ACL rupture causes structural degeneration of the joint, impairs limb function, and increases pain behaviors in a preclinical rat model of post-traumatic osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) affects over 300 million people globally[1], with a financial burden of $80 billion annually in the United States[2]. Post-traumatic osteoarthritis (PTOA) arises following acute joint injury or trauma (e.g., anterior cruciate ligament (ACL) rupture). PTOA is a particularly onerous form of OA because patients experience late stage disease pathology and total knee replacement an average of 10 years earlier than patients with primary OA[3]. A lack of disease modifying OA drugs motivates novel therapeutic interventions, yet research has largely failed to produce effective clinical outcomes. Preclinical PTOA research primarily utilizes surgically-induced PTOA in small animal models (e.g., the medial meniscal transection surgery in rats) but does not replicate the initiation of PTOA in humans after a knee injury, like ACL rupture. Non-invasive knee injury models that better recapitulate human processes have become more widespread in recent years but remain poorly characterized versus the standard surgical models. Therefore, this study leveraged a non-invasive ACL rupture in a rat model to induce PTOA and investigated quantitative cartilage and bone morphometrics[4–6] in multiple joint tissues (medial tibial plateau, medial femoral condyle, patella) via contrast-enhanced microcomputed-tomography (μCT), conducted limb pain and function testing, and assessed whole-joint histology. *We hypothesized that ACL rupture causes structural changes representative of mild PTOA pathology after 4-weeks and more severe degeneration by 8-weeks, with both timepoints accompanied by reduced injured limb weight bearing function and increased pain sensitivity.*

METHODS: Study design: Skeletally mature male Lewis rats (350 ± 11 g, Charles River) received a non-invasive ACL rupture (ACLR, n=20) or atraumatic sham procedure (n=7) to the left hind limb following local IACUC protocols. ACLR animals were euthanized after 4-weeks (n=10) or 8-weeks (n=10) and sham animals after 8-weeks (n=7). Limb pain and function were longitudinally assessed at baseline (3 days prior to injury), 4-, 6-, and 8-weeks. Evoked pain sensitivity (tactile allodynia) was measured using electronic von Frey testing (Bioseb) and the withdrawal force was recorded for both injured and uninjured (contralateral) hindpaws and repeated three times. Spontaneous pain-associated behavior was quantified by recording limb weight bearing function (Bioseb Dynamic Weight Bearing System 2.0). All limb pain and function measures were normalized to individual baselines. Structural pathology and morphometrics: Knee joint tissues were harvested and fixed in 10% neutral buffer formalin. ACL rupture was visually confirmed at this time. Following immersion in an anionic contrast agent (Conray), tibiae and femora were imaged, and joint tissue changes quantified using established[4–6] and novel evaluation techniques (n=6/group). Briefly, tibial cartilage morphometric analyses were focused on the medial 1/3 and full medial tibial plateau (cartilage volume, cartilage thickness, cartilage X-ray attenuation, osteophyte volumes, etc.), where damage was observed to accumulate. Bone volume and density was quantified in the posterior medial tibial horn. Cartilage thickness, volume and exposed bone area were calculated in the medial femoral condyles. Patellae were imaged without contrast, and bone volume and density were calculated. Histology: Whole knee joints were dehydrated, embedded in paraffin, and serially sectioned at 5 um thickness in the coronal (n=2) and sagittal (n=2) planes 4-weeks after injury, and sagittal (n=4 for ACLR and n=1 for sham) plane 8-weeks after injury (Inotiv). Sections were stained with Hematoxylin and Eosin to visualize extracellular matrix and cell nuclei or with Safranin-O and Fast Green for proteoglycan content. Representative histology qualitatively supplemented radiographs. Statistical analyses: A two-way or mixed-model analysis of variance (ANOVA) with Tukey's post-hoc analyses for multiple comparisons were used to compare across time and treatment (GraphPad Prism). Statistical significance is reported for $p<0.05$.

