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A LONGITUDINAL STUDY OF SUBCHONDRAL PLATE AND TRABECULAR BONE IN CRUCIATE-DEFICIENT DOGS WITH OSTEOARTHRITIS FOLLOWED UP FOR 54 MONTHS

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Objective. **To evaluate the sequence of changes in articular cartilage, trabecular bone, and subchondral plate in dogs with osteoarthritis (OA), 3 months, 18 months, and 54 months after anterior cruciate ligament transection (ACLT).**

Methods. **Specimens of the medial tibial plateau were analyzed with microscopic computed tomography (micro-CT) at a resolution of 60 μm, and biochemical and morphologic changes in the femoral articular cartilage were assessed.**

Results. **At 3 months and 18 months after ACLT, the articular cartilage in the unstable knee showed histologic changes typical of early OA and increased water content and uronic acid concentration; by 54 months, full-thickness ulceration had developed. Micro-CT analysis showed a loss of trabecular bone in the unstable knee, compared with the contralateral knee, at all time points. At both 18 and 54 months, the differences in trabecular thickness and surface-to-volume ratio were greater than at 3 months. Although the mean subchondral plate thickness, especially in the medial aspect of the medial tibial plateau, was greater in the OA knee than in the contralateral knee 18 months and 54 months after ACLT, these differences were not statistically significant; however, the difference was significantly greater at 54 months than at 3 months.**

Conclusion. **Thickening of the subchondral bone is not required for the *development* of cartilage changes of OA in this model. The bony changes that develop after ACLT, however, could result in abnormal transmission of stress to the overlying cartilage and thereby contribute to the *progression* of cartilage degeneration.**

In the pathogenesis of osteoarthritis (OA), interactions among all the major joint tissues, including the articular cartilage, synovium, and subchondral bone, have been implicated (1). While a large body of work has focused on the alterations in the articular cartilage in OA in humans and experimental animal

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models (1–3), the role of the subchondral bone has been studied much less extensively.

In dogs, OA can be produced by transection of the anterior cruciate ligament (ACLT) (3,4). The biochemical and metabolic changes that occur in the articular cartilage of the unstable knee mimic those seen in OA in humans (5). While prominent osteophytes develop within weeks, the morphologic changes of experimental OA in the articular cartilage develop much more slowly and remain mild for at least 3 years (5,6). In the earlier stages, both the thickness and bulk of the cartilage are increased, in association with increases in net proteoglycan synthesis and matrix proteoglycan concentration, reflecting hypertrophic cartilage repair in the unstable joint (7). Subsequently, however, full-thickness cartilage loss occurs (8).

In the canine model, standard radiographs of the unstable joint show "sclerosis" of the subchondral bone by 52 weeks after ACLT (9); by 54 months after ACLT, sclerosis is prominent. On conventional radiographs, however, overlying osteophytes can complicate the evaluation of the subchondral plate, and the resolution is not sufficient to permit quantitative examination of the trabecular bone.

We have recently used microscopic computed tomography (micro-CT) to examine the subchondral plate and trabecular bone in the guinea pig hindlimb myectomy model of OA (10). Micro-CT permits nondestructive examination of bone at a resolution of 5–75 μm (11). In the early postmyectomy period, we found thinning of the trabeculae, with increased intertrabecular distance and decreased bone fraction (percentage of bone per volume analyzed). However, with progression of articular cartilage degeneration in this model, these changes were superseded by trabecular thickening and an increase in bone fraction (10,11).

We have postulated that the cartilage changes in the canine cruciate-deficiency model of OA are accompanied by alterations in subchondral bone that are similar to those seen in the guinea pig myectomy model. Brown and colleagues (12) used a computer model to study the possible effects of subchondral bone on overlying articular cartilage and reported that changes in cartilage stress could result from alterations in subchondral bone. These bone changes have not been rigorously examined in vivo. The longitudinal study described herein documents the development and evolution of these bony changes in specimens from animals with OA.

