

# Using Liquid-Chromatography Mass-Spectrometry to Determine Metabolite Makeup Within Osteoarthritic and Healthy Synovial Fluid

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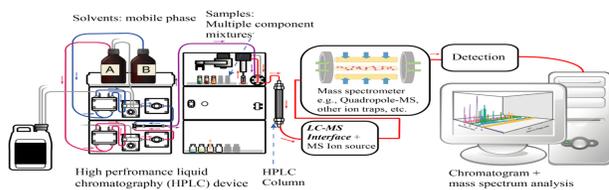


## Introduction

- Osteoarthritis (OA) is caused by the degeneration of articular cartilage found within articulated joints. This degradation leads to bone on bone interactions that become very painful as the disease progresses. The effects of this disease are non-reversible.
- Current methods for diagnosing OA include X-Rays and MRI scans. These methods are unable to diagnose the disease until it has significantly progressed and the breakdown of the cartilage can be visualized.
- Symptoms of OA cannot be cured, but are manageable through therapies such as topical creams and medications.
- Metabolomics is an emerging field of study as the last of the “omics” fields. This new field is the study of the entire metabolite population found within samples, and show exactly what is occurring within metabolic pathways.
- The use of metabolomics has shown promise in the ability to diagnose OA before symptoms progress far enough to be visualized with the current tools for diagnosis.
- This study focused on using global metabolomics in order to distinguish differences in metabolic pathways between OA and healthy synovial effusion samples. Differences that were found could possibly be used in the future as an alternative diagnostic method for earlier diagnosis of OA.

## Materials and Methods

- Samples from 11 OA patients and 5 healthy patients were obtained and metabolite extraction was used to separate metabolites from the samples.
- Extraction of metabolites included centrifugation for removal of cells and debris, then metabolites were extracted with methanol, and finally proteins were precipitated from the samples using acetonitrile.
- Liquid Chromatography Mass Spectrometry (LC-MS) was used to separate the metabolites obtained from the samples based on polarity. This data was obtained as an excel spreadsheet.
- Data were formatted for compatibility with MetaboAnalyst software. This software was used for single and multivariate statistics of the data, as well as functional analysis.



## Results

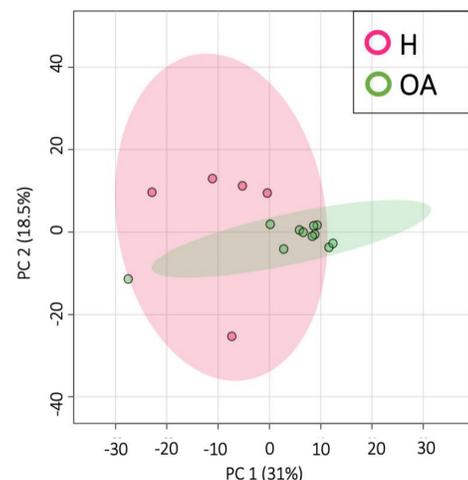


Figure 1. PCA plot showing Osteoarthritis [pink] and healthy cohorts [green].

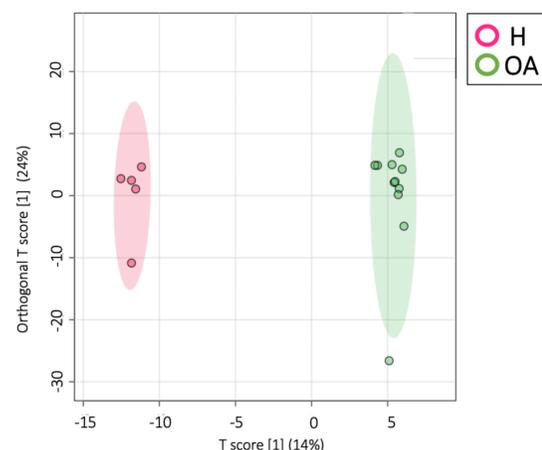


Figure 2. OPLS-DA score plot showing separation between the two cohorts: Osteoarthritis (pink) and Health (green).

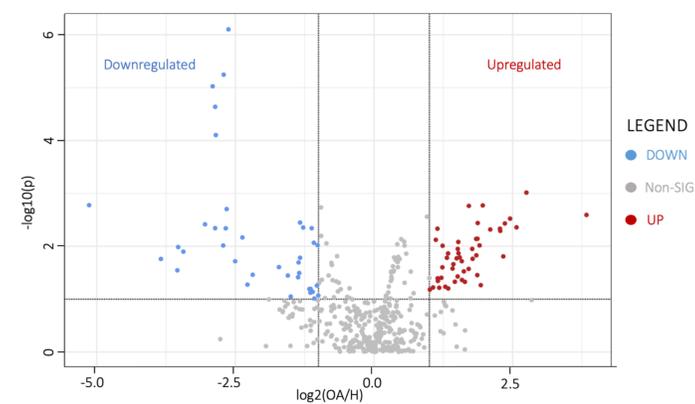


Figure 3. Volcano plot showing metabolite populations as significantly upregulated and significantly downregulated.

	Osteoarthritis (OA) n=11		
	Age	Weight	Height
Male	69	68	191
Female	68	67	185

Table 1. Data table shows the average (age, height, and weight) of OA patient for both males and females.

