Lower Limb Joint Degeneration Following Intervertebral Disc Injury and Force-Based Manipulation in a Mouse Model

Jacob Barnes[1], CJ Replogle[1], Khady Diop[1], Yuzhang Liu MS[1], Jake Klausner[1], Adam Smith[2], Jordin Marshall MS[1], Benjamin Walter PhD [1], Nathan Schilaty DC, PhD[3], Nathaniel Bates PhD[2], Sara McBride-Gagyi PhD [1], Devina Purmessur PhD [1]

The Ohio State University, Department of Biomedical Engineering1, The Ohio State University, College of Medicine, Department of Orthopedics2, University of South Florida, Morsani College of Medicine, Department of Neurosurgery, Brain and Spine

Barnes.1104@buckeyemail.osu.edu

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INTRODUCTION: Chronic low back pain (LBP) is a prevalent and debilitating musculoskeletal disorder, affecting up to 30% of U.S. adults annually and as many as 80% over a lifetime. Lumbar intervertebral disc (LIVD) degeneration as seen in aging or after localized trauma is a major contributor to LBP, but its impact may extend beyond the spine. LBP and lower-limb joint degeneration often co-occur, suggesting that spinal pathology may have downstream effects on the hips and knees through the biomechanical kinetic chain or altered circulating factors in addition to genetic predisposition. Clinical observations, such as knee-spine syndrome, indicate that degenerative changes in the lumbar spine can alter posture and load distribution at the knee, leading to pain and abnormal mechanics. LIVD annular puncture in mice produces localized matrix loss, inflammation, inflammatory cytokine release, and altered mechanics. These features are comparable to those seen in surgically induced knee degeneration models. However, whether LIVD degradation truly propagates to lower limb joints (and vice versa) or is more due to genetic predisposition is poorly established. Few small animal studies have examined joints beyond the injured location to establish correlations or causes. To address this gap and direct future research directions, this opportunistic study uses a mouse model of LIVD degeneration combined with force-based spinal manipulation (FBM) to explore their potential influences on hip and knee joint degeneration. Hip and knee joints from this existing study were analyzed alongside samples from naturally aged, uninjured animals to enable comparison across healthy, degenerative, and age-related conditions. This design removes potential effects of genetic predisposition and allows evaluation of FBM's potential to modulate or mitigate degenerative changes. We hypothesize that spinal degeneration leads to distal joint degeneration and that these effects differ with mechanical spinal interventions (i.e. FBM).

METHODS: This study utilized lower limbs from an on-going study designed to evaluate the effects of FBM on LIVD degeneration after annual puncture injury. Per the IACUC-approved protocol, 20 female mice were randomly assigned to one of 4 groups (sham surgery only, injury only, injury + pre + post FBM group, injury + post FBM group, n = 5 per group). Female mice were chosen due to the logistical requirements of the FBM treatment. An additional cohort of 48-week-old female mice (n=5) with no history of injury or FBM treatment served as a naturally aged reference group to evaluate age related joint remodeling independent of experimental intervention. IVD degeneration was induced in 21-week-old mice at the L4/L5, L5/L6, and L6/S1 levels by puncturing each disc with a 30-gauge needle and injecting with 2 µL of sterile medical-grade saline (injury groups). The same disc levels were surgically exposed for visualization without any injury (sham group). Following surgery, mice underwent a two-week recovery period without treatment. For FBM treatment, mice were anesthetized and 1 impulse (~34 N peak force, 1 ms duration) was administered at the L4/L5, L5/L6, L6/S1 levels 3 times a week. Animals in the pre + post group received FBM three times weekly for six weeks prior to IVD injury to model preventive therapy. Beginning at week 2 post-op FBM was administered three times per week until week 14 post injury (pre + post group and post group) to mimic post-injury treatment for back pain. After euthanasia and spine harvesting, the hind limbs were excised at the pelvis, keeping the hip and knee joints intact. Harvested limbs were fixed in 10% neutral-buffered formalin while at 120º. The hips and knees (n=3/group) from the right legs were microCT scanned (SCANCO µCT 50, 10 µm voxel resolution). Qualitative assessment was performed by a blinded grader using a modified Kellgren-Lawrence scale for µCT to assess articular surface morphology (0 = normal morphology, 4 = severe deformity). Quantitative volumetric morphometric analysis protocols are currently in development and will be expanded as region-of-interest and thresholding parameters are standardized. For this preliminary phase, joint space width (JSW) and subchondral bone thickness (SCB.Th) were measured from microCT reconstructions of the medial and lateral tibial plateaus of each knee. Future analyses will incorporate additional quantitative parameters, including bone volume fraction (BV/TV), trabecular thickness (Tb.Th), and trabecular number (Tb.N), along with corresponding assessment of hip joint morphology. Histology to detect micro-scale cartilage damage is planned. Statistical analysis was performed using one-way ANOVA in SPSS (IBM Corp.), with significance set at $p < 0.05$.

