

# Early regional adaptation of periarticular bone mineral density after anterior cruciate ligament injury

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<sup>1</sup>Department of Mechanical and Manufacturing Engineering, <sup>2</sup>Department of Cell Biology and Anatomy, <sup>3</sup>Department of Chemical and Petroleum Engineering, <sup>4</sup>Department of Surgery, and <sup>5</sup>Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada T2P 1N4

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**Boyd, Steven K., John R. Matyas, Greg R. Wohl, Apostolos Kantzas, and Ronald F. Zernicke.** Early regional adaptation of periarticular bone mineral density after anterior cruciate ligament injury. *J Appl Physiol* 89: 2359–2364, 2000.—The present study measured early-stage adaptation of bone mineral (BMD) in the periarticular cancellous bone of the canine knee (stifle) joint after anterior cruciate ligament (ACL) transection (ACLX). Regional changes in BMD in the tibia and femur were analyzed by using quantitative computed tomography (qCT) at 3 wk and 12 wk after unilateral ACLX to determine whether there were focal points for BMD changes and whether these changes occurred early after the induced knee injury. BMD decreased rapidly after ACLX, and the more pronounced response was in the femur. In the 3-wk group, there were decreases in BMD in the tibia and the femur, and these changes were significant in the posterior-medial region of the femur, which showed a decrease of BMD in the ACLX limb ( $-0.048 \pm 0.011$  g/cm<sup>3</sup>). In the 12-wk group, all regions in the tibia and femur exhibited significant decreases in BMD, and the average decrease was greatest in the posterior-medial region of the femur ( $-0.142 \pm 0.021$  g/cm<sup>3</sup>). The regions of pronounced periarticular cancellous BMD adaptation corresponded to observed focal cartilage defects. Early decreases in BMD in the injured knee may be related to altered loading and kinematics in the knee and may be an important link in the pathogenesis of posttraumatic osteoarthritis.

anterior cruciate ligament transected; canine; dog; knee joint; stifle joint; quantitative computed tomography

POSTTRAUMATIC OSTEOARTHRITIS (OA) subsequent to a rupture of the anterior cruciate ligament (ACL) typically involves physiological and mechanical changes to the entire organ of the knee, in particular the articular cartilage (1) and periarticular bone (28). Past research has focused considerable attention on cartilage changes, but comparatively little is known about progressive changes to the bone. The ability of cancellous bone to remodel is remarkable compared with other tissues in the knee joint due to its rich vascularity. This remodeling can precipitate structural changes in the bone that may be important in the etiology of OA.

The natural history of posttraumatic knee OA may be described as a progressive change in the joint beginning after an initial trauma leading to early-stage joint degeneration. Eventually, symptoms of OA develop, and degeneration progresses to full-scale development of the disease, or late-stage OA, which is characterized by joint space narrowing, full-thickness cartilage erosion, development of osteophytes, and increased subchondral sclerosis (8). Periarticular subchondral bone changes have been reported for patients with late-stage development of OA (14, 17, 25); however, early changes after joint trauma are less well understood. Cancellous “bone bruising” has been reported by using magnetic resonance imaging within 2 mo postinjury (30), but the cause of these changes and their structural significance are not clear.

Brandt and colleagues (5) studied degenerative changes of tissues in the knee in a canine model for posttraumatic OA by ACL transection (ACLX). Their long-term study showed that after 54 mo post-ACLX there were osteophytes formed, full-thickness cartilage erosion, and increased subchondral plate thickness that were consistent with the late-stage development of OA in humans. Dedrick and colleagues (11) investigated bone changes at 3, 18, and 54 mo post-ACLX and found an increase in subchondral plate thickness only at 54 mo, which corresponded to increased subchondral sclerosis in human patients with late-stage OA. They reported decreased cancellous bone volume ratio at all three time points, indicating that adaptation of periarticular cancellous bone occurred more rapidly than that of the subchondral plate. The rapid adaptation of cancellous bone can be attributed to its faster turnover compared with that of cortical bone (13). Although these studies (5, 11) indicated that decreases in cancellous bone volume ratio preceded thickening of the subchondral plate, it remains unclear at what stage the cancellous changes begin and whether there are regional differences in the posttraumatic joint. An early loss of cancellous bone could have structural implications that contribute to a cascading degenera-

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tion of the posttraumatic knee leading to the development of OA (5, 22).