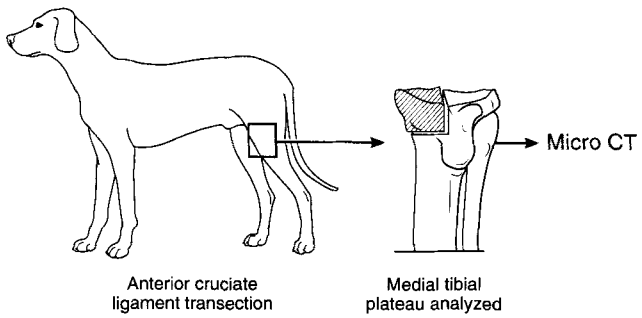


Figure 1. The medial tibial plateau was removed and scanned, using the microscopic computed tomography (Micro CT) system.

MATERIALS AND METHODS

Animals. Twelve adult mongrel dogs underwent unilateral ACLT under aseptic conditions (13). After a standard postoperative recovery period, the animals were permitted routine cage activity until they were killed, by Euthanol injection (Taylor Pharmacal, Decatur, IL), either 3 months (n = 4), 18 months (n = 5), or 54 months (n = 3) after surgery. Anteroposterior radiographs of both hind limbs were obtained at that time.

Tissue samples. After killing, both knees of each dog were aseptically removed en bloc. The joints were opened, inspected for the presence of surface irregularities and osteophytes, and then photographed. Full-thickness samples of articular cartilage (~15 mg wet weight) were taken from the central weight-bearing region of the medial femoral condyle of each knee for biochemical analysis and histologic examination (13). Cartilage from each joint was analyzed separately. The histologic appearance of, and biochemical and metabolic changes in, the femoral condylar cartilage of the 3 dogs maintained for 54 months after ACLT have been reported elsewhere (8). The proximal tibias were stored at −70°C for subsequent micro-CT scanning.

Micro-CT analysis. For analysis of the subchondral bone, each frozen tibia was placed in a vice at room temperature, and the medial plateau was removed from the remainder of the shaft (Figure 1), mounted with modeling clay onto a Plexiglas cube, and placed on the platform of the micro-CT scanner. The function of the scanner and its principles of operation have been described previously (14,15). The system at the University of Michigan has been upgraded and includes the following major components: a Feinfocus micro x-ray source (FCP-006.01; Feinfocus U.S.A., Inc., Agoura Hills, CA), a Toshiba A15764G-P1 image intensifier (North American Imaging, Newberry Park, CA), a Javelin JE-7252 CCD video camera (Javelin, Torrance, CA), and an ITI-200 1024 image processing system (Image Technology, Woburn, MA). Motion control of the specimen is directed by a VAX station II, which provides control signals to a Mac-100 motion controller (Techno, New Hyde Park, NY) and Deadal (Harrison, PA) high-resolution stages.

In this system the specimen is rotated through the 1–5-μm cone beam emanating from the x-ray source, and 129 projections are acquired. Reconstruction onto a 3-