RESULTS SECTION: Qualitative grading of knee joint morphology showed no statistically significant differences among groups (Fig 1) with the currently available sample scans (n=3/group). While not statistically significant, the mean results' trend suggests that LVID injury alone may cause knee joint degradation on par with that seen in uninjured animals 7-weeks older. Further FBM mitigates this damage, returning morphology scores almost to sham values. Preliminary microCT analysis of the tibial plateau demonstrated no statistically significant differences in subchondral bone thickness (SCB.Th) among experimental groups (Fig 2, medial plateau - $p = 0.107$, lateral plateau - $p = 0.190$). However, the overall pattern was similar to the qualitative results. The injury and aged groups exhibited the highest average thicknesses, while FBM-treated groups showed slightly reduced values, followed by the sham groups with the lowest means. This trend was observed in both the medial and lateral regions. On the other hand, JSW at the knee and hip, which is a standard clinical indicator of knee joint degeneration, was not different in the knee or hip across groups either statistically or by mean trends (Fig 3).

DISCUSSION: This preliminary analysis investigated the direct causal relationship between LIVD degeneration and distal joint degeneration as well as FBMs role in modulating those responses. Although no statistically significant group differences were detected in the data collected thus far, consistent directional trends were observed across overall qualitative assessments and bone features integrated with the articular cartilage. These patterns may indicate that LIVD injury accelerates knee damage, while FBM treatment may reduce observed bone remodeling features indicative of degradation. However, these are not corroborated by the typical clinically observed joint space narrowing. This could reflect the limited sample size and the preliminary nature of this analysis, which currently includes only three of five samples from each group. However, it could also suggest a more complex systemic mechanism. As in-depth morphometric protocols are refined and expanded to include parameters such as bone volume fraction (BV/TV), trabecular thickness (Tb.Th), and trabecular number (Tb.N), additional insight into joint adaption is expected. Integration of histological outcomes will reveal alterations in articular cartilage itself not assessable from microCT. Even if the current observed trends are refuted demonstrating no inter-joint effects, the results are significant. They would support the current methods of treating each joint in relative isolation.

SIGNIFICANCE/CLINICAL RELEVANCE: Lower back pain related to LIVD degeneration and lower limb joint degeneration are common co-morbidities. However, they are treated largely independently, and it is unclear if the concurrence is due more to genetic predisposition or a chain reaction causing cross-joint degeneration via mechanical or systemic factor alteration.

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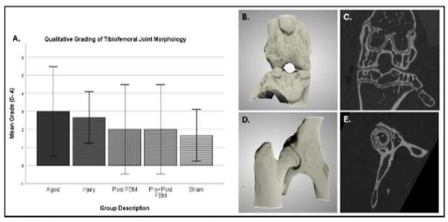

Figure 1. Qualitative grading and representative microCT reconstructions of the knee and hip joints. (A) Mean qualitative grading of knee morphology using a modified microCT based Kellgren-Lawrence scale (0 = normal, 4 = severe). Bars represent mean ± SD (n = 3 per group). Grading showed no significant differences ($p = 0.359$) but paralleled SCB.Th trends, with injury and aged groups scoring higher than FBM-treated and sham groups. (B-E) Representative microCT reconstructions illustrating joint morphology.

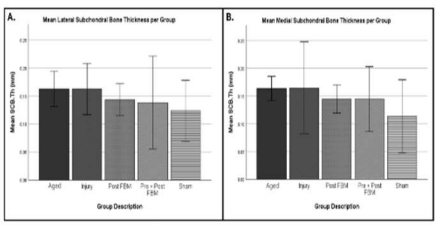

Figure 2. Mean subchondral bone thickness (SCB.Th) across experimental groups. (A) Medial tibial plateau. (B) Lateral Tibial Plateau. Bars represent mean ± SD (n = 3 knees per group). One-way ANOVA showed no significant group differences for either region (medial: $p = 0.107$, lateral $p = 0.190$). Injury and aged groups exhibited the highest mean SCB.Th, while FBM-treated groups trended lower, followed by sham controls.

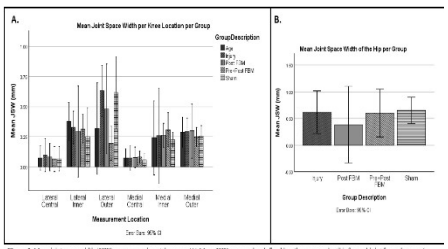

Figure 3. Mean joint space width (JSW) across experimental groups. (A) Mean JSW measured at defined locations across the tibiofemoral joint for each group (n = 3 knees per group). (B) Mean JSW of the hip joint measured along the femoral head-acetabular axis. Bars represent mean ± SD. No statistically significant differences were observed among groups ($p > 0.05$).