

Preservation of periarticular cancellous morphology and mechanical stiffness in post-traumatic experimental osteoarthritis by antiresorptive therapy

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Abstract

Background. Bone changes in experimental post-traumatic osteoarthritis occur early after transection of the anterior cruciate. The purpose of this study was to determine whether antiresorptive bisphosphonate therapy could slow or arrest the periarticular bone architecture and mechanical properties in a post-traumatic model of knee osteoarthritis.

Methods. Skeletally mature, female New Zealand white rabbits were assigned to three groups ($N = 8/\text{group}$). In two groups, anterior cruciate ligament transection was performed and half were left untreated, and the other half dosed with risidronate (0.01 mg/kg s.c. daily) for a six week period. A third group included age-matched normal controls. At the end of the six week period, all rabbits were euthanized and the femur was scanned by micro-computed tomography to assess morphology and density. Specimen-specific finite element analyses quantified the periarticular bone architecture and mechanical properties.

Findings. The untreated transected group had reduced bone volume ratio and mechanical properties compared to the controls ($P < 0.05$) and risidronate-treated transected animals ($P < 0.02$), suggesting bone conservation. Changes in bone volume ratio and mechanical properties of the risidronate-treated transected animals compared to the controls were not detected, indicating that risidronate did not improve these properties relative to the normal controls. The untreated transected group had reduced apparent cancellous bone mineral density and cortical thickness compared to transected animals treated with risidronate ($P < 0.05$).

Interpretation. Bisphosphonate therapy altered the short-term progression of periarticular bone changes including micro-structure and mechanical integrity by slowing early-stage changes to the micro-architecture. These changes following joint trauma may impact the long-term outcome of post-traumatic osteoarthritis.

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1. Introduction

Anterior cruciate ligament (ACL) knee injuries are common and are a key contributing factor in the etiology of post-traumatic osteoarthritis (OA) (McDevitt et al., 1977; McDevitt and Muir, 1976). OA is characterized by the

gradual structural changes of all knee joint tissues with the eventual full-thickness focal loss of cartilage. Past research has predominantly focused on those cartilage changes; but changes to periarticular bone which provides support to other joint tissues (i.e. cartilage, ligaments) are also important following loss of the ACL. The prevention of early progressive changes to periarticular bone and secondary supporting structures may affect internal joint loading conditions, which may in turn influence OA progression.

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The initial changes to periarticular bone following ACL transection (ACLX) in the knee are the result of an imbalance in bone remodeling stimulated by either mechanical changes, physiological changes (inflammation), or both. Bone changes have been detected early in OA disease progression (Burr and Schaffler, 1997; Radin et al., 1978) in humans (Daniel et al., 1994) and in experimental animal models (Boyd et al., 2002). These changes include altered trabecular bone architecture as early as three weeks post-ACLX, with a reduced bone mineral density (BMD), bone volume ratio (BV/TV) and trabecular thickness (Tb.Th) (Boyd et al., 2000a, 2000b). These bone changes produce a loss of mechanical integrity (Boyd et al., 2002; Wohl et al., 2001).

Changes to the secondary supporting structures (e.g. medial collateral ligament – MCL) also occur early during the disease pathogenesis of post-traumatic OA (Bray et al., 1997; Doschak et al., 2004, 2005). There is a loss of joint stability due to an increase in MCL bone complex laxity, which is correlated to an increase in MCL insertion volume (Doschak et al., 2005). However, it is unknown whether increased MCL insertion volume is the only cause for an increased MCL complex laxity, or whether a loss of mechanical integrity in the underlying trabecular bone at the MCL insertion also contributes to the greater laxity. The most common form of failure at the MCL insertion during mechanical testing is full ligament avulsion, and not mid substance ligament avulsion (Bray et al., 1992, 1997). Thus, the trabecular bone supporting the MCL may have a reduced mechanical integrity.

Bisphosphonate (BP) therapy inhibits bone remodeling (Myers et al., 1999) and may help preserve periarticular bone mechanical properties in the early stages of experimental OA. It is unlikely to be effective at inhibiting OA progression once cartilage degeneration has occurred (Spector et al., 2005); however, early prevention immediately following joint trauma may curtail the short-term bone damage. Through BP therapy, it was previously shown that BMD (measured using DXA), bone energy to failure, bone elastic modulus and MCL complex laxity (Doschak et al., 2004) were conserved in post-traumatic experimental OA. Whether MCL complex laxity was conserved solely due to a reduced MCL insertion volume (Doschak et al., 2005) or because periarticular bone architecture and mechanical integrity were also conserved remains to be determined. Recently, BP therapy has been shown to conserve bone properties in humans who have progressive OA (Buckland-Wright et al., 2007).