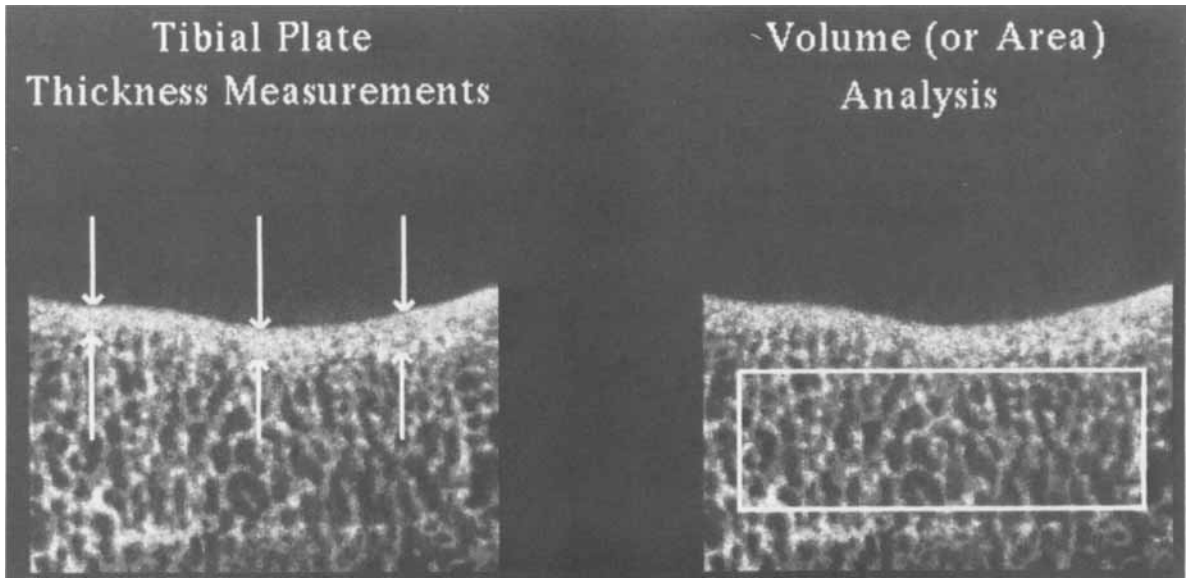


Figure 2. Thickness measurements of the tibial subchondral plate were made at 3 locations along the plateau on 10 evenly spaced sections. The trabecular bone in a rectangular volume that could be delineated immediately below the subchondral plate and above the epiphyseal scar was analyzed.

dimensional matrix is performed in a Stardent 3000 mini-super computer, resulting in the complete 3-dimensional digitization of each bone specimen. Image analysis of the reconstructed data sets was accomplished with an Apollo 4500 engineering workstation (Apollo Computer, Chelmsford, MA).

Each specimen was examined carefully for evidence of geodes and microfractures. The thickness of the subchondral plate was directly measured at 10 evenly spaced sagittal sections, from each of 3 locations (medial, central, lateral) (Figure 2), and the results for each site were averaged. Trabecular bone within the largest rectangular volume that could be drawn between the subchondral plate and the physeal scar was analyzed for bone fraction, trabecular surface:volume ratio, and trabecular plate thickness (Figure 2). The volumes of analysis in these studies varied somewhat, depending on the size of the animal; mean dimensions were 10.5 × 8.5 × 3.7 mm. The results for each specimen were normalized for volume.

Pairwise *t*-tests were used to compare samples from the OA and contralateral knees at each assessment. Analysis of variance for repeated measures, with Bonferroni adjustments for multiple comparisons, was used to examine the difference between the OA and the contralateral knee in relation to the interval after ACLT. It was necessary to use the difference between knees for this analysis since each dog was used as its own control in order to eliminate problems of varying bone density between animals at the beginning of the experiment.