Loss of periarticular cancellous bone at the knee joint is important because altered stresses in the joint tissues may result. Brown and colleagues (7) used a finite-element model to show that focal alterations in bone stiffness below the subchondral region affected stresses at the cartilage surface. This altered stress distribution would likely be exacerbated if mechanical properties of substantial portions of periarticular cancellous bone were affected (11, 25, 27, 33); therefore, quantifying the distribution of changes in mechanical properties of periarticular cancellous bone is important for understanding early-stage OA progression. These properties of bone can be estimated using quantitative computed tomography (CT; qCT), a volume-based method for three-dimensional estimation of bone mineral content per unit volume (BMD) using parallel slices of a bone and converting the attenuation measures into BMD on the basis of calibration phantoms (15, 26). The profiles of BMD distribution in the joint can be analyzed to determine regional differences in bone adaptation.

The present study was conducted as part of a coordinated research endeavor to investigate the early natural history of experimental osteoarthritis. Matyas and colleagues (22) found focal defects in cartilage that were prominent in the medial femoral condyle, but it was not clear whether regional adaptation of periarticular cancellous bone corresponded to these regions in the early stage of OA. Thus the purpose of this study was to quantify the early adaptation of periarticular cancellous BMD in the proximal tibia and distal femur after ACLX with qCT. In particular, we sought to test the hypothesis that changes in BMD occurred within the first 12 wk post-ACLX and to determine the regional differences in the BMD adaptation.

## METHODS

Skeletally mature, mixed-breed dogs ( $n = 10$ ) were assigned randomly to one of two evenly sized ( $n = 5$  each) experimental groups: 3 and 12 wk after unilateral ACLX. Dog weights ranged from 16 to 34 kg (mean 23.5 kg) and ages ranged from 1.5 to 3 yr (mean 2.5 yr). All procedures were approved by the University of Calgary Animal Care Committee. After unilateral ACLX by lateral arthrotomy as described previously (2, 22, 23), the dogs were initially housed in pens for 2 wk and then allowed to ambulate freely in large pens ( $1.5 \times 2 \times 2$  m). Food and water were provided ad libitum. Dogs rapidly returned to their preinjury level of activity; eight animals showed no distinctive limp after 2 wk, and the remaining two showed no limp after 6 wk. At euthanasia, the femora and tibia were cleaned of all soft tissue, hermetically sealed, and stored at  $-30^\circ\text{C}$  until testing.

**qCT scanning.** Estimations of BMD were made by using qCT (EMI CT5005, London, UK; 140 kVp, 28 mA, voxel size  $0.75 \times 0.75 \times 3.00$  mm,  $320 \times 320$  voxels per slice; Ref. 18). Before each scanning session, a calibration of the CT scanner was performed by scanning a series of 12 solutions of known concentrations of  $\text{K}_2\text{HPO}_4$  (0–700 mg/cm<sup>3</sup>). The density of each of these solutions was determined by Archimedes' principle so that all subsequent CT numbers determined for the

bone specimens could be converted to density values (g/cm<sup>3</sup>) (26).

For each dog, the femora of the ACLX limb and contralateral control were placed side by side in the scanner gantry with the femora tilted so the CT slice plane normals were angled at  $45^\circ$  from the long axes. This resulted in slice planes approximately parallel to the load-bearing region of the subchondral plane during the stance phase of gait (20). Thus a direct comparison of the bone density between ACLX and contralateral control was made within every transverse CT slice. The scanning procedure involved finding the most distal end of the femora and subsequently scanning at 3-mm increments, proximally, until the femoral condyles were completely scanned (typically seven scans). For tibial pairs, similar procedures were used, beginning from the most proximal position on the tibia. The purpose of scanning the entire femur and tibia in the joint region was to permit reliable selection of a scan representing trabecular bone in the joint, without partial volume effects. All raw CT data were imported into a workstation (Silicon Graphics Octane, Mountain View, CA) for analysis.

**Data analysis.** A linear calibration curve was derived from the CT scans of the  $\text{K}_2\text{HPO}_4$  calibration phantoms and applied to all the bone scans so that each voxel represented approximate BMD. Calibrations were necessary for each CT session to account for drift in the CT scanner values. For each series of femora and tibia scans, one 3-mm-thick section was chosen for analysis. For the femora series, a slice was chosen that included both condyles and the femoral groove. The location of the slice was comparable among dogs but varied depending on the size of the individual dog (ranging from 3 to 5 mm from the distal end of the femur). For the tibia, a 3-mm-thick section located  $\sim 5$  mm distal to tibial plateau was selected. The analysis of the cancellous bone was then performed on the selected slices from the femora and tibia series by using image-processing techniques.

