Both the treated and the contralateral healthy knee joint were evaluated. For pressure registration we used a data-acquisition program (MKR version 4.2; Biomed. Eng. University Medical Center Utrecht).

Bone density on X-ray

At the end of the distraction period (8 weeks), X-rays were made of both the treated and contralateral healthy joint of the treated animals (representative radiographs are presented in figure 2D and E). Using an arbitrary score from 0 to 3 for peri-articular osteopenia (0=none, 1= possible, 2= moderate, 3= severe) the X-rays were scored by two independent observers blinded to the source of the X-rays. The average score was used for statistical evaluations.

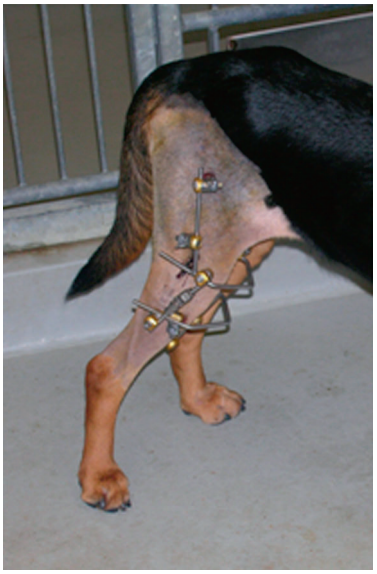

Figure 1. Canine distraction frame

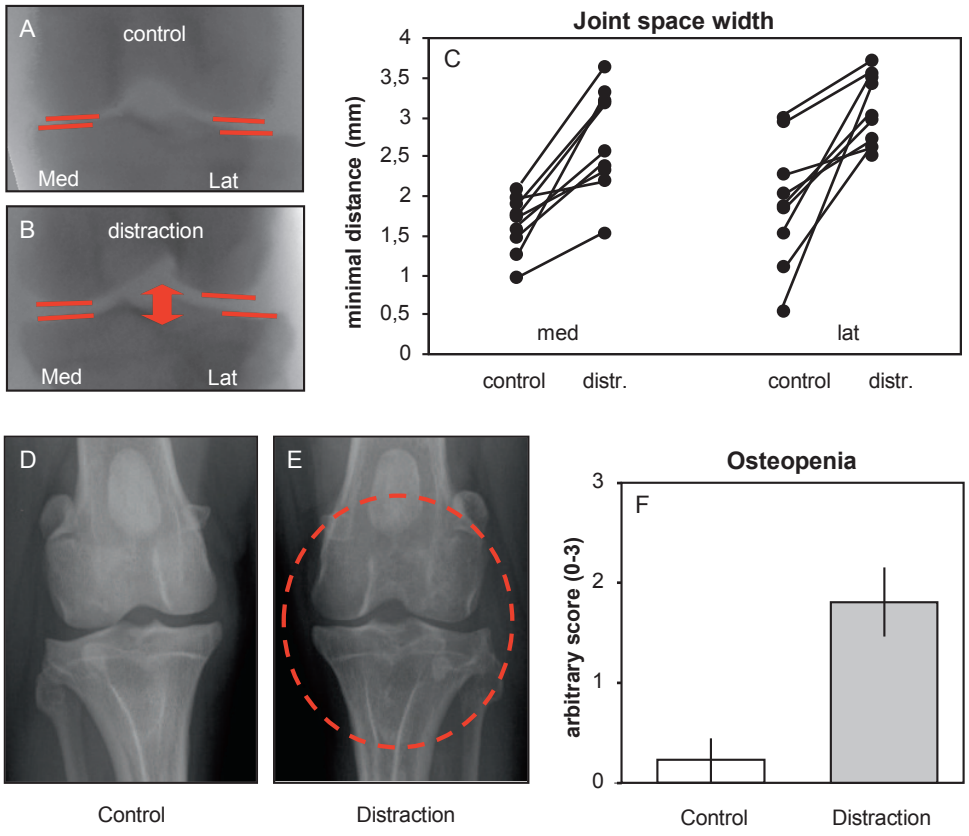

Figure 2. Conditions of joint distraction. Representative radiographs showing distraction (A and B) and osteopenia (figure D and E) for contralateral control and distracted joint, respectively. C. the increase in joint space width for both the medial (med) and lateral (lat) compartment of the distracted joint (distr.) *vs.* the control (control) is depicted (both $p<0.01$). The average presence of osteopenia of both joints is given in F ($p<0.01$).

Outcome measures

Cartilage Integrity

Macroscopic cartilage degeneration was evaluated on digital photographs by two observers unaware of the source of the photographs. Severity of cartilage degeneration of the tibia was graded according to the OARSI macroscopic cartilage damage score specific for dogs[13]. Scores of the two observers were averaged (maximum of 4) and used for statistical analysis. For histology, four samples from predefined locations of the tibia plateau (two lateral and two medial) were fixed in 4% phosphate-buffered formalin containing 2% sucrose (pH 7.0).

Cartilage degeneration was evaluated using safranin-O-fast-green iron haematoxylin-stained sections by light microscopy according to the OARSI canine cartilage score[13]. Samples were graded in random order by two observers unaware of the source of the sections. The mean score (a maximum of 36) of the medial and lateral tibia compartment of each knee joint was used for statistical evaluation.
For biochemical analysis, proteoglycan (PG)-content, one of the main components of cartilage, was determined from 8 samples taken from predefined locations. Details of biochemical analysis, using Alcian Blue staining, have been described previously [12, 14, 15]. The average of the 8 samples of each knee joint was used for statistical evaluation.
In addition, damage of the cartilage collagen (type II) was assessed from 4 samples from predefined locations by selective proteolysis using α-chymotrypsin, as described previously[16]. The percentage of denatured collagen was calculated as hydroxyproline in supernatant divided by total hydroxyproline (x 100%)[16]. The average of the 4 samples of each knee joint was used for statistical evaluation.

Chondrocyte activity
As a representative of PG-synthesis and retention of newly formed PGs, the rate of sulphate incorporation was determined ex vivo using $^{35}SO_4$ as a tracer for pulse-chase of newly formed glycosaminoglycans (GAGs). The total sulphate incorporation rate of each cartilage sample was calculated using the specific activity of the medium and was normalized for labeling time and wet weight of the explants. The release of newly formed PGs was normalized to the synthesis rate and expressed as percentage release of newly formed PGs in 3 days (% new PG release). For total release of GAGs (loss of PGs), Alcian Blue staining and precipitation techniques were used. The total amount of GAGs released (blue staining) is expressed as a percentage of the PG content (% GAG release). All techniques have been described in detail before[12, 14, 15].

Synovial inflammation
Macroscopic synovial inflammation was evaluated on high-resolution photographs of the synovium by two blinded observers. Severity of inflammation was graded by use of the OARSI macroscopic synovial inflammation score specific for dogs[13]. Scores of the two observers were averaged (maximum of 6) and used for statistical analysis.
Hematoxylin–eosin-stained histological sections of three synovial tissue samples (lateral, medial and middle) were scored by two blinded observers, by use of the OARSI histological synovial inflammation score specific for dogs[13].

Force plate analysis
Gait pattern as a measure of pain and functional ability was evaluated longitudinally by Force Plate Analysis (FPA) as described previously[17]. In short: a force plate, mounted flush with the surface of an 11 m walkway, sampled (100 Hz) peak ground reaction forces (GRFs). A computer stored signals corresponding with peak braking force (caudal direction) and peak stance force (vertical direction). Forces were normalized to body weight and

walking speed, and expressed in N/kg. A single handler guided the dogs by leash over the force plate, at a constant walking speed (1 ± 0.2 m/s). A successful run consisted of sequential, distinct paw strikes of the right front and right hind paw or the left front and left hind paw, respectively. Ten valid runs of each side were collected and GRFs were averaged for each of the four legs.

Calculations and statistics

For each joint, mean values of tibial cartilage and synovial tissue of multiple samples or scores were used as a representative value for each joint (see above). These mean values were used for statistical evaluation. Unless indicated otherwise, mean values of the animals ± SEM are presented (n=7 and 9 for OA controls and OA treated animals, respectively). The Wilcoxon rank test was used to compare data for the experimental and contralateral control joints (paired observations). Effects of distraction were evaluated by comparing the differences between the experimental OA joint and the contralateral control joint for the treated and untreated animals by use of the Man-Whitney U test (unpaired observations). Percentage differences were used for the biochemical parameters and absolute (delta) differences for the macroscopic, histological and gait pattern parameters. P values less than 0.05 were considered statistically significant.

RESULTS

Induction of OA in the Groove model

Cartilage damage

Macroscopic changes in the Groove model at 45 weeks post-surgery were similar to those observed previously[10]. Although not surgically damaged, the tibia plateau of the experimental joints showed clear cartilage fibrillation at the weight-bearing areas. Control knee joints showed healthy smooth cartilage (representative photographs are given in figure 3A and 3B). On average, the macroscopic cartilage damage was significantly more severe in the experimental joints than in the control joints (figure 3G).

These macroscopic observations were confirmed by histological analysis. The average OARSI score of the cartilage degeneration in the experimental tibia plateaus was mild but significantly higher than that of the contralateral control joints (figure 3H). There was clear damage of cartilage surface, chondrocyte clusters around the lesions and moderate loss of PGs (representative micrographs are given in figure 3D and 3E). PGs are important for the resilience of the cartilage while collagen is important for the tensile strength of cartilage. Forty-five weeks after OA induction, the PG content of the cartilage of the tibia plateaus was clearly decreased (on average -19. ± 3 %, $p<0.02$, figure 4A) coinciding with collagen damage (+1.1 ± 1.2 %, figure 4B).

These for OA characteristic cartilage integrity changes were also reflected in chondrocyte activity. Synthesis rate of PGs in the tibia plateau was higher in the experimental joints compared to the contralateral control joints (+41 ± 27 %, table 1). This for OA typical

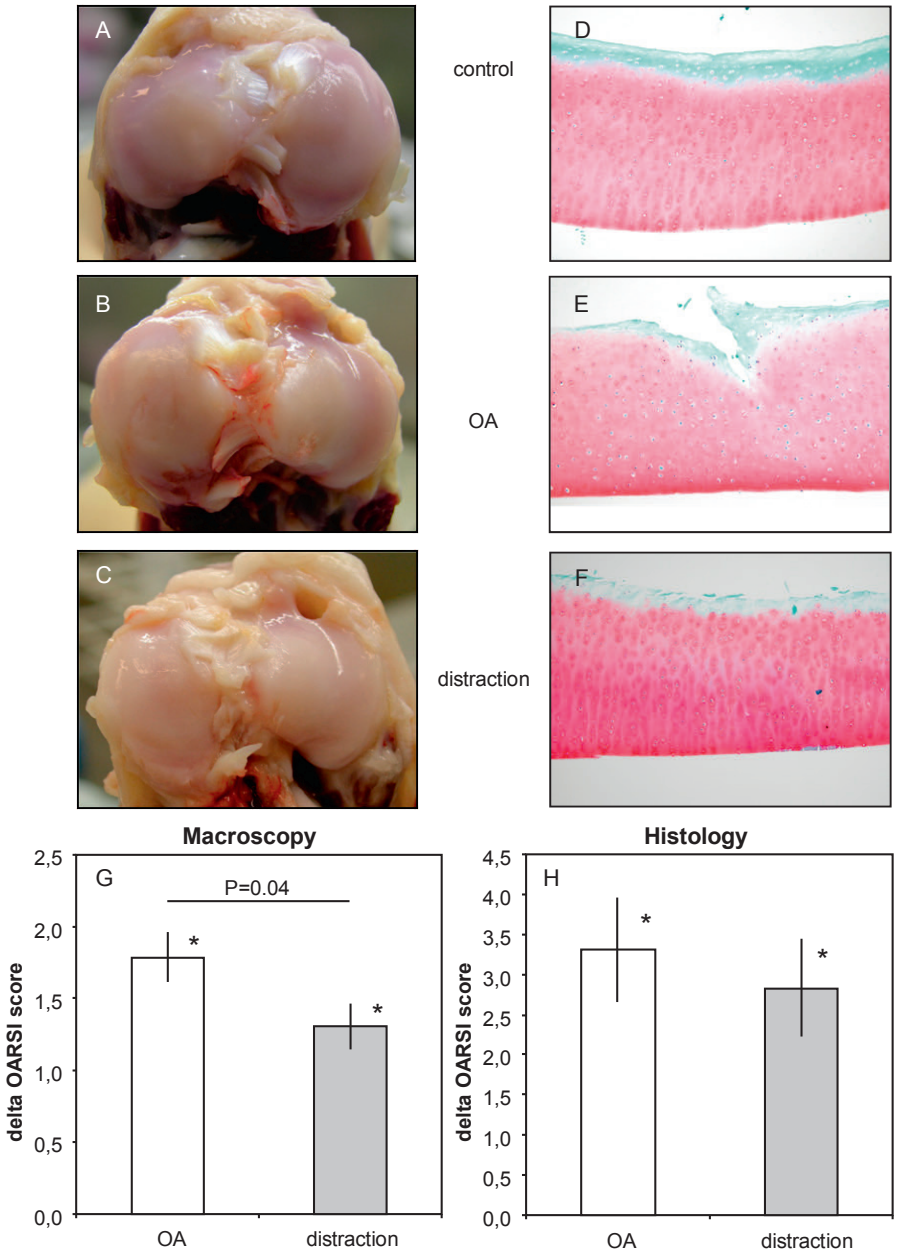

Figure 3. Cartilage damage. Representative macroscopic views of the tibia plateau of a control (A), untreated OA (B), and a treated OA (C) joint at 45 weeks of follow-up. In parallel, micrographs of the similar conditions are given (D control, E untreated OA, and F treated OA joint). G and H depict the mean values ± SEM of both the untreated OA dogs (OA, white bars) and treated OA dogs (distraction, grey bars) expressed as delta change of the OA joint compared to the contralateral control joint. Asterisk indicates a significant ($p<0.05$) difference between the OA and the contralateral control joint. P-values for the difference between the untreated OA dogs and treated OA dogs are given in the figure.

increase in PG synthesis was ineffective as the release of these newly formed PGs had also increased when compared to the control joints (+9 ± 4 % $p<0.05$, table 1). This demonstrates that there is a decreased retention of newly formed PGs in the cartilage matrix of OA joints. Also the release of the total amount of PGs (resident and newly formed) was enhanced as a result of the experimentally induced OA (+20 ± 7 %, $p<0.03$, table 1). Increased cellular activity was not the result of an increased number of cells as there was no statistical significant change in cartilage DNA content due to induction of OA (data not shown).

Synovial inflammation

In the Groove model no effusions were observed in the joints. Macroscopic evaluation of the synovial tissue showed only mild inflammation (figure 4A). This was supported by the histology. Light microscopic examination of the synovial tissue showed only mild increase of inflammation in the experimental joints when compared to the contralateral control joints (macroscopy +1.8 ± 0.4 and histology +2.7± 0.6 both $p<0.05$).

Gait pattern

The change in gait of the experimental leg compared to the contralateral control joint over the last 10 weeks is depicted in figure 6. A clear significant decrease of the two peak ground reaction forces, brake force and stance force, (-0.33 ± 0.05 N $p<0.02$ and -0.71 ± 0.12 N $p<0.03$, respectively) was seen. This indicates that there were not only tissue changes but also loss of function (increase of pain) due to OA development.

Mechanism

Conditions of joint distraction

Within 1 week after application of the distraction frame, all treated dogs were active again although not as active as before surgery. The untreated OA controls had resumed their normal loading patterns. The frame used in the present study resulted in a significantly improved gait-pattern when compared to the previous study[9] but partial unloading of the affected joint was still present. During the distraction period, all animals appeared to use their joint more or less with some flexion in the joint and partial load-bearing.

Every two weeks the amount of distraction was checked by X-rays under loading (representative X-rays are depicted in figure 2A and 2B) and the average increase in joint space width over the total 8 week distraction period is depicted in figure 1C. A clear increase in joint space width was observed suggestive for the absence or at least decrease of mechanical contact.

Also the intermittent hydrostatic fluid pressure changes during flexion and extension were presented during joint distraction. Flexion and extension of the canine knee joints revealed a comparable change in intra-articular fluid pressure in both the distracted and contralateral control joint (+4.4 ± 1.0 vs. +4.1 ± 0.7 kPa, respectively). During daily exercise, frequent motion (flexion and extension) of the treated knee was warranted.

Directly after removal of the frame, X-rays were made to evaluate the peri-articular osteopenia due to distraction. A clear osteopenia of the peri-articular bone was observed in the treated joint compared to the contralateral control joint (+1.8 ± 0.3 vs. +0.2 ± 0.4, respectively; figure 2F). Representative X-rays are depicted in figure 2D and 2E.

Cartilage damage

In all animals treated with joint distraction, macroscopic cartilage damage of the tibial plateau was less severe than that observed in the untreated OA dogs, on average statistically significant ($p<0.04$, figure 3C and 3G). This was supported by histological evaluation of the cartilage. Compared to the untreated OA dogs, a trend towards a decrease in histological cartilage damage of the tibia plateau was observed for the treated animals (figure 3F and 3H).

This improvement of cartilage integrity was even more clearly in the biochemical analysis of the PG content. Clearly, significantly less loss of PGs was seen in the treated dogs compared to the untreated OA dogs (-7± 3 % $p<0.02$ compared to untreated OA dogs, figure 4A). Also collagen damage after distraction was diminished, although not statistically significant (+ 0.3 ± 1.0 %, figure 4C).

The chondrocyte activity was positively influenced upon joint distraction treatment. Compared to the chondrocyte activity of the untreated OA dogs, the treated OA dogs showed on average a normalization of the newly formed PG release and total release (-5 ± 3 % and 5 ± 3 % respectively, both $p <0.01$ compared to the untreated OA dogs). PG synthesis rate had a tendency to normalize although this was not (yet) statistically significant.

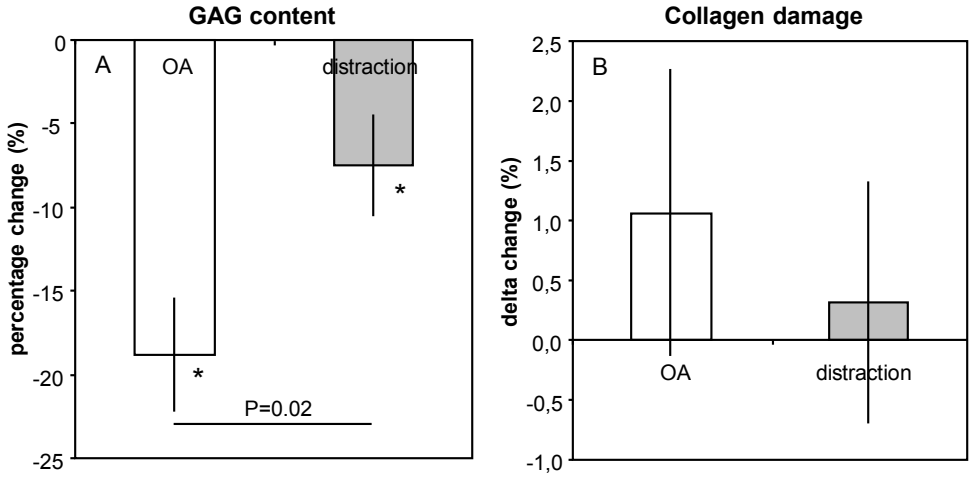

Figure 4. Mean values ± SEM of the percentage change are given for the proteoglycan content (A) and the absolute change for the collagen damage (B) of the experimental joints compared to the contra-lateral control joints. Changes for both the untreated OA dogs (OA, white bars) and treated OA dogs (distraction, grey bars) are depicted. Asterisk indicates a significant ($p<0.05$) difference between the experimental joint and the contra-lateral control joint. P-values for the difference between untreated OA dogs and treated OA dogs are given.

