Periarticular cancellous bone changes following anterior cruciate ligament injury

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1McCaig Centre for Joint Injury and Arthritis Research, Departments of 2Mechanical and Manufacturing Engineering, 6Surgery, 3Cell Biology and Anatomy, 4Pathology and Laboratory Medicine, and 5Radiology, and 7Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1

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Wohl, Gregory R., Roxane C. Shymkiw, John R. Matyas, Reinhard Kloiber, and Ronald F. Zernicke. Periarticular cancellous bone changes following anterior cruciate ligament injury. J Appl Physiol 91: 336–342, 2001.—To understand more fully the early bone changes in an experimental model of osteoarthritis, we quantified periarticular bone mineral density and bone mechanical properties in anterior cruciate ligament transected (ACLX) knee joints (4, 10, 32, and 39 wk post-ACLX) compared with contralateral joints and unoperated normal joints of skeletally mature animals. Maximal stress and energy were significantly reduced in ACLX cancellous bone from the medial femoral condyles at 4 wk postinjury. All mechanical properties (e.g., yield stress and elastic modulus) declined after 4 wk and were significantly reduced at 10 wk. ACLX bone mineral density was significantly reduced at all measured time points. Ash content was significantly reduced at 10 and 32 wk. Changes in the lateral condyles were similar but less pronounced than in the medial condyles. These bony changes accompanied the earliest articular cartilage molecular changes and preceded changes in the articular cartilage gross morphology. We suggest that these early changes in bone mechanical behavior contribute to the progression of osteoarthritis and pathogenic changes in the joint.

osteoaarthrosis; canine

END-STAGE OSTEOAARTHRITIS (OA) is characterized by articular cartilage degradation that leads to cartilage failure and, ultimately, joint failure. Changes in the bone structure around the joint (periarticular), including osteophytes, subchondral plate thickening, cyst formation, and joint space narrowing, are also prominent features of end-stage clinical OA (6, 7). In the early stages of OA, bone changes may potentially happen before cartilage changes and may contribute to the early progression of the disease (20). Understanding the early natural history of bone adaptation after a joint injury may help to develop treatments that prevent the progression of OA; but identification of early bone damage and changes (e.g., after ligament injury) is difficult and highly dependent on imaging techniques (e.g., X-ray, magnetic resonance imaging, or arthroscopy). Furthermore, because a clinical diagnosis of OA is typically made in the latter stages of the disease, the significant changes in the joint tissues by the end stages obscure the initiating causes and early tissue adaptations. Thus changes in periarticular bone in the early stages of human OA are poorly understood.

Experimental models of OA provide the opportunity to study the initiating events in OA. Considerable data have been gathered on the biochemical, cellular, and mechanical changes of articular cartilage (8, 16), but very few data describe the changes in periarticular cancellous and subchondral bone (14). Surgical transection of the anterior (cranial) cruciate ligament (ACL) of the knee (stifle) joint in dog results in joint changes that mimic human OA, progressing to the full-thickness loss of articular cartilage (3). In contrast to the degenerative changes seen in late-stage OA, early stages of the experimental OA are characterized by hypertrophic cartilage changes (1). Conversely, bone hypertrophy (subchondral sclerosis and osteophytes) is associated with end-stage OA, but the early stages after ACL injury are characterized more by periarticular osteopenia (2, 5). Increased bone remodeling develops shortly after ACLX (2, 4), and the altered periarticular bone mechanical properties may contribute to early cartilage and joint changes that lead, ultimately, to joint degeneration.

Radin and colleagues (19, 20) demonstrated, using an impulse-loaded rabbit hindlimb model, that the earliest increases in subchondral bone remodeling preceded cartilage biochemical and morphological changes, and they suggested that the resulting changes in subchondral bone stiffness contributed to OA progression. Boyd and colleagues (2) reported that morphological changes of the periarticular bone developed as early as 3-wk postinjury in the canine ACL transected (ACLX) joint, a time at which cartilage hypertrophy was just beginning to be detectable (15). Although the data of Boyd et al. (2) at 12 wk post-

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ACLX reported dramatic and progressive decrements (30–40%) in periarticular bone architecture, Dedrick and colleagues (5) suggested much more conservative decrements (7–8%) in periarticular bone morphology at 3 mo that progressed relatively little, even to the end stages of canine experimental OA. The combined data from Boyd et al. (2) and Dedrick et al. (5) suggested that, although there was a dramatic reduction in periarticular bone mass in the first 12 wk after ACLX, there was a subsequent dramatic, almost complete recovery of ACLX bone mass that was maintained through to end-stage OA. The natural history of articular cartilage after ACL transection, however, can progress to a plateau and remain hypertrophic for up to 3 yr in the canine model.

