Integrated metabolomic and transcriptomic analysis identifies joint acidosis as a defining feature of osteoarthritis

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INTRODUCTION: Osteoarthritis (OA), the most prevalent form of arthritis is characterized by progressive articular cartilage loss, synovial inflammation, and subchondral bone remodeling. Although traditionally seen as a biomechanical "wear and tear" disorder, recent work highlights metabolic and inflammatory dysregulation as central to OA progression. One poorly studied aspect of OA pathophysiology is joint acidosis, defined as a sustained decline in intra-articular pH. Clinical studies have consistently shown that synovial fluid in patients with advanced OA is more acidic than in healthy joints, with pH dropping below 6.8 in some cases. A recent study revealed localized acidification in cartilage relative to meniscus and surrounding joint compartments. Despite these findings, the functional impact of acidosis in OA remains largely unexplored, in part due to the lack of validated animal models demonstrating this metabolic phenotype in vivo. Whether acidosis is a byproduct of tissue degeneration or an active driver of inflammation and catabolism is unknown. Here, we provide the first comprehensive evidence that the destabilization of the medial meniscus (DMM) mouse model of OA develops intra-articular acidosis. Using untargeted LC-MS/MS metabolomics and transcriptomics, we establish acidosis as a defining feature of OA progression and link it to coordinated gene-metabolite networks.

METHODS: The study was approved by the IACUC committee. Male C57BL/6J mice underwent DMM or sham surgery at 12 weeks of age. Male mice were chosen exclusively in this pilot discovery study to minimize variability and maximize statistical power with limited resources. Future studies will extend to both sexes. Synovial fluid was collected from DMM and sham joints at 12-week post-surgery. Because of the small fluid volume per joint, fluid from two mice was pooled to generate sufficient volume for metabolomic profiling, representing N=6 biological samples per group analyzed as three pooled replicates. Untargeted LC-MS/MS profiling was performed, and data was processed with XCMS and MetaboAnalyst 5.0. Normalization, log-transformation, and univariate/multivariate statistics were applied. Pathway enrichment used KEGG, SMPDB, and MSEA libraries. Acidic metabolites were curated based on the presence of carboxylate, phosphate, or sulfonate moieties. RNA-seq data (GSE143447) from 12-week DMM and sham mouse joints were reanalyzed. A curated panel of 31 acid-responsive genes was grouped into four functional categories: (1) acid-base transporters, (2) hypoxia/acidosis response, (3) inflammatory mediators, and (4) metabolic enzymes. qPCR validation was performed in independent samples. Gene-metabolite correlations were calculated using Pearson's r. Acid burden plots regressed cumulative acidic metabolite intensity with gene expression. Cytoscape was used to visualize integrated gene-metabolite networks. Statistical significance was set $p<0.05$.

RESULTS: To investigate whether OA is associated with metabolic changes in the joint microenvironment, we performed metabolomics on synovial fluid from DMM and sham-operated mice. Untargeted profiling identified 87 metabolites upregulated and 16 downregulated in DMM joints compared with sham. Functional enrichment revealed alterations in amino acid metabolism, nucleotide turnover, and lipid remodeling (**Fig. 1A**). Many pathways contained intermediates with acidic groups, implicating metabolic acid stress as a component of OA progression. To directly interrogate joint acidification in the DMM model, we next performed a supervised analysis restricted to acidic metabolites. From a curated library of 103 acidic metabolites, 49 high-confidence metabolites were detected (**Fig. 1B**). Hierarchical clustering revealed distinct separation between DMM and sham. Representative acidic metabolites including 1-deoxyvaleric acid, taurodeoxycholic acid, inosinic acid, 2,4,6-trihydroxybenzoic acid, L-galactonic acid, and folinic acid were markedly elevated in DMM joints (**Fig. 1C**). Pathway analysis linked these metabolites to organic acid metabolism, one-carbon metabolism, and glycerophospholipid metabolism. To determine whether joint acidosis elicits coordinated transcriptional responses, we analyzed transcriptomics data, focusing on 31 genes linked to acid stress. Analysis revealed the induction of proton transporters (SLC16A1, SLC9A1), hypoxia markers (CA9, HIF1A), metabolic enzymes (LDHA, IDH1), and inflammatory mediators (IL1B, PTGS2). Correlation heatmaps revealed that expression of acid-linked enzymes (ENO1, ACSS2, IDH1) and inflammatory genes (IL1B, PTGS2) strongly associated with acidic metabolite abundance. Acid burden plots demonstrated positive correlation of SLC16A1 with cumulative acidic load, while PTGS2 and HIF1A exhibited negative correlations, suggesting feedback mechanisms to buffer acid stress (**Fig. 1D**). A bipartite network identified modules linking metabolites to inflammatory and metabolic genes, supporting acidosis as a coordinated multi-omic feature of OA (**Fig. 1E**).