(%) Change	OA	distraction	P=
PG synthesis rate	41 ± 27	33 ± 9	ns
Newly formed PG release	9 ± 4*	-5 ± 3	0.01
Total PG release	20 ± 7*	5 ± 6	0.01

Table 1. Chondrocyte activity parameters. Mean values ± SEM of proteoglycan (PG) synthesis rate, newly formed PG release, and total PG release expressed as percentage changes of the experimental joint compared to the contralateral control joint. Changes for both the untreated OA dogs (OA) and treated OA dogs (distraction) are depicted. Asterisk indicates a significant ($p<0.05$) difference between the experimental joint and the contralateral control joint. P-values for the difference between the untreated OA dogs and treated OA dogs are given.

Synovial inflammation

Based on both the macroscopic and histological analysis of synovial inflammation there was no difference in the treated OA dogs compared to the untreated dogs (figure 5). Note that although there is increased synovial inflammation present in both groups, the observed levels are very mild (1.5 of a total of 6 for the macroscopy and 3 of a total of 18 for the histology).

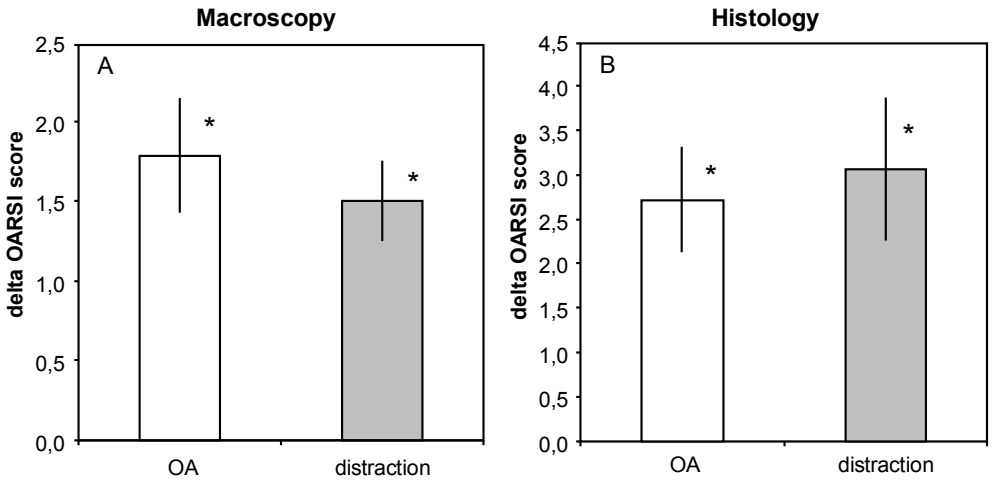

Figure 5. Macroscopic (A) and histological (B) synovial inflammation. Mean values ± SEM of the delta change are given of the experimental joint compared to the contralateral control joint. Changes for both the untreated OA dogs (OA, white bars) and treated OA dogs (distraction, grey bars) are depicted. Asterisk indicates a significant ($p<0.05$) difference between the experimental joint and the contralateral control joint.

Gait pattern

The improvement of cartilage integrity and chondrocyte activity is also reflected in a clear improvement of gait pattern, i.e. improvement in function/pain of the treated joint. Both the brake force as the stance force normalized completely in the treated OA dogs compared to the untreated OA dogs (figure 6B).

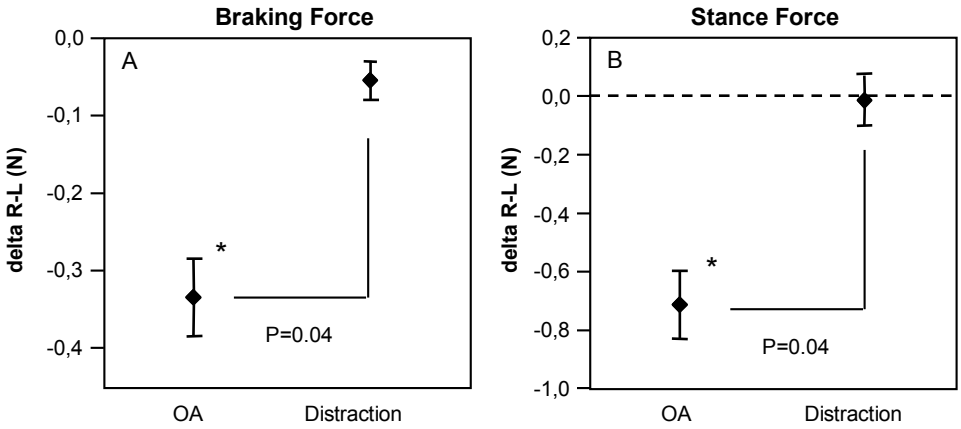

Figure 6. Force plate analyses. Delta change between the left control joint and the right experimental joint of the last 10 wks of follow-up (week 35 to 45) of the braking fore (A) and stance force (B). Mean values ± SEM are given. Changes for both the untreated OA dogs (OA) and treated OA dogs (distraction) are given. Asterisk indicates a significant difference between the experimental joint and the contra-lateral control joint. ($p<0.05$; n=4 and 5 for OA control and OA distracted group) P-values for the difference between the untreated OA dogs and treated OA dogs are given.

DISCUSSION

This interim analysis suggest that joint distraction as a treatment of experimentally induced canine knee osteoarthritis according to the Groove model results in cartilage repair activity as demonstrated by cartilage macroscopy, histology, and biochemistry, and that this tissue structure repair is accompanied by diminished pain as demonstrated by improved loading of the treated OA joint.

Distraction of the knee joint of dogs is a challenge when aiming at preservation of joint function, as during distraction dogs can easily walk on 3 limbs. As such, this study was preceded by a study to obtain the most optimal canine knee joint distraction frame (data not shown). Although weight bearing was still limited, it was significantly improved when compared to the previous study[9], and flexion within the frame appeared quit normal (within a certain range).

Joint distraction was applied after ten weeks of OA development in the canine Groove model.
After ten weeks, OA has developed, but still represents an early phase of the disease that progresses (slightly) over time[11]. As such, it cannot be distinguished whether joint distraction in this model just slows down development of progression, or that actual repair is induced. Especially in a model where loading is an essential part of OA development, unloading due to distraction might prevent progression. Optimally, the severity of joint degeneration at start of treatment should be determined. However, adding an extra group of animals has ethical and financial restrictions, and in vivo evaluation of severity of joint damage in the same group before treatment by use of biomarkers or imaging techniques is not sensitive enough. Irrespective of these considerations, the goal of establishing tissue structure modification by applying joint distraction was reached, though direct comparison with structure repair in human end stage OA remains difficult.

The actual beneficial changes in tissue structure quality corroborated the suggestion of a positive effect of joint distraction on cartilage integrity in the human clinical studies as reflected by beneficial changes in surrogate markers like MRI, X-ray and biomarkers (see also Chapter 9 and 10) [6, 7, 10]. Moreover, these findings are in line with findings in previous animal in vivo distraction studies which suggested cartilage repair activity. It was demonstrated that a combination of subchondral drilling, joint motion and distraction by an articulated external fixator promoted repair of a fresh osteochondral defect in the weight bearing area of the rabbit knee[18]. Although distraction for 4 weeks did not appear to be long enough to repair the defect, distraction for 8 and 12 weeks resulted in a good outcome based upon histological analysis of the treated knee joints. Also our own previous study using the canine ACLT model demonstrated improved chondrocyte activity of the OA cartilage as a result of joint distraction, although actual tissue repair could not be demonstrated[9]. Contrary results were demonstrated in a study using papain injection in rabbit knees as OA model where no beneficial effect was found upon joint distraction as assessed by histology of the joint[19]. It might be argued whether papain injection leads to a representative model of OA with regenerative capacity. Moreover, there was no follow-up in this study; joints were analyzed by histology directly after distraction. Which are in fact results that show resemblance to those that we found in our previous study where no cartilage repair directly after distraction could be were observed, but chondrocyte activity had improved. More recently, distraction of the rabbit knee after a complete tibia plate resection appeared to be able to regenerate the tibia plateau including cartilage[20]. A follow-up study demonstrated that gradual loading of the distracted joint improved the results even better[21]. Based on these animal studies evaluating joint distraction in the treatment of OA, it can be concluded that irrespective of the model used, there is clear data that joint distraction can lead to cartilage repair activity.

Pain and functional ability are credited as very important parameters in clinical OA research,, rather than structural changes, as patients do not suffer from lack of cartilage but

from joint pain. In canine models, gait pattern changes can be evaluated for each leg more objectively and accurately by force-plate analysis (FPA) than by visual assessments. Outcome parameters include longitudinal changes in braking, vertical stance, and propelling ground reaction forces (GRFs)[22]. Loading of a joint will be influenced by pain or other discomforts, but conversely, loading of the joint will vice versa influence cartilage nutrition, chondrocyte activation, damage[23-28] as well as cartilage repair[24]. In the present study there is clearly improvement in function of the treated joint compared to the untreated joints. This also implies that structural changes result in actual improvement of function and suggest less pain. Furthermore, this is in line with the clinical improvements seen in the human studies(chapter 8-9).

This interim analysis has clear limitations regarding power. The group sizes were calculated to be 9 and 15 for the OA control and OA treated group, respectively. As such, the results of the third phase have to be included in final analysis. At the end of the third phase also biochemical marker analyses of cartilage and bone synthesis and breakdown will be added. Moreover subchondral bone structure will be analyzed by micro-CT. When all data are collected, the changes that bone and cartilage undergo as a result of distraction, and which tissues plays a key role the clinical beneficial effect as observed in human studies can be established. Current and upcoming data will add to our understanding of the tissue structure repair activity due to joint distraction.
In summary, we can conclude from this interim analysis that joint distraction results in less cartilage damage and less pain (based on normalization of loading of the affected knee) in the canine Groove model of experimentally induced osteoarthritis, although definite complete cartilage repair (cure) could not (yet) be demonstrated. The results of this animal in vivo study corroborate the observed cartilage repair and clinical benefit in human studies. This implies that the suggestion of cartilage repair activity in clinical trials by use of surrogate markers is corroborated by the positive effects of joint distraction in an experimental model of OA on cartilage integrity parameters.

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Chapter 9

Subchondral bone remodeling is related to clinical improvement after joint distraction in the treatment of ankle osteoarthritis

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Submitted for publication

ABSTRACT

Background. In osteoarthritis (OA), subchondral bone changes alter the joint's mechanical environment and potentially influence progression of cartilage degeneration. Joint distraction as a treatment for OA has been shown to provide pain relief and functional improvement, through mechanisms that are not well understood. This study evaluated whether subchondral bone remodeling was associated with clinical improvement in OA patients treated with joint distraction.

Methods. Twenty-six patients with advanced post-traumatic ankle OA were treated with joint distraction for three months using an Ilizarov frame. CT scans were obtained prior to treatment, and at one and two years after treatment. The tibia and talus bones were manually segmented, enabling all CT datasets for a given patient to be brought into a common spatial alignment. Changes in bone density (Hounsfield Units (HU), relative to baseline) were calculated at the weight-bearing region, extending subchondrally to a depth of 10 mm. Clinical outcome was assessed using the ankle OA scale.

Results. Baseline scans demonstrated subchondral sclerosis with local cysts. At one and two years of follow-up, an overall decrease in bone density (-133HU and -123HU, respectively) was observed. Interestingly, density in originally low-density (cystic) areas increased. Joint distraction resulted in a decrease in pain (from 60 to 35, scale of 100) and functional deficit (from 67 to 36) at two years post-treatment. Improvements in clinical outcomes were best correlated with disappearance of low-density (cystic) areas ($p=0.008$).

Conclusions. Treatment of advanced post-traumatic ankle OA with three months of joint distraction resulted in bone density normalization that was associated with clinical improvement.

OA in general

Osteoarthritis (OA) is a degenerative joint disease characterized by cartilage destruction, formation of osteophytes, secondary synovial inflammation, and changes in subchondral bone. Clinical features include joint pain and stiffness, ultimately leading to loss of (joint) function. Main risk factors for OA are age, obesity, and a family history of OA. Joints most affected are spine, hip and knee[1]. Ankle OA is less common, but responsible for a significant part of the total (financial) burden of OA. The etiology of ankle OA often includes a history of joint trauma[2]. Treatment options for severe ankle OA are limited; either an arthrodesis or joint replacement, both with substantial risk of complications and adjacent joint degeneration, are the treatments of choice in end stage ankle OA[3].

Bone changes in OA

Subchondral bone changes are a distinctive feature in OA development, and they include sclerosis, cyst formation, bone attrition, bone marrow lesions (evidenced by MRI), and osteophytes. Radiographic imaging generally shows an increase in bone density, commonly referred to as subchondral sclerosis, beneath the weight-bearing joint surface. An increase in bone turnover results in higher bone volume and hypo-mineralization[4]. Locally, flattening or depression of the subchondral bony surface has often been observed, also known as bone attrition, and likely to represent bone remodeling in an area of increased loading[5]. MRI studies have shown that increased bone density (sclerosis) coinciding with excessive loading, is associated with bone marrow lesions[6]. These MRI-apparent lesions are marked by bone marrow necrosis, fibrosis, and trabecular abnormalities[7]. Bone marrow lesions may play a role in the pathogenesis of subchondral cysts, as cysts have been observed to arise within regions of marrow edema-like signal[8]. Subchondral cysts can frankly communicate with the joint space, or not, and are usually lined with fibrous connective tissue containing adipocytes and osteoblasts[9].

The role of this variety of subchondral bone changes in the development of OA is not yet clear[4], but inevitably the mechanical integrity of the joint surface is eventually disrupted and cartilage responds[10]. However, the relationship of subchondral bone changes with clinical outcome seems clearer than for the pathological changes of other damaged tissues in OA[11]. MRI studies have emphasized the importance of large bone marrow lesions[12] and the combination of bone marrow lesions and bone surface attrition[13] and their relationship with clinical features in knee OA. Subchondral cysts in the knee joint have been associated with an increased risk of knee replacement[14]. Consequently, subchondral bone has been identified as an attractive target for treatment in OA.

Joint distraction and other treatment strategies influencing bone.

There are a number of treatments that give long-term clinical improvement in severe OA, by influencing bone to widely varying degrees. One extreme is joint replacement[15], where the whole joint (including subchondral bone) is removed and with that the (unknown) source of pain. Another treatment is arthrodesis of the affected joint, where the joint surface is removed and joint function is sacrificed[16]. Other treatment methods resulting in (more or less) clinical improvement include pharmacological bone stimulation[17], osteotomy[18], and, less widely applied, joint distraction[19, 20]. Joint distraction is a surgical treatment for

advanced OA involving the use of an external fixator to unload the cartilage and underlying bone for a certain period. Joint distraction has been shown to provide long term pain relief and to improve joint function[20], through mechanisms that are not well understood.

Study goal

In light of recent studies suggesting a correlation between clinical features and subchondral bone changes in OA cohorts, and the clinically beneficial effects of joint distraction, we aimed to determine the relationship between bone density changes and clinical improvement upon treatment. In this exploratory clinical trial, the long term effects of joint distraction on longitudinal bone density changes were studied and related to clinical improvement, in young patients with severe ankle OA.

PATIENTS AND METHODS

Patients

Twenty-six patients with severe post-traumatic ankle OA were included in this prospective clinical trial, taken from a larger trial of 40 patients investigating clinical and other effects of joint distraction[21]. Suitable CT-scans were unavailable for 14 of those 40 patients (five patients withdrew, one fused before one year of follow-up, three CT-scans had severe metal artifacts, and five baseline CT-scans had technical errors).

Subjects for the primary study were selected from patients presenting with painful end stage ankle arthritis to a U.S. tertiary medical center. The criteria for selection of subjects included: symptomatic isolated, unilateral Kellgren-Lawrence[22] (KL) grade 3 or 4 ankle OA, skeletally mature and age≤ 60 years, failure of non -operative treatment > 1 year, and capacity to maintain extremity non-weight-bearing using ambulatory aids. Excluded from the study were patients who met any of the following criteria: history of inflammatory arthritis, the presence of other symptomatic joints on the ipsilateral lower extremity, contralateral ankle arthritis (KL grade 2-4), ankle or hindfoot malalignment, lived greater than 300 miles away from treatment center, current history of alcohol or drug abuse. Written, witnessed consent was obtained from all subjects using IRB approved forms.

Surgical procedure

All procedures were performed by one of two attending surgeons (AA, CS). First an arthroscopic ankle joint lavage was performed, with removal of any extra-articular anterior bony osteophytes. If the anterior osteophytes were too large to remove arthroscopically, they were removed by open means through an extension of the arthroscopic portals. No intra-articular joint debridement was performed. A circumferential external fixator was applied in a standardized fashion (Figure 1). The tibial frame was put on with the rings perpendicular to the tibia, and the foot frame was put on in line with the foot. The upper tibial ring was secured with two 5 mm half-pins, the lower one with 5 mm half pins and a crossing 1.8mm (“thin”) wire tensioned to nominally 600 N. The foot frame was then attached with a smooth thin wire transversely across the talus, two crossing thin wires across the calcaneus, and two crossing thin wires across the metatarsals, all tensioned to nominally 360 N. The

distraction rods were then placed. Intra-operatively the ankle was distracted 5 mm. The 4.8 mm internal diameter of the threaded rods connecting the rings, visualized using intra-operative fluoroscopy, was used as a radiographic guide to ensure that this amount of distraction was obtained. As the procedure was done with no tissue dissection and limited (< 1 cm) incisions, it was typically done on a short-stay inpatient admission or outpatient basis.

