Global Metabolic Analysis of Synovial Fluid Extraction Between ACL Tear, General Trauma, and Osteoarthritis Patients

Ashley Carlson and Hailey James, Department of Biology

Carroll College

Introduction

- Recent research has found a potential link between anterior cruciate ligament (ACL) injury and development of osteoarthritis (OA). Research into this relationship can be valuable as one injury can have lifelong consequences.
- The ACL is important in knee stability and has a positive correlation to the development of OA after injury. Previous research suggests this could be due to an increase in inflammation from cartilage damage and synovial fluid perturbance.
- General trauma (GT) injuries refer to knee pain without any structural damage to the joint.
- Gap in knowledge: Although a correlation between pathologies is significant, the specific metabolic pathways are uncertain.
- **Hypothesis:** GT and ACL tears will produce metabolic perturbances similar to each other due to the traumatic nature of each injury. The ACL groups should be closely related to OA, based on previous research. More specifically, ACL 1 will be more similar to GT and ACL2 will be more similar to OA.

Methods

- **Patient information:** Two ACL samples from the same patient will be used and pseudosamples (n=3) taken from ACL1, ACL2, and GT. Four OA samples are included with five healthy controls and one GT sample.
- **Extraction:** Samples undergo three rounds of centrifugation and metabolites are extracted by methanol and the acetonitrile solution was used to precipitate proteins. Dried in vacuum.
- **Liquid-Chromatography Mass Spectroscopy:** Metabolite extracts were analyzed by HPLC-MS.
- **Data Analysis:** MetaboAnalyst via univariate and multivariate statistics is used to isolate and identify perturbed pathways which is then followed by a pathway enrichment analysis to determine perturbed metabolites.

Results

Table 1. Average patient demographic data (* = overweight)

Group	Age	Height (in)	Weight (lbs)	BMI
ACL1	13	-	-	-
ACL2	14	-	-	-
OA	67	67	179	27.8*
GT	66	72	175	23.7
HC	65	67	168	26*

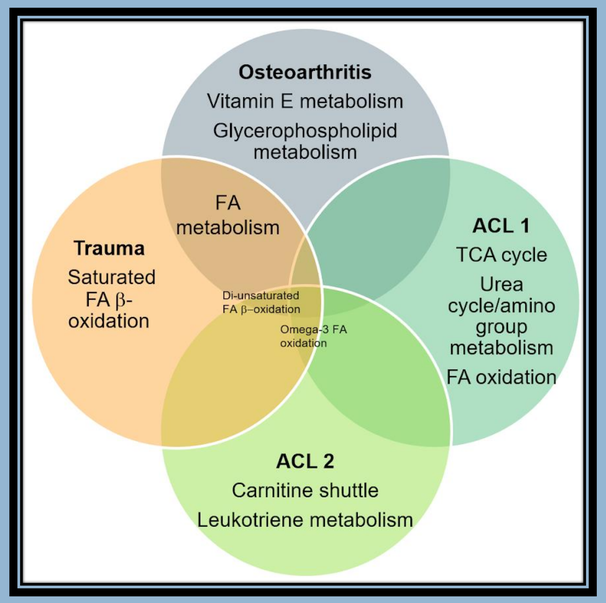

Figure 2. Venn diagram of perturbed pathways.

Table 2. Comparison of affected pathways across groups.

Pathway	ACL1	ACL2	OA	GT
Saturated FA Oxidation	-	-	-	X
Di-unsaturated FA Oxidation	-	X	X	X
FA Metabolism	X	-	X	X
Omega-3 FA Metabolism	X	X	-	X
Vitamin E Metabolism	-	-	X	-
Glycerophospholipid Metabolism	-	-	X	-
TCA Cycle	X	-	-	-
Urea Cycle / Amino Metabolism	X	-	-	-
Carnitine Shuttle	-	X	-	-
Leukotriene Metabolism	-	X	-	-

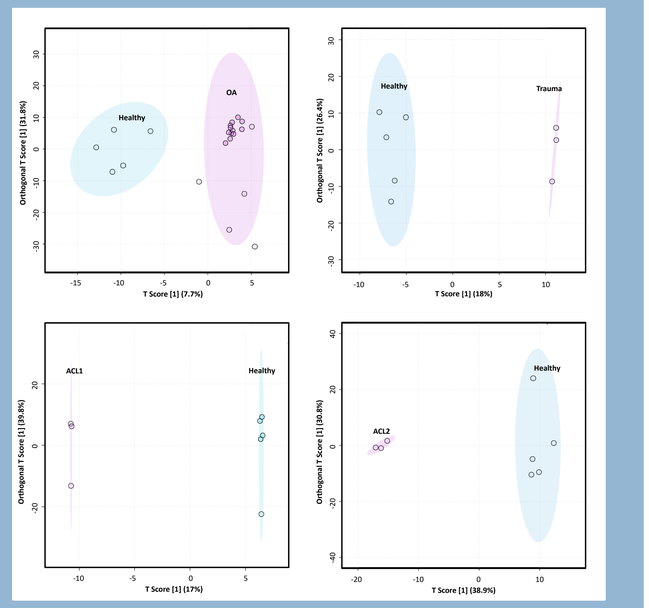

Figure 1. OPLS-DA from OA, GT, ACL1, and ACL2

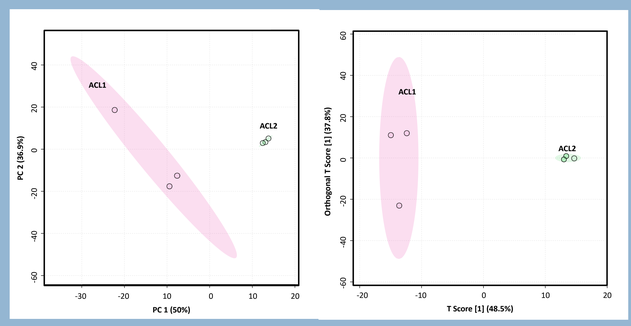

Figure 3. PCA (left) and OPLS-DA comparisons between ACL groups 1 and 2.

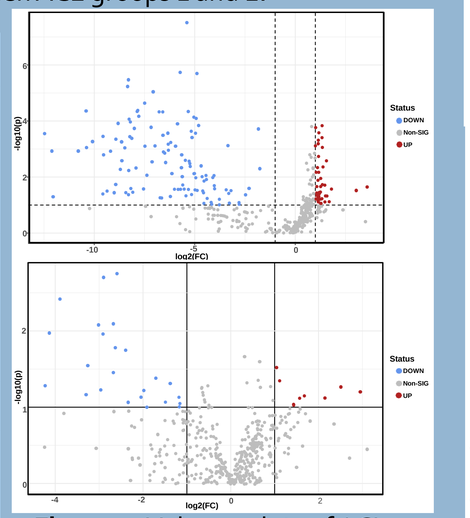

Figure 4. Volcano plots of ACL 1 (upper) and ACL 2 (lower).

Conclusion

- Most pathways had perturbances in fatty acid metabolism, which is often used by the body to reduce inflammation and as an antioxidant.
- GT, OA, and ACL2 had the most similar pathways while ACL1 perturbances were associated with acute response to injury. This may suggest that ACL2 and GT groups were trending towards development of OA while ACL1 is an immediate inflammatory response.
- Limitations: There could be a link between weight and disease development – an increase in FA concentration in obese patients could lead to OA, potentially explaining the elevated readings. Further research is required for clarification.
- Implications: Further investigations can research the inflammatory and antioxidant pathways as predictors of future disease and treatment progression.

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