Osteoarthritis and Cartilage 18 (2010) 691–698

Osteoarthritis and Cartilage

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

F. Intema †, H.A.W. Hazewinkel ‡, D. Gouwens ‡, J.W.J. Bijlsma †, H. Weinans §, F.P.J.G. Lafeber †*, S.C. Mastbergen †

† *Rheumatology & Clinical Immunology, University Medical Center Utrecht, Utrecht, The Netherlands*
‡ *Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*
§ *Orthopaedics, Erasmus Medical Center, Rotterdam, The Netherlands*

ARTICLE INFO

Article history:
Received 29 July 2009
Accepted 22 January 2010

Keywords:
Osteoarthritis
Experimental
Subchondral plate
Trabecular bone
Loading

SUMMARY

Objective: The pathogenesis of osteoarthritis (OA) includes cartilage degeneration, synovial inflammation, and bone changes. Slowly, the sequence and inter-relationship 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 anterior cruciate ligament transection (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, glycosaminoglycan (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

* Address correspondence and reprint requests to: F.P.J.G. Lafeber, Rheumatology & Clinical Immunology, UMC Utrecht, F02.127, PO Box 85500, 3508 GA, Utrecht, The Netherlands. Tel: 31-88-755-8521; Fax: 31-30-2523741.
E-mail address: f.lafeber@umcutrecht.nl (F.P.J.G. Lafeber).

1063-4584/$ – see front matter
doi:10.1016/j.joca.2010.01.004

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 etiology, 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 (±S.E.M.) of 25 ± 1 kg, were obtained from a commercial laboratory-animal breeding facility (seven males, six females; mean age (±S.E.M.) 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 8 weeks prior to the surgical induction of OA, the dogs were trained to run on a treadmill.

Induction of OA

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 parapatellar 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 3 days after surgery and antibiotics the first 5 days after surgery according to standard procedures. After a recuperation period of 2 weeks, the dogs were exercised on a treadmill for 10 min, 5 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 2 h 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 Fig. 1(a)]. 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].

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 [Fig. 2(b)]. 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].

