

Physiological and mechanical adaptation of periarticular cancellous bone after joint ligament injury

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Physiological and mechanical adaptation of periarticular cancellous bone after joint ligament injury. *J Appl Physiol* 90: 1083–1087, 2001.—The relation between blood flow and bone mineral density (BMD) of periarticular bone was examined in an in vivo model of joint instability. Eighty mature New Zealand White rabbits were randomly assigned to experimental [anterior cruciate ligament transection (ACLX)], sham-operated control, or age-matched normal control groups. Experimental rabbits underwent unilateral transection of the right anterior cruciate ligament, and the nonoperated left [contralateral (Cntra)] limb was a within-animal control. BMD and blood flow to the periarticular bone in the femoral condyles were assessed in each group at 2, 4, 6, 14, and 48 wk postsurgery, using quantitative computed tomography scanning and entrapment of colored microspheres. BMD was significantly lower (5%) in the ACLX compared with Cntra limbs. Periarticular bone blood flow in the ACLX limbs was significantly greater than in the Cntra limb (29%) in the early stages (6 wk) after injury. Up to 48 wk post-ACLX, a significant correlation was found between increased blood flow and decreased BMD in the periarticular bone of the femoral condyles in the ACLX limbs. This correlation suggested that heightened blood flow may be linked to mechanisms of bone adaptation in joints after ligament injury.

bone adaptation; blood flow; bone mineral density; anterior cruciate ligament

AFTER RUPTURE OF THE ANTERIOR cruciate ligament (ACL), joint laxity and stability are substantially altered (12). The loss of joint stability may initiate a cascade of effects leading to degradation of tissues and loss of mechanical integrity in soft and hard tissue in the unstable knee joint (5, 8, 12, 26). ACL injury leads to changes that may result in aberrant load-bearing properties of periarticular bone (2, 3, 26). Physiological and mechanical processes contribute to joint tissue adaptations, but, to date, emphasis has been placed primarily on how mechanically induced stimuli affect the post-traumatic unstable joint. Although changes in the mechanical integrity of the joint tissues may be a major

contributing factor to joint adaptation, vascular alterations (angiogenesis or hyperemia) after joint injury have also been linked to the onset and progression of posttraumatic osteoarthritis and rheumatoid arthritis (9, 15). The effects of the vascular system on the morphological behavior of bone are only incompletely understood, but alterations in perfusion can influence osteoclastic activity and mineral dynamics (10). It has been demonstrated that different loading histories can modify the perfusion of bone (14, 22), but little has been done to assess the relation between blood flow and periarticular bone structural and mechanical properties after ligament injury.

The purpose of this study was to investigate the adaptive response of femoral periarticular cancellous bone in the ACL-deficient, unstable rabbit knee joint. We sought to determine whether a relationship existed between changes in blood flow and bone mineral density (BMD) after ligament injury. The validity of using the within-animal contralateral (Cntra) limb as a control to study adaptations in bone was also examined by comparison to both sham-operated (Sham) and age-matched normal [control (Cntl)] animals. The two hypotheses tested were that 1) periarticular bone degradation induced by joint instability was correlated with tissue blood flow and 2) no significant differences in periarticular BMD and blood flow exist in Cntra, Sham, and age-matched Cntl animals.

METHODS

Overall, 80 mature New Zealand White rabbits (1 yr old) were assessed. Thirty were randomly assigned to one of five groups (2, 4, 6, 14, or 48 wk), where the time was the interval between ACL transection (ACLX) and necropsy.

Time intervals were chosen to delineate early (2, 4, 6 wk), middle, and late responses to ligament injury. A sample size of 6 was calculated with a power analysis on pilot data. With an $n = 6$ sample size and the probability of a type I error set at $\alpha = 0.05$, the probability of a type II error was $\beta = 0.19$ [average power ($1 - \beta$) across all variables was 0.81].