From currently published results, it remains unclear whether the early decrements in periarticular bone morphology persist and thereby contribute to an altered mechanical environment in the joint, or whether the bone changes are transient and contribute only to initiating factors that may trigger changes in other joint tissues. Indeed, the natural history of bone changes after ACLX remains ill defined, particularly with respect to bone mechanical function. Thus the purpose of our study was to quantify the changes in the mechanical properties and bone mineral density (BMD) in periarticular and subchondral cancellous bone during the early stages of experimental canine OA. We hypothesized that significant and progressive changes in BMD and periarticular cancellous bone mechanical properties occur rapidly after ACLX and that these decrements are sustained through the early stages of the disease.

METHODS

Animals. Twenty-nine skeletally mature, mixed-breed dogs of either sex were assigned randomly to one of four experimental groups: 4 (n = 8), 10 (n = 8), 32 (n = 8), or 39 (n = 5) wk (interval between surgery and necropsy). Five additional dogs served as nonoperated, normal controls. Animals were 2–7 yr of age [mean 3 ± 1.5 (SD) yr] and had a body mass of 16–28 kg (mean 22 ± 3.4 kg). Animals with preexisting anatomic or gait abnormalities were excluded. All animals were cared for under the supervision of a veterinarian according to the guidelines of The Canadian Council of Animal Care and with the approval of the University Animal Care Committee.

Unilateral ACLX was performed on each animal as described previously (1), with the side of the operation (left or right) randomized among animals. Briefly, the ACL was exposed and transected through a lateral arthrotomy. The nonoperated contralateral limb served as an internal control. Knee-joint laxity was tested manually before and after ACLX by “anterior drawer” (knee at 90° of joint flexion) and “Lachman” tests (at 30° of flexion). Animals were excluded if the preoperative tests were asymmetric.

Animals were housed individually in kennels (1.8 × 1.8 × 1.8 m) before and for 2 wk after surgery. Subsequently, animals were allowed to ambulate freely in outdoor pens (9.1 × 8.5 × 1.8 m) with other dogs. All dogs received food and water ad libitum.

**Experimental methods.** At necropsy (control, 4, 10, 32, or 39 wk post-ACLX), hindlimbs were disarticulated at the hip. The hip, knee, and ankle joints were assessed for macroscopic changes to the articular cartilage and other joint soft tissues. Animals with joints exhibiting unusual cartilage defects were excluded from study. Articular cartilage from the femoral condyles was carefully removed with a scalpel for biochemical, morphological, and molecular analyses (15). The triceps surae muscle group (medial and lateral gastrocnemius and soleus and associated tendons) was dissected from each leg and weighed en bloc for comparison of a wet muscle mass (a measure of muscle use and/or disuse) between the ACLX and contralateral limbs. Muscles from the dogs at the 10-wk time period were unavailable. The remaining soft tissues were removed, and femurs were wrapped in paper towels moistened with saline and stored (−30°C) until the day of testing.

The femurs were thawed, and the distal femoral condyles were scanned by using dual-energy X-ray absorptiometry (DEXA; Hologic 2000, Hologic, Waltham, MA) in a posterior-anterior direction. The condyles were submerged (5 cmH2O) to simulate surrounding soft tissues. During the scan, the femoral diaphysis was held at 55° from horizontal (posterior surface facing up) with a clamp so that the scan included a clear projection of the condyles (Fig. 1). BMD was calculated for each condyle (medial or lateral) to the depth of the intercondylar notch.