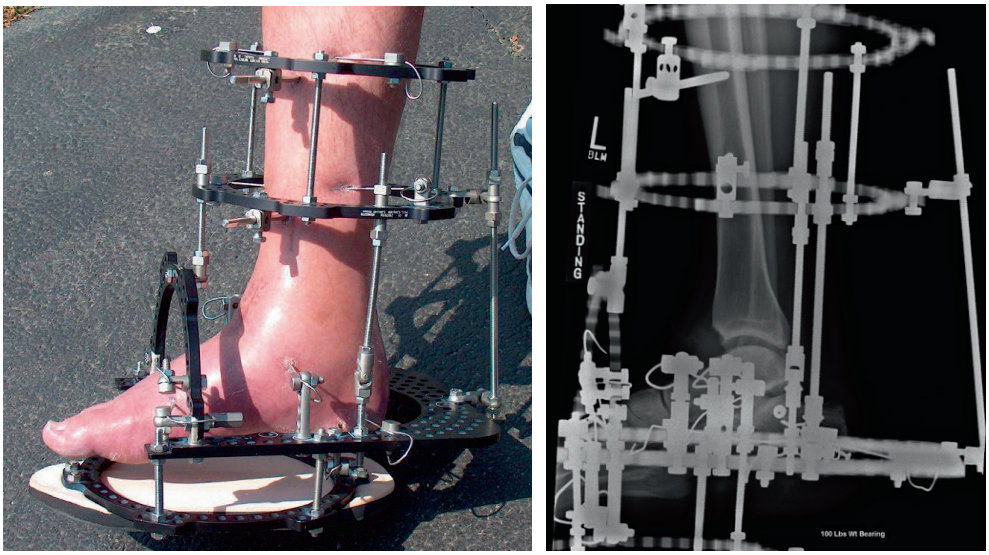


Figure 1. An Ilizarov external fixator was used to apply distraction to the ankle (left). Radiographic view of the distracted ankle (right).

Follow-up protocol

The fixators were removed between 85 and 95 days after application. The patients were gradually returned to full weight-bearing without boot immobilization by 6 months. After fixator removal, patients returned for study evaluations at 12 months and 24 months post removal.

Outcome parameters

Bone

Double-contrast multi detector row axial CT scans (Aquilion; Toshiba) were obtained at baseline (before treatment), and at one- and two-year follow-ups after treatment, to analyze joint space width[23] and bone density. Scanning parameters included 120 kVp, 75 mAs, 0.5-second-gantry rotation, 3.5 mm table travel per rotation, 1 mm section thickness, and a 512x512 matrix with in-plane pixel dimensions of 0.3x0.3. The images were reconstructed in 0.5 mm intervals.

The tibia and talus bones were manually segmented at each time point using OsiriXImaging Software (OsiriX Project; Geneva, Switzerland) with an interactive pen display (Cintiq 21UX; Wacom Technology, Vancouver, Canada). Segmentation data were then processed into continuous 3D surfaces (Figure 2A) using Geomagic Studio software (Geomagic Inc., Research Triangle Park, NC, USA). The spatial transformations for registering baseline and follow-up datasets were calculated by aligning bone surfaces using an iterative closest point algorithm in the Geomagic software. Baseline and follow up surfaces aligned well, with an average signed distance error of 0.21±0.9mm and an unsigned distance error of 0.73±0.7mm (mean±SD). Then utilizing ITK and purpose-written MATLAB code, the CT datasets for a given patient were transformed into a common spatial alignment.
Changes in bone density (in Hounsfield Units (HU), measured relative to baseline) were queried at over 30,000 discrete locations beneath the tibial and talar weight-bearing regions (Figure 2A). The measurement grid covered a subchondral patch of nominally 650 mm^2, with typically 4000 point measurements per surface (~0.17mm^2/point). Bone density was measured at 1 mm intervals beneath the bone surface, along the surface normals and extending subchondrally up to 10 mm.

Point-by-point comparison
Baseline and follow-up data were compared point-by-point over the measurement grids. For each surface point, the bone density at every 1mm interval was compared between the two time points. Further analysis bracketed data based upon the supposition that subchondral bone density in healthy joints would be expected to be >400 HU within the first 3 mm (subchondral plate) and between 100 and 400 HU in the deeper trabecular bone (4-8 mm beneath surface; Figure 2A). Any densities outside of these putative normal density ranges were considered to be abnormal (pathological). The densities of regions of bone with low density (ostensibly cystic, defined as <400 HU for the first 3 mm closest to the joint surface and <100 HU for 4-8 mm from the joint surface) at baseline were compared to the densities at corresponding locations in follow-up scans, and reported as per point changes in density.

Method Validation
To test reproducibility, two patients with CT scans that had been taken approximately two weeks apart (for reasons unrelated to the study) were analyzed using this methodology, with the hypothesis that bone densities would not have changed over that short time-period. The reproducibility analysis showed an average measured difference in distal tibia bone density of only 32±30 (mean±SD) HU and 35±19 HU, supporting the reliability of this technique.

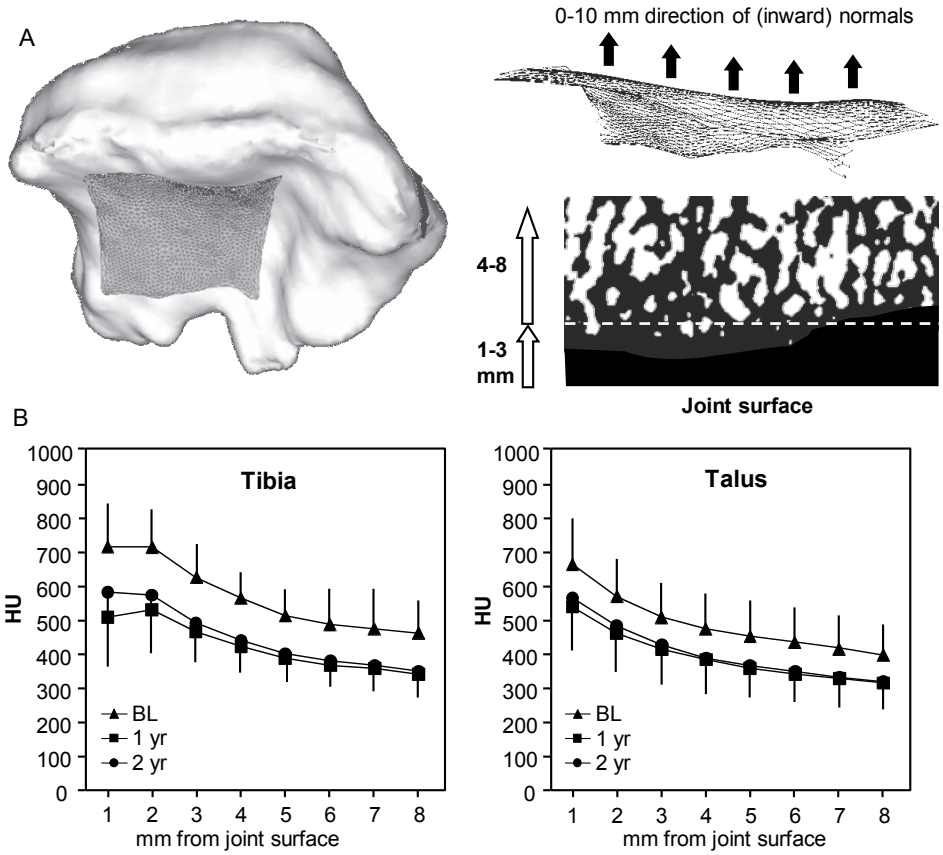


Figure 2. A. Surface created from a cloud of segmented points, with the patch placed on the weight-bearing area (upper left). Bone density calculations were performed at 1 mm intervals beneath the bone surface, along the surface normals and extending subchondrally up to 8 mm. Adjacent to the joint surface was a high density area where the cortical plate was located, gradually extending to trabecular bone with lower density (schematic drawing in the upper right). B. Mean density (+/- SD) was measured up to 8 mm from joint surface at the different time points. Density gradually decreased further from the joint surface. At 1 and 2 years of follow-up, overall density decreased in both tibia and talus. All time points differed statistically significantly from baseline (all $p<0.001$).

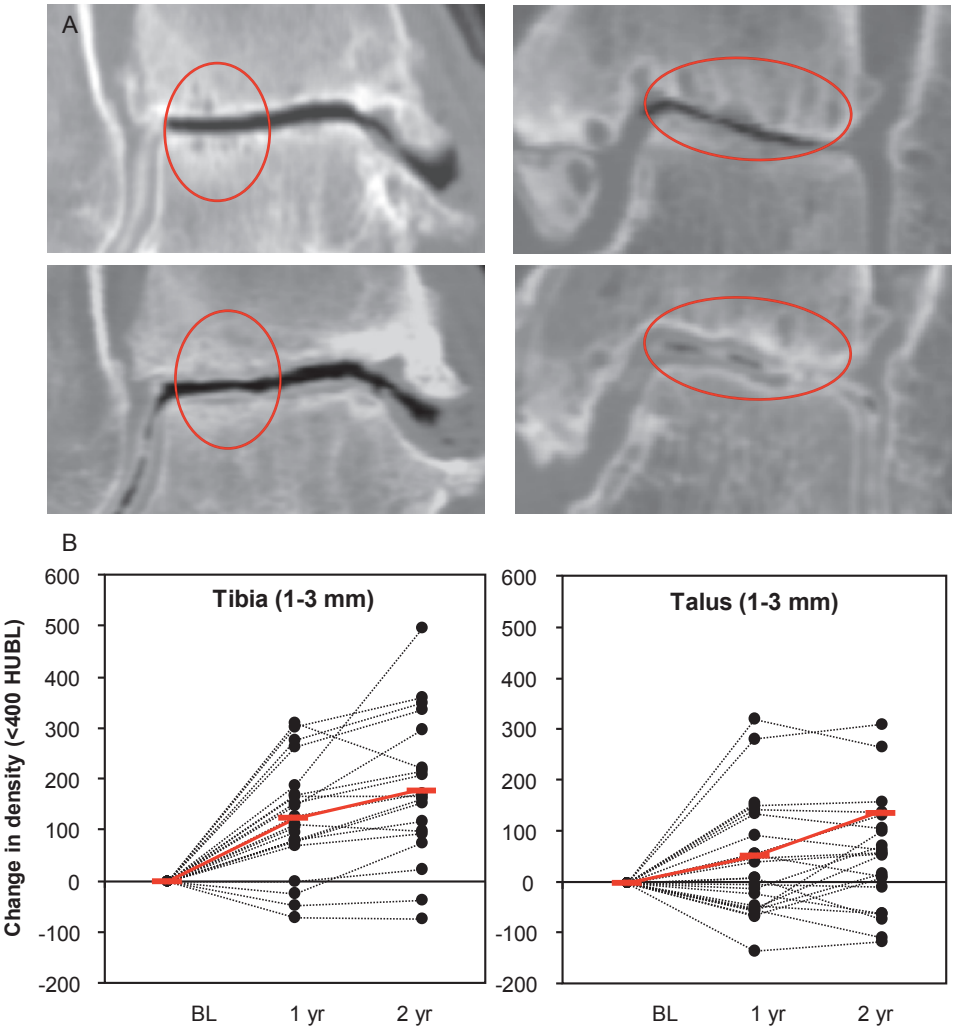


Figure 3. A. Representative CT scans of two patients before and two years after distraction, showing severe bone pathology at baseline (upper panels) with cysts and sclerosis in the weight bearing area. At 2 years of follow up (lower panels) there is a decrease of density in the sclerotic area while subchondral cysts diminished (within ovals) and the cartilage layer seemed to increase B. Change in density of the low density areas (<400 HU at baseline) for individual patients. A mean increase (solid lines) can be observed at one and two years of follow-up.

The primary clinical outcomes were changes in the Ankle Osteoarthritis Scale (AOS) score[24]; consisting of pain and disability subscales. The AOS questionnaire was completed at baseline and at one and two years after fixator removal.

Statistics

Statistical significance in changes over time for bone and clinical parameters was determined using paired parametric tests. Spearman correlations of the sum of change for tibia and talus were used to identify significant correlations.

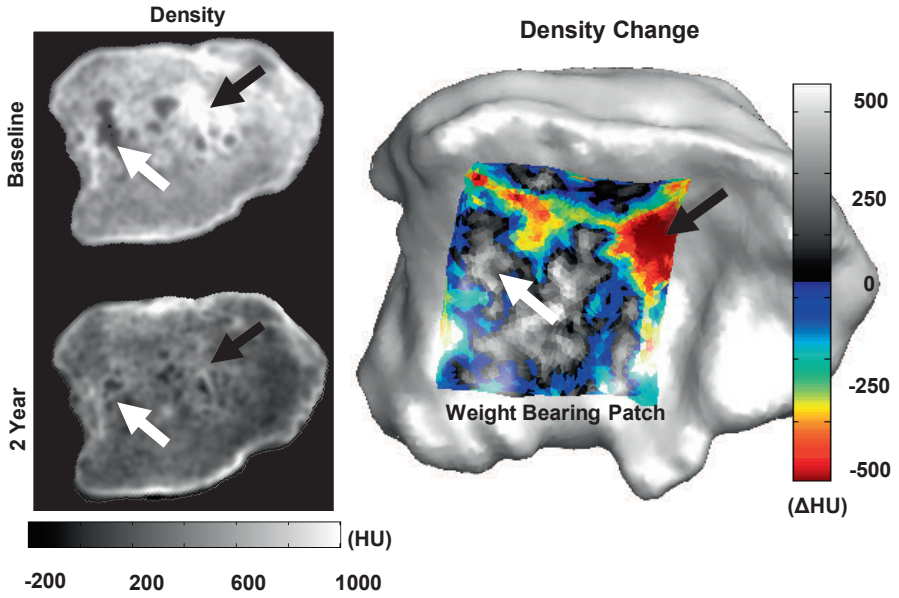


Figure 4. After two years of distraction, the normalization of bone densities is evident on CT (left). The change in bone density (2yr – baseline) in the weight bearing area of the tibia (mean from 1-3 mm depth), right, depicts the increase in density near cysts (white arrow), and a decrease in density in sclerotic regions (black arrow).

RESULTS

Recruitment and Follow-up

The study was opened for enrollment in December, 2002 and closed in October, 2006. Follow-up was completed for the last patient in March, 2009.

Bone outcome

At one year following completion of joint distraction, the average subchondral bone density for the study group was decreased. Immediately beneath the joint surface, density was approximately 700 HU at baseline, and gradually decreased with greater distance from the surface. The follow-up data followed a similar trend, but at substantially lower densities (figure 2B). Mean density over the area of 1 to 8 mm from the joint surface decreased from 569±65 HU to 435±74 HU (mean±SD; p<0.001) for the tibia and from 490±95 HU to 395±85 HU (p<0.001) for the talus. Two years after distraction, the overall decrease in bone density was still present and for the tibia statistically indistinguishable (p=0.35) from that observed at one year of follow-up (451±74 HU; p<0.001, 413±78 HU; p<0.001 for tibia and talus respectively).

A point-by-point comparison (table 1 shows mean ± SD and the 95% confidence interval) revealed that regions of bone from 1 to 3 mm beneath the joint surface with abnormally low density (< 400 HU) at baseline showed an increase in density; +123±108 HU at one and +180±143 HU at two year for the tibia, and +41±111 HU at one and +58±114 HU at two year of follow up for the talus (Figure 3B). Also, more dense regions (>400 HU) over that same volume saw a decline, and returned to more normal densities at both one and two year time points. The tibia decreased 237±111 HU and 192±117 HU, and the talus decreased 156±93 HU and 136±82 HU at these two respective time points.

		1 year					2 years				
1-8 mm		**Mean**		**SD**	**p-value**		**Mean**		**SD**	**p-value**	
	tibia	-133	±	75	0.000		-124	±	69	0.000	
	talus	-95	±	83	0.000		-88	±	83	0.000	
1-3 mm		**Mean**		**SD**	**95% CI**		**Mean**		**SD**	**95% CI**	
> 400	tibia	-237	±	111	-186	-288	-192	±	117	-139	-244
	talus	-156	±	93	-188	-194	-136	±	82	-101	-171
< 400	tibia	123	±	108	77	169	180	±	143	116	244
	talus	41	±	111	-3	84	58	±	114	9	107
4-8 mm											
> 400	tibia	-184	±	92	-141	-226	-193	±	102	-147	-239
	talus	-138	±	77	-107	-170	-127	±	80	-93	-161
< 200	tibia	144	±	133	83	206	153	±	131	94	212
	talus	92	±	100	51	133	78	±	144	17	140

Table 1. Change in density (mean ± SD in HU, p-value or 95% confidence interval in case of point-by-point comparison) compared to baseline for tibia and talus at one and two years of follow-up.

Further away from the joint surface (from 4-8 mm), a density increase at low density areas was also observed. In those regions with densities below 100 HU, there was an increase of 144±133 HU at one year and 153±131 HU at two years for the tibia. Low-density regions of the talus showed an increase of 92±100 at one year and 78±144 at two year. Regions of bone with baseline densities >400 HU at 4-8 mm beneath the joint surface showed decreases of 184±92 HU and 193±102 HU at one and two year follow-ups for the tibia and 138±77 HU and 127±80 HU at one and two years for the talus. At baseline, clustered low-density areas were surrounded by high-density areas. At two years of follow up, normalization toward a more homogenous density distribution was seen (Figure 4).

Clinical outcome

Clinical outcome was measured by use of the AOS (Figure 5A). At baseline, AOS pain was 60±3% of the maximum score and decreased to 35±4% ($p<0.001$) at one and to 35±5% ($p<0.001$) of maximum score at two year follow-up. AOS disability showed comparable results. A baseline score of 67±2% decreased to 46±5% ($p<0.001$) of the maximum score at one and 36±5% ($p<0.001$) at two years of follow-up.

Correlations

No correlation was detected between the decrease in sclerotic regions and decrease in pain ($r=-0.25$; $p=0.35$). A modest correlation was found between the lack of improvement of disability with more decrease of bone density ($r=-0.55$; $p=0.03$). Interestingly, the increase in density in cystic regions near the joint surface did strongly correlate with clinical improvement as assessed by the AOS pain and AOS disability after two years ($r=-0.65$, $p<0.01$ and $r=-0.58$, $p=0.02$ respectively; Figure 5B). The density increase in baseline low-density areas located further from the joint surface (4-8 mm) also showed a correlation with change in AOS pain ($r=-0.52$, $p=0.04$).

DISCUSSION

Summary

The present study demonstrates that treatment of advanced post-traumatic ankle OA with joint distraction produced an overall decrease of subchondral bone density, which persisted for at least two years. Subchondral bone at baseline consisted of varied regions of relatively low density (cystic) and high density (sclerotic) areas. While overall density decreased, density in cystic lesions actually increased (normalization of bone density). In addition, a correlation was found between clinical improvement and the resolution of subchondral bone cysts.