Another 30 rabbits were assigned to the Sham group to serve as age-matched sham controls, and 20 rabbits served as

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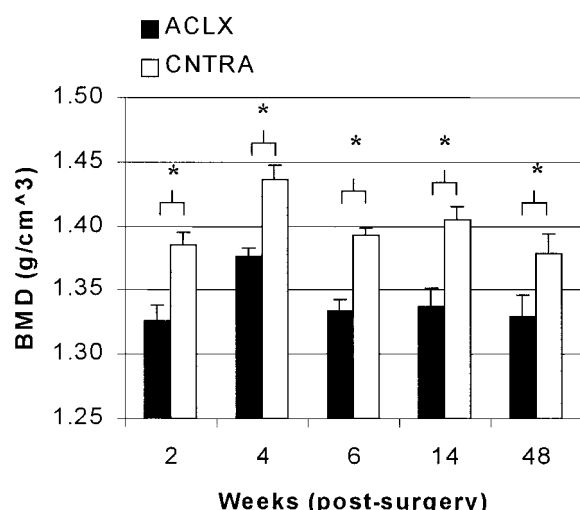


Fig. 1. Bone mineral densities (BMD) of anterior cruciate ligament-transected (ACLX) and contralateral (Cntra) limbs. Values are means $\pm$ SE. *Significant differences between the ACLX and Cntra limbs, $P < 0.05$.

age-matched Cntl animals. The Cntl rabbits received no experimental treatment at all. Sham rabbits were age-matched at 4, 6, 14, and 48 wk, and Cntl rabbits were age-matched at 2, 4, 6, 14, and 48 wk. Animals (Reimens Fur Ranche, St. Agathe, ON, Canada) were housed individually in cages (65 $\times$ 40 $\times$ 45 cm) and allowed free cage activity. Standard laboratory rabbit chow and water were provided ad libitum. All experimental protocols were approved by the Health Sciences Animal Care Committee of the University of Calgary.

Rabbits in the ACLX group underwent unilateral surgical transection of the right ACL as previously described (4), and the left (contralateral) nonoperated limb served as a within-animal control (Cntra). In the transection model, the ligament was lifted slightly before transection to allow access to the ligament without disturbing the surrounding tissue. The Sham group underwent the same procedure to simulate the transection intervention except that the ligament was only lifted and not transected (4). All rabbits had ad libitum cage activity after the surgical procedures. After the appropriate experimental epoch, the animals were anesthetized and injected, using standard microsphere techniques (18), with 15.5- μ m colored microspheres (CM; Triton Technology, San Diego, CA). Briefly, a cannula was inserted into the left ventricle via the common carotid artery. Placement was confirmed by a ventricular pressure waveform from an on-line pressure transducer. A suspension of 10.2 million CM was infused over a 30-s interval. A reference blood sample was drawn from a second catheter starting 10 s before infusion until 1 min after infusion at 3 ml/min. At euthanasia, both femurs of the ACLX group and left femurs of all other animals were removed, cleaned of all soft tissue, hermetically sealed, and stored at -30°C until testing.

Determination of BMD. Postmortem, BMD of the medial and lateral femoral condyles were determined using quantitative computed tomography (CT). Calibration of the CT scanner was performed by scanning seven known concentrations of K_2HPO_4 (0–200 g/ml). The density of each solution was determined, and all bone sample CT numbers were converted to density values. Bones were placed at a 45° incline and scanned at a resolution of 200 $\mu\text{m} \times 200 \mu\text{m} \times 2 \text{ mm}$ (Universal Medical Systems, Solon, OH). ACLX and

Cntra femurs from the same animal were placed side by side for a direct comparison within each CT slice. Scans were taken from the most distal femur and incremented proximally by 2 mm for each slice. Raw CT data were analyzed on a workstation (Silicon Graphics Octane, Mountain View, CA) with a semiautomatic algorithm (Matlab v5, MathWorks, Natick, MA) (2). A single slice, ~ 3 –4 mm proximal to the distal end of the femur and including the entire condyles and the femoral groove, was used for analysis. The entire medial and lateral condyles were analyzed separately to determine average CT values for each limb.

Determination of blood flow. Blood flow in the periarticular bone of the femoral condyles was determined by counting the trapped CM. The medial and lateral condyles were removed from each femur, decalcified for 3 days in 7 ml of 10% HNO_3 , and digested for 2 days in 7 ml of 4 M KOH at 60°C (14). The tissues were filtered through a 7- μm polyester filter (Spectra-Mesh, Spectrum, Houston, TX) and either counted directly using an epifluorescent microscope (CM < 500) or counted by spectrophotometry (CM > 500) (14, 18). Standardized blood flow (SBF; $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$) was determined by relating tissue to reference blood CM count and normalizing to the sample mass.

