Systemic Bone Loss Following Non-Invasive Joint Injury in Mice: A Potential Mechanism for Increased Fracture Risk

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Disclosures:

Introduction: Many factors contributing to increased fracture risk have been identified, but the most reliable predictor of fracture risk is a previous fracture of any kind. Subjects who have sustained a previous bone fracture are approximately 2-5 times more likely to sustain a future fracture, even after controlling for bone mineral density. This risk of future fractures increases with the number of prior fractures, and is maintained even when the previous fracture occurs early in life or at an unrelated skeletal site. There is considerable evidence that the increased risk of subsequent fracture is not constant following an initial fracture. Instead, increased fracture risk is highest in the first 1-2 years following an initial fracture, then decreases over subsequent years, but remains higher than that of the general population. The specific mechanisms and risk factors associated with this immediate and time-dependent high fracture risk are not known. It is possible that the healing response to a bone fracture or other musculoskeletal injury is associated with processes that are systemically catabolic for the skeleton, including injury-induced inflammation, decreased mechanical loading (disuse), and increased bone turnover rates. Previous fracture or musculoskeletal injury, therefore, may generally decrease the bone mass and strength of the skeleton and increase general fracture risk for the entire skeleton. However, this possible systemic catabolic adaptation to bone fracture has not been investigated.

In our lab we have developed a mouse model of non-invasive knee injury, which we have used for studying mechanisms of post-traumatic osteoarthritis development [1]. This model uses tibial compression overload to induce anterior cruciate ligament (ACL) rupture with avulsion fracture from the distal femur. Using this model, we observed a 40-44% loss of trabecular bone mass in the femoral and tibial epiphysis of the affected limb by 7 days post-injury. Significantly, we also observed a 3-12% decrease in trabecular bone volume in the contralateral knee at 7 days post-injury. This indicates a possible systemic catabolic effect of musculoskeletal injury, resulting in a loss of bone volume (and potentially bone strength) at distant skeletal sites. These data illustrate a potential mechanism by which bone fracture or other musculoskeletal injuries may actively contribute to a systemic loss of bone volume and strength, thereby increasing risk of sustaining future fractures at any skeletal site. We hypothesize that systemic loss of bone volume at distant skeletal sites following non-invasive knee injury in mice, and that this bone loss will translate to a decrease in mechanical strength.

Methods: Twenty-three mice (male C57BL/6, 10 weeks old at the time of injury) were subjected to non-invasive knee injury according to our previously published methods [2] using either a low-speed injury (1 mm/s compression; n = 8) which induces ACL rupture with an associated avulsion fracture from the femur, or a high-speed injury (500 mm/s compression; n = 8) which induces ACL rupture purely by mid-substance tear, or were sham injured (n = 7). These injury modes produce similar injuries, either with direct bone injury (low-speed) or without bone injury (high-speed). At 10 days post-injury mice were sacrificed and L5 vertebrae were removed for analysis. Vertebrae were imaged with micro-computed tomography (μCT 35, SCANCO, Brüttisellen, Switzerland) with 6 μm isotropic voxel size to quantify trabecular bone structure in the L5 vertebral body. We quantified trabecular bone volume per total volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), and apparent bone mineral density (Apparent BMD; mg HA/cm3 TV) using the manufacturer’s analysis tools. Finite element models were constructed for representative samples (n = 4/group) based on μCT scans, and were used to create estimates of apparent mechanical properties of the vertebral bodies (Fig. 1a). The hard tissue was modeled as isotropic and uniform with a Young’s modulus of 18 GPa and a Poisson’s ratio of 0.3. Models were compressed uniformly to an apparent strain of 0.5%, and the resulting stress and stiffness were calculated. The top and bottom of the models were constrained using fixed boundary conditions to simulate mechanical testing between glue-bonded platens. Analysis was performed on our BEOWULF multiprocessor using existing and specially written linear FEM software.

Results: Non-invasive knee injury resulted in reduced trabecular bone volume fraction at the L5 vertebral body using both knee injury modes relative to uninjured controls (Fig. 1b), but this reduction was greater in the low-speed injury group (-8.8%; p = 0.018). Consistent results were observed for Tb.N, Tb.Sp, and Apparent BMD, indicating that although both injury modes induced bone loss at the L5 vertebral body, the low-speed injury mode, which induces direct bone damage (avulsion from the distal femur) initiated a greater loss of bone. Finite element analysis of representative vertebral bodies from each group exhibited no significant differences in compressive stiffness or resultant stress between experimental groups (Fig. 1c).

Discussion: In this study we quantified bone loss from a distant, unrelated skeletal site (indicative of systemic bone loss) following non-invasive knee injury in mice. Consistent with our hypothesis, we found that knee injury induced by either high
speed or low speed knee injury induced bone loss resulted in trabecular bone loss from the L5 vertebral body, although the magnitude of this bone loss was greater for the low speed injury mode, which involves direct bone damage (avulsion from the distal femur). These data support the concept of bone fractures actively contributing to future fracture risk by initiating systemic bone loss. Results from this study also suggest that bone fracture may induce greater systemic bone loss than injuries that do not involve direct bone damage. This systemic bone loss following fracture may result in a decrease in bone strength at distant skeletal sites, contrary to what we observed in this study following injuries that primarily involved musculoskeletal soft tissue. The insensativity of mechanical properties of the vertebrae to change in trabecular bone mass may have resulted from the mechanical contribution of the cortical shell on the linear mechanical properties calculated in this study. Damage within vertebral bodies has been previously demonstrated to initiate within the trabecular bone, consistent with our theory of increased fracture risk arising from changes in the trabecular bone mass.

Significance: Altogether, the results from this study introduce a paradigm-changing concept that the occurrence of a bone fracture or other musculoskeletal injury may actively decrease bone strength systemically, thereby increasing the risk of future fractures. This concept represents a previously unknown and potentially important mechanism contributing to increased fracture risk, which may lead to new therapeutic targets and treatment regimens for preventing osteoporotic fractures.

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References: 1: Christiansen et al., Osteoarthritis and Cartilage, 2012
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