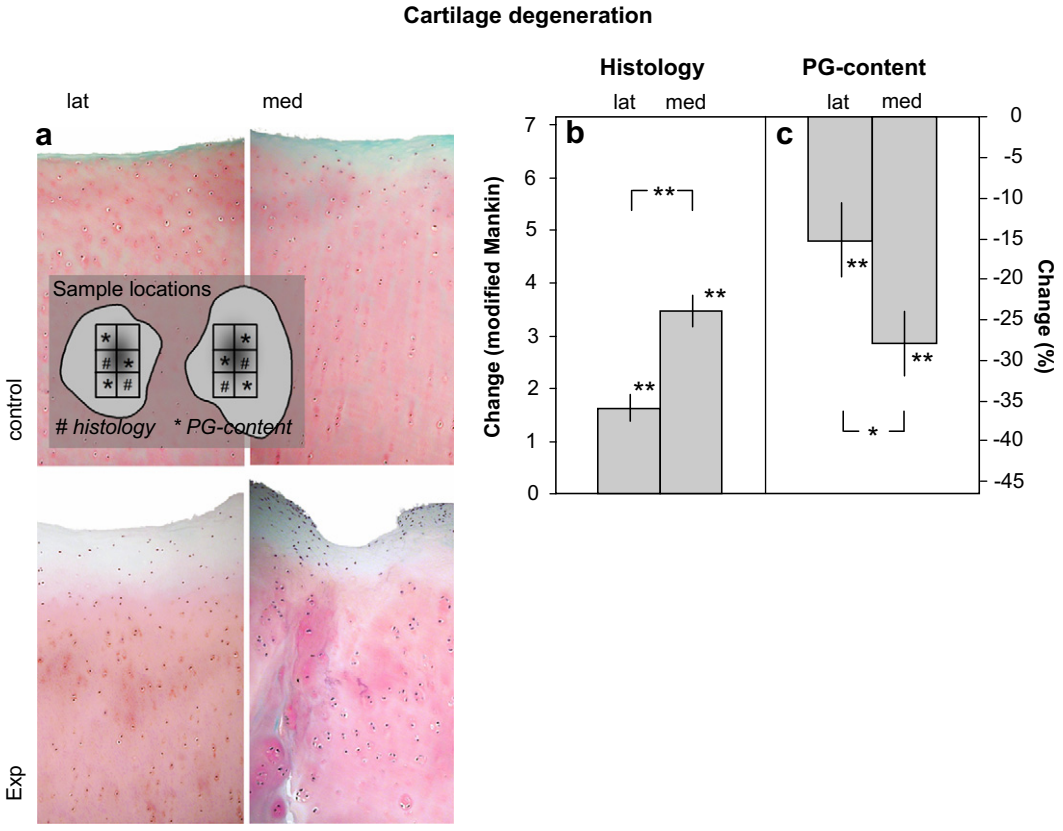

Fig. 1. a. Representative micrographs of histology. Insert: sample locations at the tibial plateau. Cartilage degeneration is represented by histological damage (b) and a decrease in PG content (c). Each bar represents the mean change (±S.E.M.) between experimental and control joint (absolute change 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 $P < 0.01$).

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

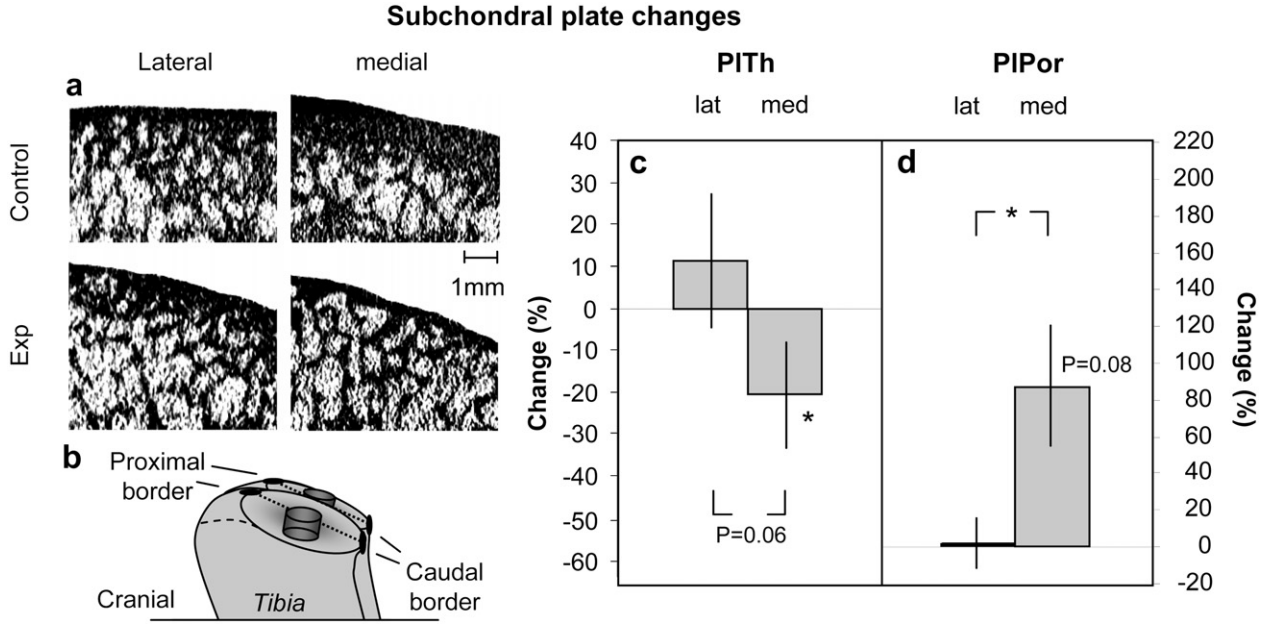

Fig. 2. Subchondral plate changes. a. Representative micro-CT images of the lateral and medial subchondral plate of the control and experimental joint. b. Cylinder location; centred on the weight-bearing area of the tibial plateau. c. Plate thickness (PlTh) and d. Plate porosity (PlPor), each bar represents the mean change (±S.E.M.) between experimental and control joint (percentage change of PlTh and PlPor) for the lateral and medial compartment (* indicates statistical significance of $P < 0.05$).

