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**OSTEOARTHRITIS and CARTILAGE**

## SHORT COMMUNICATION

# Preliminary report: increased porosity of the subchondral plate in an accelerated canine model of osteoarthritis

### Introduction/Summary

ANTERIOR CRUCIATE ligament transection (ACLT) in the dog leads to osteoarthritis (OA), with biochemical, metabolic, and morphologic changes in the cartilage of the unstable knee which closely mimic those of OA in humans. Using micro-computed tomography (micro-CT) [1], we showed that the subchondral trabecular bone became osteopenic, relative to that in the contralateral knee (CK), by 3 months after surgery, and that this persisted for as long as 54 months after ligament transection. In contrast, the thickness of the subchondral plate in the unstable knee remained normal until 18 months after surgery, during which time the articular cartilage underwent hypertrophy [2], with increases in net proteoglycan synthesis and proteoglycan concentration. However, 54 months after ligament transection, thickening of the subchondral plate was seen in association with full-thickness loss of the articular cartilage [1]. Recent histomorphometric analyses have confirmed the rapid development of osteopenia in the subchondral trabeculae in this model [3], consistent with the decrease in loading of the unstable limb which we have demonstrated by force plate analysis [4]. On the basis of the above findings we concluded that thickening of the subchondral plate is not necessary for *initiation* of articular cartilage changes in this model, although changes in the subchondral plate may be related to *progression* of the cartilage lesion.

Although full-thickness cartilage ulceration (i.e., end-stage OA) is not seen until more than 3 years after ACLT in this canine model, if the ipsilateral hind-limb is deafferented (e.g., by L4–S1 dorsal root ganglionectomy) prior to ACLT, the progression of the joint breakdown is strikingly accelerated, so that cartilage changes of end-stage OA develop within only 7–8 weeks [5]. In the present study, we used micro-CT analysis to determine whether bone changes in the accelerated model are similar to those in the neurologically intact cruciate-deficient dog. Our results show that whereas gradual thickening of the subchondral plate is a feature of OA in the standard cruciate-deficiency model, the accelerated model is characterized by thinning and increased porosity of the plate.

### Methods

Four normal adult mongrel dogs underwent left L4-S1 dorsal root ganglionectomy, followed 2 weeks later by ipsilateral ACLT [5] Postoperatively, the dogs were permitted standard activity until they were killed by an overdose of barbiturate 8 weeks after ACLT, at which time articular cartilage of both knees was removed for analysis. The medial and lateral tibial plateaus were separated from each other and from the remainder of the tibial shaft with a bone saw and were stored frozen until they were scanned as described previously [1,6,7]. A complete three-dimensional digitization of each bone specimen was examined carefully for evidence of geodes and microfractures. The cancellous bone in a $600 \times 600 \times 300$ μm volume drawn between the subchondral plate and the physeal scar was analyzed for bone volume fraction, trabecular surface:volume ratio, trabecular plate thickness, trabecular number and trabecular separation. Thickness measurements were made at peripheral, central and internal sites on each medial and lateral plateau along 10 evenly spaced coronal plane views and the 10 values obtained for each site were averaged. Paired *t*-tests were used to compare samples from the OA knee and CK.

### Results/Discussion

As reported previously in our description of this model [5], the unstable knee in each animal showed

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extensive full-thickness loss of articular cartilage, osteophytosis and marked synovitis, while the CK was grossly normal. It must be recognized, however, that CK in this model is not truly 'normal'. Even though force plate analyses did not disclose changes in the magnitude of ground reaction forces generated by CK [8], kinematic studies have shown that compensatory loading patterns develop in that joint [9]. Nonetheless, no morphologic evidence of OA is seen in either the cartilage or the bone of CK in the accelerated model, indeed, none are apparent in CK of the cruciate-deficient, neurologically-intact dog up to 54 months after the onset of joint instability, i.e., when end-stage OA is present in the unstable knee [10].

The values obtained in the histomorphometric analyses of trabecular bone and the subchondral plate of the tibial plateau in CK in the present study are similar to those reported previously for CK in the unaccelerated canine cruciate-deficiency model, in a study in which the weight of the animals, postoperative levels of activity and housing conditions were comparable with those in the present study, and the surgery was performed by the same individual (BOC) [1]. Eight weeks after ACLT, bone loss in the OA knee in the accelerated model was similar to, but less marked than, that described in the neurologically-intact, cruciate-deficient dog 12 weeks after ACLT [1]. For example, the bone volume fraction for the medial tibial plateau of the OA knee was $40 \pm 1$, whereas that for the contralateral knee was $44 \pm 4$. Corresponding values for surface : volume ratio for the OA and contralateral knee were $15.2 \pm 2.6$ and $13.2 \pm 1.5$, respectively. The loss of cancellous bone in both models is not surprising; loading of the unstable limb is diminished [8] and a significant inflammatory reaction occurs in the adjacent synovium [5].

