Doxycycline effects on mechanical and morphometrical properties of early- and late-stage osteoarthritic bone following anterior cruciate ligament injury

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Pardy, Connor K., John R. Matyas, and Ronald F. Zernicke. Doxycycline effects on mechanical and morphometrical properties of early- and late-stage osteoarthritic bone following anterior cruciate ligament injury. J Appl Physiol 97: 1254–1260, 2004; 10.1152/japplphysiol.00122.2004.—As posttraumatic osteoarthritis (OA) progresses, the mechanical and morphometrical properties of the subchondral bone change and may be linked to damage of the articular cartilage. Potentially to slow that progression, doxycycline was administered orally twice daily (4 mg·kg−1·day−1) in skeletally mature canines after anterior cruciate ligament transection (ACLX). To test if doxycycline significantly altered the structure and function of OA bone, we tested cancellous bone mechanical properties, measured bone mineral content, and analyzed bone structure by microcomputed tomography. Our investigation focused on subchondral trabecular bone changes in the medial femoral condyle at 36 and 72 wk after ACLX. Significant mechanical changes discovered at 36 wk post-ACLX were less obvious at 72 wk in both treated and ACLX groups. Doxycycline treatment conserved bone strain energy density at 72 wk. Doxycycline had little effect on the degradation of superficial osseous tissue at 36 wk post-ACLX; by 72 wk, doxycycline in an ACLX model limited subchondral bone loss within the first 3 mm of the periepiphysial bone with established OA. Significant bone loss occurred in the deeper trabecular bone for all groups. Substantial architectural adaptation within deeper trabecular bone accompanied changes in mechanics in early and established OA.

ARTHRITIS AFFECTS ONE-FIFTH of the adult population and is one of the most common causes of long-term disability (21). In posttraumatic osteoarthritis (OA), age is the most significant risk factor: the elderly are at greater risk immediately postinjury, whereas younger populations take more time to develop the disease after injury (55). Rupturing the anterior cruciate ligament (ACL) is a serious injury because of the high probability of subsequent knee OA (45).

In canines, transection of the cranial ACL (ACLX) leads to the development of posttraumatic OA (1, 12, 21, 57). Posttraumatic OA is a multifactorial disease composed of both physiological and mechanical changes within the joint after injury. Whereas a definitive diagnosis of OA in humans typically is made only after extensive joint destruction (55), changes in the joint tissues of experimental animals are detectable shortly after ACLX (28). For example, as early as 4 wk post-ACLX, both degenerative and regenerative changes are evident in the joint, including cartilage thickening, osteophytosis, and small tears in the medial meniscus (1, 12, 17, 28, 29). By 3–12 mo after transection, more extensive degenerative changes become evident, including bucket-handle tears of the medial meniscus, subchondral bone thickening, extensive osteophytosis, and surface erosions of the articular cartilage on the femoral condyles (1, 12). Eventually (4–5 yr after transection), canines exhibit full-thickness loss of articular cartilage, the hallmark pathological feature of end-stage human OA (57).

The bony changes that develop after ACLX may induce abnormal stresses in the overlying cartilage and may initiate cartilage degradation (2, 4, 8, 12, 15, 17, 22, 25, 38, 42, 56, 58). In the last decade, structural changes of subchondral trabecular bone have been documented in posttraumatic OA (ACLX model) by using microcomputed tomography (μCT) (7, 8, 17, 19, 20, 23). These studies suggest that the structural integrity of bone is important in maintaining a healthy joint, as altered mechanical properties of bone accompany joint pathology after ACLX. Specifically after ACLX, there are significant decreases in bone mineral density and trabecular thickness (TbTh), increase in trabecular spacing (TbSp) (6–8, 17, 19, 43, 54), and porosity (8, 48). Moreover, theoretical models suggest that insufficient structure in the subchondral bone may lead to the loss of cartilage and eburnation of the articular surfaces (13, 17, 30, 44, 56).

