Adipose-Derived Complement Factors Drive Knee Pain with Sham and Knee Injury in Aged Male Mice

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INTRODUCTION: Osteoarthritis (OA) is the leading cause of chronic pain and disability worldwide[1] for which there are no disease-modifying interventions and pain management strategies are inadequate. While aging is a risk factor for developing OA[2], most preclinical OA studies focus on young mice. Moreover, we have demonstrated the role of adipose tissue and secreted factors in the pathogenesis of OA and pain[3,4]. Recent work suggests adipose tissue is a depot of pro-aging mediators driving the aging milieu. Aging alters both joint tissues and systemic physiology, and adipose tissue secreted factors modulate inflammation and pain[1,3,4], and impair regenerative capacity[4]. To delineate mechanisms of OA with aging, we validated a knee injury in 18-month-old male mice and examined their adipose secretome. We posit that destabilization of the medial meniscus (DMM) surgery in aged mice induces rapid, progressive OA phenotypes, demonstrating detectable disease at 1 month post-DMM. By employing a novel TurboID proximity labeling technique to tag adipocyte-secreted proteins with high spatial precision *in vivo*[5], we will identify circulating factors secreted from aged adipose tissue. We **hypothesize** that superimposing DMM on the aging milieu will drive rapid OA progression similar to that achieved in three months post-DMM in adult mice. As a secondary hypothesis, we wish to assess if aged injured mice exhibit increased circulating complement factors secreted by fat compared to aged naïve mice.

METHODS: Chow-fed male C57BL/6J mice were challenged with unilateral DMM, sham, or no surgery (naïve) at 18 months (N=10-25/group). Baseline knee pressure-pain hyperalgesia and side-by-side limb loading were assessed using the Small Animal Algometer (SMALGO) and Static Incapacitance Test, then repeated at 2 and 4 weeks post-surgery. Whole-body composition was measured via dual-energy X-ray absorptiometry (DXA) prior to the endpoint. Prior to surgery, mice received retro-orbital injections of adeno-associated viral (AAV)-TurboID or no injection to allow targeted infection of adipose depots. The AAV-TurboID construct drives adipocyte-specific expression of TurboID, a biotinylating protein, under the adiponectin promoter (Fig. 2A). Three days before sacrifice, biotin water was administered to enable labeling of TurboID-tagged adipocyte-secreted factors. Mice were sacrificed at 19 months of age. Knee joints were harvested, processed, stained with Safranin O/Fast Green and H&E, and assessed using Modified Mankin, osteophyte, and synovitis scoring. Data were analyzed as 1- or 2-way ANOVA with Tukey's *post hoc* test. Inguinal white adipose tissue (subcutaneous), epididymal white adipose tissue (visceral), and brown adipose tissue depots were collected for V5 and Streptavidin-HRP blots to confirm AAV-TurboID expression and biotinylation prior to serum and synovial fluid untargeted proteomic profiling. Biotinylated proteins were isolated via streptavidin pulldowns and analyzed by liquid chromatography-mass spectrometry (LC-MS) (Fig. 2B). Untargeted proteomics data were processed and aligned in Spectronaut. These procedures were approved by IACUC. This study was performed in male mice as a proof-of-concept to establish feasibility of the AAV-TurboID approach in aging; aged female studies are ongoing.

RESULTS: Aged DMM mice demonstrated worsened joint structural damage, osteophyte formation, and synovial inflammation compared to sham and naïve controls (Fig. 1B), as shown by significantly increased Modified Mankin scores (Fig. 1C), osteophyte scores (Fig. 1D), and synovitis scores (Fig. 1E). At 1 month post-DMM, the extent of structural degeneration in aged mice was comparable to later timepoints typically observed in adult DMM models[1,3,4]. Significantly reduced pressure-pain thresholds were observed in DMM mice, consistent with increased knee hyperalgesia. Of interest, sham mice had significantly increased hyperalgesia compared to naïve, but less than DMM (Fig. 1F). Greater weight-bearing asymmetry was observed in DMM mice, with sham groups again presenting more asymmetry in the injured than naïve, but less than the asymmetry due to DMM (Fig. 1G). DXA analysis confirmed that total body mass (Fig. 1H) and body fat composition (Fig. 1I) did not differ significantly between groups, indicating that these histological and pain outcomes were not attributable to weight or adiposity differences. Streptavidin-HRP blot analysis confirmed robust TurboID labeling across systemic fat depots, indicating that the virus was present in systemic adipose depots (Fig. 2C). Finally, serum and synovial fluid profiling revealed distinct systemic and local proteomic signatures by injury status. DMM and sham-operated mice exhibited elevated circulating complement factor C3 (C3) (Fig. 2D) and apolipoprotein A-I (ApoA1) (Fig. 2E), while within the synovial fluid, increased levels of Transforming Growth Factor Beta-1 (Tgfb1) protein was observed compared to naïve mice (Fig. 2F). Neuron Navigator 1 (Nav1) was elevated in the synovial fluid of DMM mice (Fig. 2G), when compared to sham and naïve mice.