Therefore, the purpose of this study was to determine the effect of early BP therapy on the architecture and mechanical stiffness of periarticular bone through a morphological and finite element analysis based on micro-computed tomography. These findings could provide further insight into precisely how BP therapy influences bone in the early stages of an injury such as ACL transection, and help to determine the ability of BP to slow or prevent periarticular bone changes in post-traumatic OA.

2. Methods

Skeletally mature, female New Zealand white rabbits (Riemens Fur Ranches, St. Agathe, ON) were randomly assigned to three groups ($N = 8/\text{group}$). Bisphosphonate (RISE; risedronate, Procter&Gamble Pharmaceuticals, OH) drug dosing was achieved by sub-cutaneous (s.c.) injection to ensure the full drug dosage, in part due to concerns over poor oral bioavailability of this drug class (<2% of drug absorbed orally). The dosage of 0.01 mg risedronate per kg body mass was used as we have previously shown an effective dose–response in bone mass conservation for risedronate at this drug concentration in the NZW rabbit (Doschak et al., 2004).

The anterior cruciate ligament was transected in two groups: the first group (RISE) was dosed with risedronate, 0.01 mg/kg s.c. daily for six weeks, and the second group (ACLX) was untreated. The third group comprised unoperated normal age-matched controls (NCON). After 6 weeks, all animals were sacrificed by an overdose of sodium pentobarbital (Euthanyl, MTC Pharmaceuticals), and the distal 5 cm of the femur was dissected. Samples were placed into a polystyrene vial in 4% paraformaldehyde and capped to reduce dehydration. The samples were stored for one month prior to scanning.

All procedures were reviewed and approved by the University of Calgary Animal Care Committee, in compliance with guiding principles in the “Care and Use of Animals” published by the American Journal of Physiology.

2.1. Micro-CT

The distal 28.2 mm of the rabbit femur was scanned by μCT (vivaCT 40, Scanco Medical, Switzerland) using a standard protocol (55 kVp, 109 μA , 300 ms integration time, 38 μm isotropic voxel size). All data were Gaussian filtered (standard deviation of 1.2 voxels, support of 2 voxels) and globally thresholded (22.4% of the maximal image value) to extract the mineral phase.

2.2. Morphological and density analysis

The trabecular bone was segmented from the cortical bone using a semi-automated contouring method. The most proximal location of the subchondral plate was used as the landmark for the beginning of the region of interest containing the MCL insertion. A 7.6 mm sample (200 slices; Fig. 1) was analyzed to determine the following morphological parameters: bone volume ratio (BV/TV), bone surface to bone volume ratio (BS/BV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), connectivity density (Conn.D), and cortical thickness (Ct.Th). Trabecular bone apparent bone mineral density (BMD.Tb.App) and tissue bone mineral density (BMD.Tb), as well as cortical bone mineral density (BMD.Ct), were determined by converting the μCT Hounsfield units into equivalent units of hydroxyapatite.

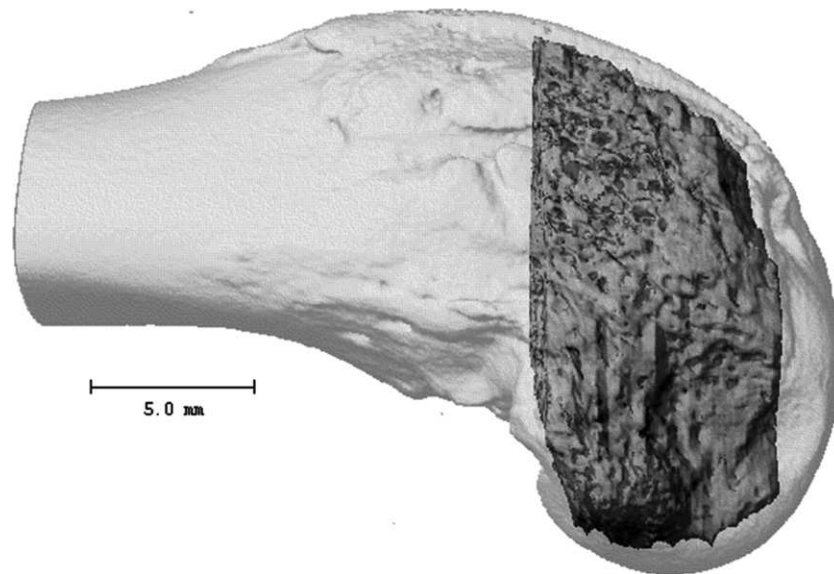


Fig. 1. Periarticular bone morphology and mechanical stiffness were analyzed on the distal femur as shown.

