

# Early unloading after ACL rupture and prior to surgical restabilization in mice slows post-traumatic osteoarthritis progression

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**INTRODUCTION:** Anterior cruciate ligament (ACL) injury is one of the most common joint injuries, with 100,000-200,000 ACL ruptures occurring annually in the United States. This type of injury leads to post-traumatic osteoarthritis (PTOA) in ~50% of individuals, which can affect patients' pain, mobility, and quality of life. Following ACL injury, ACL reconstruction is often performed, where surgeons replace the injured ligament with an autograft or allograft tissue to restore the biomechanical function of the ACL and restabilize the knee. In our previous mouse study, we found that surgical restabilization of the knee immediately after ACL injury slowed PTOA progression. However, in human patients there is typically a gap of several weeks between ACL injury and reconstruction surgery. It is currently unclear if loading or unloading the injured knee joint during this period would affect PTOA progression. Therefore, the goal of this study was to determine if one week of hindlimb unloading (HLU) following ACL injury and prior to surgical restabilization of the mouse knee joint would affect PTOA progression. We hypothesized that HLU after knee injury would slow the progression of PTOA due to diminished inflammation and tissue damage in the joint prior to restabilization, while restabilizing knee joints after one week of normal loading would accelerate PTOA progression due to the "second hit" of the surgical procedure.

**METHODS:** 103 female C57BL/6J mice (12-weeks-old at the time of injury) were used in this study. Five experimental groups were included in this study: uninjured unoperated controls (Uninj-Unop), injured-unoperated (Inj-Unop), injured with one week of hindlimb unloading prior to return to normal activity (Inj-HLU), injured with restabilization surgery after one week of normal cage activity (Inj-Restab), and injured with restabilization surgery after one week of hindlimb unloading (Inj-HLU-Restab). At Day 0, mice were subjected to non-invasive ACL injury by using a single tibial compression overload with a loading rate of 1 mm/s (ElectroForce 3200, TA Instruments). HLU mice were tail suspended immediately after injury at approximately 30 degree head-down angle, and were provided free access to food, water, and environmental enrichment. All mice were singly housed for one week after injury, after which they returned to group housing. For restabilization surgery, a bone tunnel was made in the anterior portion of the proximal tibia and a suture was passed through the tunnel and around the femoral condyle under the fabelofemoral ligaments. The suture was then tightly tied on the lateral side of the joint. 52 mice were euthanized 3 weeks after injury (2 weeks after surgery), and the remaining 51 mice were euthanized 6 weeks after injury (5 weeks after surgery). At 2, 4, and 6 weeks post-injury, mice were imaged with *in vivo* near-infrared fluorescence reflectance imaging (FRI) to determine protease activity in the joint using a commercially-available fluorescence activatable probe (ProSense 680, Perkin-Elmer). Following dissection at 3 and 6 weeks post-injury, whole knees were analyzed with micro-computed tomography ( $\mu$ CT) to measure epiphyseal trabecular bone microstructure and osteophyte volume ( $\mu$ CT 35, SCANCO Medical AG), and with whole-joint histology to grade OA progression and synovitis.

**RESULTS:** At week 3, ACL injury resulted in a loss of epiphyseal trabecular bone volume (~15% BV/TV) in Inj-Unop and Inj-HLU mice; injured mice with restabilization surgery exhibited ~30% loss of trabecular bone volume at this time point regardless of whether they had one week of post-injury HLU or normal cage activity (Figure A). At week 6 a similar pattern was observed, but with partial recovery of bone loss in all groups to less than ~20% BV/TV compared to the contralateral limb. Osteophyte formation in Inj-Unop, Inj-HLU, Inj-Restab, and Inj-HLU-Restab mice was observable at week 3, with considerably greater osteophyte volume in all injured groups at week 6. However, osteophyte volume in both HLU groups was significantly less than in normal cage activity groups (Figure B). FRI analysis of protease activity showed no differences between any experimental groups at week 2 or week 6, but significantly greater protease activity in restabilized mice at week 4 regardless of whether they had HLU or normal cage activity after injury (Figure C-D). Ongoing analyses will evaluate whole-joint PTOA progression histologically.

**DISCUSSION:** This study showed that early hindlimb unloading after ACL injury diminished PTOA progression and osteophyte formation regardless of whether or not restabilization surgery was performed, but was not able to mitigate the loss of epiphyseal trabecular bone. Restabilization surgery itself caused more trabecular bone loss than ACL injury alone. These findings are in agreement with our previous study, which showed that restabilization surgery was not able to mitigate early epiphyseal bone loss, suggesting that this bone loss is not caused by mechanical factors, but by biological factors such as inflammation. Based on the FRI results, we found that restabilization surgery caused significantly more protease-activated fluorescent signal than non-invasive ACL injury, but this greater protease activity did not accelerate PTOA progression. These data suggest that surgical procedures themselves increase the FRI signal, which may obscure the reading of protease activity within the joint. Altogether, these findings suggest that early unloading of injured limbs prior to ACL reconstruction could effectively diminish long-term PTOA progression.

**SIGNIFICANCE/CLINICAL RELEVANCE:** This study showed that hindlimb unloading of mice after non-invasive ACL injury was an effective biomechanical intervention for diminishing PTOA progression after ACL injury, while restabilization surgery after one week of normal cage activity post-injury did not diminish OA progression. The results of this study can help inform "prehab" strategies for human subjects after ACL injuries before ACL reconstruction by underlining the potential benefit of early unloading (rest) for slowing or preventing the initiation of PTOA.

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