Metabolic, Transcriptomic, Functional, and Histological Signatures of Inflammation After ACL Rupture in a Translational Model of PTOA

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INTRODUCTION: Over three billion dollars annually are spent on post-traumatic osteoarthritis (PTOA) treatments (1). PTOA pathogenesis is characterized by persistent chronic inflammation following injury and activation of acute inflammatory pathways leading to joint dysfunction, pain, and degeneration (2). The knee is the most common anatomical location of PTOA, which occurs in 50-60% of patients who experience anterior cruciate ligament (ACL) rupture (2), even following successful surgical reconstruction. ACL injuries occur in 68.6 of 100,000 people, annually (1). Given this large burden, targeting joint related acute inflammation post-ACL rupture may represent a novel therapeutic strategy to reduce chronic inflammation, degeneration, and PTOA development. Currently, no therapies exist to prevent or slow the progression of PTOA (1, 3). Polyunsaturated fatty acids (PUFAs) including omega-6 (n-6) and omega-3 (n-3) PUFAs are suggested to have anti-inflammatory effects, specifically when the ratio of n-6: n-3 approaches 1. Why hypothesize that stabilizing the n-6:n-3 ratio in the joint will reduce the severity and duration of inflammation, mitigate chronic degeneration, and subsequently reduce PTOA development post ACL injury. As a first step, this study characterizes functional mobility, fatty acid metabolism, transcriptomic profiles, and histological changes after ACL injury in mice fed a standard rodent diet consisting of 10:1 n-6: n-3 PUFAs. Future studies will test diets with 20:1 and a 1:1 n-6: n-3 ratio to determine their effect on PTOA progression.

METHODS: Unilateral ACL rupture was performed via mechanical tibial displacement in 16-week-old mice (n=5/sex/timepoint/outcome, IACUC 4665)(4). Functional Mobility: Static weight-bearing assessed hindlimb asymmetry. Fatty Acid Metabolomics: Whole joints (uninjured and injured 3, 7, and 14-d post-injury) underwent LCMS analysis of 64 PUFA metabolites. Gene Expression: Whole joints (uninjured and injured 3, 7, and 14-d post-injury) were analyzed on nCounter with a 59-gene inflammation/extracellular matrix (ECM)/fatty acid panel. Histologic scoring: Tibiofemoral joints were fixed, processed, embedded, sectioned, and stained (toluidine blue/fast green), and scored using a modified PTOA system. Statistics: One-way ANOVA (Two-way ANOVA repeated measures used for functional mobility) with Tukey's post-hoc test; α: $p<0.05$. Gene expression values were $\log_2$-transformed and normalized to housekeeping genes.

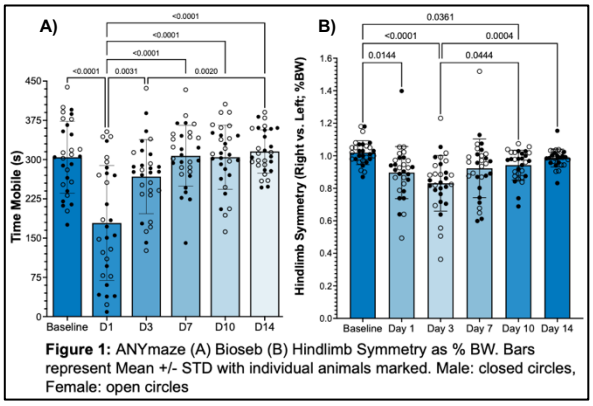

Figure 1: ANYmaze (A) Bioseb (B) Hindlimb Symmetry as % BW. Bars represent Mean +/- STD with individual animals marked. Male: closed circles, Female: open circles