2.3. Finite element analysis

The apparent and tissue level mechanical stiffness properties of the trabecular bone were estimated through a linear finite element (FE) analysis. A benefit of FE analysis is that it intrinsically relates the combined effects of many morphological properties on bone mechanics. The 7.6 mm (200 slices) sample of the medial condyle containing the MCL insertion was the same as the region of interest analyzed during the morphological analysis. Due to hardware constraints the bone samples were artificially degraded by a factor of two resulting in a voxel size of 76 μm . Finite element meshes with hexahedron elements were automatically generated using the voxel conversion approach (Müller and Rügsegger, 1995; Van Rietbergen et al., 1995). The boundary conditions for a uniaxial stress compression to 1% strain were applied along the axis of the bone: nodes were constrained to the plane defined by the

bottom surface; and similarly nodes representing the top plane could freely move laterally, and had a displacement corresponding to 1% strain applied in the axial direction. To account for differences in bone mineral density between the cohorts, a method developed for trabecular bone finite element analyses that assigns bone stiffness based on CT attenuation was used (Homminga et al., 2001),

$$E_{\text{element}} = E_{\text{tissue}} \cdot (\text{GV}_{\text{element}})^{\gamma} \quad (1)$$

where each element was assigned an elastic modulus (E_{element}) based on the relative gray-value of that element ($\text{GV}_{\text{element}}$) normalized to a range from 0 (marrow) to 1 (bone). An elastic modulus (E_{tissue}) of 15 GPa and an exponent (γ) of 1.7 was used as based from a previous study (MacNeil and Boyd, 2007), and all elements were assigned a Poisson's ratio of 0.3. The models contained up to three million elements and four million nodes and were solved on a parallel super computing system using eight processors

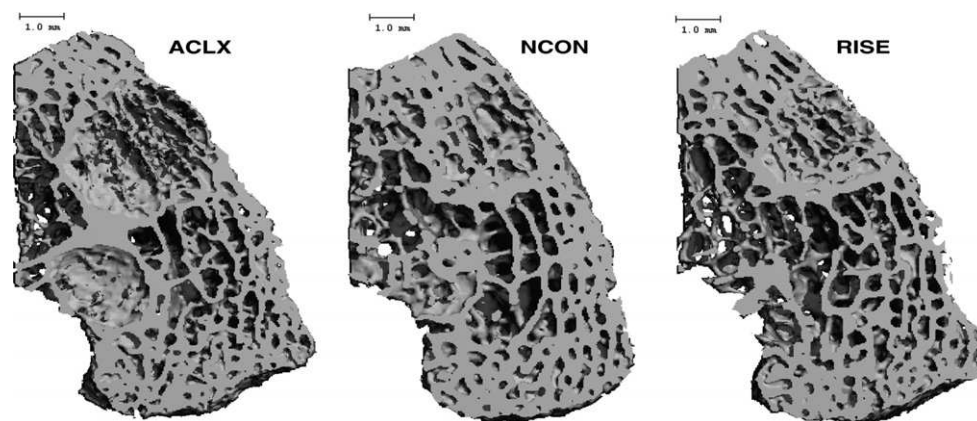


Fig. 2. A 3.8 mm crosssection of the medial condyle qualitatively illustrate the bone micro-architecture. An example is shown for the median animal (based on BV/TV) from each of the three groups: anterior cruciate ligament transected (ACLX), normal control (NCON), and bisphosphonate treated anterior cruciate ligament transected (RISE).

(1.0 GHz 64 bit processors, 4 GB of RAM per four processors). Reaction force to induce 1% strain (RF), mean von Mises stress (VM), and mean strain energy density (SED) were determined for each model.

2.4. Statistics

An one-way analysis of variance (SPSS, Chicago, IL, USA) was performed for independent variables of structure, density and mechanical parameters. Post-hoc tests (Tukey) were performed to determine differences between group means representing the three experimental groups ($P < 0.05$). The mean and standard deviations were determined for each of the measurement parameters, and statis-

tically significant differences between groups were identified.

3. Results

Macroscopically at the time of dissection, both ACLX and risedronate-dosed ACLX (RISE) groups exhibited capsular thickening, with areas of developing osteophytes. The cartilage surface at this early six weeks interval remained intact, but focal roughening was noted during dissection in several samples from both groups.