The femur was analyzed with a custom-written semiautomatic algorithm (Matlab v5: Mathworks, Natick, MA). It involved sampling the BMD in four different regions within the CT slice: the anterior-lateral, anterior-medial, posterior-lateral, and posterior-medial regions (Fig. 1). User interaction involved selecting a region on the computer screen that included one of the femora. The algorithm automatically determined the major (corresponding to the anterior-posterior axis) and minor (corresponding to the medial-lateral axis) axes of the second moment of inertia of the two-dimensional bone image and then found the weighted center of mass of each of the four quadrants. From these weighted centers, a circular core (radius 3 mm,  $21.2 \text{ mm}^3$  volume) was sampled, and means and standard errors were determined. The same procedure was repeated for each pair of femora.

The tibial CT scans were analyzed as two main regions: medial and lateral compartments (Fig. 2). The asymmetric geometry of the two-dimensional image precluded the semi-automated analysis and, therefore, a manual analysis was performed in which the user selected the centers of the medial and lateral compartments. Circular cores (radii 3 mm,  $21.2 \text{ mm}^3$  volume) were sampled, and means and standard deviations were determined. The repeatability of the mean density measurements was  $<0.002 \text{ g/cm}^3$ .

Statistical analysis of the results was done separately for the femora and tibia data. For each set, the measured BMD for each region was analyzed using pair-wise comparisons (*t*-test) to assess BMD changes at 3 and 12 wk post-ACLX. Significance was tested for a *P* value  $<0.05$ . Analysis of variance (with Tukey-Kramer adjustment to account for family-wise differences) was used to determine whether there

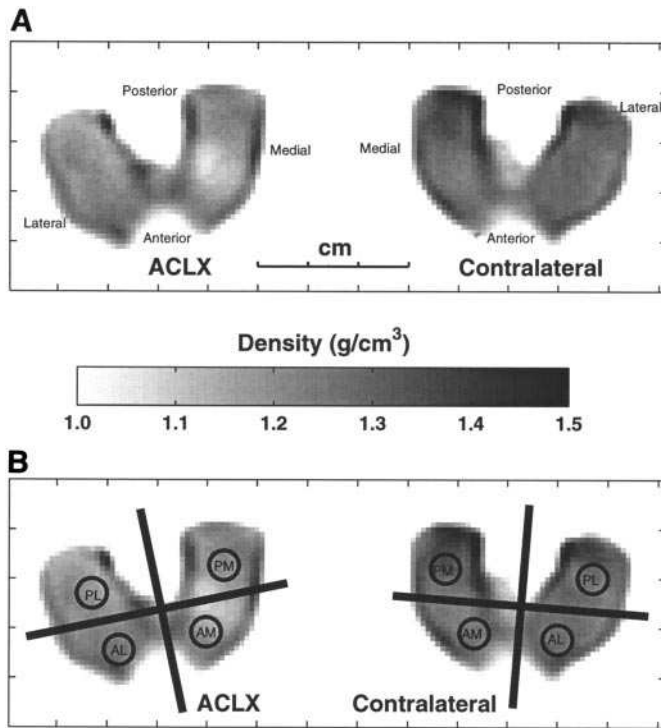


Fig. 1. Exemplar computed tomography scan of distal femur including both experimental (anterior cruciate ligament transection; ACLX) and contralateral limb (A), and the same scan after determination of 4 distinct regions and subsequent sampling within the circular regions indicated (B). The 4 regions are posterior-medial (PM), posterior-lateral (PL), anterior-medial (AM), and anterior-lateral (AL).

were regional differences in the magnitude of the changes in BMD.

## RESULTS

Periarticular bone mineral density was significantly lower ( $P < 0.05$ ) in the ACLX limb compared with the contralateral control at 3 and 12 wk post-ACLX in the femur (Fig. 3) and there was statistically lower BMD at 12 wk, but not 3 wk, in the tibia (two regions tested, Fig. 4). The differences were larger and regional variation in the differences were more pronounced at 12 wk post-ACLX than at 3 wk. Raw data for all BMD measures are presented in Table 1.