Method limitations

Patients included in this study suffered from end-stage post-traumatic ankle OA characterized by severely damaged subchondral bone. In a few cases, bone boundaries were challenging to segment. Not only were some of these ankles subject to degenerative morphological changes,

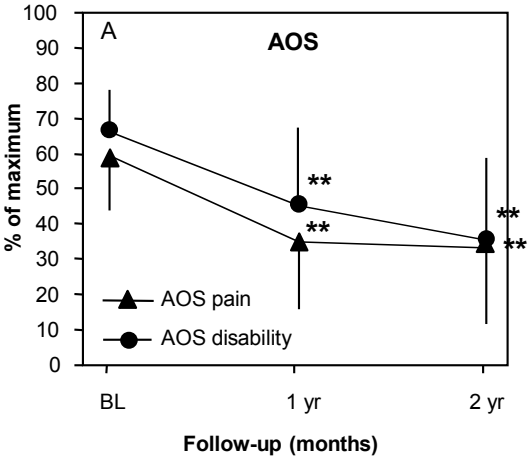
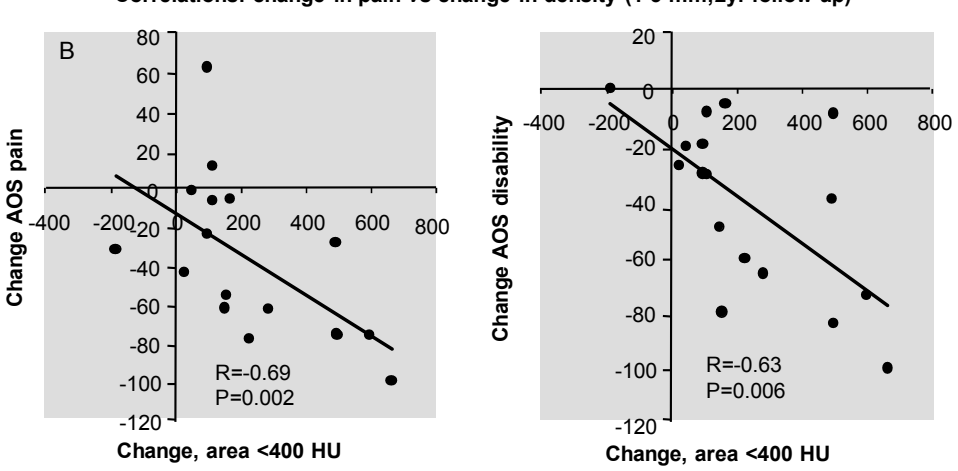


Figure 5. A. Clinical outcome presented by the AOS score (mean±SD). Subscales pain and disability are shown on a scale of 0-100 (100 being the worst outcome). ** indicate p-values <0.001 compared to baseline.
B. Correlations between percentage change in AOS subscale and change in density in low density areas (sum of change of the tibia and talus).



but bone remnants from previous fracture and hardware were also present. For these cases, registration was performed using only undamaged segments of the bone surface. While the presence of metal objects and/or fracture lines away from the joint may corrupt some volume of the CT data, no artifacts were visually apparent near the joint surface.
Analysis was done on a selected region of the joint surface that corresponded to the weight-bearing area. The baseline surface served as the datum for bone density analyses at all three time points. Over time, the contour of the joint surface may have slightly changed, and therefore density measurements relative to the joint surface would not perfectly coincide between baseline and follow up datasets. However, using a single surface as datum for

each dataset ensured that the point-by-point comparison would consistently analyze the same location (e.g. cystic regions) in the image volumes.

While the reproducibility analysis supported the validity of these methods, it is important to note that clinical CT is not the most precise modality for making absolute bone density measurements. Future studies may choose to incorporate additional measurement tools such as DXA for more robust bone mineral density measurements[25], although it would suffer from lower spatial resolution. Despite the limitation of clinical CT in terms of density precision, it provided a high-resolution data volume that enabled point-by-point comparisons to be made in 3D space. Although the absolute density measurements remain to be verified, CT was a reproducible instrument for making longitudinal comparisons.

Bone changes due to distraction

While normal trabecular bone usually exhibits a density less than 400 HU within 10mm of the joint surface, these patients had an average density greater than 400 HU at baseline. Increased subchondral density (sclerosis) was expected in these OA ankles. Within the sclerotic area, low density areas were observed (the presence of cysts, either communicating or not with the joint space), with densities <400 HU in the subchondral plate and < 100 HU in the deeper trabecular bone considered pathological.

One year after treatment with joint distraction, overall density had decreased. At 4-5 mm below the joint surface, density had decreased below 400 HU, a level deemed as 'healthy' for subchondral trabecular bone. In addition, discrete pockets of low density bone mostly resolved. The results from this study suggest that joint distraction may lead to a normalization of OA-induced pathological subchondral bone changes for a period of at least two years.

Mechanism of bone changes

In joint distraction, both cartilage and subchondral bone are unloaded for a certain period. Since bone becomes osteopenic when unloaded[26], it was not surprising to observe a decrease in bone density following distraction, although the duration of two years was somewhat unexpected. The exact mechanism for the disappearance of cysts could lie in the dramatic changes in mechanical and biochemical environment induced by distraction. Cysts represent areas of bone necrosis[9], and have the potential to not only increase but also diminish[14]. Less surrounding sclerosis and subsequently less stiff bone, may allow mechanical stimuli to reach the cystic areas and induce bone formation. This in combination with an overall increase in bone turnover might be the necessary circumstance under which cystic areas can be repaired.

A role in clinical improvement.

No positive correlations were found between globally diminished sclerosis and clinical improvement. In contrast, patients with less dramatic bone density decreases saw an improvement in disability scores. Although counterintuitive at first, this could be a result from remodeling that was stimulated by greater loading, made possible by the improvement in function.

The correlation between an increase in density of low density areas, and patient-reported outcomes suggest that the resolution of bone cysts was beneficial to clinical outcome. Cyst-

related joint pain might be caused by increased pressure and fluid flow in the subchondral bone. During loading, compression of cartilage forces fluid into the bone through the damaged subchondral plate[27]. The hydraulic conductance of osteochondral tissue has been shown to be higher in osteoarthritis[28]. When cysts and defects in the subchondral plate diminish, the subchondral bone is less subject to increased fluid flow and pressure responsible for joint pain. Especially in cystic areas (pores) close to the joint surface, within the cortical plate, an increase in hydraulic conductance might be responsible for joint pain. Bone cysts (and bone surface attrition) seem to evolve in regions of bone marrow lesions and might be the next level of bone marrow pathology in OA[8]. The relationship between bone marrow lesions as seen on MRI and clinical symptoms has already been established, and it could also be explained by increased pressure within the bone in areas of excessive loading and mechanically compromised trabecular structure[8, 29].

The results from this study show that subchondral bone density changes in response to joint distraction. Bone remodeling may lead to a more physiologically normal distribution of mechanical stresses, particularly near regions with less dense bone, which may in turn encourage cartilage repair activity and changes in the availability of pain mediators originating from bone[30-32]. In addition to bone changes, visual assessment of the CT arthrographic data suggested there to be an increase in cartilage thickness, an observation that deserves additional research. The current study showed that joint distraction started a process of bone remodeling and a subsequent improvement in clinical outcome in a series of OA patients. While further research is needed to establish efficacy before distraction can be widely implemented, these results suggest it to be a potentially effective method for treating severe OA.

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Chapter 10

Tissue structure modification in end-stage knee osteoarthritis by use of joint distraction

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Submitted for publication

ABSTRACT

Objective. Modification of joint tissue damage is challenging in late stage osteoarthritis. Few options are available for treating end-stage knee osteoarthritis other than joint replacement. This exploratory trial examined whether joint distraction can effectively modify knee joint tissue damage and has the potential to delay prosthesis surgery.

Methods. Twenty patients (<60 years) with end-stage tibio-femoral osteoarthritis were treated surgically using joint distraction. Distraction (~5 mm) was applied for two months using an external fixation frame. Tissue structure modification between baseline and one year follow up was evaluated radiographically (joint space width; JSW), by MRI (segmentation of cartilage morphology), and by biochemical markers of cartilage collagen type II turnover, with operators being blinded to time point. Clinical improvement was evaluated by Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and visual analogue scale (VAS) pain score.

Results. Radiography demonstrated a significant increase in mean and minimum JSW (2·7 to 3·6 mm and 1·0 to 1·9 mm; $p<0·05$ and $<0·01$). MRI revealed a significant increase in cartilage thickness (2·4 to 3·0 mm; $p<0·001$) and a decrease of denuded bone areas (22 to 5%; $p<0·001$). Biomarker levels showed a trend towards increased collagen type II synthesis (+103%; $p<0·06$) and decreased breakdown (-11%; $p<0·08$). The WOMAC index increased from 45 to 77 points, and VAS pain decreased from 73 to 31 mm (both $p<0·001$).

Conclusions. Joint distraction can induce tissue structure modification in end-stage knee osteoarthritis accompanied by significant clinical benefit. No current treatment is able to induce such changes. Larger, longer, and randomized studies on joint distraction are warranted.

Osteoarthritis (OA) is a degenerative joint disorder characterized by progressive cartilage damage and loss, changes in bone and other peri-articular tissues, and commonly also secondary joint inflammation. These changes in tissue structure are associated with pain, stiffness, and functional disabilities[1].

Prevalence: Knee OA affects roughly 6-10% of the adult population and is the most common form of OA, with a huge socio-economic and health care burden[2].

Current treatment options: Few options are available for treatment of end-stage knee OA and none have clearly been shown to halt or even reverse tissue structure damage[3]. Removal of pain by replacing the destroyed joint with an endoprosthesis is the currently accepted treatment option for end-stage knee OA. Consequently, the number of total knee prosthesis (TKP) is exponentially increasing in the Western world and causes major economic burden[4, 5] Over 40% of all knee replacements and up to 44% of all total knee revisions are performed in patients aged under 65[6]. Importantly, the procedure has a higher risk of failure in younger patients than in older patients[7, 8]. As such, development of alternative treatment strategies for end-stage knee OA, specifically those that can postpone a first prosthesis, are urgently needed.

Joint distraction: A surgical procedure in which the two bony ends of a joint are gradually separated to a certain extend for a certain period of time. Initially, joint distraction was used in the treatment of joint mal-alignment and joint contracture. An external fixation frame was used to actively reposition the joint and to increase range of motion. Distraction was performed to prevent damage (compression) of the joint cartilage during the forced repositioning. In some of these patients OA was present in the treated joint and an unexpected clinical improvement of the OA was observed[9, 10]. These clinical observations led us to a proof-of-concept study examining the benefit of joint distraction, by treating young patients suffering from severe ankle OA[11]. Two thirds of the patients treated for 3 months with joint distraction experienced significant clinical benefits for a period of up to ten years[12]. Based on preliminary radiographic outcome in a limited number of patients, it was suggested that joint distraction may lead to tissue structure modification as well[13].

Goal: To explore whether joint distraction can halt or reverse joint degeneration in end-stage knee OA, and whether it has the potential to delay knee replacement surgery in relatively young patients in an open, uncontrolled clinical trial.

PATIENTS AND METHODS

Patients

Twenty patients with end-stage knee OA and with an indication for knee replacement surgery were included between 2006 and 2008 according to the following criteria: age <60 years, Visual Analogue Scale (VAS) of pain ≥60 mm, radiographic signs of joint damage, and primarily tibio-femoral OA (not patella-femoral OA). Exclusion criteria were severe symptoms in both knees, a history of inflammatory or septic arthritis, severe knee mal-alignment requiring surgical correction, and inability to cope with an external fixator for two

months. The study was approved by the medical ethical review committee of the University Medical Center of Utrecht (no. 04/086). All patients gave written informed consent.

Distraction method

Two monotubes with internal coil springs (Stryker®, Monotube Triax) were placed parallel on the medial and lateral side, bridging the knee joint (figure 1A). Each monotube was fixed to two bone pins (Stryker®, 6 mm self-drilling half pins) on each end. Pinholes were placed as distant as possible from the joint line, so as not to compromise the area needed for possible future prosthesis surgery. The monotubes were placed on the bone pins and they were lengthened 2 mm, all under anesthesia. In the following three days the joint was distracted twice a day for 0·5 mm, bringing the total distraction to 5mm, which was confirmed by X-ray, and adjusted if necessary. After instructions about pin site care, daily exercise, and physical therapy, the patients were discharged from the hospital. Patients were encouraged to load the distracted joint, with full weight bearing allowed.

Every two weeks the patients returned to the hospital. At these visits the monotubes were removed temporarily. For 3-4 hours, the knee was bent in a continuous passive motion device, pain at the pin sites determining the maximum degree of flexion; on average, 25° (15-80°) flexion

and full extension was reached. The monotubes were replaced and sufficient distraction was confirmed by X-ray and adjusted if needed. In case of superficial pin tract infections, treatment with oral antibiotics was provided (flucloxacilline).

After two months, the tubes and pins were removed at day care and patients went home without imposed functional restrictions. A continuous passive motion device was provided at their home to practice flexion of the knee joint. After reaching 90° flexion (approximately one to two months after removal of the frame), the patients were advised to gain muscle strength by e.g. cycling.

Complications

Two patients suffered from lung emboli despite appropriate anti-coagulative prevention (nadroparin). Patients were admitted to the hospital for a week and treated with anti-coagulative therapy (nadroparin), after which they were discharged in good condition continuing therapy (acenocoumarol) for six months. Of the 20 patients, 17 suffered from single or multiple pin tract infections. All were successfully treated with antibiotics (flucloxacillin) for on average four weeks. One patient had to be admitted to the hospital for one week to receive antibiotics intravenously. None of the patients had any sign of osteomyelitis.

Structural outcome

Patients visited the outpatient clinic twice before treatment (baseline), every two weeks during treatment, and three, six, and twelve months after the start of the treatment.

Radiographic analysis. At all visits, weight-bearing, semi-flexed posterior-anterior radiographic views were acquired for evaluation by KIDA (Knee Images Digital Analysis)

software[14]. KIDA analyses provided minimal and mean joint space width (JSW) in both compartments and subchondral bone density using an aluminum step-wedge as a reference. Analyses were performed blinded to the order of acquisition and characteristics of the patients (ML).

Quantitative MRI analysis. At baseline and at 12 months, MRI acquisition (1·5T Philips Achieva) was performed using sequences validated for the purpose of quantitative measurement of cartilage morphology[15, 16]. Coronal images were used to segment the femoro-tibial cartilage plates, including denuded areas, the operator (SC) and quality control reader (FE) being blinded to the order of sequence (baseline *vs.* follow up). Cartilage parameters were computed using custom software (Chondrometrics Inc., Ainring, Germany). The primary structural outcomes[17] were thickness of cartilage over total area of bone (ThCtAB) and the percentage area of denuded bone (dABp). Secondary structural outcome parameter was thickness of cartilage over area of bone covered with cartilage (ThCcAB).

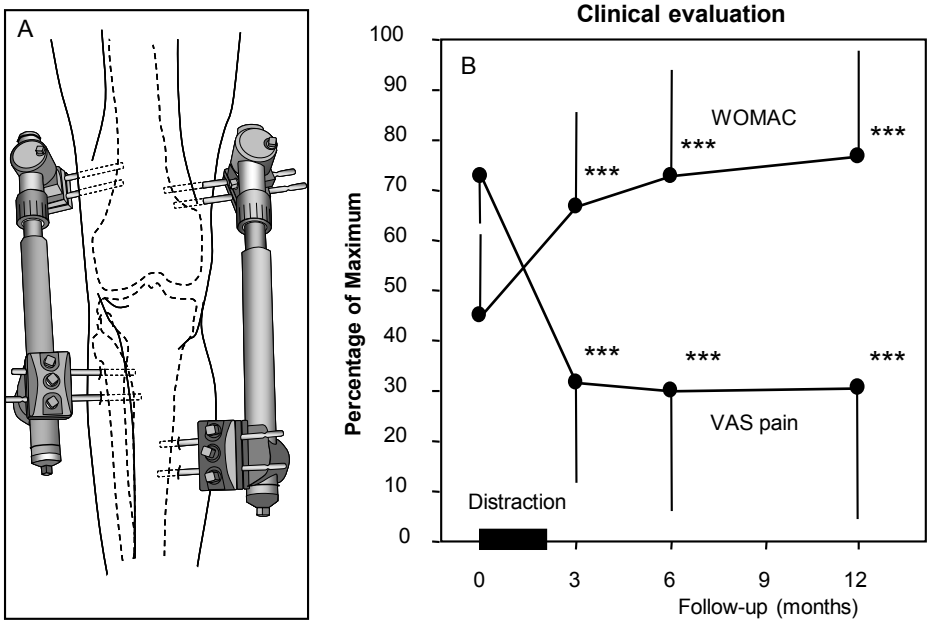

Figure 1. Drawing of the monotubes placed on bone pins bridging the knee joint. Lengthening of the tubes (approximately 5 mm) induces joint distraction. Springs within the tubes (like shock absorbers) allow restricted (3 mm) axial movement without direct joint surface contact. B. Clinical evaluation presented by the total WOMAC and VAS pain, means ± SD are given. Distraction is performed during the first 2 months, at 3 months (one month after removal of the fixator), pain has decreased and WOMAC score has increased, sustaining for at least 12 months. *** indicates statistical significance of $p<0.001$.

Biomarker analysis. Serum and urine samples were collected and stored at -80ºC. Cartilage collagen type II synthesis and breakdown were determined by serum PIIANP (Linco, EZPIIANP-53K) and urinary CTXII (Nordic bioscience, cartilaps; corrected for urine creatinine), respectively. Samples were analyzed in twofold, and longitudinal samples of one patient were assayed in one plate, to eliminate inter-kit variability (SM).

Clinical outcome

The primary clinical outcome parameter was the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC[18]), a 100 points score being the best condition). The secondary clinical outcome parameters were the visual analogue scale (VAS) on pain (0-100mm) and physical examination of the joint (pain on palpation, crepitus, pain with flexion, and joint effusion) (FI).

Statistical analysis

Parametric statistics (two-sided paired t-test) were used for all parameters, to compare whether the follow up values significantly differed from the baseline values. Spearman correlation coefficients were used to relate longitudinal changes over one year between parameters. Means ± SDs are given and $p<0{\cdot}05$ was considered a statistically significant difference (FI).

RESULTS

Twenty patients were included, eleven men and nine woman, aged 48 ±7 years. Eleven left knees and nine right knees were treated. Eighteen patients had predominantly medial, and two lateral, compartmental OA. Three, four, eleven, and two patients had a baseline Kellgren & Lawrence grade[19] of one, two, three and four, respectively.

Structural Outcome.

Radiographic analysis: The mean JSW of the most affected compartment increased from 2·7 ±1·7mm to 3·6 ±1·2mm from baseline to twelve months ($p<0{\cdot}05$; figure 2 top-left). The minimum JSW increased from 1·0 ±1·2 mm to 1·9 ±1·3mm ($p<0{\cdot}01$). Subchondral density at baseline was higher in the most affected compartment than it was in the contralateral compartment; 41±5 and 37±1mm aluminum equivalents respectively (figure 2 top-right), and decreased 5·8 ±12 mm aluminum equivalents ($p<0{\cdot}05$) in the affected compartment after one year and 2·6 ±5mm in the less affected compartment.