Statistical methods. Differences between SBF and BMD in the ACLX and Cntra limbs were assessed with ANOVA ($P < 0.05$) procedures. Similarly, differences were assessed for Cntra vs. Cntl, Cntra vs. Sham, and Sham vs. Cntl. Correlations between SBF and BMD were analyzed with a non-parametric two-tailed Spearman's rank correlation. A level of $P < 0.05$ was used to detect significant differences.

RESULTS

All data represented the average of the medial and lateral condyles because there were no significant differences between medial and lateral condylar SBF in any of the experimental groups. A significant difference, however, did exist between the medial and lateral condylar BMD data in the ACLX and Cntra limbs.

Periarticular BMD was significantly lower ($\leq 5\%$) in the ACLX compared with the Cntra limbs at 2, 4, 6, 14, and 48 wk posttransection (Fig. 1). Cntra BMD was significantly different at 4 and 6 wk post-ACLX but not significantly different from Sham BMD at 14 and 48 wk post-ACLX. There were significant BMD differences between the Cntl and Cntra limbs at 2, 4, 6, 14, and 48 wk. Cntl BMD was significantly different from Sham at 4, 14, and 48 wk but not at 6 wk post-ACLX (Table 1).

SBF in the periarticular bone was significantly greater in the ACLX than the Cntra limbs at 2, 4, and

Table 1. Bone mineral densities for Cntra, Sham, and Cntl limbs

Time Postsurgery, wk	Cntra BMD	Sham BMD	Cntl BMD
2	$1.38 \pm 0.01^*$		$1.35 \pm 0.01^{\dagger}$
4	$1.44 \pm 0.01^*$	$1.40 \pm 0.01^{\dagger}$	$1.39 \pm 0.01^{\ddagger}$
6	$1.39 \pm 0.01^*$	$1.43 \pm 0.01^{\dagger}$	$1.36 \pm 0.01^{\dagger}$
14	$1.41 \pm 0.01^*$	$1.41 \pm 0.01^*$	$1.35 \pm 0.02^{\dagger}$
48	$1.38 \pm 0.02^*$	$1.37 \pm 0.02^*$	$1.41 \pm 0.01^{\dagger}$

Values are means $\pm$ SE, given in g/cm^3 . Cntra, nonoperated left contralateral limb; Sham, sham-operated control limb; Cntl, normal control limb; BMD, bone mineral density. For each time period, group means with different symbols (*, $\dagger$, or $\ddagger$) are statistically different from each other ($P < 0.05$).

6 wk post-ACLX. SBF was not significantly elevated at 14 and 48 wk in the ACLX. At 2 wk, ACLX SBF was 97% greater than Cntra, 63% at 4 wk, 66% at 6 wk, 29% at 14 wk, and 63% at 48 wk (Fig. 2). Cntra SBF was not significantly different from Sham. Cntra SBF was also not different from Cntl at 6, 14, or 48 wk. Cntl and Sham SBF were not different at 6, 14, or 48 wk. (Table 2)

A significant negative correlation was detected between SBF and BMD at 2, 4, 6, 14, and 48 wk post-ACLX. An increase in SBF was linearly related to a decrease in BMD at all time points (Table 3).

DISCUSSION

Changes in the vascularity of periarticular soft connective tissues after injury have been suggested as influencing the mechanical properties of the tissue. Similarly, changes in periarticular bone blood flow after a joint ligament injury may be linked to mechanisms influencing periarticular bone remodeling. Few studies, however, have assessed the relation between blood flow and periarticular bone mechanical properties after joint injury. The present study demonstrated that, up to 48 wk post-ACLX, a significant negative correlation existed between SBF and BMD in the periarticular bone of the ACLX limb. Thus there is a strong link between periarticular vascular and bone mineral adaptations after joint trauma. The first hypothesis of our study, therefore, was accepted. Importantly, significant decreases in the periarticular BMD and significant increases in SBF were observed as early as 2 wk post-ACLX.