RESULTS: Voluntary functional mobility showed significantly decreased time mobile () at 1-day post-injury relative to baseline which normalized by 3-days and distance traveled at 1-, 3-, and 7-days post-injury which normalized by 10-days (data not shown). Hindlimb static weight bearing showed a significant decrease in hindlimb symmetry (indicative of injured limb off-loading) was observed at 1-, 3-, and 10-days post-injury compared to baseline which normalized by 14-days (**Fig1B).** Fatty acid metabolites of n-3 and n-6 pathways were sex and time dependently altered after injury. Cytochrome P450 pathway metabolites, including epoxyeicosatrienoic acids, were acutely upregulated after injury (data not shown). Gene expression analysis identified 45 upregulated and 14 downregulated genes with injury. Specifically, *Adamts4* and *Tlr4* expression were significantly upregulated at 3- and 7-days post-ACL injury compared to naïve controls. *Adamts4* showed a sharp increase at day 3 and remained elevated at day 7 (**Fig2A**). *Tlr4* expression also increased at day 3 and was sustained through day 7 **(Fig2B).** Synovium and joint capsule histology scores were significantly increased at 3-, 7-, and 14-days (**Fig3A)** and osteophyte severity scores were significantly elevated at 7- and 14-days post-injury **(Fig3B**) relative to naïve joints post-ACL injury compared to naïve controls.

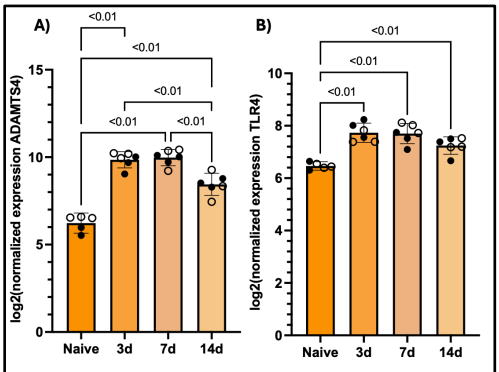

Figure 2: $\log_2$-transformed gene expression normalized to housekeeping genes, *Adamts4* (A) and *Tlr4* (B). Bars represent Mean +/- STD with individual animals marked. Male: closed circles, Female: open circles

DISCUSSION: Significant increases in limb off-loading suggests functional mobility deficits post injury. These acute changes coincided with elevated abundance of EETs which have inflammatory and vascular remodeling effects (5). *Adamts4* and *Tlr4* were significantly elevated at 3 and 7 days, reflecting rapid cartilage catabolism and sustained innate immune signaling. Histology showed increased synovium and joint capsule scores at 3–14 days and elevated osteophyte formation at 7–14 days. These results demonstrate coordinated early inflammatory and catabolic responses that precede structural joint changes, establishing a baseline for future studies on the impact of dietary PUFA ratios on PTOA development.

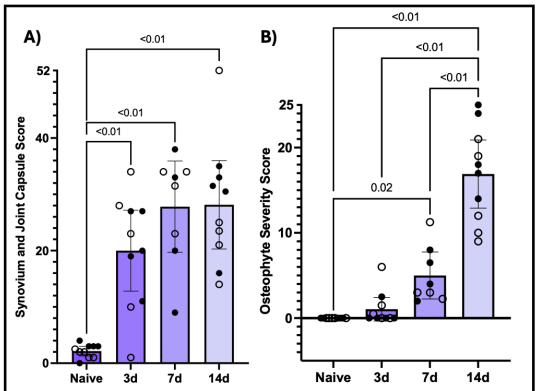

Figure 3: Histologic scores. Synovium and Joint Capsule (A). Osteophyte Severity (B).Bars represent Mean +/- STD with individual animals marked. Male: closed circles, Female: open circles

SIGNIFICANCE/CLINICAL RELEVANCE: These findings highlight the rapid inflammatory and catabolic responses that occur after ACL injury, which may drive early PTOA development. Targeting these pathways or modulating dietary PUFA ratios could offer novel strategies to reduce joint degeneration and improve outcomes following ACL rupture

REFERENCES: [1] Brown + JOT 2006; [2] Lieberthal + OAC 2015; [3] Wang + ART 2020; [4] Timkovich + ABME 2023; [5] Chen + FIP 2021

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