One cylindrical dowel (5-mm diameter) of subchondral and subjacent cancellous bone was cored from the mid-weight-bearing surface of each medial and lateral femoral condyle (Fig. 2). PBS (pH 7.4) was forced through the coring bit to moisten and cool the specimen during machining. The subchondral plate and the trabecular end were machined (130 rpm; EMCO Unimat 3, Columbus, OH) from each core to make each specimen a right cylinder 5 mm in height. Each dowel of bone was compressed to failure by using a servo...
controlled electromechanical testing system (Instron 1122, Canton, MA) at a strain rate of 50%/s (21). The load and displacement data were digitized (IBM PC; RC Computer-scope ISC-16, RC Electronics, Santa Barbara, CA) at a sample rate of 2 kHz. Strain was calculated from the displacement of the Instron crosshead during the load test. From each test, load deflection data were processed with custom software (Run Technology, Laguna, CA) to determine the load and apparent stress at the proportional limit, the maximal load and apparent stress, the modulus of elasticity, and energy absorption of the bone to the point of maximal stress (i.e., maximal energy) (21).

To measure ash content, mechanical tested bone core samples were dehydrated in acetone for 1 wk, dried for 48 h at 100°C in a muffle furnace (model 62700, Barnstead/Thermolyne, Dubuque, IA), weighed, then ashed for 48 h at 600°C, and weighed. Ash content was determined by calculating ash per unit mass of dry bone (%ash).

Statistics. BMD, ash content, and bone mechanical properties of the ACLX and contralateral joints were analyzed as within-dog pairwise data from each dog. Statistical significance (P < 0.05) at 4, 10, 32, and 39 wk was determined by using the repeated-measures analysis in a general linear model (SPSS for Windows 10.0.5, SPSS, Chicago, IL). In the analysis, the ACLX and contralateral legs were compared as repeated measures [i.e., two measures per animal, once per unit mass of dry bone (%ash).

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As animal mass and BMD correlated significantly in both the contralateral and normal control limbs (Pearson correlation, R² = 0.50, P = 0.001), a multivariate analysis incorporating bone mechanical properties, ash content, and BMD was modeled in a general linear model (SPSS) by using animal mass as a covariate. Because the general linear model in SPSS did not permit a post hoc analysis with covariates, a post hoc analysis with animal mass as a covariate was run individually (ANOVA, SPSS) at each time point (4, 10, 32, and 39 wk) by using a Bonferroni adjustment for significance to the P < 0.0125 level (i.e., P < 0.05 divided by 4 groups).

RESULTS

Animal mass. Animal body mass ranged from 16 to 28 kg (mean 21.5 ± 3.1 kg). Dogs were 22.0 ± 3.7 kg (4-wk group), 19.6 ± 2.6 kg (10-wk group), 21.0 ± 1.9 kg (32-wk group), and 23.5 ± 4.0 kg (39-wk group). The normal control dogs (25.2 ± 2.1 kg) were heavier than dogs in the other groups, and the difference in dog mass was significant between normal controls and the 10- and 32-wk groups.

Macrosopic joint changes. One animal (32-wk group) had asymmetric manual tests before surgery and medial joint capsule thickening, suggesting preexisting ACL insufficiency, which was later confirmed at necropsy. Based on discussions with the breeder, ACL deficiency was likely present for at least 1 yr (15). Data from that animal were excluded from statistical analyses. Another animal (10-wk group) was excluded at necropsy because the ACL had been incompletely transected (~5% of ACL remained at necropsy).

Gross examination of the hip and ankle joints revealed no visible macroscopic lesions on the articular cartilage of any ACLX, contralateral, or normal control limbs in any of the dogs. The articular cartilage in the contralateral and normal control knee joints appeared normal (translucent and shiny blue) at all time points. At 4 wk post-ACLX, however, the articular cartilage in the ACLX joint was dull and opaque. Medial meniscus tears were present in five of eight joints at 4 wk. At 10 wk post-ACLX, cartilage in the ACLX joints was thickened, dull, and opaque, and tears were observed in the medial menisci in six of seven joints. ACLX joints at 10 wk revealed extensive marginal osteophytes. By 32 wk, ACLX cartilage also had partial-thickness erosions on the medial femoral condyle in five of seven joints, in addition to displaced bucket-handle tears of the medial menisci in all seven joints (15). At 39 wk post-ACLX, all five ACLX joints showed extensive osteophytes, articular cartilage erosions, and tears in the medial meniscus.

