Investigating Mechanotransduction in Mouse Joints using Targeted Analysis of Central Metabolites

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INTRODUCTION: Osteoarthritis (OA) is a debilitating condition of the whole joint affecting over 600 million people worldwide[1]. Marathons runners have thicker cartilage compared to those with a more sedentary lifestyle[2]. These changes likely occur through mechanotransduction, the process by which mechanical forces are converted into biological signals. However, the short-term biological signals that link mechanical stimulation to cartilage tissue growth have yet to be fully understood. Metabolomics, the study of the small molecule metabolites, allows identification of cellular changes on a much shorter timescale than tissue changes as well as detection of specific pathways related to these changes. Targeted mass spectrometry allows us to quantify specific metabolites of interest. The metabolites in this study that are being targeted are those within central metabolism including TCA cycle as it an essential part of the chondrocyte metabolism and produces key non-essential amino acid precursors for pericellular matrix molecules like type VI collagen. Prior research finds that mice with running wheel access had differences in untargeted metabolic profiles compared to mice without wheels[3]. This previous study however didn't quantify specific metabolites and only used male mice. Many studies show sexual dimorphism when looking at joints and OA development. Improving understanding of the cellular processes that promote healthy cartilage and joints could lead to prevention and better treatment options for osteoarthritis. We hypothesize that since loading of joints supports cartilage health, mice that ran will have distinct expression of central metabolites compared to mice that did not run.

METHODS: C57BL6 mice were ordered (Charles River) in 4 groups (n=16/group) based on sex and age (22 weeks and 52 weeks). Mice within each category were randomized to either control or running groups. The mice were acclimated to cages with a locked running wheel (Med Associates Inc, ENV-047V) for 24 hours. The running group's wheels were then unlocked at 1PM and the distance ran was recorded while the control mice remained in a cage with a locked wheel. The following morning, mice were sacrificed at 8AM by cervical dislocation. Synovial fluid and tibial plateaus were extracted from a randomized hind leg. The metabolites from the synovial fluid were extracted using an 80:20 methanol:water solution overnight. The tibial plateaus were flash frozen and pulverized to increase surface area for the metabolite extraction in 100% methanol overnight. Both the tibial plateau and synovial fluid extractions were centrifuged, and the supernatant was dried down and reconstituted in 50:50 acetonitrile water. The extracted metabolites were analyzed using liquid chromatography mass spectrometry (LC-MS, Waters Synapt XS) on a C18 column. Known analytical standards of 17 key central metabolites were used to generate calibration curves to quantify metabolites within the samples. Linear models were created for each metabolite that was detected in more than 50% of samples. Variables included distance run, experimental group, age, sex and the interaction of age and sex. A step-down process was used to eliminate variables that did not impact the linear model until either all variables had a p-value of less than 0.05 or the next step would eliminate either distance run or experimental group from the model since those were the variables of interest. All p-values were multiplied by the number of linear models created for that sample type to correct for multiple comparisons.

RESULTS: After the night of running 9 of the 32 mice in the running group did not use the running wheel, forcing the creation of a third experimental group, failed to run. This most significantly affects the 22-week female group where 5 of the 8 mice did not use the running wheel. Within the tibial plateaus 10 of the 17 metabolites were present in over 50% of the samples and therefore linear models were created for the following metabolites, lactate, fumarate, malate, glutamine, glutamate, PEP, glucose/fructose, citrate, fructose-6 phosphate, and sedoheptalose-7-phosphate. There is strong evidence of increased glutamine in female mice compared to male mice when accounting for distance run and experimental group ($p<0.005$, Figure 1). There is some evidence of a difference in fumarate within the different treatment groups ($p=0.19$). 7 metabolites were present in more than 50% of the synovial fluid samples, fumarate, oxaloacetate, glutamate, glucose, citrate, fructose-6 phosphate and 6-phosphogluconate. After the linear models were performed there was strong evidence of increased fructose-6-phosphate in female mice when accounting for age treatment and distance run ($p<0.05$, Figure 2). The results of the linear models are reported in Table 1 which includes the mean and standard deviation of each of the metabolites after log transformation.