Trabecular bone architecture differences between the groups were evident by qualitative visualization (Fig. 2) and corroborated quantitatively with the morphological

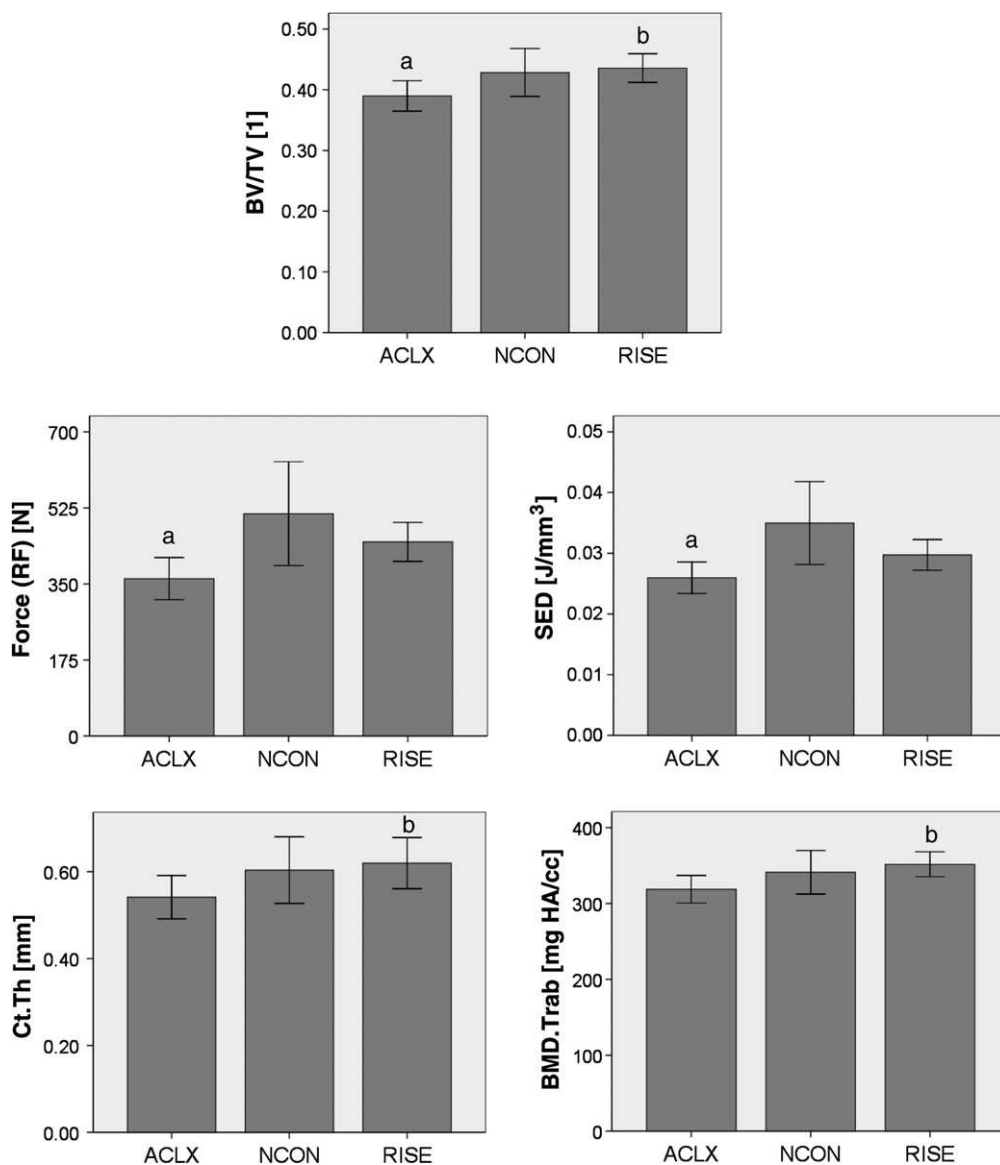


Fig. 3. Mean morphological and mechanical parameters ($\pm$ standard deviation) are shown for the three groups: anterior cruciate ligament transected (ACLX), normal control (NCON), and bisphosphonate treated anterior cruciate ligament transected (RISE). Parameters include bone volume ratio (BV/TV), reaction force to 1% strain (RF), strain energy density (SED), cortical thickness (Ct.Th) and apparent bone mineral density of the trabecular compartment (BMD.Trab). Statistically significant differences ($P < 0.05$) are indicated where 'a' indicates ACLX vs NCON, 'b' indicates ACLX vs RISE, and 'c' indicates RISE vs NCON.