External controls. Because of the significant differences in animal body mass between normal control dogs and the dogs from the 10- and 32-wk groups, correlation coefficients were calculated between the bone variables and body mass. BMD and body mass correlated significantly for the contralateral and normal control limbs (Pearson correlation, R² = 0.50, P = 0.001); variations in BMD depended in part on animal mass. Because DEXA was a two-dimensional projected bone density measure, the larger animals with larger femoral condyles likely had greater measured BMD, although their volumetric BMD may have been similar to that of the smaller animals. When we compared contralateral BMD with normal control BMD, we found that the contralateral BMD was significantly less than that of normal controls at 4, 10, and 32 wk. When body mass was taken into account, however (i.e., run as a covariate in the statistical analysis SPSS general linear model), there were no significant BMD differences between contralateral and normal control limbs. Furthermore, there were no significant differences between contralateral limbs and normal control limbs for any measured variable (mechanical properties, ash content, or BMD) at any of the four time points (Table 1). The statistical power calculated for
Table 1. Bone mineral density, ash content, and mechanical properties for cancellous bone cores from the medial and lateral femoral condyles

<table>
<thead>
<tr>
<th></th>
<th>Medial</th>
<th>Lateral</th>
<th>4</th>
<th>5</th>
<th>32</th>
<th>39</th>
<th>Normal Controls</th>
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<tr>
<td>Medial</td>
<td>CNTRA</td>
<td>36.6 ± 7.5</td>
<td>38.2 ± 2.1</td>
<td>32.1 ± 11.3</td>
<td>36.0 ± 3.5</td>
<td>42.6 ± 8.8</td>
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<td>ACLX</td>
<td>33.1 ± 3.4*</td>
<td>19.1 ± 6.6†</td>
<td>16.8 ± 2.4†</td>
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<td>CNTRA</td>
<td>33.6 ± 8.0</td>
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<td>30.1 ± 3.3*</td>
<td>23.6 ± 7.4†</td>
<td>17.5 ± 3.0†</td>
<td>21.7 ± 5.1†</td>
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<tr>
<td>Medial</td>
<td>CNTRA</td>
<td>47.2 ± 7.4</td>
<td>51.1 ± 4.1</td>
<td>43.6 ± 9.6</td>
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<td>54.1 ± 11.6</td>
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<td></td>
<td>ACLX</td>
<td>40.0 ± 4.0*†</td>
<td>28.1 ± 7.5†</td>
<td>27.9 ± 4.7†</td>
<td>30.0 ± 2.6†</td>
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<tr>
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<td>CNTRA</td>
<td>43.6 ± 8.6</td>
<td>44.2 ± 7.6</td>
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<td></td>
<td>ACLX</td>
<td>38.2 ± 3.4*</td>
<td>33.8 ± 7.6†</td>
<td>29.5 ± 4.1†</td>
<td>27.9 ± 4.3†</td>
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<td><strong>Elastic modulus, MPa</strong></td>
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<tr>
<td>Medial</td>
<td>CNTRA</td>
<td>623 ± 114</td>
<td>718 ± 110</td>
<td>573 ± 122</td>
<td>611 ± 84</td>
<td>776 ± 207</td>
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<td></td>
<td>ACLX</td>
<td>512 ± 84*</td>
<td>479 ± 70†</td>
<td>459 ± 51†</td>
<td>372 ± 81†</td>
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<tr>
<td>Lateral</td>
<td>CNTRA</td>
<td>577 ± 113</td>
<td>605 ± 112</td>
<td>611 ± 36</td>
<td>579 ± 122</td>
<td>618 ± 192</td>
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<td></td>
<td>ACLX</td>
<td>505 ± 100</td>
<td>535 ± 92</td>
<td>474 ± 50†</td>
<td>374 ± 56†</td>
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<td><strong>Maximal energy, mJ</strong></td>
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<td>CNTRA</td>
<td>293 ± 76</td>
<td>319 ± 71</td>
<td>296 ± 70</td>
<td>260 ± 35</td>
<td>337 ± 75</td>
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<td>ACLX</td>
<td>218 ± 19†</td>
<td>148 ± 53†</td>
<td>178 ± 61†</td>
<td>183 ± 20†</td>
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<td>Lateral</td>
<td>CNTRA</td>
<td>265 ± 60</td>
<td>264 ± 81</td>
<td>288 ± 27</td>
<td>248 ± 62</td>
<td>295 ± 16</td>
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<td></td>
<td>ACLX</td>
<td>216 ± 21†</td>
<td>182 ± 50†</td>
<td>194 ± 41†</td>
<td>164 ± 35†</td>
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<td><strong>Ash content, %ash</strong></td>
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<tr>
<td>Medial</td>
<td>CNTRA</td>
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<td>60.9 ± 1.8</td>
<td>62.7 ± 1.6</td>
<td>63.4 ± 1.0</td>
<td>63.5 ± 1.5</td>
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<td></td>
<td>ACLX</td>
<td>61.0 ± 1.8</td>
<td>56.8 ± 3.9†</td>
<td>59.4 ± 1.7†</td>
<td>62.5 ± 1.0</td>
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<td>60.6 ± 1.3</td>
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<tr>
<td></td>
<td>ACLX</td>
<td>60.9 ± 1.3†</td>
<td>57.2 ± 4.0*</td>
<td>60.3 ± 1.5*</td>
<td>62.6 ± 0.6*</td>
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<td><strong>BMD, g/cm²</strong></td>
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<tr>
<td>Medial</td>
<td>CNTRA</td>
<td>0.66 ± 0.05</td>
<td>0.62 ± 0.04</td>
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<td>0.62 ± 0.05†</td>
<td>0.56 ± 0.07†</td>
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<td>Lateral</td>
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<td>0.59 ± 0.05†</td>
<td>0.56 ± 0.04*</td>
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Values are means ± SD; n, No. of dogs. ACLX, anterior cruciate ligament transected; BMD, bone mineral density; CNTRA, contralateral.

