The Aging Milieu Drives Host Response to Gut Microbiome Alterations and Osteoarthritis in Male and Female Mice

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INTRODUCTION: Osteoarthritis (OA), the loss of cartilage lining of the joint, disproportionately affects the aging population. Although it is historically believed to be a consequence of mechanical loading, OA is increasingly understood to be a disease driven by systemic factors that are impacted by the aging milieu. We and others have demonstrated the role of the gut microbiome, a critical regulator of systemic inflammation in bone health and OA. However, despite the prevalence of OA in older populations, studies investigating the gut microbiome-joint axis have been largely constrained to young animals. The mechanism by which the gut microbiome may impact the aging joint remains unknown. Moreover, there is a need to understand the impact of altering the native microbiota in aged models on knee joint health, as it could be a viable treatment strategy for OA. The purpose of this study is to determine if manipulation of the gut microbiota late in life can influence age-related or spontaneous OA, and whether sex is a significant biological variable driving host response in this context. We **hypothesize** that manipulation of the gut microbiota late in life will exacerbate age-related knee OA progression in male and female mice.

METHODS: Male and female mice (n=12-20) received selective depletion of gut microbiome species through administration of antibiotics via drinking water (1 g/L ampicillin + 0.5 g/L neomycin) from weaning to 22 months of age (continuous) and compared to mice with unmodified microbiota (unaltered). To determine whether changes to the gut microbiome can impact established age-related OA, the initial group (n=17-24) experienced selective depletion from weaning until 18 months of age followed by reconstitution through fecal microbiota transplant from age- and sex-matched donors (initial). The delayed group (n=11-14) experienced selective depletion only from 18 to 22 months of age to model late-life microbiome disruption. Joints were collected at 22 months of age for structural assessment. Naturally occurring age-related changes to the cartilage and surrounding soft tissues were histologically assessed using Modified Mankin scoring system. To quantify subchondral bone sclerosis, micro-computed tomography (μCT) scans were taken of the proximal tibia. Inflammatory biomarkers in the serum were collected and quantified using multiplex ELISA. Gut microbiome composition was analyzed by shotgun metagenomics on stool samples at 22 months. Statistical analysis was performed on histological scores and μCT analysis, using non-parametric Kruskal-Wallis test with Dunn's post-hoc analysis. Mann-Whitney test was used to determine changes in inflammatory markers between initial and unaltered or continuous dosing groups. Permutational multivariate analysis of variance (PERMANOVA) was used to determine differences between Bray-Curtis beta diversity among treatment groups. Statistical significance is defined as a p-value ≤ of 0.05. Experimental procedures were approved by the Institutional Animal Care and Use Committee.

RESULTS: Male mice in the initial group demonstrate greater Modified Mankin scores compared to the unaltered and continuous groups (p=0.04, p=0.02), despite receiving a fecal microbiota transplant of the microbiomes of the unaltered mice. There was a trend towards increases in Modified Mankin scores in the delayed group compared to the continuous group (p=0.11) (Figure 1A). In female mice, the initial group had higher Modified Mankin scores compared to the unaltered group (p=0.02) and an increasing trend in Modified Mankin scores compared to the continuous group (p=0.09) (Figure 1B). Initial and delayed groups in both sexes had greater Modified Mankin scores compared to young naïve mice demarcated by the grey dashed line in Figure 1A&B). The initial group in female mice demonstrated increased subchondral plate thickening compared to the continuous group (p<0.01), although there were no differences in subchondral bone thickness in male mice (Figure 1A&B). Circulating factors of adiponectin and leptin were decreased in the male initial group (p=0.02, p=0.02) compared to the unaltered group and trending decreases in adiponectin when compared to the continuous group (p=0.15). There were no changes in insulin-like growth factor 1 (IGF-1) or IL-6 levels in male mice (Figure 1C). In female mice, the initial group had higher circulating levels of IGF-1 than the unaltered and continuous groups (p=0.04, p<0.01) and greater levels of IL-6 than the unaltered group (p=0.04) (Figure 1D). There was a trend in lowered adiponectin levels in the initial group when compared to the unaltered group, but no differences in leptin. Shotgun metagenomics of the microbiome compositions confirmed similarity between initial and unaltered groups at 22 months with distinct clustering of the continuous and delayed groups in both sexes (Figure 1E&F).