Progressive cartilage and bone destruction coincides with excessive matrix metalloproteinase enzyme activity in OA joints (16, 49, 50, 58) and proinflammatory cytokines and other tissue-damaging proteinases that can influence degradation of the subchondral bone (49, 50, 58). Hence, considerable effort has been focused on finding a pharmacological agent to limit tissue destruction that accompanies OA. One such agent, the antibiotic doxycycline, reportedly limits cartilage and bone destruction and significantly ameliorates the degenerative changes that occurred in OA joints of both animal models and humans (9, 10, 47, 51, 57, 58). Specifically, low-dose oral doxycycline appeared to reduce the rate and extent of joint pathology in the canine ACLX model of OA (57, 58). Although the exact mechanism whereby doxycycline protects against the development of OA remains unknown, preserving the structural and mechanical integrity of the subchondral bone may contribute to the maintenance of healthy articular cartilage and other joint tissues. The purpose of the present study was to quantify the structural and functional alterations of bones at different stages of experimental OA and to determine whether doxycycline had the potential to be a treatment therapy for preserving subchondral bone in the canine ACLX model of OA.

MATERIALS AND METHODS

All procedures on animals were approved by the Calgary Animal Care Committee and in accordance with the Canadian...
Council of Animal Care. In skeletally mature dogs of mixed breed and sex, the cranial ACL was transected (no. 11 scalpel blade) through a lateral arthrotomy (1). The side of the surgical limb was randomized (29), and dogs were randomly assigned to one of five groups: 1) 36-wk treated (ACLX + doxycycline), 2) ACLX 36 wk (n = 17), 3) 72-wk treated (ACLX + doxycycline), 4) ACLX 72 wk (n = 18), or 5) sham (n = 4).

Previous biomechanical and morphological studies commonly have used the contralateral stifle (knee) joint (CTLT) as internal controls for osteoarthritic (OA) preparations (1, 3, 6–8, 11, 12, 24, 29, 31–33, 36, 37, 39, 46, 48, 52–54, 57). CTLT controls provide a direct comparison within an individual animal of experimental and “normal” (noninjured) joints. Wohl and coworkers (54), however, found a “contralateral effect” in the structure and function of perarticular and subchondral bone in the canine ACLX model. Thus, as in the study of Wohl, we chose to normalize bone data from both CTLT and OA knees to bone of the lumbar spine (a site minimally affected by ACLX). In the results section, both the nonnormalized results and the spine-normalized data were entered into multivariate ANOVA analyses.

Doxycycline tablets were administered orally twice daily (50 mg/dose, −4.5 mg·kg−1·day−1) for a total of 24 wk, starting 12 wk post-ACLX (n = 9) in animals euthanized at 36 wk, and for a total of 36 wk, starting 36 wk post-ACLX in the 72-wk group (n = 9). Doxycycline treatment was provided only after the establishment of early OA, not prophylactically. The intent was to intervene after OA had been instituted. The time intervals were based on aggregate pilot data, which showed significant cartilage lesions in 50% of dogs at the 36-wk post-ACLX.

Bone cores were placed in a multiwell tissue culture plate, covered with PBS, and stored at −80°C. Following an established protocol, all cores were nondestructively loaded on a cyclic between 0 and 3 N (0.6% apparent strain) at a rate of 0.001/s before compression tests (5). After nondestructive testing, bone cores were compressed to failure at a strain rate of 0.01/s. Cores were compressed to failure, and load-displacement data were obtained (Instron 1122, Canton, MA). At the proportional limit, compressive displacement data and cross-sectional geometry were used to calculate maximal stress (MPa), maximal strain (%), elastic modulus (MPa), and strain energy density (SED) (mJ/mm³).

Microstructural Analyses

μCT. All microstructure analyses were performed on the same bone core removed from the medial femoral condyle before mechanical testing. Each specimen was scanned by using a high-resolution μCT scanner (SkyScan 1072 Desktop X-ray μCT-scanner, Aartselaar, Belgium). All medial condyle cores were scanned with an exposure time of 5.9 s, rotation step of 0.9°, frame averaging of 2, gain of 1, under a 1-mm aluminum filter producing a nominal isotropic resolution of 9.11 μm/pixel (SkyScan 1072 Desktop X-ray μCT-scanner, Aartselaar, Belgium). All images were digitized by using SkyScan 1072 operating software (TomoNT version 3c 5). Smoothing, beam-hardening correction, and postalignment algorithms resulted in 8-bit cross-sectional images (1,024 × 1,024 pixels). The same scanning criterion was used for all scans. Samples were scanned inside 0.5-mm cylindrical plastic microtubes, absorbing lower energy X-rays, thus reducing beam-hardening artifact.