*Significantly different from normal control by general linear model multivariate analysis (SPSS version 10.0.5) with a Bonferroni correction for 4 groups at \( P < 0.05 \) (\( P < 0.05/4 = P < 0.0125 \)) and animal mass as a covariate for contralateral normal control comparison.
†Significantly different from contralateral by general linear model repeated measures (\( P < 0.05 \)).

the contralateral normal control comparison was 0.74. Regression analysis revealed that none of the test variables changed significantly from 4 to 39 wk for the contralateral limbs.

Experimental animals. One bone core sample in each of the 4 wk (ACLX medial condyle) and 10 wk (contralateral lateral condyle) groups was damaged during machining, and both were excluded from statistical analyses.

BMD. BMD of the ACLX medial femoral condyles was significantly less than that of contralaterals and normal controls at 4, 10, 32, and 39 wk (Fig. 3). BMD in the lateral femoral condyles was significantly less than that of normal controls at 4, 10, 32, and 39 wk and significantly less than that of contralaterals at 4, 10, and 32 wk.

Ash content. ACLX ash content was significantly less in the medial femoral condyles at 10 and 32 wk compared with that of either contralaterals or normal controls (Fig. 4). In the lateral condyles, ACLX ash content was significantly less than that of contralaterals at 4, 32, and 39 wk and significantly less than that of normal controls at 4, 10, and 32 wk.

Mechanical properties. ACLX cancellous bone mechanical properties were significantly reduced compared with those of contralateral and normal control bones at almost every time point, particularly in the medial condyle. Statistical analyses between ACLX and contralateral bone mechanical properties revealed significant reductions in bone maximal stress in the medial femoral condyles as early as 4 wk, whereas the bone in the lateral condyles was significantly weaker by 10 wk post-ACLX. Elastic modulus of the cancellous bone cores was significantly reduced in the ACLX medial condyles by 10 wk and in the ACLX lateral condyles by 32 wk (Fig. 5). Maximal energy was significantly less in both the medial and lateral condyles by 4 wk post-ACLX. Comparisons of the mechanical prop-
erties between ACLX and normal controls showed that the ACLX medial femoral condyles had significantly less maximal stress, elastic modulus, and maximal energy at every measured time point. Although maximal energy and maximal stress were significantly reduced in the lateral condyles at all time points, the reduction in elastic modulus was not significant until 39 wk.

For normal control cancellous bone, tissue strain at the proportional limit was 8.3 ± 0.8% as measured by displacement between load platens. Strain at the proportional limit for contralateral bone cores was similar to that of normal controls at all time points and averaged 7.7 ± 1.5%. Strain at proportional limit for ACLX bone cores was significantly less than that of normal controls at 10 wk (5.5 ± 1.0%) and 32 wk (5.1 ± 0.5%). Strain at maximal load averaged 12.2 ± 1.1 and 11.9 ± 1.3% for normal control and contralateral specimens, respectively. The average maximal strain for ACLX bone cores was 11.2 ± 2.3% for all time points except at 10 wk (9.7 ± 1.7%).