Three weeks post-ACLX, a significant decrease was found in femoral BMD in the posterior-medial region (mean  $\pm$  SE:  $-0.048 \pm 0.011$  g/cm<sup>3</sup>,  $P < 0.013$ ). In the three remaining regions of the femur, decreases in BMD were observed (Fig. 3A), as well as in the tibia at 3 wk post-ACLX (Fig. 4A).

Twelve weeks post-ACLX, all four femoral regions had statistically significant differences in BMD (Fig. 3B). The largest difference was found in the posterior-medial region ( $-0.142 \pm 0.021$  g/cm<sup>3</sup>,  $P < 0.002$ ), followed by the anterior-medial region ( $-0.111 \pm 0.008$  g/cm<sup>3</sup>,  $P < 0.001$ ), posterior-lateral region ( $\pm 0.013$  g/cm<sup>3</sup>,  $P < 0.001$ ), and finally the anterior-lateral region ( $\pm 0.012$  g/cm<sup>3</sup>,  $P < 0.003$ ). The tibia also exhibited significant changes in BMD in both the lateral

( $-0.084 \pm 0.018$  g/cm<sup>3</sup>,  $P < 0.009$ ) and medial regions ( $-0.062 \pm 0.016$  g/cm<sup>3</sup>,  $P < 0.019$ ) (Fig. 4B).

## DISCUSSION

The BMD was decreased in both the distal femur and proximal tibia of the ACLX hind limb of the dog 12 wk after ACLX surgery, and these decreases appeared as early as 3 wk postsurgery. In the ACLX tibia, others have reported decreased in the cancellous bone at 3, 18, and 54 mo postsurgery (11). Our results are consistent with the data of Dedrick and colleagues (11) and add that periarticular bone adaptation was evident as early as 3 wk postsurgery and that the adaptation was more pronounced in the femur than in the tibia. We also described substantial anatomical heterogeneity in the bone changes, because the posterior-medial region of the femur exhibited significantly greater decreases in BMD compared with the other regions after 12 wk and was the only region with statistically significant decreases after 3 wk. The data from the present study clearly indicate that adaptation of BMD occurs early after a traumatic injury in this animal model.

Some aspects of the study require clarification. First, the animals were killed at distinct time points rather than as part of a longitudinal study. This was done so that cartilage samples could be concurrently analyzed (22). A longitudinal study may reduce variability, but, nonetheless, with the present experimental arrangement, statistically significant trends of decreasing BMD were strongly evident. Furthermore, accurate alignment of the left and right limbs was facilitated by

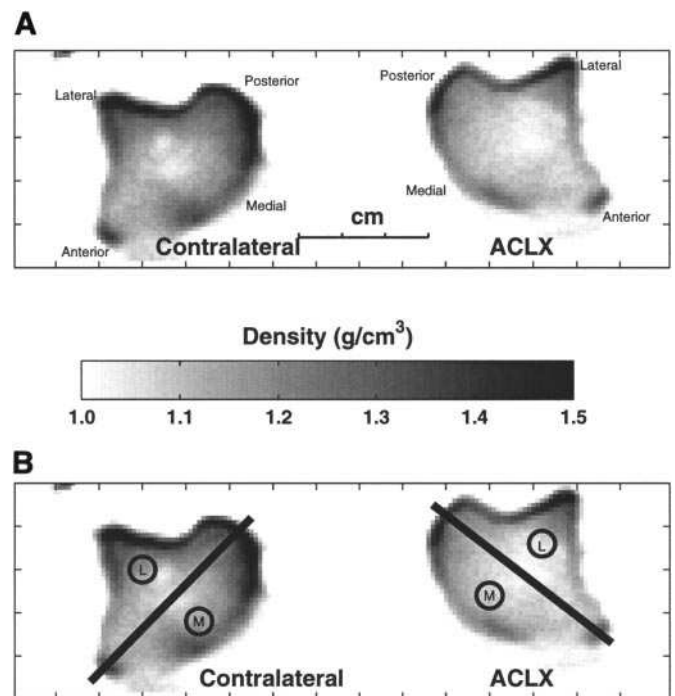


Fig. 2. Exemplar computed tomography scan of proximal tibia including both ACLX and contralateral limb (A), and the same scan after determination of medial (M) and lateral (L) regions and subsequent sampling within the circular regions indicated (B).