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.

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.

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 [Fig. 1(a)]. Histological cartilage damage in the experimental joints was statistically more severe on the medial side when compared to the lateral side [Fig. 1(b)] while there was no significant difference in histological grading between both sides of the control joint (Table I). These degenerative microscopical features were corroborated by a decrease of *PG content*, representing loss of cartilage integrity [Fig. 1(c)]. The PG content of the experimental knee was significantly lower than that of the control knee in both compartments of the tibial plateau (Table I), with statistically more severe PG-loss in the medial compared to the lateral compartment [Fig. 1(c)].

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 I). 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 [Fig. 2(c)]. 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 I), 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 [$P = 0.08$; Fig. 2(d)] whereas no change was observed in the lateral compartment. The change between the medial and lateral compartment was statistically significantly different.

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 I, Fig. 3(a)]. 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 I, Fig. 3(b and c)]. 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 I).

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 plate thickness [Fig. 4(a)]. Plate porosity correlated positively with histological cartilage damage indicating that increasing cartilage damage correlated with an increase in plate porosity [Fig. 4(b)]. The decrease in PG content showed similar correlations; a decrease in cartilage content was related to a thinning [Fig. 4(c)] and increasing porosity of the plate [Fig. 4(d)] 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.

Table I
Mean values (±S.E.M.) of the different parameters from the medial and lateral compartment of the contralateral control knee (C) and experimental (OA) knee

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

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 (Fig. 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

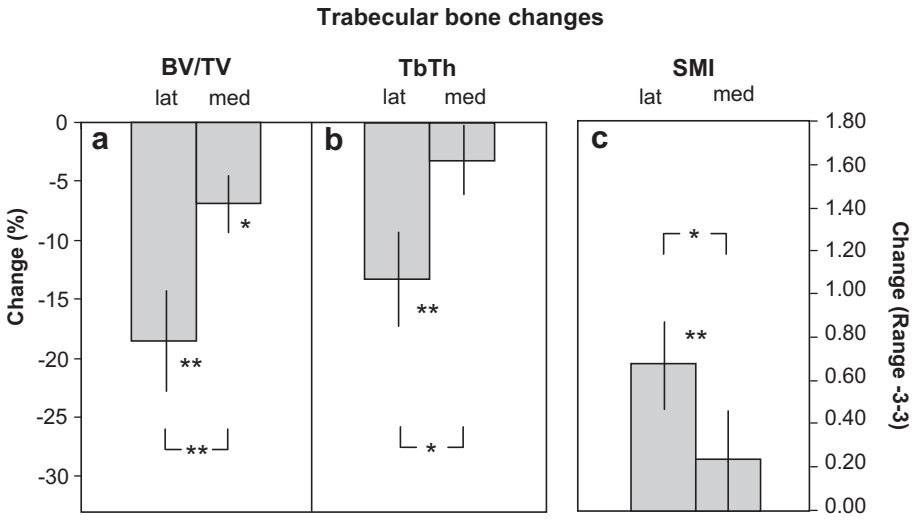

Fig. 3. Trabecular bone changes. Each bar represents the mean change (±S.E.M.) between experimental and control joint (percentage change of the fraction of (a) BV/TV and of (b) the trabecular thickness (TbTh) and (c) absolute change for the SMI) for the lateral and medial compartment each (* indicates statistical significant difference of $P < 0.05$ and ** indicates $P < 0.01$).