Because material was available for examination from only four dogs, the findings in the present study must be considered as preliminary. Nonetheless, the results were consistent among all four dogs studied, i.e., the changes in cancellous bone in the accelerated model were similar to those in the standard canine model, but changes in the subchondral plate in the two models were strikingly different. In the neurologically-intact dog, end-stage cartilage lesions in the unstable knee are associated with *thickening* of the subchondral plate [1,10], which becomes apparent about 18 months after ACLT. In contrast, in the present study, despite extensive full-thickness loss of articular cartilage, in each of the four dogs examined, a trend toward plate *thinning* was observed 8 weeks after ACLT (Table I), although, perhaps because of the relatively small number of animals studied, the difference was not statistically significant. Visual examination of the digitized images showed that the subchondral plate in the unstable knee was more porous than that in the CK (Fig. 1); indeed, at some sites the plate could be differentiated from the underlying trabecular bone only with difficulty (Fig. 2). Because the micro-CT method is not sufficiently sensitive to differentiate calcified cartilage from bone or to detect microcracks in the zone of calcified cartilage [11,12], it was not possible to determine whether the porosity of the subchondral plate was resorptive in nature, due to endochondral ossification of the zone of calcified cartilage, or to microcracks. Relevant to the above observation, it should be noted that although thickening

Table 1
*Thickness of subchondral plate 8 weeks after anterior cruciate ligament transection in four dogs which had undergone deafferentation of the ipsilateral hind-limb*

| Sampling Site | Knee | Plate thickness ($\mu$m) mean $\pm$ S.D.* | | |
|---|---|---|---|---|
| | | Location | | |
| | | Peripheral | Central | Internal |
| Medial Tibial Plateau | OA | $888 \pm 161$ | $1078 \pm 77$ | $908 \pm 540$ |
| | Contralateral | $966 \pm 205$ | $1152 \pm 148$ | $1206 \pm 64$ |
| Lateral Tibial Plateau | OA | $550 \pm 289$ | $576 \pm 349$ | $765 \pm 326$ |
| | Contralateral | $597 \pm 228$ | $909 \pm 102$ | $949 \pm 90$ |

*$P > 0.05$ for all differences between the OA knee and the contralateral knee.

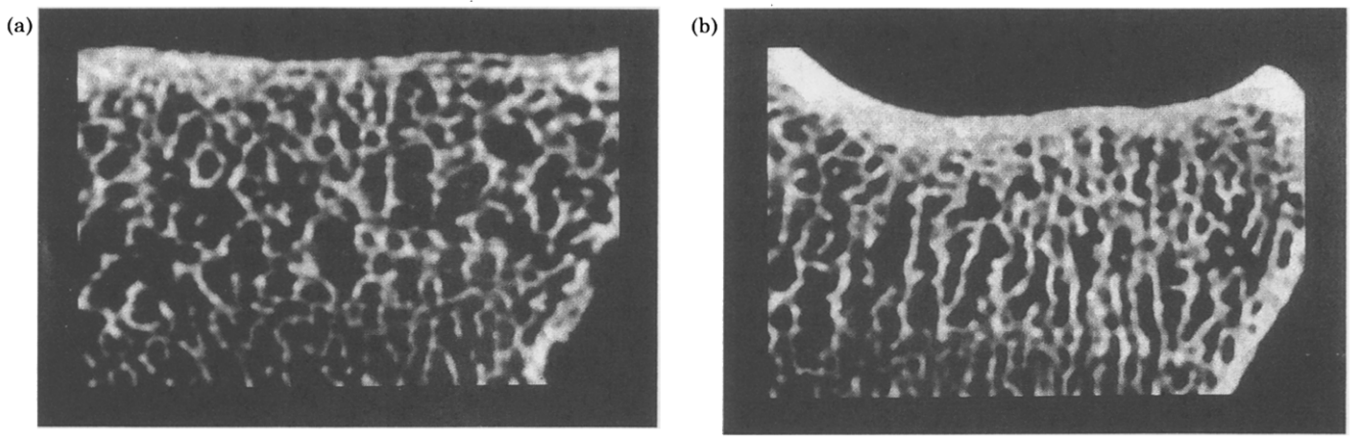


FIG. 1. Micro-CT images of medial tibial plateau from the unstable knee (a) and contralateral knee (b). The subchondral plate in the contralateral knee is intact. In contrast, the plate in the unstable knee is thin and porous. Note also the loss of trabeculae, with an increase in inter-trabecular distance in the OA knee

of subchondral bone is typical of OA, with eburnation occurring in area of full-thickness loss of articular cartilage, even eburnated surfaces may be radiographically porous. Pathologic examination of such areas may show tufts of proliferating connective tissue, often fibrocartilaginous, protruding from the marrow cavity into the joint space [13].