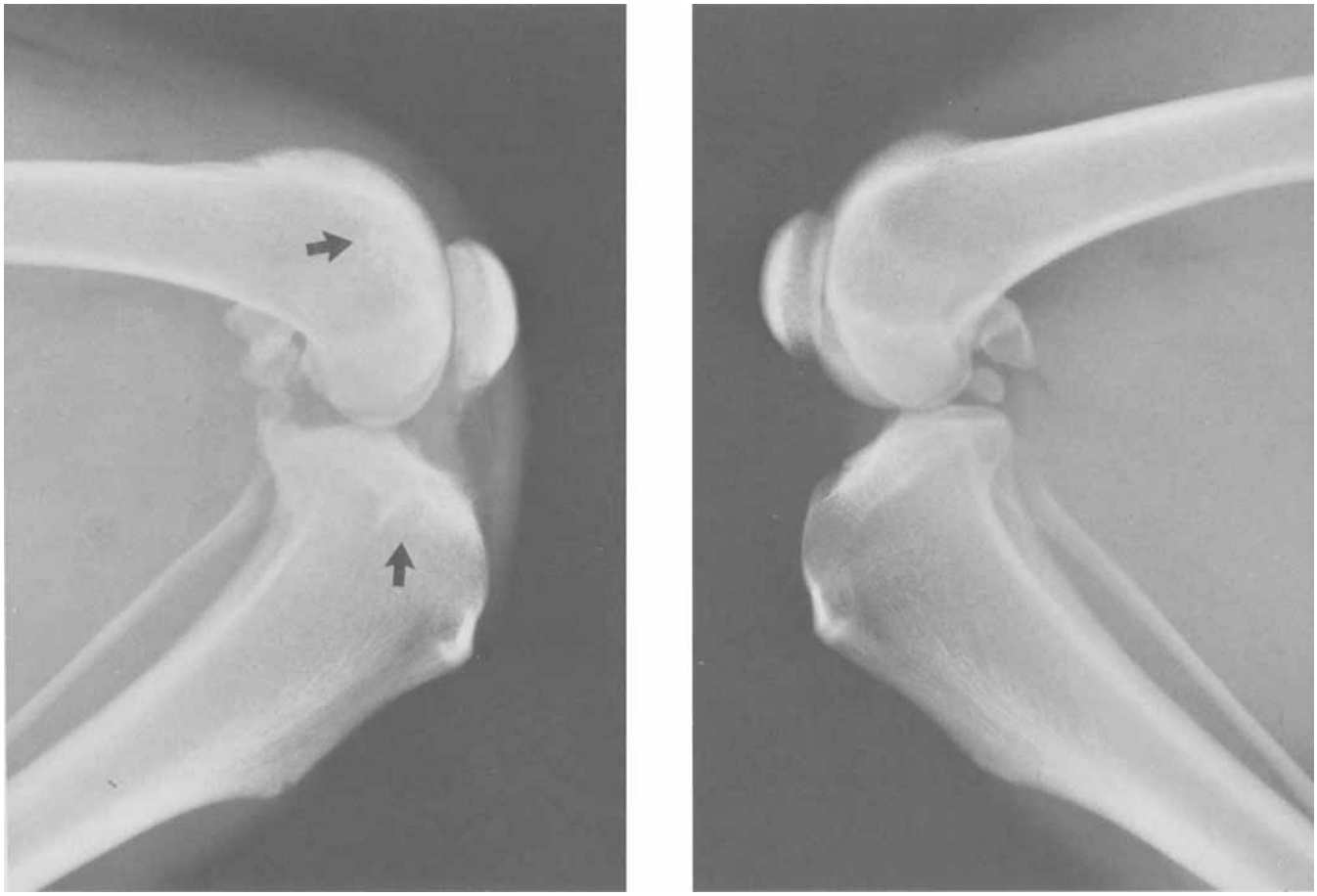
Compositional analyses of articular cartilage. The water content and uronic acid concentration in the articular cartilage were measured as described previously (16,17). The results were expressed as a percentage of the cartilage dry weight.

Histologic studies. Full-thickness samples of cartilage and underlying subchondral bone were removed from the medial femoral condyle and placed in Decalcifier I solution (SurgiPath, Grayslake, IL) for approximately 5 days. The tissue was then embedded in paraffin, and sections (6 μm) were cut perpendicular to the surface and stained with Safranin O–fast green.

RESULTS

Morphologic changes. Three months after ACLT, the articular cartilage of the unstable knee was thicker than that of the contralateral knee, but the surface showed only mild pitting of the medial femoral condyles and small marginal osteophytes. By 18 months after ACLT, remodeling had resulted in an expansion of the distal femur of the OA knee, producing a transcondylar diameter greater than that of the contralateral knee. The cartilage surface of the femoral condyle was not appreciably different from that seen at 3 months after ACLT, although the osteophytes were larger.