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

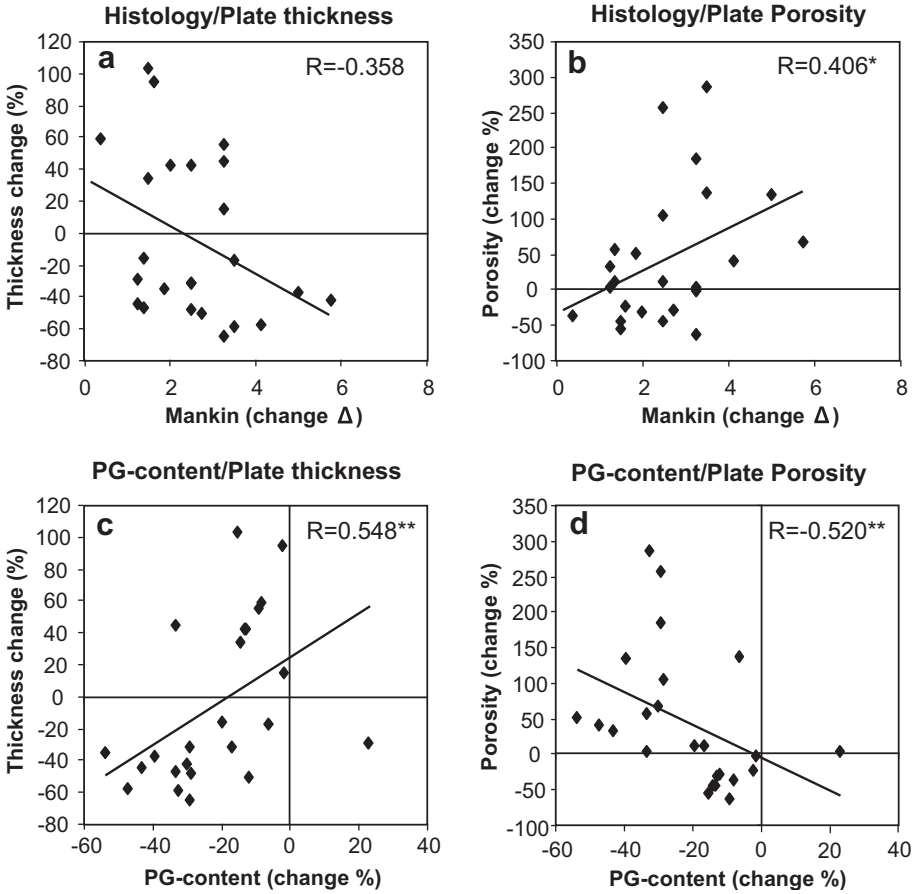

Fig. 4. Correlations between individual cartilage changes and changes of the subchondral plate for the lateral and medial compartment (* indicates statistical significance of $P < 0.05$ and ** indicates $P < 0.01$).

