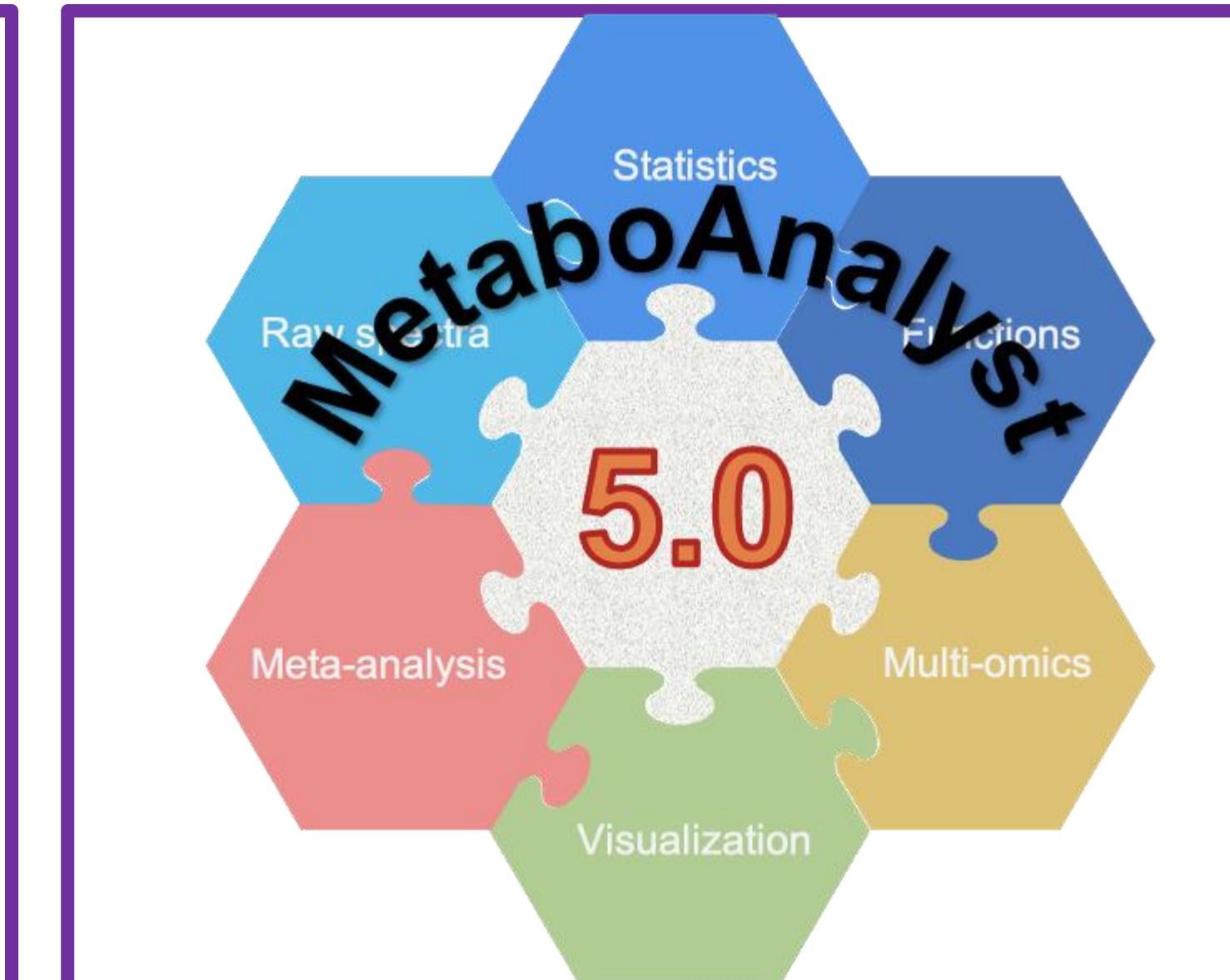


Applying Global Metabolomics to Investigate the Correlation Between BMI and Osteoarthritis

Aiden Mozer, McKenna Payne, Dr. Alyssa Hahn
 Department of Biology, Carroll College, Helena, Montana
 mpayne@carroll.edu, amozer@carroll.edu