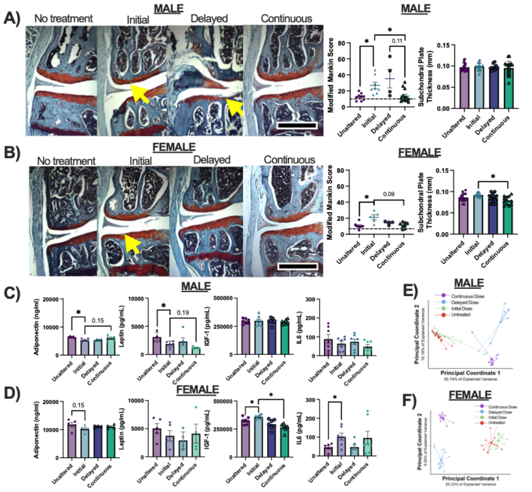

Figure 1: (A,B) Representative histological slides indicating cartilage damage (yellow arrow) male and female mice. Grey dashed line demarcates the average Modified Mankin scores in young male (Modified Mankin score = 6) and female naïve mice (Modified Mankin score = 10) at 6 months old. Initial and delayed groups have greater Modified Mankin scores compared to average young mice (n=4-13 dosage/sex). Subchondral bone sclerosis is present in the initial group in female mice (n=11-24 dosage/sex). (C,D) The initial group had changes in circulating factors when compared to unaltered and continuous groups in both sexes. (n=5-7 dosage/sex) (E,F) Initial and unaltered groups showed overlap in gut microbiota composition. Bars represent mean ± SEM. * denotes p≤0.05.

DISCUSSION: Reconstitution of a normal gut microbiota late in life accelerates age-related joint damage and is associated with alterations in systemic inflammatory factors in aged male and female mice models. Surprisingly, the initial dosing group demonstrated more severe damage in the joint despite having similar microbial composition after reconstitution. More compelling still, lifelong selective depletion of the gut microbiome in the continuous group did not exhibit any additional age-related joint damage, indicating that the microbiota resulting from the selective depletion approach (ampicillin+neomycin) may not disrupt systemic inflammation pathways that drive OA. While the delayed group exhibited some joint damage compared to young naïve mice, further samples are needed to reach a definitive conclusion about the effect of delayed dosing in spontaneous OA. Absolute magnitude of the severity of disease in females was lower than males, similar to what has been reported previously (PMID 37096632). Overall, these findings indicate that a rapid shift in gut microbiota composition, rather than the composition itself, drives joint damage in age-related OA independent of sex. Although several studies have demonstrated germ free or antibiotic-induced microbial depletion results in less susceptibility to surgically induced OA, our model takes a different approach by investigating spontaneous OA. Similar perturbations of the gut microbiome did not lead to OA damage in young mice (PMID 30240938), suggesting complex interactions between age and drastic shifts in the gut microbiota. Alterations in circulating inflammatory factors leads us to posit that systemic inflammation mediates joint damage due to late in life microbiome perturbations. While others have reported that reconstitution of a normal microbiota into germ free mice can cause transient changes in systemic factors that regulate bone (PMID: 27821775), the directionality in the relationship between inflammation, age, and the gut-joint axis remain to be clarified. Our future work will investigate the role of systemic inflammation in aged models of gut microbiome manipulation to uncover underlying causative mechanisms.

SIGNIFICANCE: Age increases the vulnerability of the joint to shifts in systemic factors caused by rapid change to the gut microbiome, further supporting the idea that OA is a systemic disease that is impacted by changes outside of the knee joint.