Multiomic Single Nucleus Sequencing And Spatial Metabolomics Reveal Metabolic Dysregulation In Fetal Chondrocytes Exposed To The Obese Uterine Environment

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Disclosures: AKO (Agathos-1,7A), KLL (N), LAB (N),TL (N), BZ (N), FG (Cytex-3A, 4 and Agathos-1,7A)

INTRODUCTION: Obesity is a primary risk factor for osteoarthritis (OA) through multiple mechanisms, including altered joint loading, low grade systemic inflammation, and disrupted systemic metabolism which impairs cartilage health (1). Furthermore, our recent study shows that the effects of obesity can be transmitted from one generation to the next, and parental obesity increases OA severity independent of postnatal diet (2). Two generations of offspring from high fat diet (HFD) fed parents were heavier, showed increased adiposity, and had an increased risk for developing injury-induced OA despite being raised on a control diet (2). These data suggest that OA has developmental origins, though the specific mechanisms linking parental obesity to offspring OA risk are unknown. Unbalanced *maternal* diet during critical windows of fetal development can shift metabolic pathways and compromise cellular function to program an increased susceptibility to cardiometabolic disease, reproductive dysfunction, and osteoporosis in the aging offspring (3). Therefore, we hypothesized chondrocytes of fetal offspring from HFD-fed dams (mothers) may display metabolic shifts that increase susceptibility to OA in adulthood.

METHODS: With IACUC approval, wildtype female C57BL/6J mice were randomly assigned to either control (Research Diets #D19052001) or omega-6 enriched HFD (Research Diets #11120105) for 12 weeks before mating and sacrificed at embryonic day 17.5 (E17.5). Fetal knee joints were collected for multiomic single nucleus assay for transposase accessible chromatin (snATAC-seq) and single cell RNA sequencing (scRNA-seq) using the 10X Chromium platform (n=3/per group). Data were aligned using cell ranger ARC v2.0.1 and QC metrics were evaluated and filtered to remove cells with n-feature counts outside 200-2,500 or mitochondrial counts greater than 5% using Seurat v4.3.0. Principle component analysis was performed on the snRNA seq data to cluster cell populations which were defined by transcription of known cell specific genes. RNA fluorescence in-situ hybridization (RNA-FISH) was used to spatially validate cluster-specific gene expression (n=4/group). Differentially expressed genes (DEGs) in the chondrocyte cluster were identified and pathway analysis was performed using EnrichR. Spatial metabolomics of E17.5 knee joints from offspring of control and HFD-fed dams was performed using the timsTOF flex MALDI-2 MetaboScape mass spectroscopy platform to visualize the presence of specific metabolites and identify metabolic shifts in chondrocytes (n=3/group).

RESULTS: Multiomic snRNA-seq of E17.5 fetal knee joints revealed 15 cell clusters which were identified based on expression of known gene markers and spatially validated using RNA FISH (Fig 1 A-C). To further understand the role of maternal diet on chondrocyte gene expression we looked at differentially expressed genes (DEGs) in the chondrocyte cluster. Our analysis revealed 141 DEGs and a pathway analysis showed multiple metabolic pathways altered in chondrocytes exposed to maternal HFD (Fig 1D). Overall, genes related to glucose metabolism were decreased, while genes related to fatty Acyl-CoA biosynthesis were increased in chondrocytes from HFD-fed dams (Fig 1E). Specifically, the upregulated *Elovl6* gene encodes a fatty acid elongase required for synthesis of fatty acids such as oleate (18:1). To validate if changes in metabolic gene expression resulted in altered metabolite presence in E17.5 chondrocytes, we performed spatial metabolomics. We observed increased presence of the oleate containing phospholipid LysoPE (0:0/18:1) in chondrocytes of offspring exposed to maternal HFD (Fig 1F).