Quantitative MRI analysis at one year showed an increase in mean ThCtAB of the most affected compartment from 2·4 ±0·6 to 3·0 ±0·5mm ($p<0{\cdot}001$) and a decrease of mean dABp from 22 ±20 to 5 ±9% ($p<0{\cdot}001$) (figure 3; including a representative pre- and post-treatment MRI image). ThCcAB showed a borderline increase from 2·9 ±0·3 to 3·1 ±0·4mm ($p=0{\cdot}062$) meaning that despite the increase in cartilage area, the overall average thickness did not decrease, which only occurs when either the newly formed cartilage is just as thick as surrounding cartilage or surrounding cartilage also thickens. Results for separate

compartments (femur and tibia; and the less affected compartment) and whole joint are provided in table 1.

Biomarkers showed an initial high increase during distraction, normalizing one month after distraction (data not shown). Changes between six and twelve months follow-up showed a trend towards a decrease of collagen type II breakdown marker CTX II (-11 ±39%; p=0·078) and an increase of collagen type II synthesis marker PIIANP (+103 ±298%; p=0·060). The average change in the ratio of PIIANP/CTXII between six and twelve months was suggestive of a net increase in collagen synthesis (p=0·056).

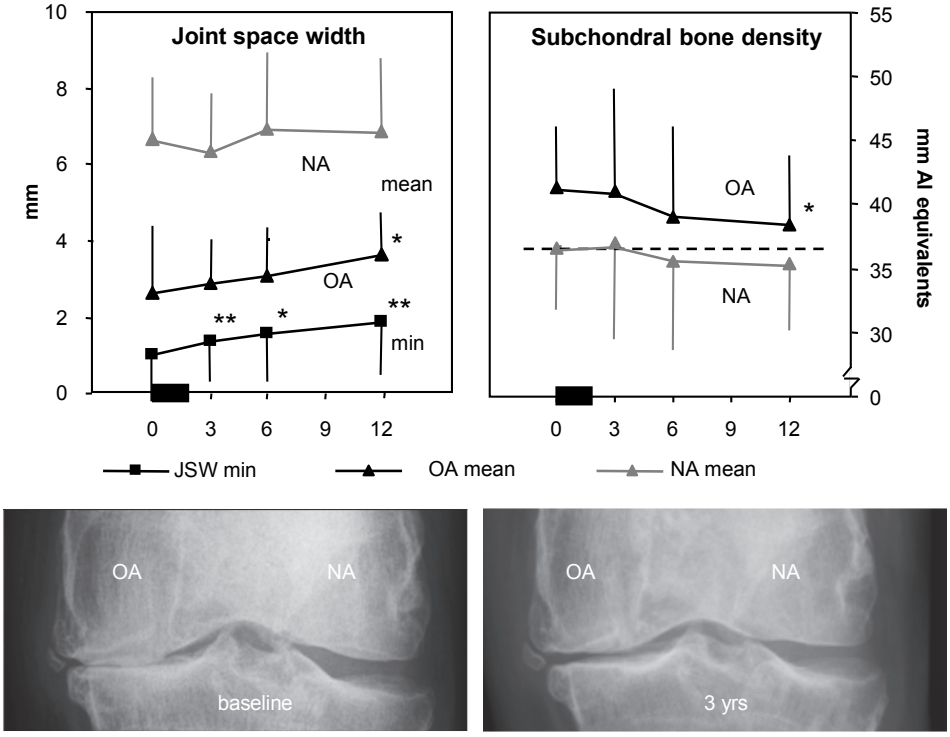

Figure 2. Joint space width (KIDA measurement). Minimum JSW (min) continuously increased after distraction. The mean JSW of the affected (OA) compartment also increased over time. The mean JSW of the less affected compartment (NA), did not change over time. Subchondral bone density (KIDA, as mm aluminium (Al) equivalents; using a reference). The affected (OA) compartment showed a decrease in bone density, the less affected compartment (NA) did not. * p<0.05, ** p<0.01. Representative radiographs before and 3 years after distraction; clear increase in JSW in affected (OA) compartment.

Clinical outcome.

The WOMAC index questionnaire showed a statistically significant improvement between baseline and twelve months follow up (figure 1B), the total WOMAC index increased from 45 ±16 points at baseline to 77 ±21 points at one year (p<0·001). Eighteen of the twenty patients showed an improvement of >10% and 16 of >25%. The individual components of the WOMAC index (pain, stiffness, and function) all improved significantly (p<0·001). VAS pain decreased from 73 ±9mm at baseline to 31 ±26mm (p<0·001) at one year (figure 1B). Physical examination of the knee showed an improvement from 46 ±22% to 75± 24% (p<0·001) of the maximum score (data not shown).

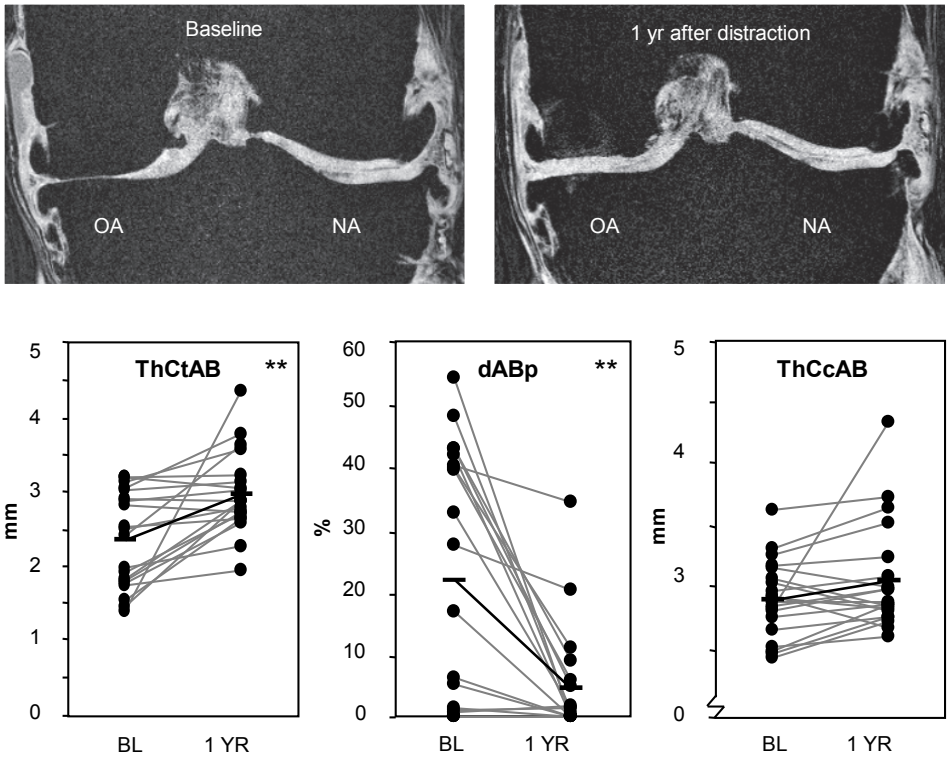

Figure 3. Representative image of single slides before and one year after treatment, showing an increase in cartilage tissue in the affected compartment. Quantitative MRI analysis of cartilage of the affected compartment of the individual 20 patients at baseline (BL) and after 1 year of follow-up (1 YR). Black lines indicate mean values. ** p<0.01. ThCtAB= thickness of cartilage divided by total area of bone, dABp= area of denuded bone, ThCcAB= volume of cartilage divided by cartilage area (p<0.062).

Correlation between structural parameters

All MRI parameters correlated positively and significantly with the increase in mean radiographic JSW (all r>0·51 and p<0·01). The increase in collagen type II synthesis marker PIIANP between 6 and 12 months correlated with the change in ThCtAB and dABp (figure 4). CTX-II change did not show such correlations. There were no clear correlations between structural and clinical parameters.

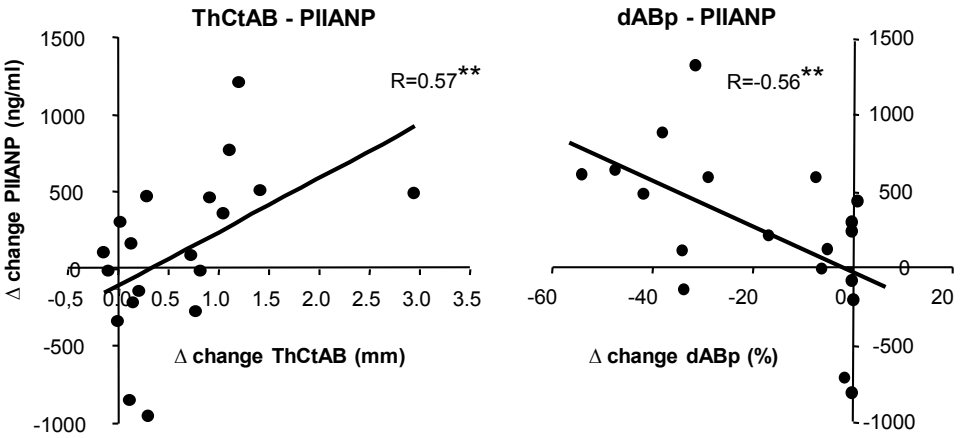

Figure 4. Correlations between the changes (compared to baseline) in cartilage thickness (ThCtAB; mm) and area of denuded bone (dABp; %) on MRI and the change in serum PIIANP (ng/ml) between 6 months and 1 year of follow-up. ** p<0.01.

DISCUSSION

This exploratory prospective open uncontrolled study demonstrates, for the first time, that joint distraction can reverse tissue structure damage in end-stage knee OA, accompanied by significant clinical benefit. Over a period of one year, two months of knee distraction was able to significantly increase radiographic JSW on weight-bearing radiographs, to increase cartilage thickness and decrease denuded bone area as evaluated by MRI, and to increase the ratio of cartilage collagen type II synthesis over breakdown as determined by biomarker analyses. These significant tissue structure changes were accompanied by improvement of functional ability and a reduction in pain. No other medical treatment is currently able to induce such changes at this stage of the disease, and no treatment is currently approved for the label of structure modification in OA at any stage.

Regenerative medicine focuses on creating circumstances under which damaged tissue recovers[20]. This study is the first to demonstrate intrinsic tissue structure repair in OA. Historically, the regenerative capacity of cartilage has been questioned due to the slow turnover rate of cartilage matrix, especially of collagen[21]. However, here we show that a

significant amount of cartilage tissue is formed within one year after the distraction, demonstrating that, under certain conditions, cartilage has regenerative capacity, even in end-stage disease.

It should be noticed that the rate of cartilage thickening (+0·9mm/year) in the present study is surprisingly fast. In general, it has been observed that the breakdown of cartilage does not exceed an average rate of 0·2mm/year[22]. Although no histological or biochemical analysis of tissue quality could be performed in this study, indirect evidence suggests cartilage of sustainable quality. X-rays are taken under full weight-bearing, demonstrating the mechanical competence of the formed tissue. In the first treated patients, X-ray evaluation several years after distraction demonstrates a sustained gain in JSW over time, as is shown in the representative X-rays of figure 2 which is taken three years after treatment. Moreover, previous studies on ankle OA distraction demonstrated a continuous increase in JSW up to five years[11], and clinical benefit up to 10 years[12]. Joint distraction in a canine ligament transection model was able to induce chondrocyte redifferentiation[23]. Preliminary data from an ongoing canine study (using the Groove model[24]) with long-term follow-up after distraction demonstrates actual repair of hyaline cartilage (personal communication). Also, the increase in the ratio of collagen type II synthesis/breakdown and the positive correlation between collagen type II synthesis and increase in the cartilage thickness on MRI, supports formation of functionally relevant hyaline (type II collagen containing) cartilage. Nevertheless, future animal and clinical studies will have to explore the compositional properties of the newly formed tissue.

MRI		**OA**			**NA**		
		BL	1 year	p	BL	1 year	p
ThCtAB	femur	1.00 ± 0.41	1.41 ± 0.30	*0.000*	2.10 ± 0.42	2.11 ± 0.41	*0.598*
(mm)	tibia	1.36 ± 0.34	1.56 ± 0.31	*0.029*	2.05 ± 0.32	2.07 ± 0.27	*0.641*
dABp	femur	27.31 ± 25.64	4.19 ± 10.22	*0.000*	0.63 ± 1.40	0.50 ± 1.39	*0.402*
(%)	tibia	16.70 ± 17.22	4.82 ± 8.33	*0.006*	0.65 ± 2.34	0.57 ± 2.54	*0.592*
ThCcAB	femur	1.33 ± 0.25	1.46 ± 0.24	*0.001*	2.11 ± 0.42	2.12 ± 0.41	*0.659*
(mm)	tibia	1.62 ± 0.24	1.64 ± 0.25	*0.653*	2.07 ± 0.30	2.07 ± 0.25	*0.698*

		Whole joint		
		BL	1 year	p
ThCtAB	(mm)	3.25 ± 0.39	3.57 ± 0.47	*0.001*
dABp	(%)	11.32 ± 10.02	2.52 ± 4.44	*0.001*
ThCcAB	(mm)	3.56 ± 0.38	3.64 ± 0.42	*0.070*

Table 1. MRI outcome for femoral and tibial side of the most affected compartment (OA, osteoarthritic) and the less affected compartment (NA, not affected) as well as for the whole joint (both compartments) of twenty patients treated for two months with joint distraction, before distraction (BL, baseline) and after one year, including 2-sided p values. Primary structural outcome parameters: thickness of cartilage over total area of bone (ThCtAB) and the percentage area of denuded bone (dABp); secondary structural outcome parameter: thickness of cartilage over area of bone covered with cartilage (ThCcAB).

Interestingly, despite the considerable improvement in both structural and clinical outcomes there was no significant correlation between them. As is known from the literature, a relationship between clinical parameters and tissue structure parameters has rarely been found, and is still an important subject of debate. A role for bone changes influencing clinical symptoms is suggested in more recent literature[25, 26]. In the present study a clear decrease (normalization) in subchondral bone density as a result of treatment was found. Also the area of denuded (potentially pain sensitive) bone decreased significantly. But also, changes in these bone related parameters did not clearly correlate with changes in pain outcome. It is reasonable to assume that the number of patients limited detection of potential relationships.
In addition, the question arises what the underlying mechanism of the observed structure repair may be? It is hypothesized that the temporary distraction prevents mechanical stress on the cartilage, prevents further wear and tear, and allows initiation of tissue repair. Joint fluid pressure changes are maintained during the distraction period, due to the springs in the distraction tubes allowing limited axial oscillation during loading and unloading of the distracted joint. These fluid pressure oscillations may provide nutrition and may trigger the cartilage cells to initiate tissue repair (re-differentiation of the diseased chondrocytes)[27, 28]. In addition, the change from a catabolic to an anabolic cartilage condition will diminish synovial activation by cartilage breakdown products and with that inflammatory activity. During distraction, the load on the bone (the biomechanical trigger for normal bone formation) is transferred through the frame instead of the subchondral bone, leading to subchondral bone resorption, which subsequently normalizes after distraction. This significant bone turnover may trigger the release of growth factors as bone matrix provides a store of resident growth factors such as TGFβs, BMPs and IGFs) that stimulate cartilage tissue repair[29, 30].
It is currently unclear which group of patients would benefit best from this treatment. In the present study, only young patients (<60 yr) with severe OA were treated, but milder cases or older patient might also benefit from the treatment. Also, it is currently unknown what the ideal duration of the distraction period is. The primary goal of the current intervention was to postpone joint replacement in relatively young patients, in order to reduce the potential numbers of revision surgeries. Follow-up studies will have to demonstrate the endurance of the structural and the clinical effect, and will have to show whether structural and clinical effects correlate, in larger populations and when follow-up is conducted over longer time periods. Larger (and longer) trials in a variety of OA populations need to be performed for optimizing the distraction treatment (duration), and for identifying those patients who profit the most and the longest from the treatment.
At present, distraction therapy is the only treatment that can reverse cartilage tissue structure damage in end-stage knee osteoarthritis accompanied by significant clinical improvement.

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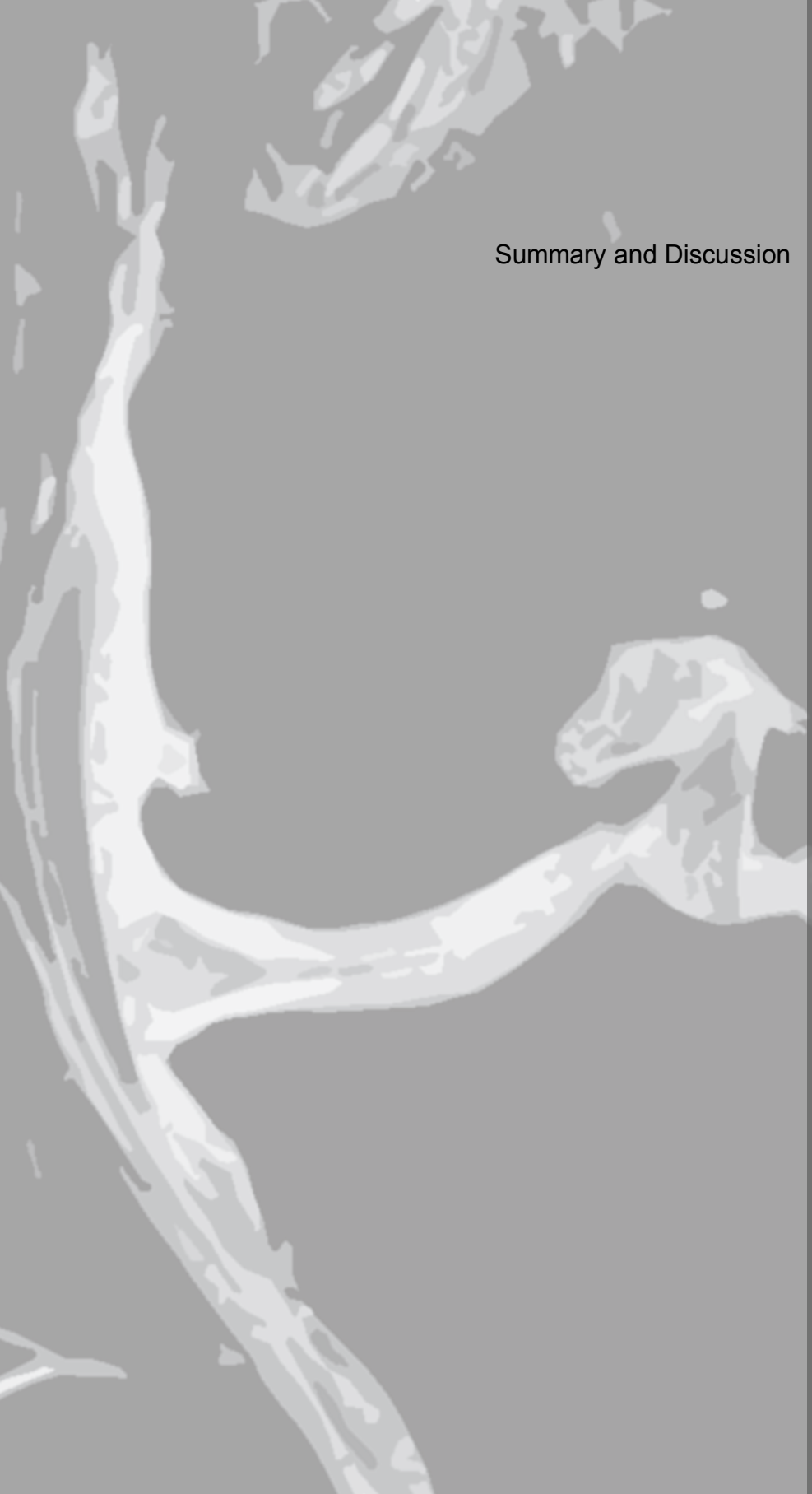

Summary and Discussion Chapter 11

SUMMARY

In healthy joints, loading adds to maintenance of the normal joint homeostasis, specifically that of cartilage and bone. Therefore, in the development and treatment of OA, overloading and unloading may significantly influence tissue homeostasis and with that integrity of cartilage and bone.
Evaluation of the effects of (un)loading on bone and cartilage homeostasis in the development of OA is hampered by the options of visualizing the changes in vivo. First of all, sufficiently sensitive markers (biochemical and imaging) for diagnosing, following progression, and evaluating treatment of OA early and late in the disease process, are lacking. Secondly, tissue samples for biochemical analysis of cartilage or architecture of subchondral bone are difficult to obtain and are largely restricted to *post mortem* material or end stage (joint replacement) disease. As such experimental animal *in vivo* models of OA can add to our understanding of the course of the degenerative process, and treatment efficacy of the disease. However, these studies require experimental models that represent (at least certain aspects of) human OA at the biochemical and mechanical level.

