Physical rehabilitation modulates pain, structural pathology, and cytokine profiles in a preclinical ACL rupture model

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INTRODUCTION: Joint injuries, such as anterior cruciate ligament rupture, increase the risk of developing osteoarthritis (OA)[1]. Exercise is essential for recovery after joint injury and reduces pain and dysfunction in OA. However, it remains unclear how exercise affects function and underlying pathology. A clearer understanding of how exercise influences OA is critical for engineering regenerative rehabilitation strategies to achieve clinically meaningful disease-modifying effects. This study leveraged a preclinical non-invasive ACL injury model in rats[2] and a therapeutic low intensity exercise intervention (treadmill walking)[3] to link pain and dysfunction with local (cartilage, bone, synovium) and systemic (inflammatory cytokines) features of OA disease progression. *We hypothesized that walking exercise would improve pain-related behaviors (tactile allodynia, limb weight bearing), which would correlate to attenuated osteophyte volume, cartilage lesions, and subchondral sclerosis and increased systemically circulating anti-inflammatory cytokines (IL-10, IL-4).*

METHODS: Study design: Adult male Lewis rats (373 ± 12g) were selected for their propensity to rapidly develop OA and trained to run at 10 m/min for 30 min through a 2-week acclimation protocol on a custom treadmill system. Animals were reintroduced to exercise 5 weeks after OA induction (Fig. 1A). Knee injury: A single compressive overload (55 ± 5 N, loading rate 5 N/s) was applied to the left hindlimb limb to induce ACL rupture (ACLr) or an atraumatic Sham procedure (n=7-10/group, N=28). Pain assessment: Pain related behaviors were measured at baseline, 4-, 5-, 6-, and 8-weeks. Evoked pain response was quantified using the electronic von Frey (Bioseb) assay to record a paw withdrawal threshold in hind paws. Spontaneous pain behaviors were measured via limb weight bearing during 5 minutes of spontaneous roaming (Bioseb). Knee joint hyperalgesia was recorded with an algometer applied carefully to the joint (Bioseb). Pain-related outcomes are reported as relative change from baseline. Structural pathology: Animals were euthanized at 8 weeks and fixed hindlimbs were either dissected, immersed in an anionic contrast agent and scanned with microcomputed tomography (Scanco) or prepared for histology (H&E). Structural changes (cartilage, subchondral plate, osteophytes) in the medial tibiofemoral compartment were quantified with validated CT evaluation methods[3] and established synovium histomorphometries were adapted to the rat[4]. Inflammatory biomarkers: Blood was collected prior to euthanasia and assayed for inflammatory biomarkers with a multiplexed magnetic beading sorting assay (ThermoFisher). Statistical analysis: A two-way or mixed-model analysis of variance (ANOVA) with appropriate post-hoc analyses for multiple comparisons were used to compare time and treatment groups ($p<0.05$) (GraphPad Prism). Statistical models (sPLS-DA, Random Forest) were generated to identify salient OA structural pathologies and cytokines (MetaboAnalystR), with top contributing features regressed against pain-related behaviors to identify pathologies associated with chronic pain.

RESULTS: ACL rupture: ACL rupture significantly increased pain sensitivity and reduced weight bearing compared to baseline measures and Sham control groups through 8-weeks. Typical OA pathogenesis was observed throughout the medial tibiofemoral compartment and patella after ACL injury with no degeneration in Sham controls (Fig. 2A). Synovitis and calcified cartilage scores were significantly greater after ACLr compared to Sham. Cartilage thickness and volume and osteophytes significantly increased after ACL rupture in the medial tibiofemoral compartment compared to Sham. Tibial, femoral, patellar bone volume and thickness were significantly reduced by ACLr. ACLr significantly reduced multiple inflammatory cytokines (e.g., IL-1β). Exercise intervention: Exercise significantly ameliorated pain sensitivity by 8-weeks after ACL rupture, with no effects on weight bearing. Histomorphometry showed that intimal synoviocytes were better preserved in ACLr+Exer compared to ACLr. Exercise significantly reduced osteophyte volume compared to ACLr controls and non-significantly attenuated pathological cartilage volume increases in the tibial medial 1/3 plateau (Fig. 2B), with no effect on bone metrics. Inflammatory cytokines (e.g., G-CSF, IFN-ɣ) (Fig. 2C) were modestly increased in ACLr+Exer compared to ACLr and Sham controls, without significant changes to anti-inflammatory cytokines (e.g., IL-10). Statistical model: Hierarchical Clustering first visualized clusters of ACLr, ACLr+Exer and controls groups (Euclidean distance) when considering all structural morphometrics (Fig. 3A). The top five contributing structural pathologies and biomarkers were then selected from a Random Forest ensemble (2000 trees, 7 features/tree) (Fig. 3B) and included for PCA analysis (Fig. 3C). Treatment groups clearly separated along Principal Component 1 with ACLr+Exer phenotypes shifting towards Sham controls, although there was variability in responsiveness. Pain correlations: Granulocytic and monocytic chemokines (e.g., MIP-2, CXCL2, GM-CSF) had moderate-strong significant correlations with pain sensitization (Fig. 3D). Medial tibiofemoral bone density had significant moderate-strong positive correlations to pain sensitization and limb weight bearing whereas medial cartilage quality had negative correlations.