DISCUSSION: We previously showed both *parental* (2) and *maternal* (4) obesity increases OA severity in adult offspring in a sex-specific manner (2). Here we show the *maternal* obese environment by itself alters key developmental and metabolic pathways in fetal chondrocytes of their offspring. These results are in line with others showing maternal obesity shifts metabolism in the liver, heart, adipose, and bone tissues (3). Chondrocytes are avascular and rely primarily on glycolysis for energy production. However, under inflammatory conditions chondrocytes dynamically shift metabolic pathways to further increase glycolysis and decrease mitochondrial respiration and fatty acid oxidation (1,5). It is theorized these shifts may be adaptive mechanisms to increase energy requirements under inflammatory conditions (5). Therefore, we reason that early metabolic insults may program decreased metabolic plasticity in chondrocytes throughout the lifespan to increase susceptibility to OA. However, it is yet to be determined if metabolic shifts in the chondrocytes exposed to the maternal obese environment persist into adulthood. Alternatively, many metabolites are key co-factors for the epigenetic maintenance machinery (6) and future work is necessary to determine if metabolic shifts in chondrocytes exposed to maternal HFD epigenetically program intergenerational OA susceptibility.

SIGNIFICANCE: By demonstrating OA has developmental origins rooted in metabolic disfunction, our data has unlocked a novel therapeutic treatment window for treating metabolic dysfunction and OA initiation.

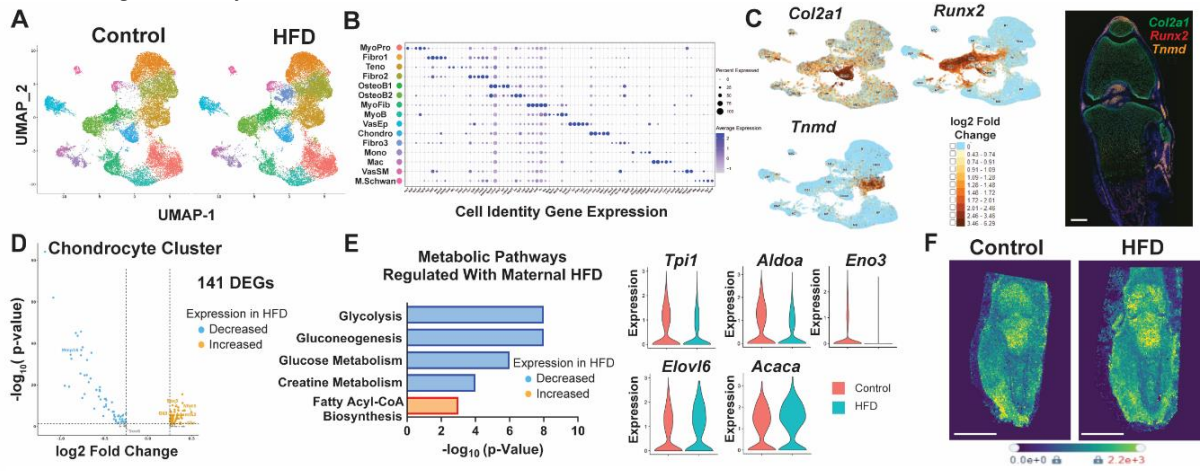

Figure 1. A) UMAP cell clusters in fetal knee joints from control and HFD dams and **B)** dot plot showing expression of cell identity genes driving cluster assignment. **C)** Cluster expression and RNA FISH of key genes expressed in cartilage (*Col2a1*), bone (*Runx2*), and tendon (*Tnmd*) (scale bar 200µm). **D)** DEGs and **E)** pathway analysis showing key changes in metabolic genes and **F)** spatial metabolomics showing increased LysoPE (0:0/18:1) in fetal chondrocytes from HFD dams (scale bar 1mm).

REFERENCES: (1) Batushansky+2022 (2) Harasymowicz+2019 (3) Oestreich+2017 (4) Oestreich+ ORS 2023 (5) Arra+2023 (6) Harvey 2019