Image analysis. To investigate if bone morphology changed in OA femurs, morphometrical analyses were performed on two regions of the bone-core μCT images (stacks). All 8-bit scan outputs were converted from tagged information file format into bitmap file format by using Cone Beam CT-Reconstruction program (version 2.11 SkyScan 1072 desktop X-ray μCT scanner, Aartselaar, Belgium). Bitmap images were imported into Tview (SkyScan BMP image visualization) to establish the threshold of trabecular bone. The more superficial of two bone stacks (stack 1) comprised 150 serial images taken from the distal medial condyle core, beginning 0.09 mm (10 pixels) below the subchondral bone, measuring 1.63 × 5.00 mm (Fig. 1). The second set of serial images, stack 2, was taken 0.46 mm (50 pixels) from the end of the first stack (Fig. 1). Stack 2 comprised trabecular bone located ~2 mm below the medial condyle articulating surface. Stack 2 cores were 549 pixels long and 549 pixels in diameter (5.00 × 5.00 × 5.00 mm) (Fig 1). A top-down approach, starting from the subchondral surface and moving deeper into the trabecular bone, produced a standard and repeatable method of choosing stack origins between samples.

All images were globally thresholded, passed through a median filter, and cropped to 5 mm in diameter cylindrical volume by using ImageJ 1.27z (http://rsb.info.nih.gov/ij/). Micromorphological information was obtained from image stacks, allowing three-dimensional parameters of bone microstructure to be calculated, including bone volume (BV) ratio (BV divided by total volume (TV) = BV/TV) (%), bone surface (BS) ratio (BS divided by total BV = BS/BV) (mm−1), TbTh (mm), TbSp (mm), and trabecular number (TbN) (number/mm).

Statistics

Statistical analyses were performed on all groups (36-wk treated, 36-wk ACLX, 72-wk treated, 72-wk ACLX, and 36-wk sham-operated control). Body mass was a covariate for all statistical measures. Pearson’s correlation explored the relations between parameters of bone mechanics and morphometry. Within-animal evaluation of CTLT controls was done by using a multivariate ANOVA with Bonferroni post hoc tests (P ≤ 0.0125). CTLT limbs were compared...
among groups and with respect to a lumbar spine control. Normalized CTLT control data were obtained by dividing all CTLT data by corresponding lumbar spine data. Statistical evaluations between ACLX and CTLT limbs were carried out through a repeated-measures analysis general linear model. Within-subject and between-subject analyses were performed with \( P \leq 0.05 \) (SPSS for windows 11.0; SPSS, Chicago, IL).

RESULTS

No animals were excluded from this study due to a preexisting cruciate ligament insufficiency or because of surgical complications. Data from one animal from the 36-wk ACLX group were unsalvageable because the subchondral bone was damaged during preparation. At euthanasia, right and left triceps surae and plantaris were weighed (g). No significant differences were detected in muscle masses between ACLX and CTLT limbs for all groups.

Mechanical and morphometrical data were significantly correlated for bone mineral density and the morphometrics of bone in stack 1 (BV/TV, BS/BV, and TbTh). Between stack 1 and stack 2, significant correlations were found for BV/TV \( (r^2 = 0.67) \), BS/BV \( (r^2 = 0.69) \), TbTh \( (r^2 = 0.65) \), TbSp \( (r^2 = 0.61) \), and TbN \( (r^2 = 0.55) \).

Spine measures revealed no statistical differences among groups. The results of the femoral analyses using spine-normalized data were not significantly different from the nonnormalized data, and thus only the raw (nonnormalized) data are presented here.

Macrostructural Analysis

Mechanical testing. Mechanical data were analyzed by using repeated measures with a between- and within-subject evaluation \( (P \leq 0.05) \). One sample was excluded from the 72-wk ACLX group because the bone core was cut too short while removing subchondral bone.