DISCUSSION: Exercise after ACLr improved pain sensitization without affecting limb weight bearing or knee algesia and reduced clinically relevant structural pathology (osteophytes). Inflammatory cytokines and chemokines were counterintuitively increased with exercise, which may implicate a systemic immunomodulatory effect of exercise. Our statistical model identified key structural and biological markers modified by ACL rupture and physical rehabilitation. Structural and biological correlations to pain behaviors suggest that it is immunomodulatory biomarkers rather than structural pathology that may mediate improvements in pain sensitization frequently observed with rehabilitation.

SIGNIFICANCE/CLINICAL RELEVANCE: It remains largely unknown how physical rehabilitation modifies OA pathology and pain sensing, which challenges engineering novel therapies to enhance the regenerative potency of exercise. This work suggests **1.** Exercise therapeutically targets osteophytes and may initiate immunomodulatory pathways, which correlate with reduced pain sensitization and **2.** Novel regenerative rehabilitation interventions should consider targeting subchondral pathologies, which were unaffected by exercise, and further enhancing endogenous inflammation resolution processes.

REFERENCES: [1]Brophy+2014, JBJS, [2]Pancheri+, 2025 Under Review, [3]Kaiser+2024, Osteoarthr Cartil, [4]Obediat+2024, Osteoarthr Cartil

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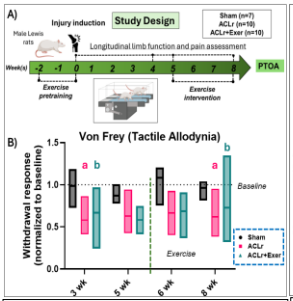

Figure. 1: **(A)** Animals received an ACL injury and then a low intensity exercise intervention **(B)** ACLr increased pain sensitivity at all timepoints. ACLr+Exer recovered to baseline at 8-weeks. Letters denote statistical difference from Sham Mixed-effects model with Tukey's test ($p<0.05$).

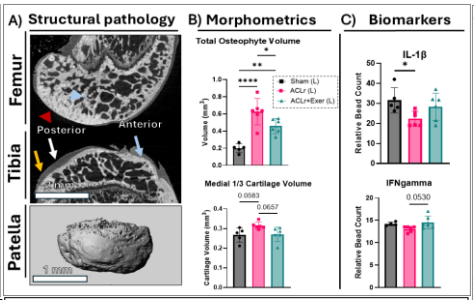

Figure. 2: (A) Representative μCT micrograph of medial tibiofemoral compartment. Tibia: White = cartilage thickening, yellow = bone degeneration, blue = osteophytes. Femur: White = cartilage thickening, Red = cartilage lesion, Blue = sclerosis. Scale bar = 1-2 mm. (B) Total osteophytes were significantly attenuated and cartilage volume was moderately (non-significantly) reduced by exercise. (C) Pro-inflammatory cytokines were increased with exercise. One-way ANOVA with Tukey's Test ($p<0.05$).

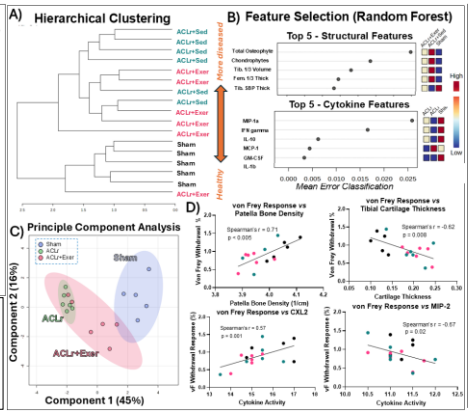

Figure 3: (A) Initial data visualization with hierarchical clustering to show Euclidean clustering of the disease hierarchy. (B) Top structural and cytokine features were identified with a Random Forest ensemble for Feature Selection (C) Top features were selected for PCA visualization and show a shift towards Sham with ACLr+Exer (D) Structural pathology and biomarkers regressed against pain related behavior and assessed with Spearman's Correlations ($p<0.05$).