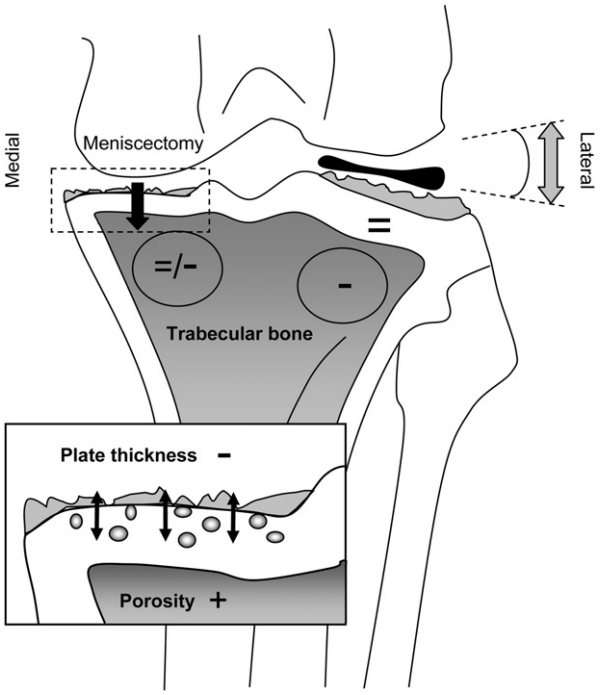

Fig. 5. Schematical overview of changes in the ACLT-meniscectomy model. Cartilage damage is present in both the medial and lateral compartment, but more severe on the medial side where the meniscus is removed. Thinning and increasing porosity of the subchondral plate coincides with cartilage degeneration 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.

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.

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 trabecular 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 ACLT 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.

Conflict of interest

All authors have no conflicts of interest.

Acknowledgements

This study is financially supported by the Anna Fund and the Dutch Arthritis Association.

References

1. Martel-Pelletier J. Pathophysiology of osteoarthritis. Osteoarthritis Cartilage 2004;12(Suppl A):S31–3.
2. Moskowitz RW. Osteoarthritis. 4th edn. Philadelphia: Lippincott Williams & Wilkins, a Wolters Kluwer Business; 2007.
3. Ameye LG, Young MF. Animal models of osteoarthritis: lessons learned while seeking the "Holy Grail". Curr Opin Rheumatol 2006;18:537–47.
4. Lories RJ. Joint homeostasis, restoration, and remodeling in osteoarthritis. Best Pract Res Clin Rheumatol 2008;22:209–20.
5. Burr DB. Anatomy and physiology of the mineralized tissues: role in the pathogenesis of osteoarthrosis. Osteoarthritis Cartilage 2004;12(Suppl A):S20–30.
6. Bobinac D, Spanjol J, Zoricic S, Maric I. Changes in articular cartilage and subchondral bone histomorphometry in osteoarthritic knee joints in humans. Bone 2003;32:284–90.
7. Day JS, Ding M, van der Linden JC, Hvid I, Sumner DR, Weinans H. A decreased subchondral trabecular bone tissue elastic modulus is associated with pre-arthritic cartilage damage. J Orthop Res 2001;19:914–8.
8. Ding M, Odgaard A, Hvid I. Changes in the three-dimensional microstructure of human tibial cancellous bone in early osteoarthritis. J Bone Joint Surg Br 2003;85:906–12.
9. Blumenkrantz G, Lindsey CT, Dunn TC, Jin H, Ries MD, Link TM, *et al*. A pilot, two-year longitudinal study of the interrelationship between trabecular bone and articular cartilage in the osteoarthritic knee. Osteoarthritis Cartilage 2004;12:997–1005.
10. Chappard C, Peyrin F, Bonnassie A, Lemineur G, Brunet-Imbault B, Lespessailles E, *et al*. Subchondral bone microarchitectural alterations in osteoarthritis: a synchrotron micro-computed tomography study. Osteoarthritis Cartilage 2006;14:215–23.