Quantitative CT can detect significant bone density changes between experimental and contralateral femoral condyles of experimental arthritic and ACLX canine models (2, 24), and the difference between ACLX and Cntra BMD in this study at 2 wk was similar to that at 4, 6, and 14 wk, suggesting degradation of BMD

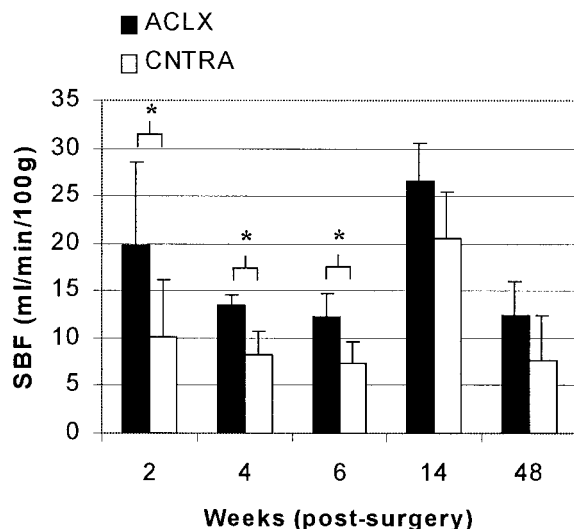


Fig. 2. Standardized blood flows (SBF) of ACLX and Cntra limbs. Values are means $\pm$ SE. *Significant differences between the ACLX and Cntra limbs, $P < 0.05$.

Table 2. Standardized blood flows for Cntra, Sham, and Cntl limbs

Time Postsurgery, wk	Cntra SBF	Sham SBF	Cntl SBF
2	10.08 $\pm$ 6.03*		28.49 $\pm$ 26.15†
4	8.27 $\pm$ 2.43*	12.58 $\pm$ 2.91*	21.60 $\pm$ 10.93†
6	7.33 $\pm$ 2.41*	6.05 $\pm$ 0.87*	11.75 $\pm$ 4.64*
14	20.64 $\pm$ 4.82*	14.94 $\pm$ 5.82*	25.84 $\pm$ 9.51*
48	6.52 $\pm$ 4.78*	9.32 $\pm$ 7.27*	2.14 $\pm$ 0.52*

Values are means $\pm$ SE, given in $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$. SBF, standardized blood flow. For each time period, group means with different symbols (* or †) are statistically different from each other ($P < 0.05$).

was an early response to joint instability (14). At 48 wk post-ACLX, BMD was still significantly lower in the femoral condyles of the ACLX limb, suggesting that the early changes in BMD persisted for an extended period of time.

SBF was significantly elevated in the periarticular cancellous bone of the ACLX limb from 2 to 6 wk post-ACLX. Although SBF was somewhat elevated at 14 and 48 wk, it was not significant, suggesting changes in SBF were an early response to joint injury and instability of the joint and may be reversible with time. The greatest difference between ACLX and Cntra SBF at 2 wk post-ACLX would have been commensurate with the increase in metabolic demands of the bone after initial injury. Peak angiogenic activity may have occurred at 14 wk post-ACLX.

Previous research has reported a significant increase of absolute flow in the distal epiphyses of arthritic joints (25) compared with normal joints in dogs. The observed increase in flow in our present study may have been linked to an increased flow through native vessels and/or increased flow through neovascular vessels generated by angiogenic activity (17) because both can be associated with tissue remodeling (6, 11). The microsphere method did not distinguish between angiogenesis or vasodilation as the source of the increase in blood flow. Others, however, have shown a larger number of 50- μm CM trapped in the distal femoral epiphysis relative to the number of 15- μm CM, suggesting intraosseous arteriolar dilation could be a factor in increased flow in the arthritic epiphyses (25).