DISCUSSION: This study provides the first validated evidence of joint acidosis in a preclinical OA model. While human studies showed reduced synovial pH, mechanistic insights were limited by lack of an animal model. Our findings demonstrate that DMM joints accumulate acidic metabolites beyond lactate, including amino acid- and lipid-derived acids, thereby broadening the concept of an "acidic metabolome." At the gene level, OA joints activated transporters, hypoxia pathways, and inflammatory mediators, consistent with adaptive responses to acidic stress. Integration revealed dense gene-metabolite networks, showing that acidosis is not a passive byproduct but an actively regulated process shaping inflammation and catabolism. This mirrors mechanisms reported in RA and cancer where acidic niches amplify tissue destruction. Our pilot design used only male mice for consistency, justified given the proof-of-concept nature of this work. Pooled synovial fluid represented N=6 biological samples per group, transparently reported here. Future studies with larger cohorts and both sexes are essential to assess variability and sex-specific responses.

SIGNIFICANCE: We establish the DMM model as the first validated in vivo system for joint acidosis in OA, integrating metabolomic and transcriptomic data. These findings reframe OA as a disease with metabolic and acid-base dysregulation. The model provides a platform to test whether correcting pH imbalance modifies OA progression, develop biomarkers of acidic metabolites, and design interventions targeting acid-sensing pathways and transporters. In summary, this study reframes OA as a disease with metabolic acidosis at its core and establishes the DMM model as a robust experimental platform for mechanistic and therapeutic studies.

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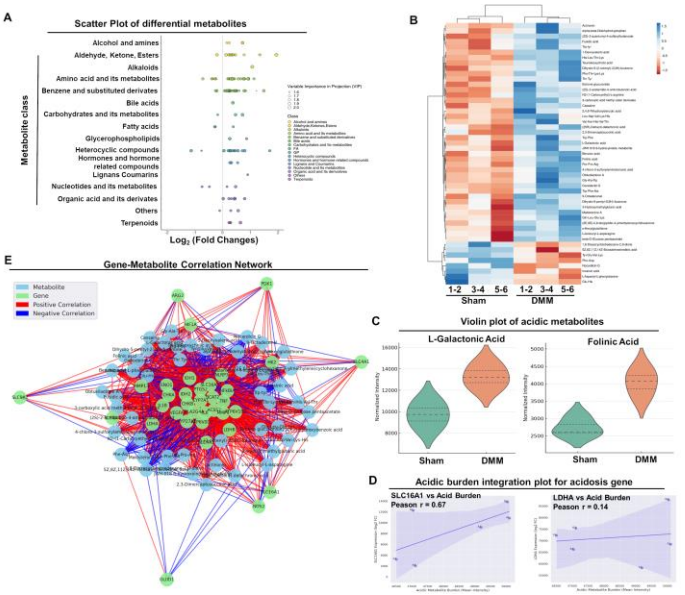

Figure 1. Integrated metabolomic and transcriptomic analysis reveals acidosis-associated alterations in OA joints (A) Scatter plot highlights enrichment of acidic metabolite classes; (**B**) Heatmap shows distinct clustering of acidic metabolites in DMM versus sham joints; (**C**) Violin plots demonstrate increased levels of representative acids; (**D**) Acid burden plots reveal correlations between metabolite intensity and acidosis-linked genes; (**E**) Gene-metabolite network illustrates coordinated acidosis-driven regulatory modules.