and mechanical data (Fig. 3 and Table 1). The ACLX group had significantly reduced BV/TV compared to the NCON group ($P < 0.05$) as well as to the RISE group ($P < 0.02$), suggesting bone conservation. There was no difference in BV/TV detected between the RISE and NCON groups ($P = 0.88$) indicating that risedronate did not improve the BV/TV. Also, the ACLX group had reduced Ct.Th and apparent trabecular bone mineral density compared to the RISE group ($P < 0.05$), but it should be noted that no significant differences between the NCON and ACLX groups for Ct.Th and apparent trabecular bone mineral density were detected. Overall, the morphological parameters exhibited a consistent non-significant trend of conservation using BP therapy.

The finite element analysis revealed that the ACLX group had significantly reduced stiffness as reflected by the reduced reaction force required to induce 1% strain compared to the NCON group ($P < 0.05$; Fig. 3). No difference between the RISE group and the NCON group was detected, suggesting no change in mechanical stiffness in the BP treatment group. Additionally, at the tissue level, the ACLX group had significantly reduced mean SED compared to the NCON group ($P < 0.01$).

4. Discussion

This study shows that early BP therapy helps conserve bone architecture (BV/TV) and mechanical properties (stiffness) of periarticular bone. These data, when evaluated in the context of our previous work showing that normal MCL complex laxity was maintained in our BP treated experimental OA model (Doschak et al., 2004,2005); suggest that conservation of bone mechanical stiffness may have played an important role. Applying BP treatment early after injury had the effect of slowing the short-term degeneration of the periarticular bone in this model of post-traumatic OA.

The trends of the bone morphology measurements were consistent, but only BV/TV and Ct.Th of all the morphological parameters measured showed statistically significant effects. Biological variance and our small sample sizes likely impacted our ability to detect significant effects consistently, and for an unknown reason the biological variability was particularly high in our normal control group as represented by the standard deviations (Fig. 3). Furthermore, the time period of only six weeks may also explain why some of the trends toward conservational effects of BP therapy were not always statistically significant. The variability was also evident in our observation of visible osteoarthritic changes (i.e. meniscal tears and osteophyte formation; Fig. 4) in the ACLX compared to other groups indicating disease progression varied among animals. Although we standardized the regions of interest for the morphological and finite element analyses as much as possible, small differences in animal size and morphometry also may have added variability in the measurements. Nevertheless, important morphological parameters (i.e., BV/TV, Ct.Th) and mechanical parameters (i.e. reaction force) indicated that BP conserved periarticular bone in comparison to the ACLX group despite the short experimental timeframe and small cohorts.

Our finding of conservation of BV/TV with BP therapy is consistent with results from other studies using a rat model (Hayami et al., 2004) and a canine model (Myers et al., 1999) of OA. The results of our study also indicated that the conservation of trabecular bone we observed led to an effect on mechanical stiffness. This is of interest because the trabecular bone in the distal femur plays a role in supporting the subchondral plate and therefore the conservation of mechanical stiffness may have a significant effect on loading experienced by the overlying cartilage (Radin and Rose, 1986). Another impact of maintaining stiffness is that ligament insertion sites may be protected from reduction of mechanical integrity, and this is consistent

Table 1

Mean and standard deviation of the morphological, density and mechanical properties from the anterior cruciate transected (ACLX), normal control (NCON) and risedronate-treated ACLX animals ($N = 8/\text{gp}$)

	ACLX	NCON	RISE	P-Values
BV/TV (%)	38.99 (2.52)	42.83 (3.94)	43.58 (2.37)	a($P = 0.05$), b($P = 0.02$)
BS/BV (mm^{-1})	8.42 (0.90)	7.87 (0.98)	7.89 (0.81)	
Tb.N (mm^{-1})	1.43 (0.15)	1.51 (0.25)	1.57 (0.16)	
Tb.Th (mm)	0.26 (0.02)	0.26 (0.02)	0.27 (0.02)	
Tb.Sp (mm)	0.74 (0.11)	0.71 (0.20)	0.64 (0.09)	
Conn.D (mm^{-3})	5.09 (0.96)	4.76 (1.47)	5.33 (1.16)	
Ct. Th (mm)	0.54 (0.05)	0.60 (0.08)	0.62 (0.06)	b($P = 0.05$)
BMD. Tb(App) (mg/cc HA)	318.7 (18.2)	341.1 (28.8)	351.6 (16.4)	b($P = 0.02$)
BMD. Tb (mg/cc HA)	734.0 (11.7)	743.3 (15.5)	746.0 (9.4)	
BMD.Ct (mg/cc HA)	712.9 (19.3)	788.3 (16.1)	726.6 (22.6)	a($P < 0.01$), c($P < 0.01$)
RF (N)	362.0 (48.5)	511.8 (119.6)	446.9 (45.0)	a($P < 0.01$)
VM (MPa)	8.90 (0.66)	10.85 (1.28)	9.81 (0.54)	a($P < 0.01$)
SED (J/mm^3)	0.026 (0.003)	0.035 (0.007)	0.030 (0.003)	a($P < 0.01$)