Muscle mass. Wet muscle mass in the ACLX limb was significantly less than that in contralateral limbs at 4, 32, and 39 wk. No samples were available at 10 wk.

DISCUSSION

Periarticular cancellous bone changes may contribute to the development of OA in the ACL-deficient knee. Although osteophytes and subchondral sclerosis are hallmarks of end-stage OA, little is known about the early bone changes that may contribute to the progression of OA. Thus our purpose was to quantify the changes in the mechanical properties and BMD in

![Fig. 3. Bone mineral density (BMD) from medial (A) and lateral (B) femoral condyles. Values are means ± SD. BMD from the contralateral limbs (Contra) was not significantly different from that of normal controls (Norm) at any time point. BMD from anterior cruciate ligament transected (ACLX) limbs was significantly reduced compared with that of normal controls (• P < 0.05) and contralaterals (* P < 0.05).](image)

![Fig. 4. Ash content of bone from medial (A) and lateral (B) femoral condyles. Values are means ± SD. Ash content from the contralateral limbs was not significantly different from that of normal controls at any time point. Ash from ACLX limbs was significantly reduced compared with that of normal controls (• P < 0.05) and contralaterals (* P < 0.05).](image)

![Fig. 5. Elastic modulus of bone from medial (A) and lateral (B) femoral condyles. Values are means ± SD. Elastic modulus of bone from the contralateral limbs was not significantly different from that of normal controls at any time point. Elastic modulus of bone from the ACLX limbs was significantly reduced compared with that of normal controls (• P < 0.05) and contralaterals (* P < 0.05).](image)
periartricular and subchondral cancellous bone through the early stages of experimental OA. We hypothesized that significant changes would occur in BMD and subchondral cancellous bone mechanical properties after ACLX.

To determine whether the contralateral was an appropriate internal control for changes in the ACLX limb, we also compared the contralateral data with that of normal controls. When normal control and contralateral limbs were compared, there were significant differences in BMD at 4, 10, and 32 wk. There was, however, a significant correlation between BMD and animal body mass, and the normal control dogs were significantly heavier than dogs in the 10- and 32-wk group. BMD determined by DEXA was a two-dimensional projection, and, therefore, a greater quantity of tissue in the anterior-posterior direction (direction of the scan) in the larger dogs would generate a higher BMD value, even though the mineral per unit volume may not have been different. To control for this significant difference in body mass, we statistically compared contralateral limbs and normal control limbs with body mass as a covariate. Once body mass was taken into account as a covariate, there were no significant differences between contralateral and normal control variables at any time point. Thus, for the variables measured in this study, the contralateral limb was an appropriate internal control.

There were some limitations associated with our mechanical tests. Trabeculae at the ends of the bone core samples were not constrained, which may have produced a nonlinear stress-strain curve (11) and an underestimation of the elastic modulus (measured from the linear portion of the curve) (11, 18). Furthermore, strain was measured from crosshead displacement on the material testing system. A systematic error from the unconstrained sample ends and crosshead displacement was evident in the strain values obtained from the mechanical tests. Although mechanical properties of cancellous bone from the canine femoral condyles have not been published previously, compression tests on canine humeral cancellous bone cores (9) reported mean maximal strains of 1.4% strain in normal control animals compared with our values ranging from 7.3 to 16.3% strain. Such discrepancies were due largely to exaggerated strain in the nonlinear “toe” region of the stress-strain curve. Mean elastic modulus values from the same study of canine humeral cancellous bone cores (9) were reported as 1,490–2,110 MPa for normal control animals and as a mean as low as 410 MPa for dogs with forelimbs immobilized for 16 wk. Our mean elastic modulus values were comparatively low (e.g., for medial condyles: normal control, 776 MPa; contralateral, 633 MPa; ACLX, 465 MPa), which may have been related to our strain calculation. Mean maximal strength for cancellous bone from the canine humerus was reported as 13–21 MPa (9). Our maximal stresses were greater (e.g., for medial condyles: normal control, 54 MPa; contralateral, 47 MPa; ACLX, 32 MPa), likely related to the higher loading rate [Kaneps et al. (9), −1% strain/s; present study, 50% strain/s].

