Micro-computed Tomography Comparison of Trabecular Bone Changes in Rabbits Following Surgical Transection of Anterior Cruciate Ligament and Menisci or Traumatic Impact to the Tibiofemoral Joint

Hannah Pauly¹, Blair Larson¹, Keith Button², Charlie DeCamp², Roger Haut², Tammy Donahue, PhD¹.
¹Colorado State University, Fort Collins, CO, USA, ²Michigan State University, East Lansing, MI, USA.

Introduction: Posttraumatic osteoarthritis (PTOA) develops when a joint sustains a traumatic injury and it is characterized by the degradation of joint structures [1]. The chronic advancement of OA following an anterior cruciate ligament (ACL) tear has been studied through the use of a surgical ACL transection model in various animals [2,3]. Although most transection models demonstrate the progression of OA, they fail to accurately address the occult damages to the surrounding soft tissues and bone along with meniscal damage that occur as a result of trauma. In addition to the rupture of the ACL, damage to the surrounding tissue structures in the knee likely contributes to the functional degeneration of knee joint. Clinically, osteochondral lesions occur in over 80% of ACL injury cases [4] and it is hypothesized that the traumatic loading sustained during injury induces significant bone damage which contributes to the progression of OA [5].

The goal of this study was to compare changes in femurs and tibias that occur as a result of two models of ACL injury. The first model is a modified ACL transection model (mACLT) in which the ACL undergoes a full surgical transection and the medial and lateral menisci each undergo a partial transection. The second model of ACL injury is a closed-joint traumatic loading model (ACLF) [6]. Our hypothesis was that the traumatic loading model of ACL injury would result in more pronounced changes to the subchondral bone of the impacted knee than the mACLT model.

Methods: Twelve skeletally mature Flemish Giant rabbits (5.5 ± 0.5 kg) were used for this study. The study was approved by Michigan State University and Colorado State University All-University Committees on Animal Use and Care. While under anesthesia the right limb of six animals underwent a complete surgical transection of the ACL and partial transections of the medial and lateral menisci each undergo a partial transection. The second model of ACL injury is a closed-joint traumatic loading model (ACLF) [6]. While under anesthesia a gravity accelerated mass was dropped onto the right flexed knee. The tibia was left unrestrained to allow for anterior tibial translation.

Animals were sacrificed twelve weeks post-injury and femurs and tibias were immediately harvested and fixed in buffered formalin. Each bone was scanned in formalin via micro-computed tomography (Scanco µCT, Scanco Medical AG, Brüttisellen, Switzerland) with a resolution of 25µm. Four spatially distributed cylindrical volumes of interest (VOI) were identified for each tibia and femur. The location of the VOIs was standardized for each femur and tibia and each VOI began immediately under the subchondral bone plate and consisted entirely of trabecular bone. Using the µCT analysis software measurements of the following were obtained: volumetric bone mineral density (BMD), bone volume fraction (bone volume/total volume, BV/TV ), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp). Paired sample t-tests were performed on the data to compare injured limbs to uninjured limbs.

Results: All ACLF animals suffered a ruptured ACL and had meniscal tears. Twelve weeks after injury the femurs and tibias from the ACLF animals showed a significant decrease in bone mineral density (p<0.05) when compared to the unaffected control limb (Figure 1). In the affected femurs of ACLF animals there was also a significant decrease in bone volume fraction, the number of trabecular struts, and the thickness of trabecular struts (p<0.05). The separation of trabecular struts also increased significantly in impacted femurs of ACLF animals (p<0.05). In mACLT animals no significant changes were seen in the bones of the affected
Discussion: Based on several µCT measures, rabbit knee joints subjected to a traumatic compressive load demonstrated significant decreases in quantity and quality of trabecular bone. The decrease in bone quantity was manifested by a decreased bone volume as well as a decrease in the number and thickness of trabecular struts and increased trabecular strut separation. Decreased bone mineral density measurements indicated a decrease in bone quality. Clinically, decreased bone volume and bone mineral density are associated with an increased likelihood of fractures [7,8]. Although there was a similar trend for a decrease in bone quantity and quality, potentially due to altered joint loading in injured limbs of both models, there were no significant changes in the bones of rabbits subjected to a surgical transection of the ACL and menisci. The degree of difference in bone changes post-injury between ACLF and mACLT treatment groups was thought to be due to occult damages to surrounding tissues in the knee joint that occur due to the compressive impact load. The initial traumatic compressive load has been shown to cause occult microcracks in the bone [6] and continued loading of these injured knees may result in an accelerated progression of change in the underlying bone.

The significant bone changes found with the ACLF model is supported by clinical findings from studies investigating the progression of OA in humans. Clinical studies have found hypomineralization of bones in OA patients and a longitudinal loss of bone mineral density that is typically associated with the progression of OA [9,10]. Additionally, studies of arthritic human bones have found significant decreases in bone volume and trabecular thickness [11]. Based on the present data, the ACLF model may provide a more clinically relevant method for studying the progression of PTOA due to the acute occult damages induced to surrounding tissue structures within the knee joint which are consistent with clinical observations of OA in humans. Future studies with the ACLF and mACLT models will analyze bones at four and eight weeks post trauma to track longitudinal changes in the joints. Additionally, histological analyses will be used to assess how the thickness of subchondral bone plate is affected in the ACLF and mACLT models.

Significance: These findings suggest that traumatically induced ACL tears in a rabbit model may be used to better understand clinical progression of post traumatic osteoarthritis and develop more effective treatments.

Acknowledgments: Funding for this research was provided by the National Institutes of Health: National Institute of Arthritis and Musculoskeletal and Skin Disease (AR060464).
