verified using UPARSE algorithm. Annotation and classification hierarchy were analyzed using RDP classifier, PyNAST and GreenGenes database. Serum lipidomic profiles were characterized using gas chromatography along with tandem mass spectrometry. Chondrocytes and calvarial osteoblasts were isolated from 3-day-old mice. Chondrocytic makers and osteogenic gene expression were quantified using RT-quantitative PCR.

Results: Old mice showed severe articular cartilage erosion and sparse subchondral bone microstructure. Likewise, OARSI scores of articular compartment were increased together with significant reductions in bone mineral density (BMD), trabecular volume (BV/TV, %) and thickness (Tb.Th/mm) of subchondral bone. Microorganisms-derived short-chain fatty acids, like butyric acid, caproic acid, and caprylic acid, etc. were significantly decreased in age mice, whereas long-chain fatty acid levels were higher than young mice. Microbiome profiles of 28 families of microorganisms were significantly altered in aged mice. Of them, microorganisms related to probiotics, like Lactobacillacea, were reduced, whereas bacteria relevant to metabolic syndrome, like Lachnospiraceae, were upregulated. In vitro, butyrate treatment attenuated extracellular matrix proteoglycan underproduction and aggrecan expression loss in inflamed chondrocytes, as well as ameliorated mineralized matrix accumulation and osteogenic gene expression in inflamed osteoblasts.

Conclusions: Gut microbiome is correlated with age-mediated articular cartilage and subchondral bone loss. Dysbiosis alters short-chain fatty acid metabolism, which is important to stabilize extracellular matrix production in chondrocytes and osteoblasts to sustain cartilage and subchondral bone integrity. This study offers a productive insight into the communication of gut microbiome to host chondrocytes and osteoblasts in the development of joint degeneration. Control of short-chain fatty acid metabolism in joint microenvironment has the perspective of remedial effects to ameliorate age-induced joint damage.

4
DISTINCT MURINE CARTILAGE MICROBIAL DNA SIGNATURES ARE SEEN IN HIGH FAT DIET-INDUCED OBESITY AND AGING

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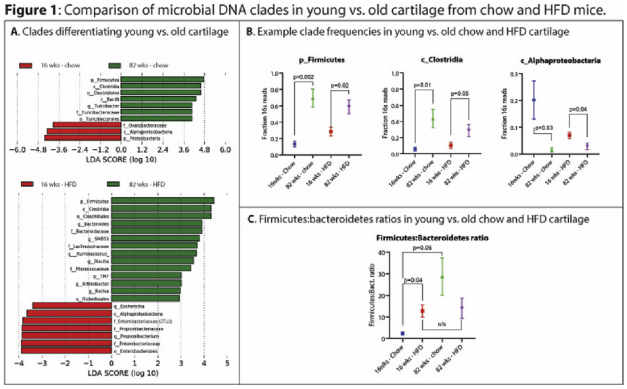
Purpose: The strongest nongenetic risk factors for primary knee OA are advanced age and obesity. We have previously demonstrated that human cartilage exhibits a microbial DNA signature which changes in association with the development and progression of knee OA. In this study, we hypothesized that aging and obesity, known to cause gut microbiome shifts, would also produce shifts in cartilage microbial DNA patterns in mice.

Methods: Young (8 week old, n=10) and aged (74 week old, n=10) male C57BL/6J mice were divided into equal groups and randomly assigned to chow (NIH-31) or high fat (RD D12492, 60% kcal fat) diet for 8 weeks. Mice were then sacrificed at 16 weeks and 82 weeks of age and knee articular cartilage collected using sterile technique and flash frozen in liquid nitrogen. Cartilage was cryogenically ground using a Bertin cryolys evolution and DNA extracted using a Qiagen DNEasy kit. The V3 and V4 regions of the bacterial rRNA gene were amplified using a high-fidelity DNA polymerase and Illumina Nextera XT indices attached. All plasticware and PCR reagents were decontaminated by UV exposure and dsDNAse treatment, respectively. Deep sequencing of amplicons was performed on an Illumina HiSeq. Operational taxonomic units (OTUs) were assigned in QIIME 1.9.1 using the Greengenes 13_8 97% representative reference set. Group composition differences were compared by Linear Discriminant Analysis Effect Size (LEfSe, LDA-effect sizes ≥ 2 or ≤ -2 were considered significant) following rarefaction.