These limitations in our mechanical testing protocol likely resulted in some systematic errors, but our experimental design included both contralateral and normal controls for bone mechanical properties, and our conclusions are based on relative changes that demonstrate a clear effect. Our data showed significant reductions in periarticular BMD and periarticular cancellous bone mechanical properties in the ACL-deficient knee compared with those of normal control limbs and contralateral limbs at 4, 10, 32, and 39 wk post-ACL transection. The medial condyle, in particular, revealed significant decrements in BMD and mechanical properties at all time points. The changes in the lateral condyle were less dramatic than those in the medial condyle and, in the case of elastic modulus, did not occur until 32 wk after surgery.

There were significant changes in ACLX ash content at 10 and 32 wk in the medial condyle and at all time points in the lateral condyle. The relatively small change in ash content, however, particularly in the medial condyle, suggested that the BMD changes in the ACLX limbs were due primarily to a loss of bone mass and, to a lesser extent, a change in bone ash content. Boyd and colleagues (2) found significant reductions in trabecular thickness and relative bone volume and significant increases in trabecular separation and the trabecular surface-to-volume ratio in the proximal tibia as early as 3 wk post-ACLX. By 12 wk post-ACLX, the magnitude of these bone-loss-related changes had progressed and was significant in both the proximal tibia and femoral condyles (2). The data from the study of Boyd et al. support our finding that the early bone changes we observed were due primarily to loss of bone mass and suggest further that the bone loss was due to trabecular thinning.

The greatest reductions in BMD, ash, and mechanical properties happened in the 4- to 10-wk time period. In subsequent weeks (32 and 39 wk), the differences between ACLX and contralateral values were either maintained or even decreased, particularly in the medial condyle, suggesting some recovery after the initial response to injury. Although the magnitudes of changes reported by Dedrick and colleagues (5) were dramatically less, they found that bone loss at 3 mo did not recover, even 54 mo after injury. Instead, the medial portion of the tibial subchondral plate at 18 mo was significantly thicker than at 3 mo. The significant difference in subchondral plate thickness persisted and progressed to the central region of the tibial plateau through to 54 mo, at which time the canine joints had frank osteoarthritides with deep cartilage ulcerations, fibrillations, and tidemark duplication with vascular invasion (5). Our data confirmed that the early loss of cancellous bone mass corresponded to a significant loss in cancellous bone stiffness.

It is unclear whether the early bone changes were adaptive or pathological in nature. The loss of bone mass in the ACLX limb was likely due, in part, to a reduction in limb loading (i.e., relative disuse). The significant reduction in muscle mass in the ACLX limbs suggested that, as noted before in ACL-deficient limbs.
dogs (12, 17, 22), the dogs in the present study altered their limb use. Vascular changes in the joint (6), however, likely also contributed to an increase in bone turnover and bone loss. Regardless of the nature of the early bone changes, such changes may play an important role in the initiation of OA. Furthermore, the increased thickness of the subchondral plate in later stages after ACLX may be an adaptive response to compensate for the early loss of stiffness in the underlying cancellous bone.

Recent clinical studies suggest that periarticular bone loss also happens in humans after ligament injury or during treatment of the injury. Leppälä and colleagues (13) measured joint BMD of patients with surgical treatment of complete ACL rupture at <4 wk after injury (presurgical baseline) and 4, 8, and 12 mo postsurgery. By 4 mo, there were significant losses in patellar (−17%), distal femoral (−21%), and proximal tibial (−14%) BMD in the injured limb compared with presurgical baseline, and these losses persisted at 12 mo postsurgery. Karvonen and colleagues (10) reported significantly lower BMD in the knees of patients with mild idiopathic OA (radiographic grades of 0, 1, and 2) compared with normal controls.

Thus the reduction of bone mass in the canine model mimicked human clinical findings. The bony changes that we observed accompany and/or precede the earliest molecular and biochemical changes in the articular cartilage in the canine model (15). We suggest that the early loss of bone mass, in conjunction with vascular changes throughout the joint, may initiate damage and adaptation in the articular cartilage. Subsequent attempts by the bone to recover lost bone mass not only may be ineffective, but may also exacerbate cartilage changes by altering mechanical properties of the subjacent subchondral plate.

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