Introduction:

- Osteoarthritis is a degenerative joint disease that causes degradation of articular cartilage and creates an imbalance between the hypertrophy and atrophy of periarticular bone.
- Onset of symptoms and modern diagnosing techniques are recognized to only work after irreversible degradation has occurred.
- Current research suggests that osteoarthritis is a metabolic disease, often linked to metabolic syndrome, type-2 diabetes, and dyslipidemia.
- Obesity is an important risk factor that can increase risk of developing osteoarthritis by up to five times and a 33% increased risk of joint replacement surgery.
- This study utilizes global metabolomic profiles to identify specific trends in metabolite concentrations in osteoarthritis patients with respect to BMI in order to support existing data of potential osteoarthritis biomarkers and further understand the overall pathogenesis of osteoarthritis.

Methods:

- Samples of synovial fluid were extracted from 15 patients, frozen, and thawed on ice to prepare for extraction.
- Samples centrifuged to remove cells and debris
- Metabolites extracted from samples with methanol
- Proteins precipitated with acetonitrile
- Metabolite extracts were analyzed by HPLC-MS
- Data collected was analyzed in MetaboAnalyst via PCA, OPLS-DA, and Volcano plots. Enrichment pathway analysis was performed to determine metabolic pathways

Instrumentation:

- Agilent 1290 Ultra Performance Liquid Chromatography (UPLC) system (Agilent, Santa Clara, CA) with a Cogent Diamond Hydride HILIC column (MicroSolv, Eatontown, NJ)
- Agilent 6538 Q-TOF mass spectrometer (Agilent, Santa Clara, CA)
- Elution gradient
- Mass spectrometry detects metabolites with a resolution of ~20,000 and accuracy of 5 ppm.

Data and Results:

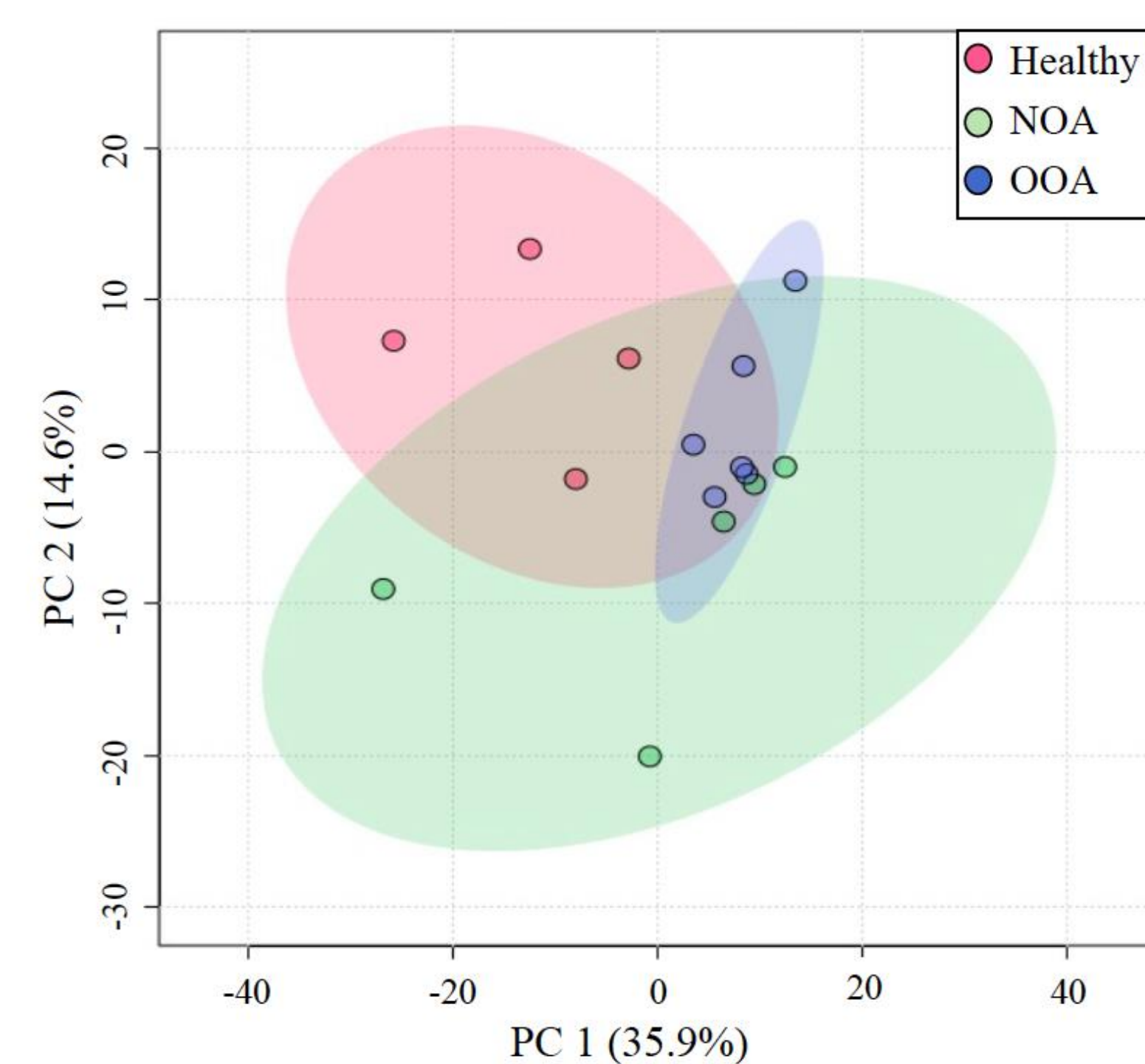


Figure 1. Principal Component Analysis of Osteoarthritic and Healthy Patients. Depicts the natural groupings of osteoarthritic and healthy patients based on global metabolomic profiles.

