Characterization of metabolomic profiles in late and early Osteoarthritis patients

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Introduction

- Osteoarthritis (OA) is a progressive joint disease that currently has no cure and is the most common form of arthritis, affecting over 250 million individuals worldwide.
- Researchers are focusing on identifying metabolic pathways associated with OA in order to categorize its highly variable phenotypes.
- Using metabolomics to identify biomolecules in OA would help with stage diagnosis and give a clearer picture of effective treatment methods and symptom management for diagnosed individuals.
- **Hypothesis:** Patients with early OA will display Gly-Ser, amino acid, and collagen degradation pathways that can be compared more closely to healthy patients, rather than patients with late OA.

Methodology

- **Populations:** Synovial fluid samples were taken from 5 healthy individuals, 3 individuals with late OA, and 1 individual with early OA. Pseudo-samples were made from the early OA sample.
- **Extraction:** Metabolites were extracted from samples with 80% v/v methanol, centrifuged, and dried down. Proteins were precipitated with 5x solution of aq. acetonitrile solution. After a final centrifugation, samples were sent to MSU Bozeman for further analysis.
- **Metabolite Analysis:** An Agilent 1290 Ultra Performance Liquid Chromatography (UPLC) system with a Cogent Diamond Hydride HILIC column was used to perform liquid chromatography and an Agilent 6538 Q-TOF mass spectrometer was used for the liquid chromatography mass spectrometry measurements.
- **Statistical Analysis:** Was performed using Metaboanalyst software. Unsupervised PCA was utilized first to determine if OA groups naturally cluster. Then, OPLS-DA was used to determine VIP scores to find significant pathways that differentiate between group pairings.

Results

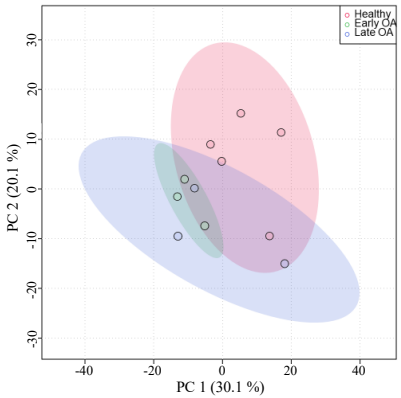

Figure I. Principal Component Analysis shows unsupervised clustering of groups: healthy (pink), early (green), and late (blue). Plot accounts for 50.2% of the variability between groups.

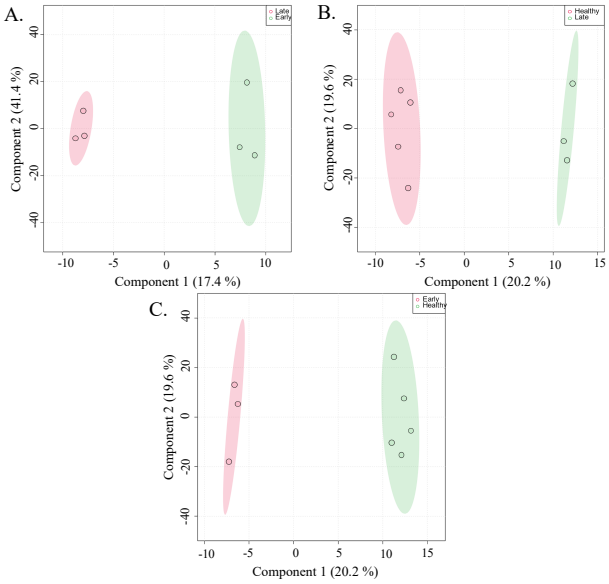

Figure 2. OPLS-DA comparisons between groups (A) late/early, (B) healthy/late, and (C) early/healthy. Total percent variability accounted for by each plot is as follows: (A) 58.8 %, (B) 39.8 %, and (C) 39.8 %.

Table I. Perturbed metabolic pathways identified via pathway enrichment of significant metabolite features using OPLS-DA VIP scores. Significant metabolites were given P-values equal to 1. Only pathways with false discovery rate-corrected P-values < 0.2 are reported.

Metabolic Pathway	Hits	Sigs	P-Value
Chronic vs. Acute			
Vitamin B6 (peradoxine) metabolism	4	4	0.13942
Fatty Acid activation	6	4	0.15265
Carnitine shuttle	12	6	0.15733
Saturated fatty acid beta-oxidation	5	3	0.1709
Di-unsaturated fatty acid beta-oxidation	3	2	0.18988
Vitamin E metabolism	3	2	0.18988
Fatty acid metabolism	3	2	0.18988
Glycerophospholipid metabolism	3	2	0.18988
Pyrimidine metabolism	3	2	0.18988
Acute vs. Healthy			
Urea cycle/amino group metabolism	5	4	0.16924
Vitamin B6 (peradoxine) metabolism	4	3	0.18745
Monounsaturated fatty acid beta-oxidation	2	2	0.19826
Chronic vs. Healthy			
No perturbed metabolic pathways detected.			

Conclusions

- Unsupervised clustering suggests metabolic pathways differ between OA and healthy patients.
- Supervised clustering allowed for pairwise comparison and identification of specific pathways that were significantly different between groups. This allowed for an observed distinction between late OA/healthy and late/early groups, with no statistically significant difference between the early/healthy grouping.
- Late OA and healthy comparisons identified statistically significant differences in the Urea cycle/amino acid metabolism. Amino acids are the building blocks of enzymes, proteins, and cell growth and reproduction. Disruption in this pathway could be a cause of the characteristic OA inflammation.
- As well as amino acid metabolism, analysis of late and early OA comparisons identified differences in lipid metabolism, lipids being a main component of synovial fluid that acts as lubricant and nutrient transport system. Disruption of lipid metabolism may also be a cause of inflammation.

Acknowledgments

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