µCT Analysis of Subchondral Bone After Intra-Articular Injection of an Indoleamine 2,3-Dioxygenase–Galectin-3 Fusion Protein

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INTRODUCTION: Osteoarthritis (OA) is a heterogenous disease characterized by the degeneration of all joint tissues. Understanding underlying mechanisms, such as metabolic changes that precede joint pathology, may lead to the development of more targeted OA treatments. Subchondral bone plays a key role in OA progression, as its thickening disrupts joint alignment and accelerates joint deterioration. Shifts in bone metabolism alter bone structure, and over time disorganized bone formation accumulates, capturing a history of disease progression and intervention effects. To investigate the relationship between joint metabolism and subchondral bone remodeling, we employed an enzyme therapy to induce reproducible metabolic shifts in the joint. We developed the fusion protein, indoleamine 2,3-dioxygenase-1 (IDO-1) linked to galectin-3 (Gal3), or IDO-Gal3 which shifts the global synovial fluid metabolic profile by converting tryptophan into kynurenine. Our prior work following an intra-articular (IA) injection of IDO-Gal3 showed reduced inflammation and pain modulation in rodents, although cartilage damage was unchanged. We also observed some potential to reduce trabecular bone sclerosis; however, because these measurements were taken after bone decalcification for histology, accurately assessing the connection between joint metabolism and subchondral bone remodeling requires more advanced and rigorous methods that preserve bone tissue and provide more accurate bone data. In this study, we used µCT analysis to evaluate if metabolic shifts following IA injection of IDO-Gal3 can preserve subchondral bone structure in the context of OA.

METHODS: All animal experiments were approved by the University of Florida IACUC. OA was induced in male and female five-month-old Wistar rats (n=44/sex) *via* transection of medial meniscus and medial collateral ligament in the right knee. Naïve rats (n=8) did not undergo any surgical procedures. Three days after surgery, one group received 11.25 µg of IDO-Gal3 *via* IA injection and the other received saline as a vehicle control. A cross-sectional approach was used, with rats euthanized at 48 hours, 1 week, and 4 weeks post-injection. Operated knees were collected at 1 and 4 weeks for *ex vivo* µCT scanning. Images were acquired using 80kVp/125µA, 1mm aluminum filter, 1k resolution, 19.0-µm magnification, 0.5° rotation step, and 180° tomographic rotation. Subsequent image analysis was conducted to quantify subchondral bone in the medial tibial plateau at the joint loading region (**Fig. 1A-C**).

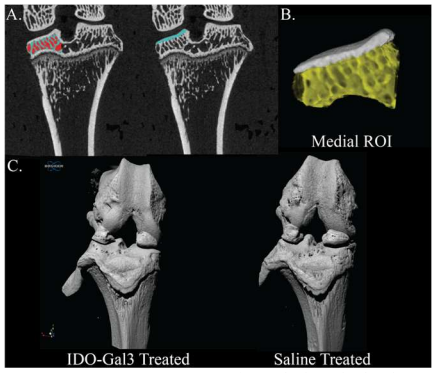

Fig 1. Assessment of subchondral bone microarchitecture. **A.** Posterior view of selected regions of interest (ROIs) in subchondral trabecular bone (left) and the subchondral cortical plate (right); **B.** 3D model of both ROIs shown in Fig. 1A; **C.** Volume rendering of the whole knee joint.

RESULTS: Quantitative µCT analysis of the medial subchondral bone structure showed that bone remodeling was affected following IA IDO-Gal3 injection, but contradictory findings made it difficult to draw a clear conclusion. Morphometric data are plotted as mean ± 95% confidence intervals. In both males and females, trabecular number appeared higher in IDO-Gal3 rats at 4 weeks, suggesting a healthier trabecular structure, whereas trabecular number in saline rats appeared to decrease from week 1 to week 4, consistent with OA-related trends (**Fig. 2A**). However, IDO-Gal3 rats showed increased trabecular thickness accompanied by decreased trabecular separation at weeks 1 and 4 compared to saline and naïve rats, suggesting bone is becoming sclerotic (**Fig. 2B-C**). Although trabecular bone volume did not differ between treatments at week 1, IDO-Gal3 rats of both sexes had higher values at week 4, further suggesting an increase in bone formation (**Fig. 2D**). Finally, connectivity density was lower in IDO-Gal3 rats of both sexes compared to saline rats and naïve rats at both weeks 1 and 4, indicating disorganized and weak bone formation (**Fig. 2E**). Qualitative assessment of the µCT images revealed varying degrees of sclerosis and osteophyte formation in surgical rats regardless of treatment.