	OPLS-DA Pathway Enrichment		
	Total	Detected	Significant (VIP score >1)
Carnitine shuttle	72	12	7
Fatty acid activation	74	6	5
Fatty acid oxidation	35	4	3
Mono-unsaturated fatty acid beta-oxidation	19	2	2
Di-unsaturated fatty acid beta-oxidation	26	3	2
Vitamin E metabolism	54	3	2
Omega-3 fatty acid metabolism	39	3	2
Aspartate and asparagine metabolism	114	4	2
Urea cycle/amino group metabolism	85	5	2
De novo fatty acid biosynthesis	106	5	2
Valine, leucine and isoleucine degradation	65	5	2
Arginine and proline metabolism	45	5	2
Omega-6 fatty acid metabolism	55	5	2
Squalene and cholesterol biosynthesis	55	1	1

Table 2. OPLS-DA pathway Enrichment

Upregulated
Squalene and cholesterol biosynthesis
Carnitine shuttle
Bile acid biosynthesis
Vitamin E metabolism
Valine, leucine and isoleucine degradation
Butanoate metabolism
Vitamin E Metabolism
Urea Cycle/amino group metabolism
Arginine and Proline Metabolism
Glycerophospholipid Metabolism
Fatty acid activation
Pyrimidine metabolism
Aspartate and asparagine metabolism
Downregulated

Table 3. Volcano plot pathway enrichment analysis of upregulated and downregulated metabolites

## Conclusion

- A total of 63 metabolites within 14 pathways were detected according to the OPLS-DA pathway enrichment.
  - Of these 63 detected metabolites, 36 significant metabolites with a VIP score > 1 were identified (Table 2).
- The volcano plot showed different metabolic pathways that were down or up regulated in OA samples compared to healthy samples (Table 1).
- The data showed that there is a difference between the two cohorts using a supervised statistic and pathway enrichment analysis.
- This research shows that earlier detection of OA in patients may be a possibility using metabolomics as a diagnostic tool.

## References

- Bai, B., & Li, Y. 2016. Combined detection of serum CTX-II and comp concentrations in osteoarthritis model rabbits: An effective technique for early diagnosis and estimation of disease severity. *Journal of Orthopedic Surgery and Research*, 11(149).
- Carlson, A. K., Rawle, R. A., Adams, E., Greenwood, M. C., Bothner, B., & June, R. K. (2018). Application of global metabolomic profiling of synovial fluid for osteoarthritis biomarkers. *Biochemical and Biophysical Research Communications*, 499(2), 182–188.
- Chen, D., Su, X., Wang, N., Li, Y., Yin, H., Li, L., & Li, L. 2017. Chemical isotope labeling LC-MS for monitoring disease progression and treatment in animal models: Plasma Metabolomics Study of osteoarthritis rat model. *Scientific Reports*, 7(1).
- Clish C. B. (2015). Metabolomics: an emerging but powerful tool for precision medicine. *Cold Spring Harbor molecular case studies*, 1(1).
- Finehout, E. J., & Lee, K. H. 2004. An Introduction to Mass Spectrometry Applications in Biological Research. *Biochemistry and Molecular Biology*, 32(2), 93–100.
- Huang, Z., He, Z., Kong, Y., Liu, Z., & Gong, L. 2020. Insight into osteoarthritis through integrative analysis of Metabolomics and transcriptomics. *Clinica Chimica Acta*, 510, 323–329.
- Hugle, T., Kovacs, H., Heijnen, I. A. F. M., Daikeler, T., Baisch, U., Hicks, J. M., & Valderrabano, V. 2012. Synovial fluid metabolomics in different forms of arthritis assessed by nuclear magnetic resonance spectroscopy. *Clinical and Experimental Rheumatology*, 30.
- Kim, S., Hwang, J., Xuan, J., Jung, Y. H., Cha, H.-S., & Kim, K. H. (2014). Global metabolite profiling of synovial fluid for the specific diagnosis of rheumatoid arthritis from other inflammatory arthritis. *PLoS ONE*, 9(6).
- Ma, T., Zhang, Z., Song, X., Bai, H., Li, Y., Li, X., Zhao, J., Ma, Y., & Gao, L. 2018. Combined detection of Comp and CS846 biomarkers in experimental rat osteoarthritis: A potential approach for assessment and diagnosis of osteoarthritis. *Journal of Orthopedic Surgery and Research*, 13(1).
- Mayo Foundation for Medical Education and Research. (2021, June 16). *Osteoarthritis*. Mayo Clinic.
- Mayo Foundation for Medical Education and Research. (2020, June 17). *Swollen Knee*. Mayo Clinic.
- Pang, Z., Chong, J., Zhou, G., de Lima Morais, D. A., Chang, L., Barrette, M., Gauthier, C., Jacques, P.-É., Li, S., & Xia, J. 2021. Metaboanalyst 5.0: Narrowing the gap between raw spectra and functional insights. *Nucleic Acids Research*, 49(W1).
- Somashekar, B. S., Kamarajan, P., Danciu, T., Kapila, Y. L., Chinnaiyan, A. M., Rajendiran, T. M., & Ramamoorthy, A. 2011. Magic angle spinning NMR-based metabolic profiling of head and neck squamous cell carcinoma tissues. *Journal of Proteome Research*, 10(11), 5232–5241.
- Sophia Fox, A. J., Bedi, A., & Rodeo, S. A. (2009). The basic science of articular cartilage: Structure, composition, and function. *Sports Health: A Multidisciplinary Approach*, 1(6).
- Yu, S. P., & Hunter, D. J. (2015). Managing Osteoarthritis. *Australian Prescriber*, 38(4), 115–119.

## Acknowledgements

We would like to thank Dr. Alyssa Hahn for her guidance and support throughout this research project. We would also like to thank the Carroll College Biology department for the funding and resources for this project.