RESULTS: Functional outcomes: ACL rupture significantly decreased the paw withdrawal threshold in injured (ipsilateral) limbs 4- and 6-weeks post-injury compared to baseline and sham measurements, indicating increased pain sensitization (Fig. 1A). Uninjured (contralateral) limbs had a significantly increased paw withdrawal threshold 6-weeks after injury compared to baseline levels. Tactile allodynia measurements returned to baseline 8-weeks post-injury, with no changes to sham groups. ACL rupture significantly reduced weight bearing on injured limbs at all timepoints compared to sham and baseline levels (Fig. 1B), with correspondingly increased weight bearing on uninjured contralateral limbs. Sham procedure did not affect weight bearing behavior. Structural pathology: Degenerative PTOA-associated changes were qualitatively observed in tibiae (Fig. 2A) and femora (Fig. 2B) at 4- and 8-weeks post-injury, with no pathology evident in sham groups. Marginal osteophytes were observed in patellae at 8-weeks. Structural morphometrics: Cartilage thickness in the medial 1/3 and osteophytes were significantly higher at 4- and 8-weeks post-injury compared to sham (Fig. 3). Bone volume significantly decreased in the medial posterior horn versus sham (Fig. 3). Full-thickness cartilage lesions and exposed bone appeared at 4- and 8-weeks. Quantitative patellar analyses are ongoing. Histology: Representative micrographs at 4-weeks corroborate increased cartilage thickness in medial tibial plateau and thinning of femoral condyle cartilage and show increased synovial thickness and meniscal inflammation (Fig. 3). 8-week histology remains ongoing.

DISCUSSION: Non-invasive ACL rupture impaired limb-weight bearing behavior, with measurable pain behaviors 4- and 6-weeks after injury. Degenerative changes in cartilage and bone characteristic of PTOA arise by 4-weeks post-injury, with more severe pathology by 8-weeks. This degeneration occurs throughout the joint, with specific effects dependent upon tissue compartments. Results provide quantitative measures of previously qualitatively described pathological changes observed by other groups[7–9] and establish functional metrics for rat ACL rupture preclinical models.

SIGNIFICANCE/CLINICAL RELEVANCE: Clinical PTOA is a whole joint disease. Recognizing this, we quantified progressive degenerative changes in multiple joint tissues and limb pain and function after noninvasive ACL rupture, establishing a suite of analytical metrics to assess novel therapeutic strategies and investigate PTOA etiology.

REFERENCES: [1]Peat+2021, Osteoarthr Cartil; [2]Dieleman+2020, JAMA; [3]Brophy+2014, JBJS; [4]Reece+2018, Osteoarthr Cartil, [5]Willett+2016, Osteoarthr Cartil; [6]Brown+2020, JOR; [7]Maerz+2016, Osteoarthr Cartil

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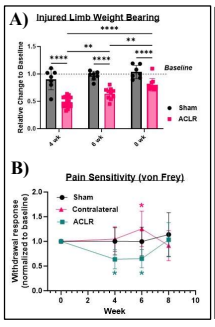

Fig. 1: **(A)** ACL rupture reduces weight bearing in injured limbs after 4-, 6- and 8-weeks compared to sham and baseline (statistics not shown). **(B)** Increased injured limb pain sensitivity (mechanical allodynia) is observed 4- and 6-weeks after ACL rupture and returns to baseline by 8-weeks, with no effects to sham. Mixed-effects model with Tukey's posthoc test ($p<0.05$). *p=0.05, **p=0.005, ***p=0.0005, ****p=0.00005

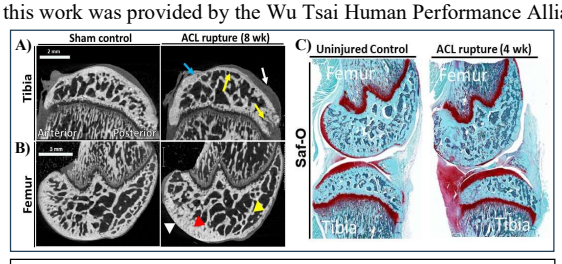

Fig. 2: Representative μCT sagittal radiographs in **(A)** tibiae and **(B)** femora with sham or ACL rupture. **(A)** Tibia: white arrows = cartilage thickening, yellow arrows = bone degeneration, blue arrows = osteophyte. **(B)** Femur: White triangle = cartilage lesion, yellow triangle = subchondral thinning, red triangle = subchondral sclerosis. Scale bar = 2-3 mm. **(C)** Representative whole joint histology at 4 weeks after ACL rupture or uninjured contralateral limbs. Red = proteoglycan content.

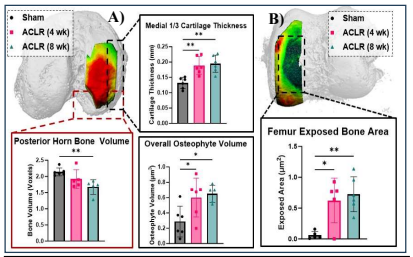

Fig. 3: 3D μCT reconstructions **(A)** ACL rupture increased cartilage thickness and osteophytes and reduces bone volume in tibiae. **(B)** Injury induces full-thickness cartilage lesions and exposed bone in femora. One-way ANOVA with Tukey's posthoc ($p<0.05$)