DISCUSSION: This study provides a step toward understanding how joint loading associated with wheel running affects profiles of central metabolites. While this sample size did not reach statistical significance when comparing the distance run and experimental group there is still some evidence of metabolite differences, particularly in the fumarate. The lack of results may result from the timeframe used for this study: as metabolites are very quick to change it is possible that a shortened timeframe may be necessary to capture the changes. Additionally, because this study relied on voluntary running of the mice an additional experimental group was forced to be made for the non-runners. This decreased the power of the study particularly with relation to the young female mice where only 3 of the 8 mice that had an unlocked wheel ran.

SIGNIFICANCE/CLINICAL RELEVANCE: Results from this study continue to support the importance of sex-based differences when studying osteoarthritis. While significant changes weren't found when comparing running groups to the control groups further research in this area is necessary to understand the cellular level changes caused by loading joints.

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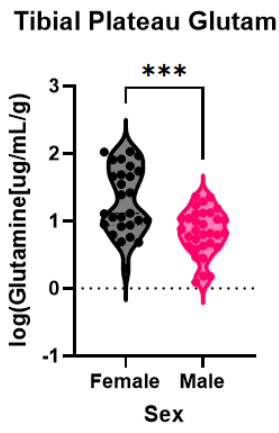
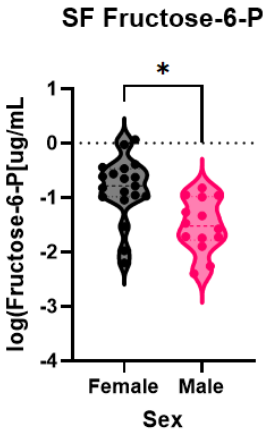
REFERENCES: [1]Steinmetz, Jaimie D., et al. *The Lancet Rheumatology* 5.9 (2023): e508-e522. [2] Zhang, Yanjing, et al. *Quantitative Imaging in Medicine and Surgery* 14.8 (2024): 6036. [3]Hahn, Alyssa K., et al. *Osteoarthritis and Cartilage Open* 4.1 (2022): 100228.

FIGURES:





Tibial Plateau Metabolites

Metabolite	Transformed Mean of Running Mice	Transformed Mean of Control Mice	Adjusted Age p-value	Adjusted Sex p-value	Adjusted Age and Sex interaction p-value	Adjusted Treatment p-value	Adjusted Distance Run p-value
Lactate	2.168+/-0.725	2.288+/-0.746	x	x	x	1	0.5
Fumarate	2.188+/-0.799	2.453+/-0.408	x	x	x	0.19	0.25
Malate	1.920+/-0.393	1.987+/-0.339	x	x	x	1	1
Glutamine	0.976+/-0.479	1.081+/-0.443	x	0.00341*	x	1	0.4
Glutemate	2.143+/-0.569	2.216+/-0.460	x	1	x	1	0.73
PEP	0.313+/-0.152	0.291+/-0.130	x	0.69	x	1	1
Glucose	1.566+/-0.350	1.607+/-0.346	x		x	1	0.394
Citrate	1.087+/-0.436	1.049+/-0.538	0.8284	1	1	1	1
Fructose 6 Phosphate	0.939+/-0.729	1.080+/-0.638	x	x	x	0.39	0.16
Sedoheptulose -7-Phosphate	0.202+/-0.229	0.168+/-0.312	x	x	x	1	1
Synovial Fluid Metabolites							
Fumarate	-1.046+/-0.419	-1.017+/-0.477	1	x	x	0.2862	1
Oxoloacetate	-0.919+/-0.150	-0.934+/-0.105	x	x	x	x	1
Glutamate	-0.249+/-0.322	-0.487+/-0.373	1	1	1	1	1
Glucose	0.569+/-1.071	0.633+/-0.983	1	x	x	x	1
Citrate	-0.980+/-0.405	-1.094+/-0.317	1	x	x	x	0.6706
Fructose 6 Phosphate	-0.922+/-0.526	'-1.245+/-0.672	1	0.04862*	x	1	1
6-Phosph-Gluconate	-2.099+/-0.049	-2.087+/-0.077	0.371	x	x	1	1