EFFECT OF ACLX SURGERY. No statistical differences in mechanical properties were observed between the short-term and long-term ACLX groups (36-wk vs. 72-wk ACLX), but significant differences were found within animals of both 36- and 72-wk groups. The maximal stress of femoral bone cores was significantly less in ACLX limbs compared with CTLT controls at 36 wk but not at 72 wk (Table 1). The 72-wk ACLX showed similar reductions in stress, but there was greater variance within the group (Table 1). Maximal strain was significantly lower in ACLX limbs at 72 wk but not at 36 wk. A significant decrease in elastic modulus was noted at 36 wk but not at 72 wk post-ACLX (Table 1). Proportional SED was significantly greater for CTLT controls at 36-wk and 72-wk post-ACLX in ACLX specimens (Table 1).

EFFECT OF DOXYCYCLINE TREATMENT. No statistical differences were observed between doxycycline-treated groups for

Table 1. Macrostructural measures of bone mineral content, maximal stress, maximal strain, elastic modulus, and strain energy density

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Limb</th>
<th>Maximal Stress, MPa</th>
<th>Maximal Strain, %</th>
<th>Elastic Modulus, MPa</th>
<th>SED, mJ/mm³</th>
<th>BMC, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>36-wk Treated</td>
<td>ACLX</td>
<td>16.17±3.66*</td>
<td>3.85±0.99</td>
<td>749.48±213.46*</td>
<td>0.31±0.02*</td>
<td>45.55±5.23*</td>
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<td></td>
<td>CTLT</td>
<td>20.68±2.29</td>
<td>3.45±0.66</td>
<td>966.67±217.15</td>
<td>0.36±0.01</td>
<td>53.46±2.68</td>
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<tr>
<td>36-wk ACLX</td>
<td>ACLX</td>
<td>13.94±2.80*</td>
<td>4.64±2.25</td>
<td>616.93±275.94*</td>
<td>0.32±0.02*</td>
<td>44.70±2.86*</td>
</tr>
<tr>
<td></td>
<td>CTLT</td>
<td>21.23±1.53</td>
<td>3.36±0.68</td>
<td>1,136.22±261.05</td>
<td>0.36±0.01</td>
<td>54.75±2.44</td>
</tr>
<tr>
<td>72-wk Treated</td>
<td>ACLX</td>
<td>16.86±2.87*</td>
<td>3.34±0.51</td>
<td>999.20±335.32</td>
<td>0.28±0.01</td>
<td>46.37±4.08</td>
</tr>
<tr>
<td></td>
<td>CTLT</td>
<td>20.30±3.30</td>
<td>2.77±0.93</td>
<td>1,358.85±542.25</td>
<td>0.28±0.02</td>
<td>50.36±5.14</td>
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<tr>
<td>72-wk ACLX</td>
<td>ACLX</td>
<td>17.55±4.76*</td>
<td>3.03±0.76*</td>
<td>917.15±308.47</td>
<td>0.27±0.02*</td>
<td>46.48±6.34</td>
</tr>
<tr>
<td></td>
<td>CTLT</td>
<td>20.54±3.21</td>
<td>4.03±1.16</td>
<td>955.06±340.40</td>
<td>0.41±0.02</td>
<td>51.68±4.69</td>
</tr>
<tr>
<td>Sham</td>
<td>ACLX</td>
<td>21.32±0.89</td>
<td>3.45±0.89</td>
<td>965.90±208.96</td>
<td>0.37±0.00</td>
<td>52.63±3.33</td>
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<tr>
<td></td>
<td>CTLT</td>
<td>20.58±2.60</td>
<td>3.43±0.91</td>
<td>1,061.43±431.47</td>
<td>0.35±0.01</td>
<td>53.48±2.15</td>
</tr>
</tbody>
</table>

Values are means ± SD for transection of the anterior cruciate ligament (ACLX) and contralateral (CTLT) limbs. SED, strain energy density; BMC, bone mineral content. Oral dosing of doxycycline was provided to 36-wk and 72-wk treated cohorts. *Significant difference between limbs, \( P \leq 0.05 \).
all mechanical parameters. ACLX bone cores displayed significant decline in maximal stress for 36- and 72-wk groups (Table 1). A significant reduction in elastic modulus was found at 36 wk but did not persist in 72-wk post-ACLX animals, although a nonsignificant but similar trend was observed in ACLX limbs (Table 1). Doxycycline-treated animals had a significant reduction in SED at 36 wk, but those results were not found at 72 wk post-ACLX in treated animals (Table 1).