In this thesis, the first step was to answer the question: *Is cartilage damage in the canine Groove model representative for cartilage degeneration in human clinical OA?*
Certain aspects of cartilage integrity and proteoglycan turnover observed in the canine Groove model (a recently developed model suggested to mimic human OA) were compared with those observed in human clinical osteoarthritis **(chapter 2)**. Generally appreciated, but for the first time demonstrated by direct comparison, canine and human cartilage differed significantly with respect to absolute proteoglycan content and turnover. However, changes due to experimentally induced joint degeneration or osteoarthritis in the canine and human cartilage, respectively, were very similar. Despite species-specific differences in cartilage characteristics in human OA and canine joint degeneration, similarities in changes due to the degenerative process prevailed. These results show that the canine Groove model, a large, surgically induced experimental model of (knee) OA is a suitable model for studying the development and treatment of OA, at least with respect to the evaluated characteristics.

In OA, besides cartilage changes, bone changes (sclerosis, cysts, osteophytes, attrition, and bone marrow lesions) can be observed and are suggested to be interrelated with the cartilage degenerative processes. Bone changes can be present in either the subchondral cortical plate or further away from the joint in the subchondral trabecular bone. In the development of OA, it is unclear when bone changes become evident and whether they subsequently induce cartilage changes. Therefore the question: *What is the sequence of subchondral bone changes in the process of osteoarthritis and do these changes differ between the unilateral ACLT and Groove model?* was addressed in **chapter 3**. The Groove model as a surgically induced cartilage damage model (a onetime trigger) and the ACLT model as a (permanent) joint instability model, both lead to very comparable cartilage damage over time. In this study, cartilage integrity was analysed by GAG-content and

grading of histological damage, while bone changes were evaluated by architectural changes quantified by micro-CT analysis. Both models showed the anticipated comparable cartilage changes. Significant repair activity was still present at 3 weeks but clearly cartilage degeneration predominated at 10 and 20 weeks. An early reduction of the thickness and an increased porosity of the subchondral plate could be observed at 3 weeks, very consistently in the two different canine models of OA: the Groove model and the ACLT model. In contrast, the changes in the subchondral trabecular bone at the tibial epiphysis in the Groove model were relatively limited and inconsistent over time whereas these changes in the ACLT model were clear and consistent with up to 20% loss in bone volume fraction. Interestingly, similar significant changes in the corresponding architectural parameters in the metaphysic area, further away from the joint, were observed in the ACLT model but not in the Groove model. In addition, differences in osteophyte formation between the Groove model and the ACLT model could be observed, with the Groove model clearly showing a slower development of these bony outgrowths. The quick and consistent significant loss of subchondral plate thickness and increase in plate porosity in both models, cannot be explained by unloading (the opposite would have been anticipated) and strongly suggests that an interplay between cartilage and subchondral bone plate is part of the etiologic process in osteoarthritis.

Results from this small pilot study were the basis for a larger study where the changes in bone and cartilage were analyzed more thoroughly. In the previous study, unilaterally induced OA models were compared with the contralateral joint as a control in a limited number of animals over 3 time points. In the follow-up study both models were compared as bilaterally induced OA models with a sham group as control, limiting variations in loading due to unknown load transfer to the contralateral (healthy) hind limb.

The bilateral ACLT model has been described previously. The bilateral Groove model had to be validated against the unilateral variant. In **chapter 4** the following question was addressed. *Are cartilage changes in the bilateral Groove model of OA comparable to those found in the unilateral model?* It was demonstrated that a bilateral canine model of OA provides reliable macroscopic, histological, and biochemical OA-related degenerative changes in cartilage accompanied by mild synovial inflammation in a period of 20 weeks post-surgery. Features of OA were very comparable to those observed in the unilateral version of the model. The bilateral model has the advantage that it allows evaluation of local (intra-articular) treatment with the advantage of an internal OA animal control. This results in a reduction of the number of animals needed without loss of statistical power, or in the advantage that smaller differences can be detected with the same number of animals. For systemic treatment the unilateral version has the advantage that an internal non-OA control is present, limiting the number of animals needed to design a proper study. The bilateral canine Groove model might be the OA model of choice in case local chondroprotective treatment has to be evaluated.

With the bilateral Groove model validated, the next question could be answered: *What are the similarities and discrepancies in subchondral bone structure in two differently induced canine models of osteoarthritis?* In this study described in **chapter 5** a sham surgery group was used as a reference. It was demonstrated that thinning of the subchondral plate coincides with degenerative changes in articular cartilage independent of the model used and as such to be considered an intrinsic part of the OA process. This is in contrast to the subchondral trabecular bone changes that were clear in the ACLT model but hardly present in the Groove model and that coincided with similar changes in the metaphyseal bone. Consequently, subchondral trabecular bone changes might not be an intrinsic part of the cartilage degenerative process and are differently regulated in different models (due to different causes) of OA. Conclusively, the mechanical unloading of the bone is causative in early subchondral trabecular bone changes rather than the degenerative process itself.

The observed differences in subchondral bone changes in two bilateral models of OA with similar cartilage degeneration could have resulted from the difference in induction of OA. Therefore, a validation study was performed using experimentally induced OA in a single joint with asymmetrical joint loading, the ACLT-meniscectomy model **(chapter 6)**: *Is in the canine ACLT-meniscectomy model, thinning of the subchondral bone directly related to cartilage damage?* This model shows relatively unloading in the lateral compartment and more loading stress in the medial compartment, due to the created varus angle, in addition to overall unloading of the affected limb due to the joint instability. This model is characterized by predominantly cartilage degeneration in the meniscectomized medial compartment with less damage in the lateral compartment. The significant changes in cartilage degeneration in the more loaded medial compartment coincided with severe thinning of the subchondral plate. There was a lack of plate thinning in the lateral compartment coinciding with only moderate cartilage degeneration. These results are in contrast to the decrease of trabecular volume, mainly present in the unloaded lateral compartment. At the medial side, local peak loading might compensate for overall joint unloading, preventing a decrease in subchondral trabecular volume at the medial side.

Overall, from the results of the first part of this thesis (specifically chapters 3, 5 and 6) it can be concluded that plate thinning and loss of subchondral trabecular bone are differently regulated and are a localized phenomenon within the osteoarthritic joint. In early OA the thickness of the subchondral plate decreases, a phenomenon related to cartilage damage. Trabecular changes are independent of cartilage damage and anticipated to be related to unloading. Whether these phenomena also occur in early human OA has to be established. Irrespectively, when designing and interpreting studies interfering with bone turnover in treatment of experimentally induced and human OA, the above should be taken in account

The second part of this thesis deals with the potential of unloading in the treatment of osteoarthritis. **Chapter 7** questions: *Is unloading of joints, including joint distraction, useful in treatment of osteoarthritis?* This chapter provides an overview of the literature describing the

effects of unloading in the treatment of OA. There is ample, but indirect support that unloading indeed might be effective in reducing pain and slowing down structural joint damage. Clinical efficacy was described due to partial unloading by footwear and bracing (non-surgical), focal unloading by osteotomy (surgical), and more generalized unloading by joint distraction. However, current literature shows that further research (appropriate high quality clinical trials) and analysis (clinical as well as pre-clinical and fundamental) are needed to understand, validate, and refine the different approaches of unloading to treat osteoarthritis. In this chapter it was also concluded that joint distraction as a treatment of osteoarthritis is the ultimate form of transient unloading. Joint distraction is a surgical method to 'unload' the osteoarthritic joint for a certain period of time to facilitate tissue repair activity. In the final 3 chapters the effects of joint distraction on structure modification was studied.

The first question in this part of the thesis was: *Has joint distraction structure modifying properties in addition to clinical benefit when studied in the canine experimentally induced OA?* As described above, evaluation of tissue repair is hampered by the limited options to visualize tissue repair in vivo, due to the lack of sufficiently sensitive markers. As such, effects of joint distraction on degenerated cartilage were evaluated in the canine Groove model of OA (**chapter 8**). This model allows for prolonged follow-up after transient intervention without introducing joint instability. After development of experimental OA over a period of 10 weeks, animals were treated or left untreated as control for 8 weeks with a custom designed distraction frame. All conditions supposed to be critical for efficacy of distraction (absence of mechanical wear and tear, intermittent fluid pressure changes during distraction, and peri-articular bone turnover) were achieved in this model. Twenty five weeks after removal of the frames (or follow-up in the control group), cartilage integrity was evaluated. Interim analyses (results from the first 2 of 3 parts of the study) revealed already (despite the limited power) significantly less cartilage damage in the distracted OA group compared to the control OA group as measured by biochemical and histochemical evaluation. Moreover, joint loading, reflecting clinical symptoms, remained significantly impaired in the OA control group over time but completely normalised after distraction in the OA group. A clear beneficial cartilage structure modifying effect of temporary full unloading of the joint by applying joint distraction has become evident.

After showing the beneficial effect of joint distraction on joint structure in an experimental model of OA, we studied OA in two exploratory clinical trials. Joint distraction has already shown to induce clinical benefit in the treatment of severe ankle OA. However, the rational for this clinical benefit is unclear. Recent literature has pointed out changes in subchondral bone as the best candidate for causing pain in OA. As such, c*an subchondral bone density changes explain the clinical benefit of ankle distraction?* The effects of joint distraction on subchondral bone were evaluated in patients with severe end stage ankle osteoarthritis. The two year follow-up of all treated patients demonstrated prolonged clinical efficacy. In this study the effects of joint distraction on subchondral bone changes was evaluated by use of

CT data gathered at baseline, and 1 and 2 years post treatment **(chapter 9)**. The study demonstrated that treatment of advanced post-traumatic ankle OA with joint distraction produced an overall decrease of subchondral bone density, which persisted for at least two years. Subchondral bone at baseline consisted of varied regions of relatively low density (cystic) and high density (sclerotic). While overall density decreased (less sclerosis), density in cystic lesions actually increased (normalization of bone density). A correlation was found between clinical improvement and the resolution of these subchondral bone cysts. As such a clear beneficial bone structure modifying effect of temporary full unloading of the joint by applying joint distraction was observed.

The final goal was to demonstrate cartilage structure modification in a clinical study in end stage OA. In case of end stage knee OA, total knee prosthesis is at present the treatment of choice. As a result of aging of the population, the number of total knee prosthesis is dramatically increasing. Severe end-stage knee OA is therefore a major socio-economic problem. Subsequently, demonstrating cartilage repair activity and clinical benefit in the treatment of OA, would have a major impact on healthcare. Thus the question was: *Does joint distraction result in cartilage regeneration in osteoarthritic knees?* The exploratory prospective open uncontrolled study demonstrated, for the first time, that joint distraction can reverse tissue structure damage in end-stage knee OA, accompanied by significant clinical benefit **(chapter 10)**. Over a period of one year, two months of knee distraction was able to significantly increase radiographic JSW on weight-bearing radiographs, to increase cartilage thickness and decrease denuded bone area as evaluated by MRI, and to increase the ratio of cartilage collagen type II synthesis over breakdown as determined by biomarker analyses. These significant tissue structure changes were accompanied by improvement of functional ability and a reduction of pain. No other medical treatment is currently able to induce such changes at this stage of the disease,

Overall, from the results of the second part of this thesis, described in chapter 7 to 10, it can be concluded that temporary unloading of osteoarthritic joints clearly is clinically beneficial and may lead to tissue structure repair at the level of bone and cartilage in (at least end stage) osteoarthritis.

DISCUSSION
(considerations, speculations, and suggestions to ultimately achieve cure of disease)

Loading joints
Joints are designed to withstand forces necessary for functional movement. This implies that they can resist a certain amount of loading and shear stresses. In fact, a certain amount of loading and movement is needed to keep joints in shape. In case of complete immobilization, joint atrophy occurs[1].
During joint loading, the surfaces are pressed together and fluid is forced from the cartilage matrix while the shape of the tissue alters. The load from the cartilage is transported to the bone underneath the cartilage. During unloading of the joint, cartilage absorbs the fluid that was lost during loading, due to its osmolaric gradient, thereby normalizing its shape. Chondrocytes and subchondral bone cells respond to the mechanical triggers by increasing or decreasing cartilage and bone matrix turnover, thus fitting the amount of load that is given[2, 3]. As such, the cartilage and bone matrix can adapt to different loading patterns[4]. Presumably, bone can adapt quicker than cartilage. As a result of pathological loading conditions, the delicate balance between synthesis and breakdown can become disturbed, even to a point, probably earlier for cartilage, where spontaneous repair becomes difficult or even impossible.

Unloading healthy joints
In case of prolonged unloading, the fluid flow in and out of cartilage is lost. As a result, cartilage cells lack the proper physiologic trigger and nutrition to maintain homeostasis; cartilage thickness decreases in the absence of normal joint loading[5]. Moreover, investigators have described deterioration of biochemical and mechanical properties of the cartilage during immobilization[1]. In addition to cartilage changes as a result of unloading, unloading of bone results in osteopenia. Bone cells diminish the bone formation process whereas the counterbalancing bone breakdown proceeds[6].

Overloading joints.
Overloading of cartilage, either locally or generalized, will damage the cartilage matrix structure. Overloading can have many causes. It can be the result of joint malalignment where certain joint areas have to withstand the amount of load that would be distributed over the whole joint surface in a healthy mechanical situation[7]. Focal overload is also generated when cartilage defects have occurred by joint trauma. Joint congruity is lost and the cartilage is subjected to local peak loads[8]. Generalized overloading can occur during situations of intensive sporting or physical challenging occupation[9]. More weight, as in obese people, also results in intensified loading of the weight bearing joints[10].
Cartilage has a natural repair capacity. Chondrocytes can respond by increasing matrix synthesis and breakdown to repair damaged cartilage. However, due to the relatively slow turnover rate of adult cartilage (a relative limited number of cells are responsible for an

abundant amount of extracellular matrix), it is questioned to what extent this repair activity remains functional. In case of osteoarthritis (OA), apparently this mechanism has failed.
In addition to cartilage damage due to excessive overloading, bone structure can also be damaged in case of overloading. Microscopic cracks occur in the subchondral plate and underlying trabecular bone[11]. Osteocytes and osteoblasts are stimulated to increase bone synthesis because more bone is required to answer to the increased loading demand. An altered loading pattern may be anticipated by changing bone architecture and adapting to such changes[12]. In OA, we see an inadequate increase of bone turnover, with finally more, but less mineralized and subsequently weaker bone. The mechanism of bone adaptation does not keep up with the altered biomechanical en biochemical environment and finally fails.
In OA, joint degeneration could be the result of a failed attempt by all tissues to establish a functional repair. Why do these mechanisms fail? The answer to this question might be the 'holy grail' to treatment of OA, possibly leading to cure of the disease or at least a slowing down of the degenerative process.

Loading in early OA
In the first part of this thesis early cartilage and bone changes are evaluated in canine models of osteoarthritis. It becomes clear from these studies that, early in the disease, in addition to cartilage damage, thickness of the underlying subchondral plate has decreased, while loss of trabecular bone is the result of joint unloading as a consequence of pain or instability. Loss of mechanical resilience of the overlying cartilage due to the arthritic process leads to a condition of increased bone loading. This in turn was expected to lead to stronger bone, and a thicker plate was to be expected. The absence of changes of the underlying trabecular bone pleads against an effect of unloading due to OA induction. Apparently, it is not a mechanical signal that induces this initial plate thinning in early OA. It could be that the damaged cartilage gives a biochemical signal to the underlying bone that causes bone resorption.
It is tempting to speculate that this bone response is initiated by the cartilage to soften its base (foundation), preventing excessive load and generating a mechanical environment that still allows cartilage repair. However, it might also just be an inadequate pathological response of the affected joint, due to abrupt excessive load on the subchondral bone induced by the impaired biomechanical condition of the overlaying cartilage. Either way, the change of underlying bone will, on its turn, affect the overlying cartilage.
Thinning of the subchondral plate was accompanied by an increase of plate porosity. Increased porosity might open the door for the transport of all kind of biochemicals including pain mediators and cytokines that promote cartilage destruction. On the other hand, increased porosity might also add to nutrition of cartilage. Either way, mechanically or biochemically, early subchondral plate changes can have a positive or a negative influence on the 'struggling' cartilage.

Intervening with bone turnover in this early phase of disease to achieve normal subchondral plate structure might be interesting to explore, but as long as the role of early plate thinning is not clear, this pathway could just as well give disappointing results.
The role of early loss of trabecular bone in some models of OA, which is attributed to unloading, is unclear. Cartilage degeneration is not related to these early effects of unloading and the role of early loss of trabecular bone on sclerotic changes in more advanced OA has to be subject of further studies.
Despite the insight that the studies on bone and cartilage in this early phase of OA has given, more and preferably longitudinal studies have to be performed in animal models and results need to be confirmed in early human OA. Once pathologic mechanisms of bone and cartilage interaction can be solidly described, possible targets can be identified, and treatments applicable for early OA developed and evaluated.

Loading in late OA
Later in the process of OA, the subchondral plate increases in thickness[13]. At what moment during OA development, the decrease of the subchondral plate thickness turns into an increase, which mechanisms are involved, and whether this has indicated a point of no-(spontaneous) return, is unknown. Further longitudinal studies (in animal models) are needed to study this. When an increased thickness of the plate, and underlying trabecular bone occurs, cartilage will sense increased stresses, leading to further damage of the already impaired cartilage tissue. Consequently, the further loss of mechanical properties of the cartilage will lead to further increase of mechanical load of the underlying bone leading to a vicious circle. Subchondral bone changes cause venous obstruction, scarring, and new bone formation. Changes in pH and chemicals further damage the already degenerated tissue[14], and although the exact mechanisms in development are still unclear, these changes might also be important in development of bone cysts. Bone cyst may come in contact with the cartilage and at that stage, the biomechanical properties of the joint are completely disturbed.