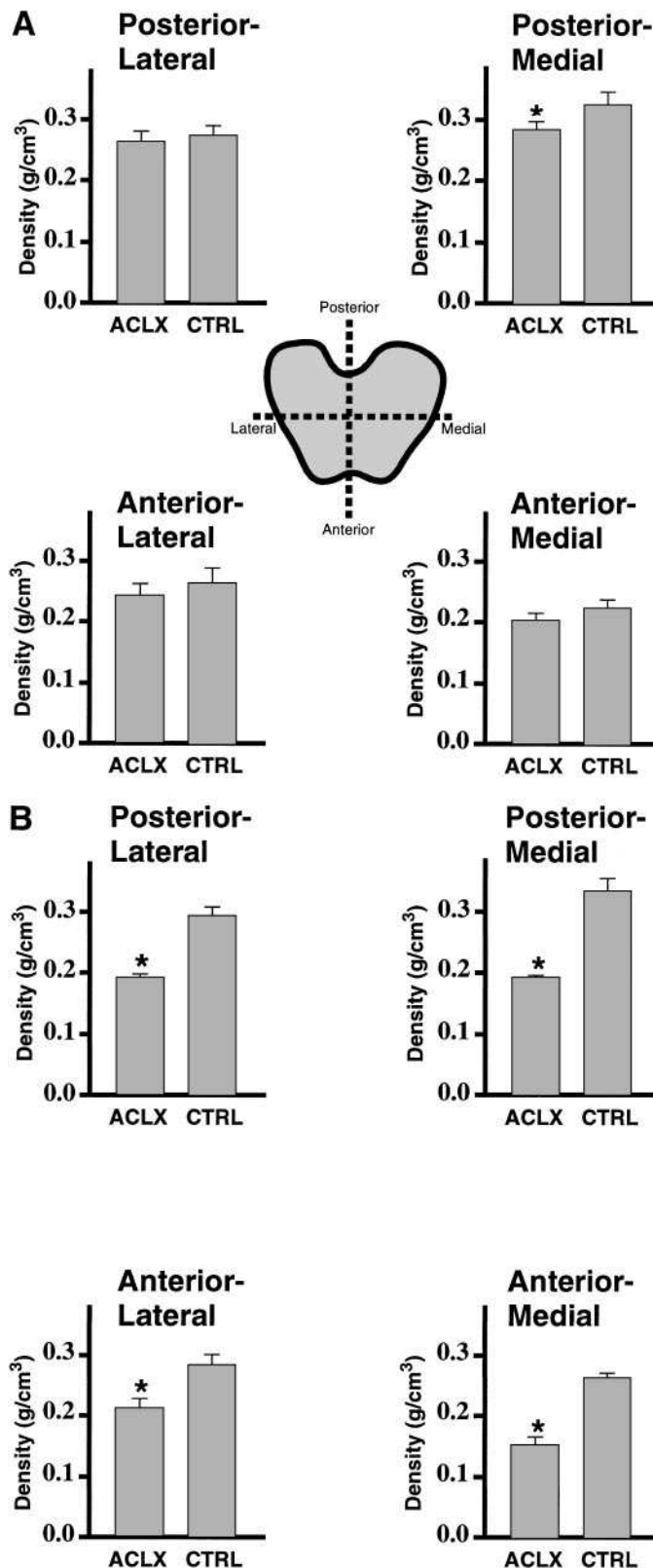


Fig. 3. Bone mineral density (BMD) in 4 regions of the femur showing mean  $\pm$  SD BMD for the ACLX and control (CTRL) limb 3 wk post-ACLX (A;  $n = 5$ ), and 12 wk post-ACLX (B;  $n = 5$ ). \*Statistically significant ( $P < 0.05$ ).

removal of surrounding soft tissue to allow comparisons of identical scan locations in the ACLX and contralateral limb. The technique used in this study of single-energy qCT is well suited for such intra-animal comparisons because the effect of variable presence of fat in the bone is eliminated (12). Second, the assumption that the contralateral limb is unaffected and remains a normal control is somewhat controversial, but several studies support that it is suitable in the canine ACLX model for OA (6, 22, 24). The advantage of using the contralateral limb as a control is that it reduces experimental artifacts introduced by making inter-animal comparisons. Finally, the method of qCT provides scalar measures of BMD within a series of three-dimensional volumetric regions (i.e., voxels). A qCT analysis can be used to infer mechanical properties of the bone and thus can be useful in a clinical setting for diagnoses. However, the voxels approximate bone matrix as a continuum and do not reveal information about the detailed bone microstructure. Analysis of microstructural adaptation during the development of OA would require high-resolution CT scanning (29).