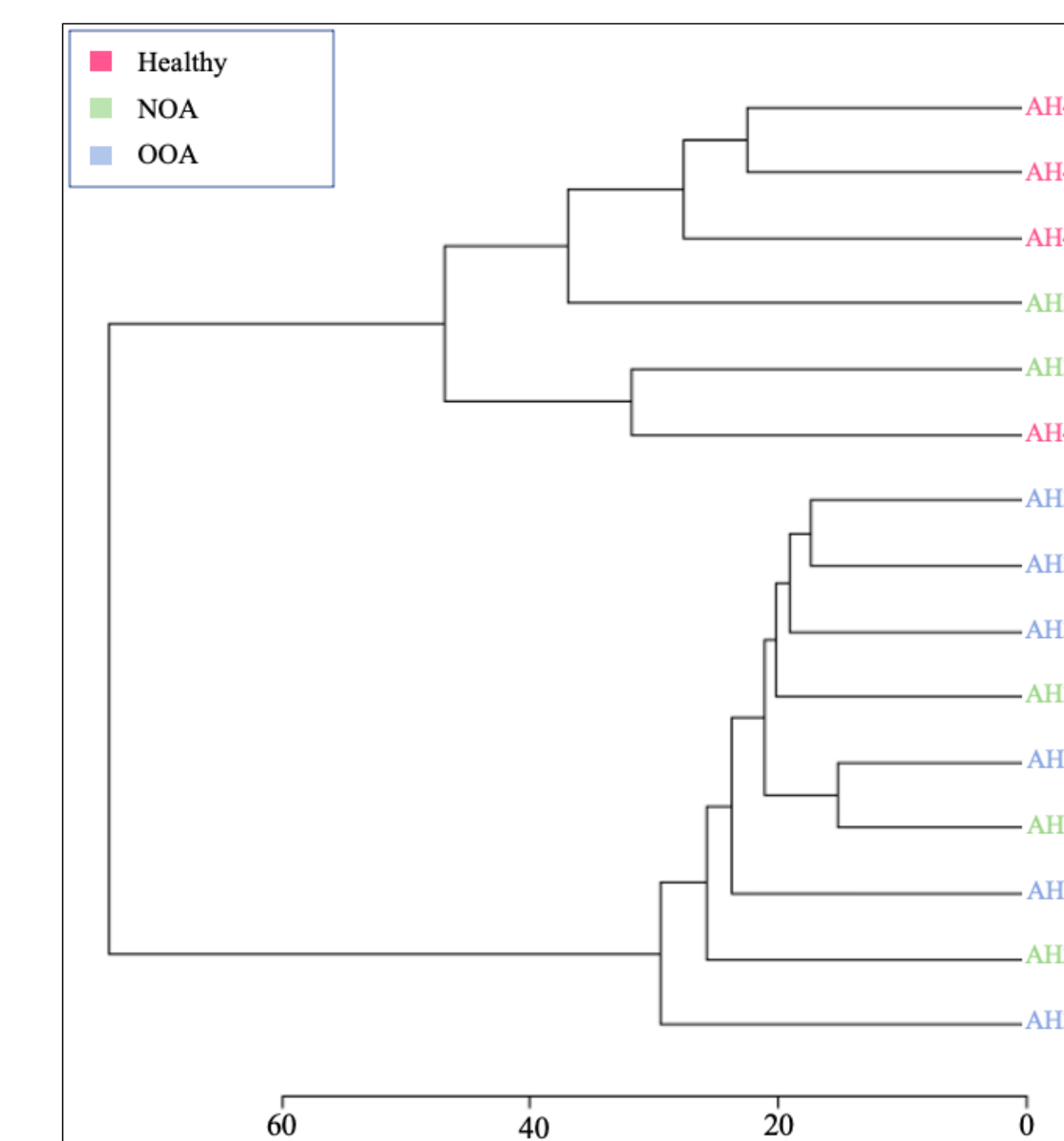


Figure 2. Dendrogram. A dendrogram depicting the groupings based on metabolic profiles. Healthy patients mixed with low BMI OA patients are grouped together, while high BMI and some low BMI OA patients are grouped as well.