BMC. No statistical differences existed between sham-operated and CTLT control limbs for all groups. Correlations showed BMC to be related significantly and positively to mechanical properties of OA osseous tissue, while assessing the natural history of OA progression. The canine model of OA consists of ACLX, with the possibility of limiting the natural history of OA, while assessing the treatment intervention potential of doxycycline. The loss of cartilage is the final stage of the OA process, and the present study explored whether doxycycline treatment altered the structural properties of the periarticular bone following ACLX, with the possibility of limiting the natural history of articular cartilage degradation in this model.

The underlying etiology of OA in humans remains unknown, and results from animal models need to be treated with caution as the underlying mechanism and physiology may differ between species. Nonetheless, animal models facilitate analyzing the natural history of OA progression. The canine

<p>| Table 2. Stack 1 and stack 2 bone volume ratio, bone surface ratio, trabecular thickness, trabecular spacing, and trabecular number morphometrical measures |
|---------------------------------|-----------------|------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>Cohort</strong></th>
<th><strong>Limb</strong></th>
<th><strong>BV/TV, %</strong></th>
<th><strong>BS/BV, mm⁻¹</strong></th>
<th><strong>TbTh, mm</strong></th>
<th><strong>TbSp, mm</strong></th>
<th><strong>TbN, mm⁻¹</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stack 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36-wk Treated</td>
<td>ACLX</td>
<td>28.06 ± 6.40</td>
<td>23.83 ± 3.30</td>
<td>0.07 ± 0.01</td>
<td>0.40 ± 0.05</td>
<td>2.12 ± 0.20</td>
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<td>41.97 ± 5.89</td>
<td>16.82 ± 2.51</td>
<td>0.10 ± 0.00</td>
<td>0.35 ± 0.03</td>
<td>2.24 ± 0.18</td>
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</tr>
<tr>
<td>72-wk Treated</td>
<td>ACLX</td>
<td>39.60 ± 7.63</td>
<td>17.26 ± 3.09</td>
<td>0.10 ± 0.01</td>
<td>0.38 ± 0.04</td>
<td>2.11 ± 0.16</td>
</tr>
<tr>
<td>CTLT</td>
<td>42.75 ± 8.92</td>
<td>15.96 ± 3.43</td>
<td>0.11 ± 0.02</td>
<td>0.38 ± 0.07</td>
<td>2.09 ± 0.27</td>
<td></td>
</tr>
<tr>
<td><strong>Sham</strong></td>
<td>ACLX</td>
<td>36.80 ± 1.05</td>
<td>18.51 ± 2.54</td>
<td>0.09 ± 0.01</td>
<td>0.37 ± 0.04</td>
<td>2.21 ± 0.23</td>
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<tr>
<td>CTLT</td>
<td>37.83 ± 3.98</td>
<td>17.98 ± 3.34</td>
<td>0.10 ± 0.02</td>
<td>0.37 ± 0.03</td>
<td>2.19 ± 0.20</td>
<td></td>
</tr>
<tr>
<td><strong>Stack 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36-wk Treated</td>
<td>ACLX</td>
<td>25.73 ± 6.34</td>
<td>23.18 ± 5.35</td>
<td>0.08 ± 0.02</td>
<td>0.47 ± 0.06</td>
<td>1.86 ± 0.19</td>
</tr>
<tr>
<td>CTLT</td>
<td>37.85 ± 5.28</td>
<td>16.32 ± 3.26</td>
<td>0.11 ± 0.02</td>
<td>0.40 ± 0.05</td>
<td>2.00 ± 0.20</td>
<td></td>
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<tr>
<td>72-wk Treated</td>
<td>ACLX</td>
<td>27.23 ± 2.93</td>
<td>20.91 ± 1.85</td>
<td>0.08 ± 0.00</td>
<td>0.46 ± 0.03</td>
<td>1.86 ± 0.12</td>
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<td>CTLT</td>
<td>38.56 ± 2.66</td>
<td>15.30 ± 0.91</td>
<td>0.11 ± 0.01</td>
<td>0.42 ± 0.03</td>
<td>1.90 ± 0.11</td>
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<tr>
<td><strong>Sham</strong></td>
<td>ACLX</td>
<td>28.42 ± 2.40</td>
<td>20.12 ± 1.76</td>
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<td>1.87 ± 0.15</td>
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<td>37.94 ± 8.08</td>
<td>16.19 ± 3.93</td>
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</tr>
<tr>
<td>72-wk ACLX</td>
<td>ACLX</td>
<td>29.56 ± 6.46</td>
<td>20.30 ± 4.03</td>
<td>0.09 ± 0.02</td>
<td>0.45 ± 0.05</td>
<td>1.91 ± 0.15</td>
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<tr>
<td>Sham</td>
<td>ACLX</td>
<td>38.31 ± 5.65</td>
<td>15.73 ± 2.46</td>
<td>0.11 ± 0.02</td>
<td>0.41 ± 0.03</td>
<td>1.97 ± 0.11</td>
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<tr>
<td>CTLT</td>
<td>35.92 ± 4.39</td>
<td>16.52 ± 3.10</td>
<td>0.10 ± 0.02</td>
<td>0.43 ± 0.03</td>
<td>1.90 ± 0.14</td>
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</table>