Alterations in perfusion may influence mineral dynamics and deposition and resorption of bone (10). The observed increase in blood flow may have stimulated

Table 3. Differences in SBF and BMD between ACLX and Cntra limbs

Time Postsurgery, wk	SBF, $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$	BMD, g/cm^3	R
2	9.80	0.06	0.76*
4	5.20	0.06	0.79*
6	5.72	0.06	0.66*
14	13.98	0.07	0.71*
48	4.84	0.05	0.83*

ACLX, anterior cruciate ligament transected. *Significant linear correlations, $P < 0.05$.

osteoclastic activity, resulting in an increase of bone resorption (1, 14) or an increase in mineral deposition (21). In a recent human study, surgically treated, complete ACL rupture patients (12 mo after injury) had a significant decrease (21%) in the BMD of the distal femur compared with baseline (19). The rabbit model develops osteoarthritis with full thickness ulcerations in the articular cartilage as early as 8 wk post-ACLX (27). Although the physical properties of soft tissues in the joint demonstrate degenerative changes, implying an increase in bone turnover, BMD was not increased at 48 wk post-ACLX in the present study. After rupture of the ACL there can be an increase in bone turnover, and the combination of greater bone resorption than deposition (23) and the presence of a large proportion of newly formed and presumably less mineralized (1) bone would result in lower BMD values.

It is unknown, however, whether vascular adaptations preceded osteoarthritis-induced bony adaptation or whether the blood flow changes were secondary to the heightened cellular activity associated with tissue remodeling (17). Altered loading of bone after joint ligament injury leads to bone turnover that may stimulate an increase in blood flow to carry the necessary nutrients to the new bone. Blood flow, under the influence of external loading, has also been considered a possible mechanism controlling bone remodeling (16).

Using the contralateral limb as an internal control could be advantageous in future experiments to reduce the number of animals required and interanimal variability. Biochemical and morphometric studies of articular cartilage have used the contralateral limb as a control (27), but questions have remained as to whether it was a valid control for bone studies, because loading changes occurred after surgery (3). For example, Gilbertson (13) reported increased uptake of fluorochrome labeling in the contralateral knee of the ACLX canine model.

Changes in limb (un)loading after ACLX may result in stimulation of bone formation in the contralateral (uninjured) limb. O'Connor and colleagues (22) did not observe an increase in the peak vertical ground reaction forces generated in the contralateral limb in conjunction with the 50% decrease in the unstable limb, but the total force generated by the contralateral limb over the entire stance phase may have increased and not been accounted for in their reported data (3). In contrast, Brandt and colleagues (3) found radionuclide uptake in sham-operated animals similar to baseline levels at 5.5 wk postsurgery, suggesting the surgical intervention in the canine did not have an effect on bone turnover at that time point.

In the present study, the significant differences between Sham and Contra BMD at 4 and 6 wk post-ACLX suggested that the Contra limb may not be an appropriate control when studying early responses to joint injury. Thus the second hypothesis of our study was rejected. Alterations in the loading patterns of the Contra limb (7) may have been one factor to explain the differences between the Sham and Contra limbs at these earlier time points. The lack of significant difference

between Sham and Contra BMD and SBF data at 14 and 48 wk post-ACLX, however, suggested that using the Contra limb as a within-animal control may be appropriate in studying later response to joint injury as others have suggested (3). The Contra limb was appropriate to use as a control when studying SBF because there were no differences between the Contra and Sham limbs at any time point. Cntl and Contra BMD were significantly different at all early time periods but were not different at 48 wk post-ACLX. For SBF, Cntl and Contra were significantly different at 2 and 4 wk post-ACLX, suggesting that the contralateral limb may be appropriate as a control only when studying blood flow at later time periods after injury.

One potential limitation of our experimental protocol was the use of microspheres to quantify blood flow in relatively low-flow tissues. Researchers have shown, however, that an accurate estimate of bone blood flow can be obtained with 150–250 microspheres per bone sample (20). The number of microspheres per condyle in this study was, on average, >200 with a relative error <10% (20). Although variability existed in the blood flow data of this study, significant differences were detected. Another potential limitation with BMD measurements between different animals would be different quantities of fat in the bone. This may be a concern for interanimal but not for intra-animal comparisons. ACLX, Contra, Sham, and Cntl were scanned side by side to reduce potential errors due to comparison of different regions of bone.

Within this context, significant BMD and SBF differences were detected between the ACLX and Contra limbs. An increase in SBF was significantly correlated to a decrease in BMD in the periarticular bone after ACLX. Our data suggested that skeletal tissue degradation, specifically within the cancellous periarticular bone, was linked to joint instability and that alterations in the vascular supply were related to aspects of bone adaptation and remodeling dynamics in the ACL-deficient joint. This information may have potential clinical implication in the treatment of ACL injury.

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