Because the ground reaction forces generated by the unstable limb are greater in the deafferented

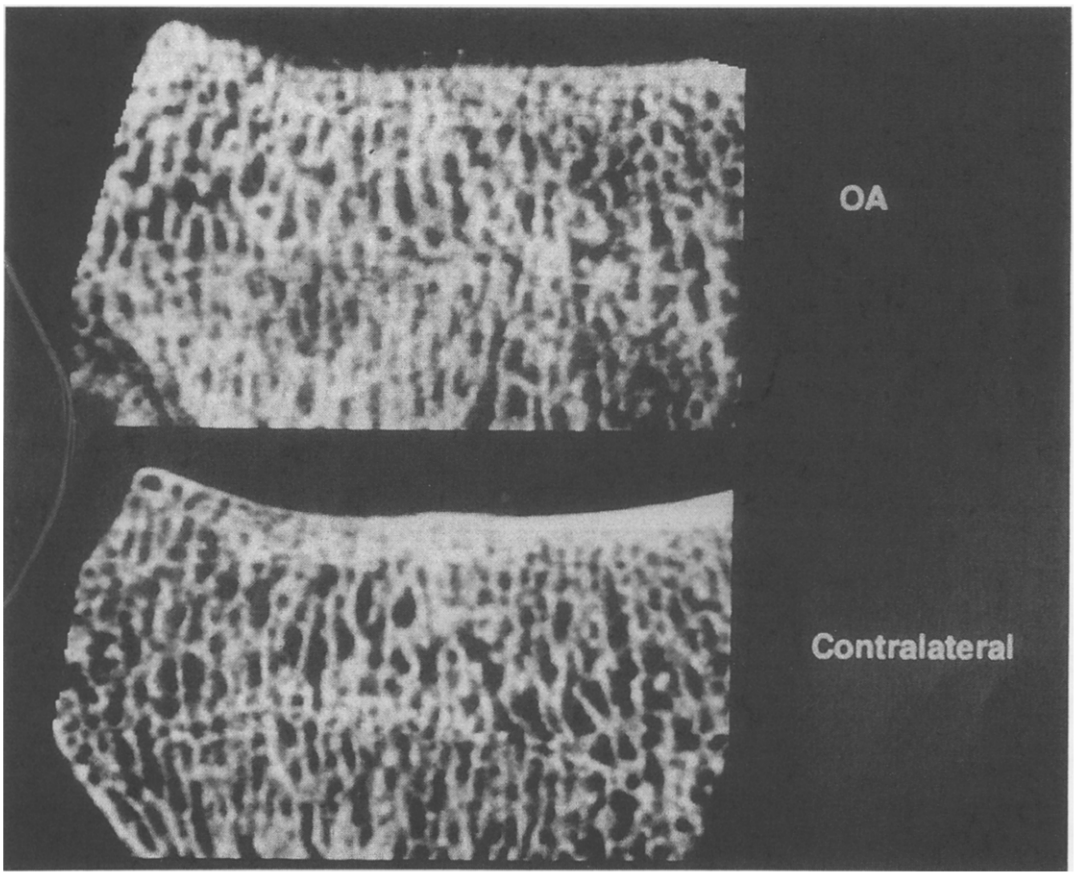


FIG. 2. Micro-CT images of lateral tibial plateau from the unstable knee and contralateral knee. Note the wispy appearance of the superficial aspect of the subchondral plate and loss of integrity of the plate from the OA knee. The distribution between the plate and the cancellous bone in the OA knee is not sharp, as it is in the contralateral knee, where the plate is dense and intact, with a sharply demarcated surface. The peripheral thinning of the plate is normal.

animal that in the neurologically-intact dog [8], thinning of the subchondral plate in the accelerated model is presumably not due to greater unloading of the deafferented limb. On the other hand, synovial inflammation in the OA knee of the deafferented dog is more intense than that in the neurologically-intact dog [10] and, even within the brief duration of this study, could have stimulated resorption of the subchondral plate [14]. The possibility that acute thinning of the plate is detrimental to the overlying articular cartilage, perhaps by increasing mechanical stress or strain [15] during loadbearing, warrants consideration. That the bony alterations in the two models may have different mechanical effects on the overlying cartilage, and that biologic interactions between the bone and other connective tissues within the unstable joint may not be comparable, should be taken into account in studies with the acceleated model.

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