The cartilage changes in the 3 animals examined 54 months after ACLT were strikingly different. Deep ulceration of the cartilage on the central weight-bearing region of the medial femoral condyle was



A B

Figure 3. Lateral radiographs of the unstable (**A**) and contralateral (**B**) knees 18 months after anterior cruciate ligament transection. Note prominent subchondral sclerosis of the femoral condyle and the tibial plateau (**arrows**), and the prominent posterior tibial osteophyte in **A**.

apparent. In 2 of the 3 specimens, a wide area of subchondral bone was exposed (8).

Radiographic changes. Radiographs of the contralateral (stable) knees revealed normal features in every animal at all time points. The cruciate-deficient knees of the dogs killed 3 months after ACLT also appeared radiographically normal. However, 18 months after ACLT, the OA knee had osteophytes and subchondral sclerosis of the medial tibial plateau (Figure 3). These changes were more prominent at 54 months post-ACLT.

Cartilage histology. Histologic changes in the articular cartilage 3 months after ACLT consisted of mild disruption of surface integrity, with loosening and increased prominence of the tangential layer of collagen fibers, and rounding of the normally flattened superficial layer of chondrocytes. Samples of cartilage obtained from the OA knee 18 months after ACLT were remarkably similar to those obtained at 3 months, except that the surface changes were somewhat more marked and mild cloning of chondrocytes (up to 5 cells per cluster) was apparent. In striking contrast, 54 months after ACLT, extensive fibrillation, duplication of the tidemark, with capillary invasion, and more prominent cloning of chondrocytes had developed (8).

Composition of the articular cartilage. The water content of cartilage from the unstable knee was approximately 5% greater than that from the contralateral knee, as observed previously in this model (4,7). The uronic acid concentration in the OA cartilage was uniformly increased 3 months and 18 months after ACLT (26% and 78% of the value for the contralateral knee, respectively), which reflects hypertrophic "re-

Table 1. Changes in thickness of the subchondral plate of the medial tibial plateau in dogs subjected to ACLT*

Interval after ACLT	No. of dogs	Knee	Subchondral plate thickness (mean ± SD μm)		
			Medial	Central	Lateral
3 months	4	OA	990 ± 77	975 ± 57	1,125 ± 90
		Contralateral	1,110 ± 77	1,155 ± 102	1,140 ± 89
18 months	5	OA	1,020 ± 159	1,008 ± 172	1,104 ± 151
		Contralateral	972 ± 89	1,020 ± 147	1,104 ± 131
54 months	3	OA	1,700 ± 346	1,500 ± 262	1,320 ± 60
		Contralateral	1,160 ± 125	1,280 ± 227	1,260 ± 216

* The between-knees difference in plate thickness in the medial portion of the plateau was significantly greater at 18 months and 54 months after anterior cruciate ligament transection (ACLT) than at 3 months after ACLT ($P = 0.002$, by analysis of variance). Similarly, in the central region of the plateau, the between-knees difference was significantly greater at 54 months after ACLT than that at 3 months ($P = 0.013$). OA = osteoarthritis.

pair'' activity of the chondrocytes (7). Fifty-four months after ACLT, however, when full-thickness loss of cartilage had developed, the uronic acid concentration in the OA knee of 1 dog was nearly the same as that in the contralateral knee (3.8 and 3.7 μg/mg dry weight, respectively), while in another dog it was lower (2.6 and 3.2 μg/mg dry weight, respectively) (8).

Thickness of the subchondral plate. Examination of the micro-CT scan images of the OA knee revealed a trend toward thickening of the medial and central regions of the subchondral plate with increasing time after ACLT. At 54 months after ACLT, the mean plate thickness was nearly twice that at 3 months (Table 1). This difference was not statistically significant, however, probably because of the small number of animals per group. The between-knee difference in the thickness of the plate in the medial portion of the plateau at 18 months and at 54 months after ACLT was significantly greater than that seen at 3 months after ligament transection ($P = 0.002$ in each case). The magnitude of the between-knee difference in plate thickness in the central region of the plateau was also significantly greater at 54 months than at 3 months ($P = 0.013$).