Values are means ± SD for ACLX and CTLT limbs. BV/TV, bone volume/total volume; BS/BV, bone surface/bone volume; TbTh, trabecular thickness; TbSp, trabecular spacing; TbN, trabecular number. Oral dosing of doxycycline was provided to 36-wk and 72-wk treated cohorts. *Significant difference between limbs, *P* ≤ 0.05.
stifle joint, although smaller, is generally similar in structure to the human knee (27). There is ongoing debate about the appropriateness of using the CTLT limb as a control to study the effects of ACLX. For example, to compensate for the loss of stability within the ACLX joint, there can be a significant reduction in mean peak vertical force, mobility, and joint angles in the affected limb (36, 52), which forecasts an immediate increase in the use of an animal’s CTLT limb. However, long-term studies suggest the reuse of the CTLT, with no significant differences by 26 wk post-ACLX (11, 36, 52). That was borne out in the present study by the similarity in the mass of the plantar flexors from both ACLX and CTLT control hindlimbs.

The results of the present study revealed that BV/TV and BS/BV for stack 1 were slightly (but not significantly) lower in CTLT controls at 72 wk than in sham limbs. Periods of disuse produced osteopenia and atrophy from a lack of bone and muscle use (18). Muscular forces are prime contributors to dynamic bone stimulation and influence bone adaptation. A lack of significant difference between the right and left triceps surae and plantaris complex suggested that all animals regained routine use of the ACLX limb after surgery. Those results reinforced the efficacy and reliability of the CTLT limb as an appropriate, within-animal control measure.

Through the μCT data, the trabecular changes were revealed as OA progressed. Periarticular medial femoral condyles exhibited decreased BV/TV, TbTh, and TbN compared with the proximal tibia, predicting less bone metabolism in the femur (40). On the other hand, in OA, a decrease in BV/TV and TbTh and an increase in TbSp and TbN have been reported (7, 40). As mineral is removed, trabeculae become thinner, resulting in a loss of BV, and larger trabeculae begin to decay in the midsubstance, increasing the number of weaker struts. Similar results were observed in deeper (stack 2) trabecular bone samples for all groups. Greater BS/BV suggested elevated resorption as a result of a reduction in trabecular plate thickness, minimizing surface loss.

As others have noted, our ACLX limbs had significant bone loss, which is symptomatic of OA (7, 8, 17, 20, 41). Stack 1 from 36-wk treated and 36- and 72-wk ACLX groups showed a significant drop in BV, BV ratio, and TbTh and a significant increase in TbSp and BS ratio. In the doxycycline-treated groups, the significant differences between ACLX and CTLT control limbs in stack 1 at 36 wk did not persist in the 72-wk doxycycline-treated animals. During the established phase of OA, doxycycline may have facilitated bone conservation.