The mechanisms responsible for the prominent BMD decreases found in the early stage of experimental OA are not clear, and it is probable that multiple factors play a role, given that the entire knee organ is affected by the joint injury. Among the potential mechanisms are physiological changes such as altered blood flow and bone remodeling. Trauma to the joint associated with transection of the ACL can increase blood flow (16) that can stimulate osteoclasts and increase bone resorption, particularly in the more rapidly remodeling cancellous bone (13). Another potential mechanism is related to mechanical changes that occur in the ACLX knee, such as altered loading conditions and increased joint laxity.

In the ACLX dog, the changes in loading patterns and kinematics have been well documented (9, 10, 20, 24, 32). The ACLX limb loading is reduced to  $\sim 35\%$  of preoperative levels 2 wk after surgery and slowly increases to 50–60% within 6–12 wk (20, 24). Concurrently, the kinematics are altered predominately during the stance phase, which includes an anterior tibial displacement relative to the femur with each step at paw strike (20, 31). These studies demonstrate that gait adaptation occurs after ACLX and suggest that joint instability and reduced loading are associated with the early-stage development of OA in the dog model. Joint instability, which alters specific regions of contact patterns and stress distribution within the ACL-deficient joint (3, 4), may explain why in the present study we found that early changes in BMD were not uniform and that regions such as the posterior-medial aspect of the femur clearly exhibited greater loss of BMD than other regions in the joint. The nonuniform decrease in BMD indicated that the changes were not simply due to osteopenia. However, further experiments would be necessary to establish a relationship between in vivo joint mechanics, including contact area and stress distributions, and regional adaptation of BMD in the ACLX joint.

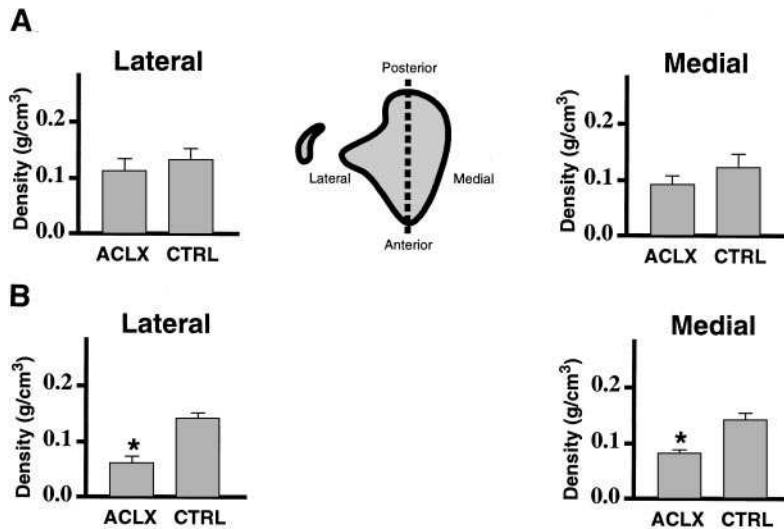


Fig. 4. BMD in 2 regions of the tibia showing mean  $\pm$  SD BMD for the ACLX and CTRL limbs 3 wk post-ACLX (A;  $n = 5$ ), and 12 wk post-ACLX (B;  $n = 5$ ). \*Statistically significant ( $P < 0.05$ ).

The results from the present study suggested that cancellous adaptation occurred concurrently with cartilage wear in the ACLX dog model for OA. Cartilage degeneration in the early stage of this experimental model for OA followed a consistent pattern of the entire cartilage surface having an increasingly opaque and swollen appearance, with focal meniscal tears and surface erosions (22). Surface erosions were not yet at full thickness after 12 wk, but appeared predominantly on the medial femoral condyles (22), which coincided with the region of greatest decrease of BMD. Although the cartilaginous changes are not limited to the medial femoral condyles, the predominant cartilage wear coincides with the greatest BMD decreases found in the present study.