Analysis of trabecular bone. In each animal, micro-CT measurement of subchondral trabeculae revealed a difference in bone fraction in the OA versus the contralateral knee. In every sample obtained 3 months, 18 months, or 54 months after ACLT, there was less bone in the trabecular region of the OA knee than in the contralateral knee (Table 2).

Morphologic measurements confirmed that trabeculae in the OA knee were more slender and had a higher surface:volume ratio than those in the contralateral knee at 18 and 54 months ($P < 0.05$) (Table 2). At both 18 months and 54 months after ACLT, the between-knee differences for trabecular thickness and surface:volume ratio were significantly greater than the differences in these parameters 3 months after ACLT ($P = 0.001$ in both cases).

No trabecular microfractures were seen, al-

Table 2. Changes in trabecular bone after anterior cruciate ligament transection (ACLT)*

Interval after ACLT	No. of dogs	Knee	Bone fraction	Surface:volume ratio	Trabecular plate thickness
3 months	4	OA	39 ± 3	13.9 ± 0.6	8.7 ± 0.4
		Contralateral	42 ± 4†	12.8 ± 0.6	9.5 ± 0.5
18 months	5	OA	37 ± 2	14.9 ± 0.6	8.1 ± 0.3
		Contralateral	40 ± 3†	12.0 ± 0.6†	10.0 ± 0.5†
54 months	3	OA	37 ± 3	15.0 ± 0.8	8.0 ± 0.4
		Contralateral	40 ± 1	11.6 ± 0.5†	10.4 ± 0.4†

* The between-knees difference in surface:volume ratio (mm^2/mm^3) and trabecular plate thickness (μm) at 18 months and 54 months after ACLT was significantly greater than that at 3 months ($P = 0.001$, by analysis of variance). Bone fraction is the percentage of bone per volume analyzed.
† $P < 0.05$ versus osteoarthritic (OA) knee, by paired t-test.

though they may not have been visible at the magnification used. Geodes were noted in the subchondral weight-bearing region of the OA knees of 2 of the 3 dogs maintained 54 months after ACLT. In 1 of these 3 dogs, 1 very small geode was found below the tibial spine, in a non–weight-bearing region, of the contralateral knee. In contrast, geodes were not observed in any of the animals at 3 or 18 months after ACLT.

DISCUSSION

At 18 months and 54 months after ACLT, conventional radiography revealed subchondral sclerosis in the tibia of the knee with OA. This change was reflected by the micro-CT analysis, which showed slight increases in the thickness of the subchondral plate, compared with the contralateral knee, at those time points (Table 1). The between-knee differences both 18 months and 54 months after ACLT were significant compared with that at 3 months ($P = 0.002$) (Table 1). This is consistent with the results of histomorphometric analyses of the femoral condyles, which showed marked thickening of the subchondral plate in the medial condyle of the cruciate-deficient knee at 54 months after ACLT (8).

Below the thickened subchondral plate, loss of subchondral trabecular bone was noted at all 3 time points in each animal. This decrease in trabecular bone mass in the tibia of the OA knee, as detected by micro-CT, is consistent with the approximately 30% reduction in load bearing by the unstable limb, as demonstrated by force-plate analysis (18), which we have shown to persist for at least 45 months after ACLT (19). It is also possible that postoperative synovitis (20), with its accompanying increase in blood flow, may have contributed to the loss of trabecular bone in the OA knee. We have shown that synovitis remains prominent 54 months after ACLT (8).

In normal bone, the turnover of trabecular bone is more rapid than that of cortical bone (21). Thus, loss of bone from the trabeculae would occur more quickly in response to unloading or increased blood flow than would loss of bone from the subchondral plate, which is more similar to cortical bone in this respect. It is particularly notable, therefore, that even in the presence of load reduction and synovitis, the thickness of the subchondral plate in the OA knee *increased* while the quantity of trabecular bone *decreased*.