Statistically significant differences between groups are indicated based on an ANOVA and Tukey post-hoc test ($P < 0.05$).

(a) ACLX vs NCON, (b) ACLX vs RISE, (c) RISE vs NCON.

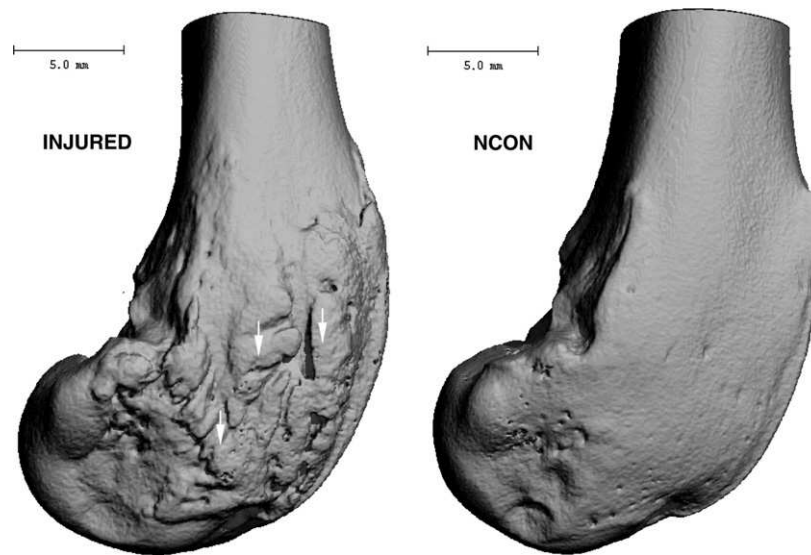


Fig. 4. Osteophytes (white arrows) were evident on all anterior cruciate ligament transected animals including both RISE and ACLX groups but not control animals. A typical example from the injured groups (left) and controls (right) are shown. The presence of osteophytes in both RISE and ACLX groups suggest that treatment did not prevent their formation.

with the finding that BP therapy reduced MCL complex laxity that has been observed with ACLX in the rabbit experimental model (Doschak et al., 2005). The conservation of laxity could have important implications for long-term joint health because joint laxity can lead to altered internal mechanical loading (i.e. contact patterns) which has been suggested to be a factor in the long-term development of OA (Boyd and Ronsky, 1998; Herzog et al., 1998).

Short term use of BP therapy has been previously shown to increase tissue bone mineral density by increasing secondary mineralization (Durchschlag et al., 2006; Kishimoto, 2006). Although a significant difference was not found between the trabecular tissue densities by μ CT, the density analysis did show a significantly reduced trabecular bone apparent density in the ACLX group compared to the RISE group. The material properties assigned to each finite element were based on the density of that voxel Eq. (1) (Goulet et al., 1994; Hodgkinson and Currey, 1992); thus we maximized our ability to detect mechanical differences by inclusion of both structural and density in our analysis. Therefore, the small changes found in trabecular bone apparent and tissue mineral density likely had a significant effect on the conservation of overall mechanical properties we showed as illustrated by the reaction force resulting from 1% applied strain.

In conclusion, the effects of BP therapy on the post-traumatic ACLX joint were detected in the short term (six weeks) indicating that normal bone loss either due to short-term limb disuse or injury-related inflammation has been slowed. Bone conservation may have important repercussions on long-term outcome of OA indirectly by preventing joint laxity (i.e. MCL laxity) and maintaining mechanical support for articular cartilage, however further work is needed to understand if there are long-term

benefits. Nevertheless, intervention in the early-stage following ACLX effected bone conservation and indicates that in post-traumatic OA BP therapy may be beneficial for protecting against damaging bone changes.

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