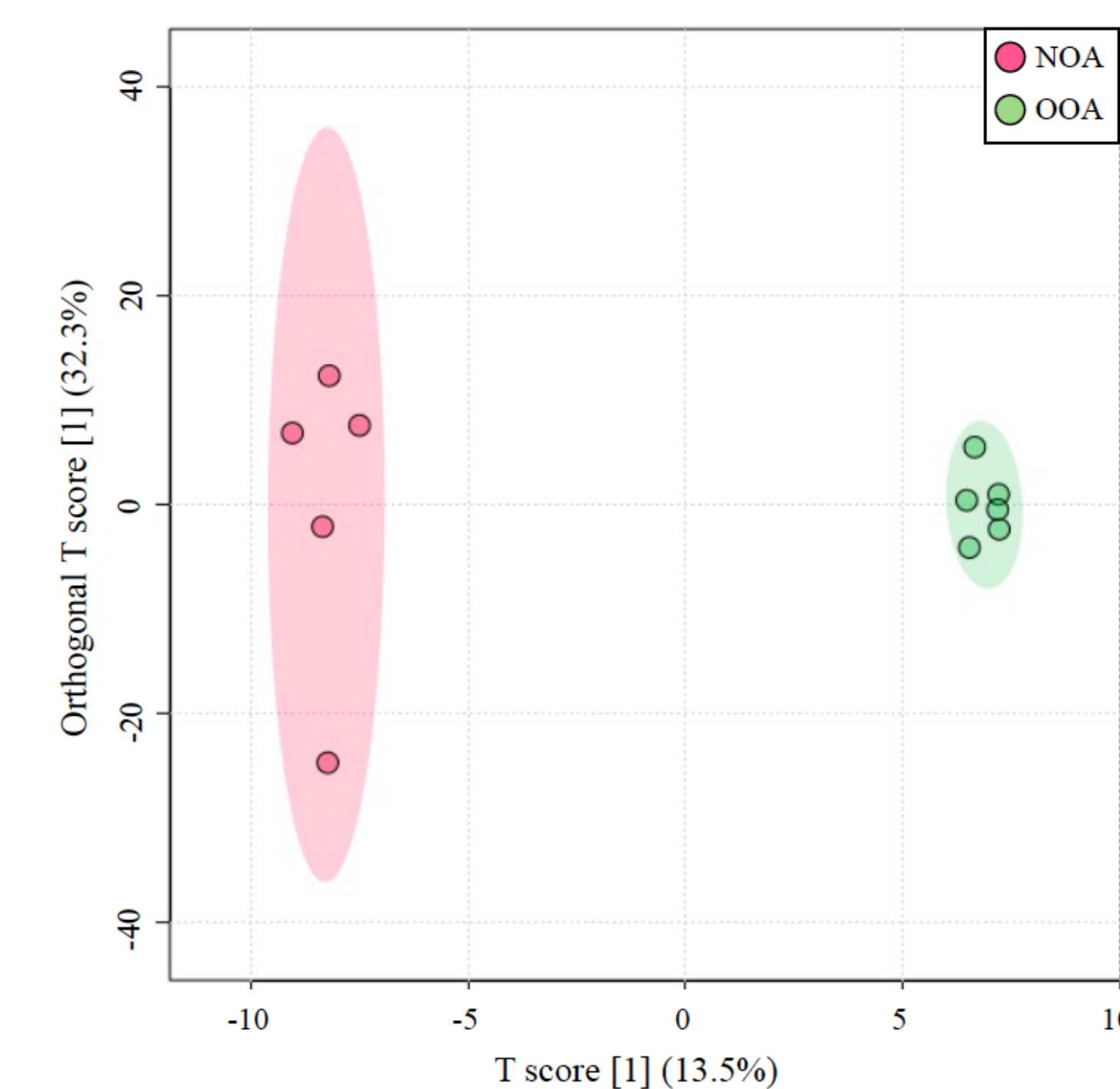


Figure 3. Orthogonal-Partial Least Squares Discriminant Analysis. OPLS-DA performed on patients with higher BMI's vs. those with lower BMI's to tease out differences in metabolites taking class membership into account

OPLS-DA Pathway Enrichment			
	Total	Detected	Significant (VIP Score > 1.5)
Carnitine Shuttle	72	12	2
Arginine	45	5	2
Saturated Fatty Acids Beta-Oxidation	36	5	2
C21-Steroid Hormone Biosynthesis and Metabolism	112	4	2
Alanine and Aspartate Metabolism	30	2	2
Tyrosine Metabolism	160	11	1
Fatty Acid Activation	74	6	1
Linoleate Metabolism	46	6	1
De Novo Fatty Acid Biosynthesis	106	5	1
Urea Cycle/Amino Group Metabolism	85	5	1

Table 1. OPLS-DA pathway enrichment analysis.