There are similarities between the progression of posttraumatic OA in humans and the canine experimental model. Typically, in posttraumatic OA, the predominant clinical feature described is an increase in subchondral plate thickness (14, 17). This observation in clinical studies is typically made on patients who have suffered an initiating event such as an ACL

rupture much earlier, already present with symptoms of OA, and can be classified as late-stage OA. In the canine model of posttraumatic OA, an increase in subchondral plate thickness also occurs in the late stages (54 mo post-ACLX) (11), at which point other symptoms of the developed disease, such as full-thickness cartilage erosion, are already well established. In the early stage, the present study reported a decrease of periarticular cancellous BMD after ACLX. Although clinical studies investigating early bone changes after a traumatic injury are uncommon due to practical limitations, it has been shown that decreases in BMD occur within the first year of the initiating event (21) and that altered loading patterns likely play an important role (19). Therefore, it appears that the periarticular cancellous changes occur in two stages: a decrease in cancellous BMD in the early stage, followed by an increase in subchondral plate thickness in the later stage. This progression of the disease is consistent with clinical findings and with findings in experimental models of OA such as that used in the present study. If subchondral plate thickening was a response to weak-

Table 1. *Experimental qCT measures after conversion into BMD*

| Dog No.      | Femur BMD, g/cm <sup>3</sup> |                   |                  |                 | Tibia BMD, g/cm <sup>3</sup> |             |
|--------------|------------------------------|-------------------|------------------|-----------------|------------------------------|-------------|
|              | Posterior-medial             | Posterior-lateral | Anterior-lateral | Anterior-medial | Medial                       | Lateral     |
| <i>3 wk</i>  |                              |                   |                  |                 |                              |             |
| 1            | 0.257/0.311                  | 0.282/0.287       | 0.188/0.283      | 0.188/0.205     | 0.032/0.147                  | 0.080/0.094 |
| 2            | 0.301/0.386                  | 0.273/0.288       | 0.242/0.323      | 0.246/0.261     | 0.098/0.174                  | 0.166/0.157 |
| 3            | 0.233/0.253                  | 0.192/0.208       | 0.182/0.165      | 0.180/0.171     | 0.081/0.031                  | 0.084/0.131 |
| 4            | 0.310/0.341                  | 0.295/0.301       | 0.288/0.276      | 0.221/0.235     | 0.132/0.143                  | 0.159/0.200 |
| 5            | 0.277/0.329                  | 0.288/0.287       | 0.223/0.265      | 0.181/0.225     | 0.084/0.107                  | 0.049/0.081 |
| <i>12 wk</i> |                              |                   |                  |                 |                              |             |
| 1            | 0.200/0.335                  | 0.188/0.247       | 0.193/0.281      | 0.147/0.257     | 0.078/0.119                  | 0.039/0.116 |
| 2            | 0.178/0.374                  | 0.187/0.322       | 0.206/0.273      | 0.128/0.267     | 0.052/0.121                  | 0.022/0.170 |
| 3            | 0.200/0.333                  | 0.174/0.275       | 0.254/0.298      | 0.162/0.263     | 0.087/0.136                  | 0.070/0.149 |
| 4            | 0.186/0.259                  | 0.206/0.325       | 0.158/0.223      | 0.196/0.285     | 0.093/0.122                  | 0.047/0.124 |
| 5            | 0.198/0.370                  | 0.173/0.292       | 0.217/0.331      | 0.118/0.236     | 0.068/0.188                  | 0.115/0.155 |

Values are bone mineral density (BMD) of the experimental (anterior cruciate ligament transection) limb over the contralateral limb for the 3-wk ( $n = 5$ ) and 12-wk ( $n = 5$ ) specimens for 4 regions of the femur and 2 of the tibia. qCT, quantitative computed tomography.

ened cancellous bone, it suggests that early prevention of BMD loss may be important to prevent the long-term development of periarticular adaptation.

The present study quantified significant adaptation of cancellous bone that occurred in an experimental model of posttraumatic OA. It demonstrated that there were regional variations in the change of BMD after surgical transection of the ACL, and there were significant changes in the femur as early as 3 wk post-ACLX. The change progressed to significant levels in the femur and tibia after only 12 wk, and the largest change was in the posteromedial region of the femur, which also coincided with the focal cartilage defects. The BMD decreased rapidly and very early after joint trauma, and the finding suggests that early preventative measures may be necessary to suppress the cascading degenerative process that contributes to the development of OA.

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