Variations in trabecular structure were related to the location from which the cores were taken and the depth of analyzed material. Closer to the medullary cavity, trabecular bone blends into the marrow cavity, and, consequently, TbSp and TbTh increased, whereas TbN and BV/TV decreased (Fig. 2). Joint contact forces during movement can be dissipated by cartilage and then transformed from shear stress into tensile and compressive forces by the periarticular trabecular bone (15). Stresses at the surface are highest, whereas, with increasing depth, the stress is distributed through a larger volume of bone. Brown et al. (13, 14) used a nonlinear two-dimensional finite-element model to predict that bone changes within 3 mm from the articulating surface influenced the ultimate integrity of overlying cartilage. Similar experimental results (30) were recently reported to validate the results of Brown and colleagues (13, 14). Those results placed emphasis on bony changes of the subchondral plate. In the present study, stack 1 was located 1.30 mm below the articulating joint surfaces and was 1.37 mm in length. Therefore, morphometrical changes at that location fell within the depth proposed to influence significantly the function of articular cartilage. Morphometry in stack 1 varied between groups, and significant morphometrical changes were observed in ACLX limbs for both 36-wk groups (treated and ACLX) and 72-wk ACLX groups. The 72-wk treated groups, however, maintained bone structure in the superficial region on the femoral bone. Doxycycline appeared to reduce trabecular deterioration within the first 3 mm of subchondral bone. In a previous study, the immediate prophylactic use of doxycycline was shown to reduce markedly the severity of OA in weight-bearing medial femoral cartilage, directly after injury, and held out to 6 wk (58). While the mechanism of cartilage preservation in that study remained to be defined, the present study supported the concept that maintaining the subchondral trabecular structure (within 3 mm of the chondral surface) may be a contributory factor to protecting the articular cartilage from degradation.

Alterations in bone mechanics post-ACLX can begin as early as 4 wk and extend to 72 wk (54). In the present study, both 36-wk groups (treated and ACLX) showed significant trabecular mechanical changes in maximal stress, elastic modulus, and proportional stain energy density. By 72 wk, mechanical results showed greater variability within groups. ACLX limbs had significantly less maximal stress, whereas elastic modulus was not significantly different than CTLT limbs. OA joints in 72-wk ACLX doxycycline-treated animals showed no differences in SED when compared with their normal counterparts. Consequently, per unit area, trabecular bone in 72-wk treated cores maintained their ability to store compressive energy.

BV fraction of trabecular bone tissue is a major predictor of its compressive mechanical properties (35), and the organization of individual trabeculae is important in predicting the mechanical properties of cancellous bone. A strong correlation existed between the BV/TV and maximal stress of stacks 1 and 2. Nafei and colleagues (34) suggested that apparent OA bone...
loss generated a loss of bone mechanical properties. ACLX limbs at 36-wk treated and ACLX groups showed a significant reduction in maximal stress and proportional SED. Maximal stress was conserved in 72-wk ACLX animals, and doxycycline treatment led to maintenance of SED in the 72-wk treated group.

Doxycycline had little effect on the mineral content of subchondral bone (BMC). BMC significantly dropped for 36-wk groups, but similar findings were not observed at 72 wk. Wohlr et al. (54) found similar early mineral changes in OA joints at 36 wk post-ACLX, but BMC reductions in ACLX limbs were not significant in 72-wk cohorts. After a substantial loss of mineral content early in the progression of OA at 36 wk, at 72 wk post-ACLX, BMC was maintained.

**Conclusion**

Bone mechanics and OA tissue structure were altered after ACLX. At 36 wk post-ACLX, significant periarticular bone remodeling was apparent, but these adaptations were not as obvious at 72 wk post-ACLX. Substantial architectural adaptation within deeper trabecular bone accompanied changes in bone mechanics in the early and established OA. To maintain normal cancellous bone after a traumatic injury, intervention may be focused most effectively on preventing trabecular bone alterations.

Early therapeutic use of doxycycline following ACLX had little effect on the mechanics, morphometry, and preservation of underlying subchondral bone. On the other hand, prolonged doxycycline treatment had a significant effect on the superficial (0–3 mm) subchondral bone in late OA (72 wk). The mechanism(s) by which doxycycline may preserve cartilage or bony structure has yet to be established. Results from the present study, however, amplify the integral role of subchondral and periarticular bone in OA progression, while establishing a role for reduction of bone remodeling with doxycycline treatment after OA development.

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