11. Batiste DL, Kirkley A, Laverty S, Thain LM, Spouge AR, Holdsworth DW. Ex vivo characterization of articular cartilage and bone lesions in a rabbit ACL transection model of osteoarthritis using MRI and micro-CT. Osteoarthritis Cartilage 2004;12:986–96.
12. Botter SM, van Osch GJ, Waarsing JH, Day JS, Verhaar JA, Pols HA, *et al*. Quantification of subchondral bone changes in a murine osteoarthritis model using micro-CT. Biorheology 2006;43:379–88.
13. Botter SM, van Osch GJ, Waarsing JH, van der Linden JC, Verhaar JA, Pols HA, *et al*. Cartilage damage pattern in relation to subchondral plate thickness in a collagenase-induced model of osteoarthritis. Osteoarthritis Cartilage 2008;16:506–14.
14. Boyd SK, Muller R, Leonard T, Herzog W. Long-term periarticular bone adaptation in a feline knee injury model for post-traumatic experimental osteoarthritis. Osteoarthritis Cartilage 2005;13:235–42.
15. Hayami T, Pickarski M, Zhuo Y, Wesolowski GA, Rodan GA, Duong le T. Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. Bone 2006;38:234–43.
16. Sniekers YH, Intema F, Lafeber FP, van Osch GJ, van Leeuwen JP, Weinans H, *et al*. A role for subchondral bone changes in the process of osteoarthritis; a micro-CT study of two canine models. BMC Musculoskelet Disord 2008;9:20.
17. Pond MJ, Nuki G. Experimentally-induced osteoarthritis in the dog. Ann Rheum Dis 1973;32:387–8.
18. Frost-Christensen LN, Mastbergen SC, Vianen ME, Hartog A, DeGroot J, Voorhout G, *et al*. Degeneration, inflammation, regeneration, and pain/disability in dogs following destabilization or articular cartilage grooving of the stifle joint. Osteoarthritis Cartilage 2008;16:1327–35.
19. Marijnissen AC, van Roermund PM, TeKoppele JM, Bijlsma JW, Lafeber FP. The canine 'groove' model, compared with the ACLT model of osteoarthritis. Osteoarthritis Cartilage 2002;10:145–55.
20. Lafeber FP, Vander Kraan PM, Van Roy JL, Huber-Bruning O, Bijlsma JW. Articular cartilage explant culture; an appropriate in vitro system to compare osteoarthritic and normal human cartilage. Connect Tissue Res 1993;29:287–99.
21. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. II. Correlation of morphology with biochemical and metabolic data. J Bone Joint Surg Am 1971;53:523–37.
22. Waarsing JH, Day JS, Weinans H. An improved segmentation method for in vivo microCT imaging. J Bone Miner Res 2004;19:1640–50.