Treatment of OA and joint repair.
In Osteoarthritis, a joint disease that indisputably depends in its early and late phases on joint mechanics (loading) and its effects on joint metabolism, very little has been accomplished so far in the field of prevention or early treatment. Symptom (pain) control in general fails to be effective over a longer period of time. Altering disease progression seems far away[15]. There are several flaws in studying different treatment methods, as were defined by Felson in 2007[16]. First of all, we fail to define the right outcome parameters. Related to the complex, over time changing, interaction between bone and cartilage, this is not surprising. When aiming at protecting the cartilage (a non-innervated tissue) from further damage, it is not to be expected that this will lead to immediate pain relief. Secondly, in addition to a proper chemical environment, a favorable mechanical environment must be created in a disease that depends for an essential part on biomechanics. Thirdly, in this complex disorder, consisting of changes in several tissues and overall joint homeostasis[17],

treating only one component is probably insufficient[18]. Cartilage is often the main target of treatment, but with severely damaged subchondral bone, ligaments, capsule, with inflammation and impaired muscle conditions, curing cartilage alone will not cure the whole joint. Numerous examples like cartilage tissue engineering approaches that only focus on cartilage repair, anti-inflammatory medication only focusing on the inflammatory component, or bisphosphonate treatment only focusing on bone, reflect interventions targeting just an isolated part of a complex problem in which changes in several tissues are interrelated.

Joints distraction

From the above, it is clear that creating a favorable mechanical environment plays an important role in attempting to cure the joint. In OA, the mechanical properties of the joint at the macro- and micro-environment are severely disturbed. In end stage disease, overloading of cartilage and bone are a continuous stimulus for progressive joint degeneration. Thus, unloading the joint might be an essential condition to stop the progression of disease and to allow repair activity to become functional.

The second part of this thesis deals with the potential benefit of joint unloading, with joint distraction being the ultimate way of unloading a joint. Joint distraction is a surgical procedure in which the two bony ends of a joint are gradually separated to a certain extend for a certain period of time. An external fixation frame bridging the joint, attached to both bone ends, is used to widen the joint space. With joint distraction, loading of bone and cartilage is diminished within the distraction frame. A circumstance is created where an important driving force of joint degeneration, mechanical overload, is temporarily removed. This more favorable mechanical condition may be a prerequisite to induce tissue repair[16].
It should be stressed that joint distraction creates a condition different from complete immobilization, which is known to be detrimental to the joint. During distraction, loading of the affected joint is encouraged. The combination of loading and flexibility within the distraction frame preserves fluctuation of intra-articular fluid pressure as naturally occurring during normal loading and unloading. This condition, which might be crucial for nutrition and stimulation of the mechanically unloaded cartilage, suggests an important difference compared to complete immobilization.

Although suggested by previous studies[19], the present thesis describes for the first time that by performing joint distraction in the treatment of severe OA, cartilage can re-grow, both in surface area and in thickness, and subchondral bone abnormalities (sclerosis and cysts) can to a certain extent normalize. In addition, pain decreases as a result of the treatment, related to joint normalization.
Creating circumstances under which an essential component of OA (overload) is diminished, even without additional treatment, apparently results in joint regeneration by using the intrinsic repair capacity of the different joint tissues. The source of the chondrocytes responsible for, and adding to this repair process is speculative. Undifferentiated (stem) cells might originate from bone or even synovial tissue as has been suggested[20]. They may also

derive from the remaining cartilage before start of treatment. The significant bone changes during distraction, a process starting with inducing osteopenia to finally normalization of bone characteristics, might be designated as an important promoter of cartilage repair activity. Moreover, it may be the factor that relates the observed structure modification to ultimate pain relief.

Clinical benefits and joint distraction

Joint distraction in the treatment of OA results in a reduction of pain in addition to structural joint changes. Despite the small group sizes treated thus far, it is shown that this reduction in pain is correlated to the disappearance of subchondral bone cysts. The source of pain in OA has been subject of many studies[21]. A most likely candidate seems to be the subchondral bone. Studies showing correlations between large bone marrow lesions on MRI and pain confirm this idea[22]. Bone marrow lesions and bone cysts represent areas of overloading, resulting in bone necrosis[23, 24] which might cause pain in a highly innervated organ like bone. Future larger studies might demonstrate a relationship between clinical benefits and covering of the denuded bone areas by new cartilage as a result of distraction.

Future studies

Whether joint distraction can indeed reverse the degenerative process of OA and lead to actual cure, or just causes temporary relief is unknown. Long term beneficial effects have been demonstrated in the treatment of severe ankle OA[25]. However, the difference between cure and delay may depend on the original course of the disease. This course may have been different in each of the treated patients. Selection of patients that will benefit in the long term might therefore be essential in future studies. Additionally, in the more holistic approach of this generalized joint disease, there will be a role for additional therapies, pharmacological therapies aiming at disease modifying activity (DMOADs) or tissue engineering approaches. The efficacy of these approaches might improve when the biomechanical environment also favors tissue repair. Applying other cartilage repair strategies in addition, or even concurrent with joint distraction, might be a useful future approach to improve treatment of the disease specifically in subgroups, depending on the original trigger for the disease.

The success of joint distraction thus far, might shade the strong motivation and persistence that is needed from patients to tolerate this still relatively invasive surgical treatment. Patients have to endure an external fixator for a period of at least two months. Not only is mobility severely diminished (bound to wheel chair or crutches for a substantial period of time), a significant number of patients is subjected to painful pin tract infections which require antibiotic treatment (with possibly side effects). Although never observed in all ankle and knee distraction patients thus far, a severe complication might be osteomyelitis which requires long time antibiotic treatment. Additionally, the influence of pin tract infection on the potentially subsequently needed final placement of an endoprosthesis is not clear yet. The reversibility of the induced osteopenia by distraction is not yet determined and might also

interfere with successful joint replacement therapy if still needed, specifically in case of failure shortly after distraction.
Fortunately, no cases of osteomyelitis have occurred yet, neither in the studies on ankle distraction, nor in the one on knee distraction, more than 100 in total. To diminish the changes of interference with future endoprosthesis, when beneficial results of joint distraction do not last, the bone pins are placed distant from the region involved in knee replacement surgery. More effort using strict treatment protocols to prevent pin tract infections might be helpful. Longer follow-up and additional studies are needed to determine the duration of clinical benefits and whether placement of a joint endoprothesis following distraction involves an increased risk of infection or loosening of the prosthesis.

In the end stage of OA, arthroplasty is still the gold standard. The operating techniques and functional designs of endoprostheses have evolved in the last decades and the new artificial joints appear to last longer and allow a more active lifestyle. Nevertheless, complications such as aseptic or septic loosening can be severe and may even necessitate arthrodesis or, in rare instances, amputation. With the increasing demand for a cost-effective treatment method which stimulates participation in society quickly, arthroplasty answers these demands. However, as yet, the lifespan of artificial joints, specifically applied relatively early in an active life, is still limited. Revision surgery with less favorable results and more costs, is expected to increase exponentially in the near future.
Ideally, interventions aiming at prevention of end-stage osteoarthritis hold the promise to be superior to joint replacement. Joint distraction (possible in combination with other cartilage repair strategies, by medication, or surgically) has the potential to play a role in treating (young) patients with OA, and might delay, or even prevent arthroplasty.

In conclusion: this thesis demonstrates the sequence and nature of early bone and cartilage changes in experimental models of OA, and underlines the role of mechanical loading in the development of degenerative joint changes. Additionally this thesis demonstrates that mechanical unloading in the form of joint distraction is the first treatment that actually induces structural changes to bone and cartilage in addition to clinical benefits by creating a mechanical environment where natural repair mechanisms can be functional.

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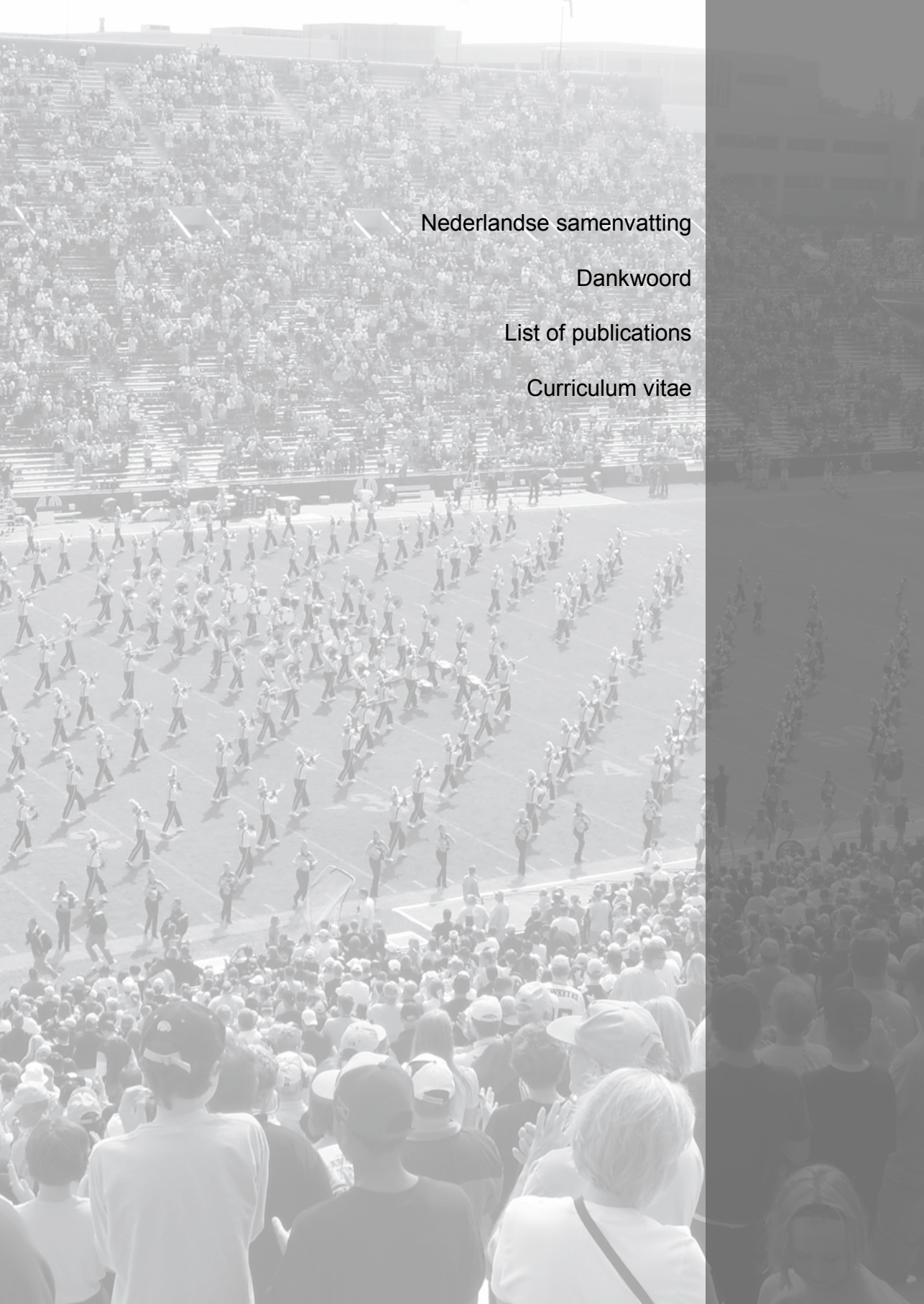

NEDERLANDSE SAMENVATTING

Artrose

Artrose is een aandoening van de gewrichten die zich presenteert met pijn en stijfheid. Als gevolg hiervan is er een verminderde functie van het aangedane gewricht. Daarbij kan er ontsteking voorkomen die zich kenmerkt door toename van pijn en zwelling van het gewricht. De gewrichten die het meest zijn aangedaan zijn die van heupen, knieën, rug, voeten en/of handen, maar artrose kan in principe voorkomen in alle gewrichten.

Er zijn verschillende risicofactoren voor het ontstaan van artrose waarbij leeftijd de belangrijkste is. Een andere belangrijke risicofactor is de aanwezigheid van overmatige belasting. Dit kan zijn in de vorm van overgewicht waarbij hele gewrichten te zwaar worden belast, maar ook lokaal in een gewricht kan er sprake zijn van overbelasting. Voorbeelden hiervan zijn een afwijkende belastingsas (bijvoorbeeld O of X benen), instabiliteit van een gewricht na letsel aan de banden, of een lokale beschadiging van het kraakbeen en/of het onderliggend bot waarbij er verhoogde belasting is van omringende weefsel.

Gewrichten zijn de scharnierpunten van het benige skelet en bestaan uit botuiteinden bedekt met kraakbeen. Naast de vorm van het gewricht, zorgen gewrichtskapsel, banden en spieren voor de stabiliteit en bewegingsmogelijkheden. Wanneer er zich klachten voordoen kenmerkend voor artrose, zijn er vaak een aantal veranderingen in het gewricht die op röntgenfoto's zichtbaar gemaakt kunnen worden. Er treedt een versmalling op van de gewrichtsspleet, de afstand tussen beide botuiteinden, welke een afname van kraakbeendikte weergeeft. De botuiteinden vertonen een toename van botdichtheid. Daarnaast ontstaan er botuitstulpingen aan de randen van het gewricht, zogenaamde osteofyten.

Een andere mogelijkheid om een beeld te krijgen van de weefsels in het gewricht is met behulp van MRI. MRIs kunnen de verschillende soorten weefsels van het gewricht, zowel bot als kraakbeen in beeld brengen. Dit in tegenstelling tot röntgenfoto's die alleen de harde weefsels (botten) tonen. Op MRI kenmerkt artrose zich o.a. door schade aan het kraakbeen (verminderde dikte en zelfs gaten) en veranderingen in de structuur van het onder het kraakbeen gelegen bot (botsclerose, botcysten en beenmerglaesies).

Bij artrose is er niet alleen een afname van de hoeveelheid kraakbeen maar de samenstelling van het kraakbeen verandert ook. Kraakbeen bestaat uit collagenen, proteoglycanen en voor 80% uit water. Collagenen zorgen voor de stevigheid en proteoglycanen voor de elasticiteit en opname van water. Bij artrose is er een beschadiging van het kraakbeenoppervlak en er ontstaan scheurtjes in het weefsel. Het collagene netwerk wordt verbroken en vervolgens verliest het kraakbeen proteoglycanen. Hiermee raken de mechanische karakteristieken die voor een goed functionerend gewricht zo belangrijk zijn verloren.

De mechanische eigenschappen van bot zijn te wijten aan biologische kwaliteit van het weefsel, en voor het grootste deel aan de opbouw van de botbalkjes. Deze architectuur van de botbalkjes kan nauwkeurig worden geanalyseerd door middel van micro-CT (hoog

resolutie röntgenbeelden vanuit verschillende hoeken waaruit een 3D beeld kan worden gereconstrueerd). Deze methode is alleen beschikbaar voor een klein volume weefsel. Bij klinisch ernstige artrose zijn de botbalkjes in volume en aantal toegenomen. De kwaliteit van de botbalkjes blijkt echter ook te zijn veranderd. Ze bevatten minder kalk en zijn minder goed in staat om krachten te absorberen.

Het bestuderen van artrose is zoals beschreven in bovenstaande analysemethoden, vaak niet mogelijk zonder een deel van het gewrichtsweefsel weg te nemen. Het wegnemen van weefsel is niet gewenst bij patiënten met artrose aangezien dit het gewricht verder kan schaden. In het onderzoek naar de ontwikkeling en behandelmogelijkheden van artrose is de ontwikkeling van diermodellen voor artrose dan ook noodzakelijk. Een aantal modellen voor artrose is ontwikkeld in vooral grotere diersoorten zoals de hond, waarbij de biomechanica het meest vergelijkbaar is met die van de mens. Het meest gebruikte model is het ACLT (anterior cruciate ligament transection) model waarbij de voorste kruisband wordt doorgesneden en instabiliteit van het gewricht zorgt voor de ontwikkeling van gewrichtskenmerken representatief voor artrose. Ook bij de mens is dit een oorzaak voor artrose.

Behandeling
De behandeling van artrose is lastig. Dit komt deels doordat het ontstaan van schade in het gewricht een traag proces is en pas laat ontdekt wordt. Kleine veranderingen in het gewricht kunnen niet makkelijk gedetecteerd worden. Pijnstilling, fysiotherapie en gewichtsverlies zijn methoden om in ieder geval de klachten te verminderen. Of het de ontwikkeling van weefselschade daadwerkelijk remt is onduidelijk. Eigenlijk zijn er nog geen behandelmethoden die onomstotelijk bewezen hebben het ontstaan van weefselschade te vertragen, laat staan te herstellen.
Verschillende operatieve methoden worden toegepast om de overbelasting van het gewricht te doen verminderen. Een voorbeeld is een osteotomie, waarbij de belasting op het beschadigde kraakbeen wordt verminderd.

Een nieuwe, en minder vaak toegepaste methode is gewrichtsdistractie. Bij gewrichtsdistractie worden de gewrichtsoppervlakken een klein stukje van elkaar getrokken door middel van een frame rond het gewricht dat met pennen bevestigd is aan het bot. Gedurende een periode van 2-3 maanden wordt het kraakbeen en onderliggend bot mechanisch ontlast. Studies naar gewrichtsdistractie als behandeling van enkelartrose hebben laten zien dat enkele maanden distractie resulteert in langdurige vermindering van klachten. Tevens waren er aanwijzingen voor vermindering van gewrichtsschade. Een dergelijke behandeling zou het plaatsen van een gewrichtsprothese mogelijk kunnen uitstellen en wellicht zelfs afstellen. Vooral jonge mensen met ernstige artrose zouden van de behandeling met gewrichtsdistractie kunnen profiteren.

Proefschrift

Dit proefschrift kan worden ingedeeld in twee delen. Het eerste deel betreft vooral de interactie tussen kraakbeen en bot in de ontwikkeling van artrose. Het tweede deel richt zich op de behandeling van artrose door middel van ontlasting (gewrichtsdistractie).

Voor het bestuderen van artrose vroeg in de ontwikkeling, wordt gebruik gemaakt van het honden Groove model voor artrose. In het kraakbeen van het bovenbeen in de knie worden chirurgisch een aantal krassen aangebracht. In de loop van enkele weken treedt er degeneratie op van het gehele gewricht, inclusief het kraakbeen van de knie dat onbekrast is.