DISCUSSION: The superimposition of aging and DMM uniquely captures rapid OA progression in one month, producing similar damage levels observed over three months in adult mouse studies. These data demonstrate that the aging milieu can accelerate injury-driven damage and pain. Surprisingly, in this context, sham surgery is not a control for pain, as the sham group demonstrated significant increases in knee hyperalgesia and static limb loading in sham-operated aged mice compared to naïve, but less than DMM. Using TurboID proximity labeling, we are able to specifically trace adipose-derived proteins in circulation and within the joint. Notably, the two most significantly increased factors due to DMM or Sham in serum are C3 and ApoA-I. This demonstrates that factors driving OA disease and pain originate from adipose tissue and are enmeshed with aging. Moreover, these findings are consistent with our previous work demonstrating the role of fat-secreted complement factors in OA pathogenesis and pain. While ApoA1 has been implicated previously in OA pathogenesis as a damage-associated molecular pattern in the joint[6], we observe increased systemic ApoA1 with DMM. Systemic increases in ApoA1 are observed in cognitive decline, including Alzheimer's disease, with aging, which is consistent with our emerging hypothesis that knee injury may drive organismal aging processes within and beyond the joint. Synovial fluid increases in Nav1 and Tgfb1 were increased with sham and DMM. These two factors have been implicated in OA, aging, obesity, and pain previously[7,8]. Nav1.7 and Nav1.8, isoforms of the Nav1 sodium channel, have known roles in OA pain[9], although long-term strategies to target these channels have mixed success[10], and new drugs that target these pathways are under active investigation. Nav1 increases in both sham and DMM groups demonstrate that the aging milieu can confer vulnerability to the local environment of the joint, and induce pain-related factors in OA. It is unknown how fat-secreted C3 and ApoA1 can drive Nav1 or Tgfb1 signaling in the joint, but is the subject of further studies. Together, these findings reveal a potentially causal axis in which systemic aging and adipose-derived factors exacerbate OA joint pathology, and in particular, pain. The links between complement signaling, ApoA1, Tgfb, and Nav1 remain to be clarified, but offer new druggable mechanistic links between adipose tissue in the pathophysiology of OA pain and structural damage in aging.

SIGNIFICANCE/CLINICAL RELEVANCE: These data establish a rapidly occurring aged knee injury model as a physiologically relevant framework to interrogate age-dependent mechanisms of OA structure and pain. We demonstrate that the aging milieu drives joint degeneration and pain through systemic adipose-derived factors. These findings position adipose tissue as a critical mediator of OA pathogenesis, identify candidate circulating proteins as actionable targets for which FDA drugs exist, and provide a translational foundation for clinically relevant, age-specific therapies aimed at preserving healthy aging and joint health, mitigating chronic pain, and addressing the broader systemic consequences of OA on health span in older patients.

REFERENCES: [1]Collins+ PNAS 2021; [2]Goldberg+ Immunol Rev; [3]Tjandra+ Bioxriv 2025; [4]Collins+ Sci Adv 2025; [5]Wei+ Nat Chem Biol 2020; [6]de Seny+ PLoS One 2015; [7]Malfait+ Clin Geriatr Med 2022; [8]Kaya+ Bone Rep 2022; [9]Fu+ Nature 2024; [10]Miller+ Arthritis Rheumatol 2017.

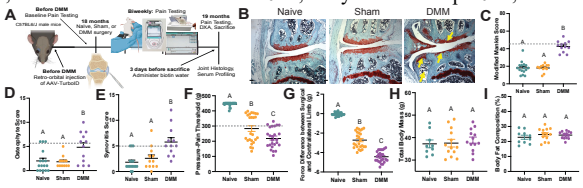

Figure 1. (A) Experimental design, (B) representative images of Safranin O/Fast Green-stained medial tibial sections of 19-month-old mice with no surgery (naïve), sham, or DMM, (C) Modified Mankin scores, (D) osteophyte scores, (E) synovitis scores, (F) pressure-pain thresholds, (G) static limb loading differences, (H) total body mass, and (I) body fat composition. Dotted lines on graphs represent median scores from adult cohorts. Data were analyzed as 1- or 2-way ANOVA with Tukey's *post hoc* tests (N=10-25/group). P<0.05 between groups is indicated by letters "A", "B", and "C." Scale bars indicate 400μm.

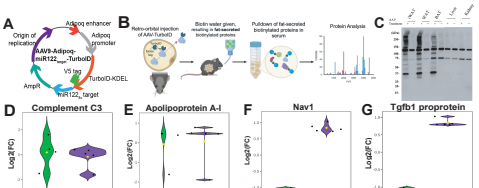

Figure 2. (A) AAV-TurboID construct map, (B) TurboID experimental design, (C) Streptavidin-HRP blot reveals adipocyte-specific infection, (D-E) protein factors define the serum and (F-G) synovial fluid profiles of aged male mice with knee injury (N=4-5/group).