DISCUSSION: This work aimed to build foundational knowledge for metabolically reprogramming bone remodeling at the knee. Although the epiphyseal structural results suggest some effect of IDO-Gal3 on bone, the findings remain inconclusive. There are several limitations that could explain why unclear shifts in bone were observed with more accurate measurements compared to prior work [1]. Metabolism is an important contributor to OA pathology, but it does not exist in isolation; OA is a multifactorial disease influenced by the health of all joint tissues. Moreover, severe bone structural changes typically occur in later stages of OA, and this study prioritized quantifying metabolic changes by selecting earlier timepoints. It is possible that bone damage was not severe enough to reveal conclusive differences between treatments. Additionally, IDO-Gal3 primarily shifts the tryptophan pathway, which has not been studied in depth in bone. It is theorized that kynurenine and its metabolites affect bone tissue in many distinct ways, potentially explaining the ambiguous results observed in this study. Our future work will evaluate synovial fluid metabolomic profiling using HPLC-MS to confirm shifts in synovial fluid metabolism following injury and treatment; however, later studies should include extended time points to allow sufficient time for pathological remodeling to develop. Although the enzyme influenced bone remodeling in a variable manner, previous work confirms that IDO-Gal3 shifts joint metabolism and impacts inflammatory and pain pathways, reinforcing the role of tissue metabolism in OA progression and its potential as a therapeutic target.

SIGNIFICANCE: The delicate balance between bone metabolism and its pathological adaptations provides a framework for studying enzyme therapies. By shifting joint metabolism, these therapies can help elucidate the role of specific metabolic pathways in the knee that drive OA pathological changes.

REFERENCES: [1] Partain et al., 2023, Arthritis Research and Therapy.

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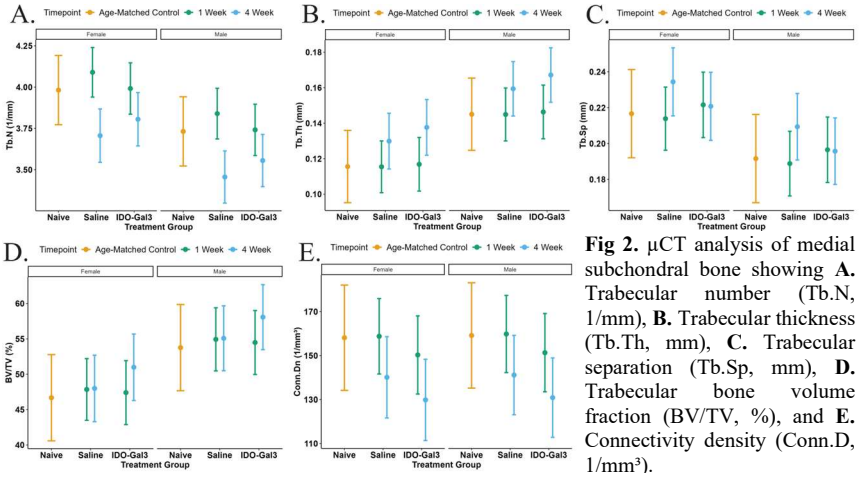

Fig 2. µCT analysis of medial subchondral bone showing **A.** Trabecular number (Tb.N, 1/mm), **B.** Trabecular thickness (Tb.Th, mm), **C.** Trabecular separation (Tb.Sp, mm), **D.** Trabecular bone volume fraction (BV/TV, %), and **E.** Connectivity density (Conn.D, 1/mm³).