23. Hildebrand T, Laib A, Muller R, Dequeker J, Ruegsegger P. Direct three-dimensional morphometric analysis of human cancellous bone: microstructural data from spine, femur, iliac crest, and calcaneus. J Bone Miner Res 1999;14:1167–74.
24. Hildebrand T, Ruegsegger P. Quantification of bone microarchitecture with the structure model index. Comput Methods Biomech Biomed Engin 1997;1:15–23.
25. Bikle DD, Halloran BP. The response of bone to unloading. J Bone Miner Metab 1999;17:233–44.
26. Boyd SK, Muller R, Zernicke RF. Mechanical and architectural bone adaptation in early stage experimental osteoarthritis. J Bone Miner Res 2002;17:687–94.
27. Pardy CK, Matyas JR, Zernicke RF. Doxycycline effects on mechanical and morphometrical properties of early- and late-stage osteoarthritic bone following anterior cruciate ligament injury. J Appl Physiol 2004;97:1254–60.
28. van den Berg WB. Lessons from animal models of osteoarthritis. Curr Opin Rheumatol 2001;13:452–6.
29. Boyd SK, Muller R, Matyas JR, Wohl GR, Zernicke RF. Early morphometric and anisotropic change in periarticular cancellous bone in a model of experimental knee osteoarthritis quantified using microcomputed tomography. Clin Biomech (Bristol, Avon) 2000;15:624–31.
30. Lindsey CT, Narasimhan A, Adolfo JM, Jin H, Steinbach LS, Link T, *et al*. Magnetic resonance evaluation of the interrelationship between articular cartilage and trabecular bone of the osteoarthritic knee. Osteoarthritis Cartilage 2004;12:86–96.
31. Messent EA, Ward RJ, Tonkin CJ, Buckland-Wright C. Tibial cancellous bone changes in patients with knee osteoarthritis. A short-term longitudinal study using Fractal Signature Analysis. Osteoarthritis Cartilage 2005;13:463–70.
32. McDermott ID, Amis AA. The consequences of meniscectomy. J Bone Joint Surg Br 2006;88:1549–56.
33. Lorenz H, Wenz W, Ivancic M, Steck E, Richter W. Early and stable upregulation of collagen type II, collagen type I and YKL40 expression levels in cartilage during early experimental osteoarthritis occurs independent of joint location and histological grading. Arthritis Res Ther 2005;7:R156–65.
34. Botter SM, Glasson SS, Hopkins B, Clockaerts S, Weinans H, van Leeuwen JP, *et al*. ADAMTS5-/- mice have less subchondral bone changes after induction of osteoarthritis through surgical instability: implications for a link between cartilage and subchondral bone changes. Osteoarthritis Cartilage 2009;17:636–45.
35. Wu DD, Burr DB, Boyd RD, Radin EL. Bone and cartilage changes following experimental varus or valgus tibial angulation. J Orthop Res 1990;8:572–85.
36. Hwang J, Bae WC, Shieu W, Lewis CW, Bugbee WD, Sah RL. Increased hydraulic conductance of human articular cartilage and subchondral bone plate with progression of osteoarthritis. Arthritis Rheum 2008;58:3831–42.
37. Karsdal MA, Leeming DJ, Dam EB, Henriksen K, Alexandersen P, Pastoureau P, *et al*. Should subchondral bone turnover be targeted when treating osteoarthritis? Osteoarthritis Cartilage 2008;16:638–46.
38. Kwan Tat S, Amiable N, Pelletier JP, Boileau C, Lajeunesse D, Duval N, *et al*. Modulation of OPG, RANK and RANKL by human chondrocytes and their implication during osteoarthritis. Rheumatology (Oxford) 2009;48:1482–90.
39. Sanchez C, Gabay O, Salvat C, Henrotin YE, Berenbaum F. Mechanical loading highly increases IL-6 production and decreases OPG expression by osteoblasts. Osteoarthritis Cartilage 2009;17:473–81.
40. Appleton CT, McErlain DD, Pitelka V, Schwartz N, Bernier SM, Henry JL, *et al*. Forced mobilization accelerates pathogenesis: characterization of a preclinical surgical model of osteoarthritis. Arthritis Res Ther 2007;9:R13.
41. O'Connor BL, Visco DM, Rogers PI, Mamlin LA, Brandt KD. Serial force plate analyses of dogs with unilateral knee instability, with or without interruption of the sensory input from the ipsilateral limb. Osteoarthritis Cartilage 1999;7:567–73.
42. Radin EL, Rose RM. Role of subchondral bone in the initiation and progression of cartilage damage. Clin Orthop Relat Res 1986;34–40.
43. Saag KG. Bisphosphonates for osteoarthritis prevention: "Holy Grail" or not? Ann Rheum Dis 2008;67:1358–9.
44. Myers SL, Brandt KD, Burr DB, O'Connor BL, Albrecht M. Effects of a bisphosphonate on bone histomorphometry and dynamics in the canine cruciate deficiency model of osteoarthritis. J Rheumatol 1999;26:2645–53.
45. Agnello KA, Trumble TN, Chambers JN, Seewald W, Budsberg SC. Effects of zoledronate on markers of bone metabolism and subchondral bone mineral density in dogs with experimentally induced cruciate-deficient osteoarthritis. Am J Vet Res 2005;66:1487–95.
46. Behets C, Williams JM, Chappard D, Devogelaer JP, Manicourt DH. Effects of calcitonin on subchondral trabecular bone changes and on osteoarthritic cartilage lesions after acute anterior cruciate ligament deficiency. J Bone Miner Res 2004;19:1821–6.
47. El Hajjaji H, Williams JM, Devogelaer JP, Lenz ME, Thonar EJ, Manicourt DH. Treatment with calcitonin prevents the net loss of collagen, hyaluronan and proteoglycan aggregates from cartilage in the early stages of canine experimental osteoarthritis. Osteoarthritis Cartilage 2004;12:904–11.