Our findings are supported by those from the recent studies of human OA femoral heads conducted by Grynpas et al (22), who found hypomineralization with increases in all osteoid parameters in OA bone as compared with both young and age-matched controls. This hypomineralization would likely be interpreted by our micro-CT system as decreases in the bone fraction and in the trabecular size, since the unmineralized osteoid would not be identified by the x-ray beam. In the current study, we did not examine mineralization, which should be considered in future examinations of the subchondral region.

In the OA knee, subchondral trabecular loss may have an important effect on mechanical stress in the overlying articular cartilage. Greater bone density is associated with greater bone compressive strength and lesser density with diminished bone strength (23). Although it has been suggested that an increase in subchondral stiffness is important in the pathogenesis of OA, it is clear from our data that thickening of both the subchondral plate and trabeculae is not required to initiate the cartilage changes of OA in this canine model (24–26). Indeed, cartilage alterations typical of OA were seen 3 and 18 months after ACLT despite trabecular *thinning*.

Indeed, the loss of bony subchondral support itself may have led to cartilage breakdown by altering the stress in the overlying articular cartilage. Brown et al (12), using a computer model of the ovine proximal tibia, examined the mechanical consequences of softening as well as stiffening of the subchondral trabecular region. Their findings suggest that either stiffening or softening would change the mechanical stresses in the overlying cartilage. The late tendency toward thickening of the subchondral plate observed in our study may represent an attempt to normalize mechanical stress in the cartilage without replacing the trabecular support.

For the purpose of examining bone alterations by micro-CT, each dog in this study served as its own control. As noted previously, the amount of tibial bone at the outset of the study may vary significantly from animal to animal, due to factors such as nutritional and exercise history, age, and sex. Micro-CT studies of bone from normal adult dogs have shown as much as a 4% difference between the right and left knees with respect to the morphologic parameters we examined (27). Hence, at the time of ACLT, some of the dogs in the present study presumably had more bone in the operated knee than in the contralateral knee; the degree of osteopenia that developed was therefore all the more notable.

This longitudinal study of the subchondral plate and subjacent trabecular bone in a canine model of OA has revealed results that differ somewhat from those of previous studies. Histomorphometric studies of bone from the femoral condyles of the unstable knee at 54 months post-ACLT showed increases in the number and the area of the trabeculae and a decrease in the separation of the trabeculae (8). It should be noted, however, that the analyses in the previous study were performed on trabeculae immediately subjacent to the femoral subchondral plate, whereas in the present study, the micro-CT analysis examined trabeculae throughout the entire area between the plate and the physeal scar on the medial tibial plateau. Although Brown and coworkers suggested that the influence of trabecular support is minimized at a distance greater than 3 mm from the tidemark, that conclusion was based exclusively on a mathematical model (12), whereas we examined the tissues themselves. Furthermore, when we examined smaller areas, trabecular loss was found in all regions of the OA knee, which parallels our findings in the entire sample.

The micro-CT analysis used in the present study was also used in a guinea pig model of OA, in which initial thinning of the subchondral trabeculae, followed by trabecular thickening, was demonstrated (10,11). The reason for the difference in the bone response during development of OA in that model as compared with the canine ACLT model is unclear; however, the guinea pigs were skeletally immature and we examined the femoral heads, whereas the dogs were skeletally mature and we examined the tibial plateaus. Furthermore, the guinea pig model was produced by an extraarticular tenotomy and myectomy, while the canine model was produced by ACLT which was performed via arthrotomy. These differences could account for the differences in the late bone response between the two models.

Our observations confirm the involvement of both the subchondral plate and the trabecular bone in this model of OA, and raise questions about the differential contribution of bone to the development and progression of the cartilage pathology in OA. If the bone changes and their effects on stresses in the overlying cartilage are not addressed therapeutically, attempts to normalize cartilage alterations by the use of growth factors (28), chondroprotective drugs (29), or other pharmacologic agents in the treatment and prevention of OA may be fruitless.

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