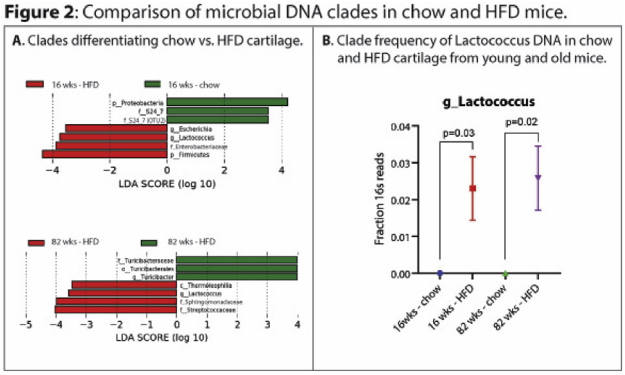
Figure 1: Comparison of microbial DNA clades in young vs. old cartilage from chow and HFD mice.



Results: Several clade differences were seen comparing young and old cartilage. In both HFD and chow animals, aging was associated with expansion of DNA from members of the phylum *Firmicutes* (LDA ES 4.8, p=0.009 for chow young vs. old, LDA ES 4.4, p=0.04 for HFD young vs. old, Figure 1A,B), specifically class *Clostridia* (LDA ES 4.6, p=0.009 for chow, LDA ES 4.3, p=0.03 for HFD, Figure 1A,B) , along with reductions in DNA from members of class *Alphaproteobacteria* with age (LDA ES 4.3, p=0.02 for chow, LDA ES 3.6, p=0.04 for HFD, Figure 1A,B). Comparing chow to HFD young mice, phylum *Proteobacteria* was expanded in chow (LDA ES 4.2, p=0.03) whereas members of phylum *Firmicutes* were expanded in HFD mice (LDA ES 4.3, p=0.02, Figure 2A), specifically including genus *Lactococcus* (LDA ES 3.7, p=0.005 Figure 2 A,B). Comparing chow to HFD old mice, chow were enriched in order *Turicibacterales* (LDA ES 4.0, p=0.02, Figure 2A), whereas HFD were enriched in class *Thermoleophilia* (LDA ES 3.5, p=0.02, Figure 2A) and genus *Lactococcus* (LDA ES 3.6, p=0.02, Figure 2A,B). We also noted significant increases in the Firmicutes:Bacteroidetes ratio in both HFD and aging (chow young vs. old 2.3±0.7 vs. 29±9, p=0.05, young chow vs HFD 2.3±0.7 vs. 13±3, p=0.04) Figure 1C.

Conclusions: Cartilage microbial DNA patterns vary with nongenetic OA risk factors, including obesity and aging. Previous studies have shown increases in the Firmicutes:Bacteroidetes ratio in the gut microbiome associated with both obesity and aging, we identified a similar pattern within articular cartilage. Future studies should investigate the relationship between cartilage microbial DNA patterns and the gut microbiome, and any direct pathophysiological relationships between cartilage microbial DNA and OA pathogenesis, particularly given the immunogenicity of certain bacterial DNA motifs and emerging literature regarding chronic intraarticular innate immune activation and OA.

Figure 2: Comparison of microbial DNA clades in chow and HFD mice.



5
SYSTEMIC INCREASE OF PRO-INFLAMMATORY MONOCYTES CAUSED BY WESTERN DIET FEEDING IS INDEPENDENT OF IL-1β AND NOT REFLECTED IN INFLAMED SYNOVIUM DURING COLLAGENASE INDUCED OA

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Purpose: Metabolic syndrome is a risk factor for osteoarthritis (OA), which suggests metabolic regulation of OA pathology. Western diet feeding of mice has been shown to result in systemic reprogramming of monocytes to become hyperreactive, which was dependent on IL-1β. Even though we previously showed that IL-1β is not important for the development of pathology in collagenase-induced OA development (CiOA), this might be different in a CiOA model where monocytes are metabolically rewired. Our lab previously showed that Western