Prebiotic Manipulation of the Gut Microbiome Confers Protection Against Osteoarthritis in Obese, Type 2 Diabetic Mice

Eric Schott1,2, Christopher Farnsworth1,2, Sara Soniwala1, Madison Doolittle1, Jun Zhang, Alex Grier1, Steven Gill1, Robert Mooney1,2, Michael Zusick1,2
1Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester NY, 2Center for Musculoskeletal Research, University of Rochester Medical Center, Rochester NY.

INTRODUCTION: Obesity-induced type 2 diabetes (T2D) is a major risk factor for the development of osteoarthritis (OA), as 47.3% of T2D patients have arthritis, and 66% of individuals with OA are either obese or obese/T2D. Despite the known association, the mechanism linking this comorbid condition has not been fully elucidated. Our recent data suggest that in obesity/T2D increased systemic inflammation may play a major role in disease progression. Both published and preliminary data establish that compared to non-obese, non-T2D OA patients, synovium from obese/T2D OA patients is inflamed with an increased presence of macrophages and monocytes. Intriguingly, recent evidence suggests that in obesity/T2D, the gut microbiome is altered, with an increase in bacterial phyla associated with inflammation and lymphocyte activation, leading to other pathologic states. Studies have shown that this obese gut microbiome can be altered by prebiotic supplementation, reverting the bacteria to those found in a lean, healthy gut. These findings lead us to hypothesize that prebiotic supplementation provides protection from OA in obese/T2D mice.

METHODS: Male C57BL/6 mice were fed a lean or high fat diet (HF) for 12 weeks, at which point they were provided a lean or high fat diet supplemented with a control non-digestible fiber (cellulose) or experimental prebiotic (oligofructose). After 2 weeks on the prebiotic diet, OA was surgically induced by a meniscal injury (DMM), and the mice were continued on the supplemented diets for 12 weeks. At the onset of prebiotic diets, fecal samples were collected and frozen every two weeks for 16S rRNA sequencing, and mice were weighed weekly. Before completion of the experiment, mouse body composition was analyzed by DEXA scan, and metabolic status was investigated by glucose tolerance test. 12 weeks after injury, mice were sacrificed, at which point serum, knee joints, small intestine, and colon samples were collected. Serum samples were analyzed for cytokine levels, free fatty acid and endotoxin content. Fecal samples were analyzed for bacterial abundance by 16S rRNA sequencing. OA status was determined by a modified OARSI scoring system, and additional outcomes including cartilage area, chondrocyte numbers and osteophyte size were measured by histomorphometric analysis.

RESULTS: Obese mice supplemented with the oligofructose prebiotic had improved metabolic status and decreased serum cytokine levels, while maintaining an equal weight and similar body fat percentage compared to those on the control cellulose supplement. Oligofructose supplementation provided protection against cartilage degeneration (Fig. 1A) and synovial hyperplasia in mice on a high fat diet, as indicated by improved OARSI (Fig. 1B) and synovial (Fig. 1C) scores, as well as increased femoral and tibial unciliated cartilage areas (Fig. 1D), increased Safranin-O positive chondrocytes, reduced frequency and size of osteophytes, and decreased hypertrophic chondrocyte numbers. Even in lean animals, oligofructose provided some protection against OA, with an increased number of Safranin-O positive chondrocytes, increased presence of macrophages and monocytes. Oligofructose supplementation provided protection against OA progression in obese mice, with a marked increase in the abundance of species in the microbial phylum Actinobacteria (Fig. 1E), with Bifidobacterium pseudolongum, a commensal gut microbe associated with decreased inflammation, primarily responsible for this increase. A decrease in the ratio of Bacteriodes/Firmicutes was detected in the HF cellulose group, consistent with a proinflammatory shift in gut microbiome populations (Fig. 1E). Supplementation with oligofructose reversed this effect of the HF diet, providing further protection against systemic inflammation.

DISCUSSION: Obese/T2D patients have an increased risk of developing OA; however, as we and others have shown, increased biomechanical loading due to increased body mass does not explain this effect. Rather, increased systemic inflammation leads to tissue specific effects at the knee joint including increased local inflammation, synovial hyperplasia, immune cell infiltration, and cartilage degeneration. We hypothesized that this increased systemic inflammation stems from an alteration to the gut microbial flora as a result of the HF diet, and that prebiotic supplementation can mitigate these effects. Mice supplemented with the oligofructose prebiotic displayed a marked increase in the abundance of Bifidobacterium pseudolongum, a commensal gut Actinobacteria microbe associated with decreased inflammatory effects. Furthermore, the Bacteriodes/Firmicutes ratio was increased in oligofructose, a shift that is suggested to provide additional anti-inflammatory benefits. Regarding OA, oligofructose supplemented mice had improved OARSI scores compared to cellulose controls, as well as increased cartilage area, decreased synovial hyperplasia, and fewer osteophytes. As the greatest reduction in systemic inflammation was in the obese mice in response to oligofructose, it is possible that the protective effect of the prebiotic is due to the Bifidobacterium’s ability to crowd out organisms that typically dominate in the HF diet environment and are pro-inflammatory, leading to reduced overall systemic inflammation. Overall the switch to predominance by Bifidobacterium pseudolongum coupled with the increased Bacteriodes/Firmicutes ratio in oligofructose supplemented mice may support protection against the pro-inflammatory state that exists in the HF diet. With these findings, alteration of the gut microbiome could prove to be a useful therapeutic strategy to combat the degenerative processes involved in OA progression.

SIGNIFICANCE: Obese/T2D patients have a significantly elevated risk of developing Osteoarthritis, which is the most prevalent disease in the US, and currently has no cure. Alteration of the gut microbiome could be a major step forward in transforming the treatment options for this debilitating disease, and further studies investigating mechanism, as well as the effects of other pro- and prebiotics are essential.