In *hoofdstuk 2* wordt bestudeerd of het honden Groove model representatief is voor humane artrose. Het blijkt dat na 20 weken ontwikkeling van artrose in het Groove model, er significante kraakbeenschade is opgetreden. Wanneer er wordt gekeken naar absolute mate van kraakbeenschade in het Groove model en bij humane artrose, dan is de schade bij humane artrose ernstiger. Wordt echter gekeken naar de verschillen in ten opzichte van gezond honden of menselijk kraakbeen dan blijken de relatieve verschillen voor de experimenteel veroorzaakte artrose gelijk te zijn met die van humane artrose. Hetzelfde geldt voor de veranderingen in de activiteit van de kraakbeencellen en het verlies van kraakbeen proteoglycanen. Het verschil met de controlegroep is in het honden Groove model vergelijkbaar met humane artrose. Ondanks basale verschillen tussen hond en mens zijn de veranderingen ten gevolge van artrose vergelijkbaar en is het honden Groove model representatief voor humane artrose.

Bij artrose is het bekend dat er kraakbeen en botveranderingen optreden. Wat echter niet duidelijk is, is wanneer de botveranderingen ontstaan en wat de aard van de botveranderingen is vroeg in de ontwikkeling van artrose. *Hoofdstuk 3* beschrijft een eerste studie naar bot- en kraakbeenveranderingen op verschillende momenten vroeg in de ontwikkeling van artrose in twee diermodellen, het honden Groove model en het honden ACLT model. Beide modellen laten dezelfde mate van kraakbeenschade zien. Het blijkt dat ook in beide modellen de dikte van de botplaat onder het kraakbeen afneemt, al zeer vroeg in de ontwikkeling van artrose. Opvallend is echter dat afname van volume en dikte van de onder de botplaat gelegen botbalkjes alleen in het ACLT model optreedt. Dit gebeurt op een later moment dan de afname van plaatdikte. De conclusie is dat vroeg in de ontwikkeling van artrose er een afname is van dikte van de onder het kraakbeen gelegen botplaat. De veranderingen in botbalkjes lijken echter onafhankelijk van de ontwikkeling van kraakbeenschade. De resultaten van deze inventariserende studie gaven aanleiding tot het uitvoeren van een tweede studie naar botveranderingen in relatie tot de ontwikkeling van kraakbeenschade. Om zo min mogelijk verstorende factoren te hebben is er gekozen voor modellen met artrose in beide knieën (bilaterale modellen), met als controlegroep een groep dieren die dezelfde behandelingen hebben ondergaan maar waarbij geen artrose is geïnduceerd (sham groep).

Alvorens met deze studie te starten was het noodzakelijk om het bilaterale honden Groove model te valideren. *Hoofdstuk 4* beschrijft het bilaterale honden Groove model en vergelijkt

dit met de unilaterale variant. Er blijft dat het bilaterale model vergelijkbare kenmerken laat zien waarbij er zelfs méér kraakbeenschade optreedt dan het unilaterale model.
In *hoofdstuk 5* worden botveranderingen in het bilaterale Groove model en ACLT model vergeleken met een sham groep. In deze studie wordt bevestigd dat afname van dikte van de botplaat onder het kraakbeen samengaat met kraakbeenschade, terwijl afname van botbalkjes alleen in het ACLT model voorkomt. Het lijkt zeer waarschijnlijk dat afname van botbalkjes simpelweg het gevolg is van verminderde belasting in dit specifieke model. Ondanks het gebruik van bilaterale modellen zorgt de instabiliteit en pijn aan het gewricht voor verminderde belasting.
Om dit idee te bevestigen is gekeken naar verhoogde en verminderde belasting in één gewricht in een derde studie. Alle biochemische omstandigheden zijn in dat geval gelijk en eventuele verschillen in botveranderingen zijn zeer waarschijnlijk te wijten aan mechanische veranderingen. Voor deze studie (*hoofdstuk 6*) werd er gebruik gemaakt van het ACLT-meniscectomie model. Naast het doorknippen van de voorste kruisband waardoor instabiliteit ontstaat, werd ook de meniscus aan een binnenkant (mediaal) van het gewricht verwijderd. Hierdoor verandert de belastingsas van het gewricht, met overbelasting aan de binnenzijde en ontlasting aan de buitenzijde. Het resultaat was dat de binnenzijde zich kenmerkte door ernstige kraakbeenschade en sterke afname van de dikte van de botplaat. Aan de buitenzijde van de knie was er minder kraakbeenschade en was de plaatdikte niet afgenomen. Wel was nu juist aan die kant de dikte van de botbalkjes afgenomen. Dit fenomeen bevestigt de hypothese dat het volume van de botbalkjes afneemt als gevolg van ontlasting en niet het directe gevolg is van kraakbeen degeneratie. Het zou mogelijk kunnen zijn dat belasting van het aangedane kraakbeen noodzakelijk is voor afname van de botplaat aangezien alleen bij verhoogde belasting een afname van de botplaat optreedt. Dit wijst naar een biochemische oorzaak voor de afname aangezien mechanisch gezien het bot juist dikker en sterker moeten worden.

Het tweede deel van dit proefschrift richt zich op ontlasting als (onderdeel van) de behandeling van artrose.
Allereerst wordt in *hoofdstuk 7* een uiteenzetting gedaan van de behandelmethoden voor artrose die tot dusver ontwikkeld zijn waarbij ontlasting van het aangedane gewricht geïnitieerd wordt. Voorbeelden zijn het gebruik van inlegzooltjes en braces, maar ook osteotomie is een voorbeeld van ontlasting van het meest beschadigde deel van het gewricht. Gewrichtsdistractie wordt uitgebreid behandeld en alle onderzoeken tot dusver bekend, inclusief onderzoek in diermodellen worden uiteengezet. Er kan geconcludeerd worden dat ontlasting van het aangedane gewricht leidt tot afname van klachten en dat gewrichtsdistractie een veelbelovende behandelmethode van artrose is.
In *hoofdstuk 8* wordt onderzocht of gewrichtsdistractie naar klinische verbetering ook daadwerkelijk structurele veranderingen tot gevolg heeft. In het honden Groove model wordt gewrichtsdistractie toegepast om het effect op kraakbeenkwaliteit te meten. Het blijkt dat als gevolg van gewrichtsdistractie, er minder kraakbeenschade optreedt. Bovendien blijken de dieren hun aangedane poot beter te gaan belasten. In experimentele artrose resulteert

gewrichtsdistractie dus in structureel veranderingen van het kraakbeen en vermindering van pijn.

Hoofdstuk 9 behandelt de botveranderingen die plaatsvinden als gevolg van distractie bij ernstige artrose van de enkel. Klinische symptomen van artrose zijn vaak lastig te relateren aan schade aan het gewricht. Veranderingen van het onder het kraakbeen gelegen bot laten tot op heden de grootste relatie zien met klinische symptomen. In deze studie wordt een verklaring voor de klinische verbetering als gevolg van gewrichtsdistractie gezocht in veranderingen in het subchondrale bot. Het blijkt dat als gevolg van gewrichtsdistractie er over het geheel genomen een afname is van botdichtheid. Het blijkt echter ook dat gebieden die reeds een lage botdichtheid hadden (botcysten), een stijging laten zien van densiteit. De toename van densiteit in cysteuze gebieden laat een duidelijke correlatie zien met de klinische verbetering. Geconcludeerd wordt dat gewrichtsdistractie tot re-modellering van subchondraal bot leidt wat gerelateerd is aan klinische verbetering.

Dit proefschrift sluit af (*Hoofdstuk 10)* met een klinische trial waarin gewrichtsdistractie voor het eerst wordt toegepast bij ernstige artrose van de knie. Ook hier leidt gewrichtsdistractie tot een duidelijke klinische verbetering. Maar veel belangrijker is dat er ook duidelijke veranderingen in het kraakbeen waar te nemen zijn. Op röntgenfoto's is er een significante toename gemeten van de gewrichtsspleet. Deze toename wordt tevens gezien op MRI's in de vorm van een toename van kraakbeendikte. Ook op plekken waar de kraakbeenlaag volkomen is verdwenen heeft zich een nieuwe laag kraakbeen gevormd; kraakbeen dat in ieder geval voldoende stevig is om de gewrichtsspleet te laten toenemen in belaste toestand. Ook de analyse van biochemische markers in bloed en urine voor de aanmaak en afbraak van kraakbeen suggereren kraakbeenregeneratie. Ontlasten van een artrotisch gewricht in de vorm van gewrichtsdistractie leidt tot klinische verbetering en is de eerste behandeling die daadwerkelijk weefselherstel laat zien.

DANKWOORD

Gedurende vier jaar promotieonderzoek is het opvallend hoe veel mensen zich hebben ingespannen, op welke manier dan ook, om mij te steunen om dit proefschrift te voltooien. Graag wil ik allen bedanken en enkele in het bijzonder.

Floris, hooggeleerde dr. FPJG Lafeber, het is één grote achtbaanrit geweest om jou als promotor te hebben. Ik wil je ontzettend bedanken voor alles wat je me geleerd hebt en ik heb met veel plezier met je samen gewerkt. Jouw creativiteit en volharding zijn de basis voor ons prachtige onderzoek. Een onderzoek waarvan ik trots ben dat ik het heb mogen uitvoeren, zelfs al heeft het me bij de afdeling Reumatologie gebracht, een zeer onwaarschijnlijke route voor een orthopeed in wording. Ik hoop zeker nog eens met je te sparren over de wetenschap of over welk onderwerp dan ook.
Daarnaast wil ik ook mijn andere promotor, professor Castelein, Rene, hartelijk bedanken voor de steun bij dit onderzoek. Zonder deze steun was het absoluut niet mogelijk geweest om in dit tijdsbestek alle patiënten te behandelen en de studie uit te voeren.
Professor Bijlsma, Hans, ook al heb je je positie als promotor afgestaan, ik wil je toch bedanken voor het aandeel dat je in mijn promotietraject gehad hebt. Jouw inbreng op de werkbesprekingen heb ik als zeer prettig ervaren en toonde betrokkenheid bij mijn project.

Doctor van Roermund, Peter, bedankt dat jij mijn co-promotor wilde zijn. Het is een groot plezier geweest om met jou samen te werken. Jouw positiviteit, vrolijkheid en enthousiasme voor dit onderzoek werkten aanstekelijk.
Dan is er natuurlijk mijn andere co-promotor Simon. Siem, niet alleen co-promotor, maar ook onderzoeksmaatje, barmaatje op congressen en neutrale derde in discussies met Floris. Bedankt dat je altijd voor me klaarstond en mij advies kon geven als doorgewinterde kraakbeen- en diermodelonderzoeker. Daarnaast was chillen met jou langs de boulevard in Miami of op een boot tussen de alligators toch wel één van de leukste momenten van mijn promotietijd.

Margot en Sarita, mede-AIO's, paranimfen. Jullie hebben mijn AIO tijd absoluut een stuk leuker gemaakt. Sariet, AIO van het eerste uur. Altijd heel fijn om bij jou mijn hart te luchten, gezelschap te hebben op het balkon en veel te kunnen lachen met jou. Margot, fijn dat er eindelijk iemand is binnen de Reumatologie die ook wat minder genuanceerd is, jammer van het ochtendhumeur, maar dat neem ik op de koop toe. Bedankt dat je altijd om mij lacht, zorgt dat er op het lab over voetbal gepraat wordt en zorgt dat de minder assertieven onder ons nog eens wat leuks met elkaar afspreken. Ik verheug me er nu al op om jullie beide straks links en rechts van met te hebben staan en om samen een drankje op de goede afloop te doen.
Dan wil ik natuurlijk de overige AIO's bedanken, en dat zijn er veel tegenwoordig. Allereerst Nathalie, mijn congresmaatje, inmiddels heb je het kraakbeen achter je gelaten en ben je AIO af. Bedankt voor de leuke tijd en dat ik al je kraakbeenkennis met je mocht delen.

Marije, Q -maatje, bedankt voor al het gezellige kletsen zodat de dagen achter de computer wat minder saai werden. Tineke, onze Friese akela, fijn om te weten dat er altijd iemand meer uren maakt dan ieder ander op het lab. Marlies, eindelijk iemand om alle patiëntensores mee te delen. Jos, het toonbeeld van vrolijkheid, wellicht een dooie mus, maar….. bedankt. Angela, thanks voor het brengen van absoluut heel veel extra gezelligheid binnen de AIO-groep, en voor iets van concurrentie tijdens Singstar. Verder natuurlijk Monique, Erwin, Laurens en Frederique, ik wil jullie ook allemaal bedanken voor de gezelligheid van alledag, maar ook tijdens de etentjes, het flow-boarden, Singstar-avondjes, tivoli-feestjes, lab-uitjes, etc. Karen weet je waar je aan begint? Bij voorbaat dank voor het voortzetten van dit prachtige project.

Ook wil ik graag de postdocs bedanken. Allereerst natuurlijk Anne Karien, mijn voorganger, zonder jouw voorwerk zou ik met de klinische studie niet half zo ver zijn gevorderd. Bedankt voor alle goede adviezen en dat ik altijd bij je kon binnenlopen. Joel, bedankt voor de gezelligheid als kamergenoot en voor de wetenschappelijke input vanuit andere hoek. Paco, bedankt voor de adviezen op het vlak van de statistiek.

Marieke, Kim, Marion, Angelique en Arno, bedankt dat jullie als analisten altijd klaar stonden om wat dan ook te regelen. Of het nou ging om last minute biomarker analyses, een dagje force platen of even een kleine onenigheid sussen betreffende histologie. Marieke, bedankt voor de inwijding in de kraakbeenanalyse en het was altijd weer een aangename verassing als je weer een dagje op het lab kwam. Joline, ik had me geen betere kamergenoot (invalidentoilet genoot) kunnen wensen.

Michiel, Tanja, Dory en Jurjen, mijn studenten, allemaal bedankt voor het aandeel in mijn project dat jullie tijdens de stages geleverd hebben.

Behalve mijn naaste collega's, wil ik ook de mensen van andere afdelingen of instituten bedanken met wie ik heb mogen samenwerken voor mijn onderzoek. Allereerst professor Weinans, Harry, ik denk dat we trots kunnen zijn op de resultaten van de twee onderzoeksprojecten en bijbehorende publicaties. Bedankt dat we gebruik konden maken van uw expertise en faciliteiten.
Professor Hazewinkel, Herman, bedankt voor de samenwerking vanuit verschillende hoeken en voor uw input welke tot alvast één mooie publicatie heeft geleid. Daarnaast stond ik elke keer weer versteld van hoe snel u dingen kunt regelen binnen uw instituut. Wij hebben daar dankbaar gebruik van gemaakt.
Ank en Arie wil ik graag bedanken voor het eindeloze geduld tijdens de force plate metingen. Ik denk dat slechts weinige beseffen hoeveel, soms frustrerende, uren er in deze analyses zitten. De honden waren ontzettend lief en knuffelbaar, maar ook eigenwijs, koppig en vaak slimmer dan wij.
Voor de uitvoering van de hondenstudie was een goede samenwerking met het GDL zeer belangrijk. Ik wil dan ook de medewerkers van het GDL, Hans, Nico, Hester, Elly, Janny,

Helma, Wendy, Tamara en Romy, allen bedanken voor de support, voor het meedenken bij de meer complexe experimenten en voor de flexibiliteit waardoor altijd alles toch nog op tijd rond kwam.

Dit onderzoek heeft me ook naar Iowa City gebracht waar ik enkele maanden heb doorgebracht. Don, Tom, Thad and Ned, thank you all so much for the successful collaboration and warm welcome in Iowa City. The work that we have done together turned out to be one of my favorite projects with surprisingly interesting results.
Een andere buitenlandse samenwerking is er geweest in verband met de expertise op het gebied van kraakbeenbeeldvorming. Felix, Sebastian, thank you for making time to analyze the MRIs at such short notice and for giving input regarding the manuscript on the knee distraction study.

Ondanks mijn aanstelling bij de afdeling Reumatologie, heb ik vanwege persoonlijke interesse en de orthopedische aard van mijn onderzoek, ook mijn werk kunnen delen en presenteren tijdens de werkbesprekingen van de afdeling Orthopedie. Ik wil Wouter, Daan en Laura bedanken voor de support vanuit orthopedische hoek voor mijn project.

Er zijn een aantal afdelingen die mij ondersteund hebben bij mijn onderzoekswerkzaamheden. Graag wil ik iedereen van de divisie Beeldvorming bedanken voor de flexibiliteit voor het maken van de MRIs, foto's, en natuurlijk de snelle aanlevering ervan. Aart en Frank van de pathologie, bedankt dat wij altijd weer even mochten 'inbreken' tijdens jullie drukke werkzaamheden. Sylvia en Gerrit, bedankt voor de inzet als fysiotherapeut om de patiënten zoveel mogelijk te laten belasten en buigen. Medewerkers van de polikliniek Orthopedie en betrokkenen van het OK-complex, bedankt voor het mogelijk maken van de klinische studie.
Daarnaast wil ik iedereen die ik niet specifiek genoemd heb, maar die ook heeft medegewerkt aan mijn onderzoek, graag bedanken.

Het laatste jaar heb ik doorgebracht als AIOS Orthopedie in het Meander MC. Ik wil mijn collega's aldaar bedanken voor de interesse in mijn promotie en voor de support.

En dan nu het thuisfront, paps, mams en Maarten, ja hoor, het is eindelijk af, ik beloof niets, maar verwacht zeker meer tijd voor bezoekjes aan de Moerbeek en aan knallende optredens van Feather and the White. Bedankt voor alle steun en interesse.

Dan rest er nog één. Steven, promoveren in de avonduren, je hebt het geweten. Van carrièreman naar huisman, ik zal het weer van je overnemen. Bedankt voor de steun en dat je er altijd voor me bent. Je bent alles voor me. Nu kunnen we op vakantie.

LIST OF PUBLICATIONS

1. Lafeber FPJG, Intema F, van Roermund PM, Marijnissen ACA. Unloading joints to treat osteoarthritis, including joint distraction. Current Opinion in Rheumatology 2006; 18(5):519-525
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CURRICULUM VITAE

Femke Intema was born on October 10th in 1980 in Niedorp. In 1998 she finished secondary school at the 'Gemeenschappelijke Scholengemeenschap Schagen'.
She started that year with the study Medicine at the University of Utrecht. As part of this study she participated in a research project at the department of Gynecology of the University Medical Center Utrecht (UMCU) called 'Clinical applications of cell free fetal DNA from maternal plasma'. She finished Medicine in 2005 with an interimship at the department of Orthopedics at the UMCU.

In 2006 she started as a PhD student at the department of Rheumatology & Clinical Immunology in collaboration with the department of Orthopedics which resulted in her thesis entitled "Loading and unloading in the development and treatment of osteoarthritis" (Prof. F.P.J.G. Lafeber, Prof. R.M. Castelein). Part of this work was performed during a two month work visit at the 'Orthopedic Biomechanics Laboratory' at the 'University of Iowa, USA' (Prof. T.D. Brown). Femke has also participated in research on "loading in the development of the Groove model", which is not part of this thesis.

In 2009, after slightly more than three years as a researcher, she has started as an orthopedic residence, after being accepted for the specialism of Orthopedics at the UMCU.