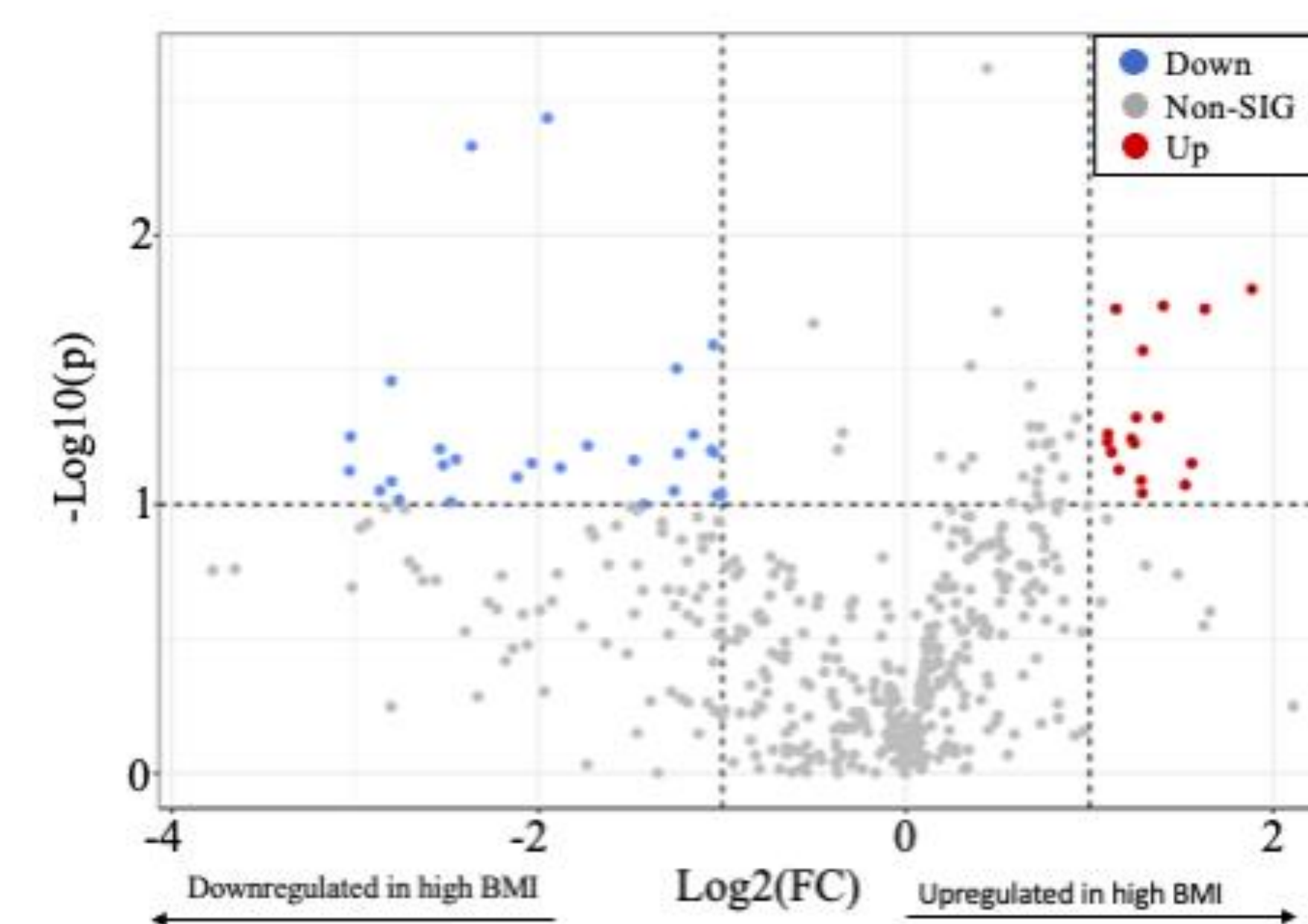


Figure 4. Volcano Plot. Demonstrates metabolites that are upregulated and downregulated based on significance.

Volcano Plot Pathway Enrichment			
Upregulated in Overweight OA vs. Normal OA			
	Total	Detected	Significant
Tyrosine Metabolism	160	11	1
Arginine and Proline Metabolism	45	5	1
Purine Metabolism	80	4	1
Aspartate and Asparagine Metabolism	114	4	1
Pyrimidine Metabolism	70	3	1
Downregulated in Overweight OA vs. Normal OA			
C21- Steroid Hormone Biosynthesis and Metabolism	112	4	2
Carnitine Shuttle	72	12	1
Linoleate Metabolism	46	6	1
Urea Cycle/Amino Group Metabolism	85	5	1
Saturated Fatty Acid Beta-Oxidation	36	5	1

Table 2. Volcano plot pathway enrichment analysis.

Conclusions:

- Samples were clustered within respective cohorts. Metabolic profiles show some clear separation between healthy and osteoarthritic patients. Similar profiles were seen in osteoarthritic patients separated by their BMI's with some variation (**Figure 1 & 2**).
- Metabolomic profiles reveal distinct metabolites that contribute to variation between patients with higher BMI's and patients with lower BMI's. (**Figure 3**).
- Enrichment analysis shows similar metabolic pathways derived from OPLS-DA and volcano plot (**Figure 3 & 4**). Compared to lower BMI patients, higher BMI patients showed upregulation in pathways involved with amino acid and nucleic acid metabolism. Additionally, they show downregulation in pathways involved with hormones, fatty acids, and β -oxidation (**Table 2**).

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