Variability in Unique metabolomic profile of skeletal muscle in chronic limb threatening ischemia

This project is focused on a cross-sectional analysis of non-PAD controls and CLTI patients undergoing either a vascular intervention or undergoing limb amputation was performed and involved a detailed assessment of the limb muscle metabolome using semi-solid and solution state nuclear magnetic resonance spectroscopy. It was hypothesized that patients undergoing limb amputation would present with altered muscle metabolite features compared with non-PAD controls.

**Abstract**

Chronic limb threatening ischemia (CLTI) is the most severe manifestation of peripheral atherosclerosis. Patients with CLTI have poor muscle quality and function and are at high risk for limb amputation and death. The objective of this study was to interrogate the metabolome of limb muscle from CLTI patients. To accomplish this, a prospective cohort of CLTI patients undergoing either a surgical intervention (CLTI Pre-surgery) or limb amputation (CLTI Amputation), as well as non-PAD controls were enrolled. Gastrocnemius muscle biopsy specimens were obtained and processed for nuclear magnetic resonance (NMR) based metabolomics analyses using solution state NMR on extracted aqueous and organic phases and 1H magic angle spinning (HR-MAS) on intact muscle specimens. CLTI Amputation specimens displayed classical features of ischemic/hypoxic metabolism including accumulation of succinate, fumarate, lactate, alanine, and a significant decrease in the pyruvate/lactate ratio. CLTI Amputation muscle also featured aberrant amino acid metabolism marked by elevated branched chain amino acids. Finally, both pre-surgery and amputation CLTI muscles exhibited pronounced accumulation of lipids, suggesting the presence of myosteatosis, including cholesterol, triglycerides, and saturated fatty acids. Taken together, these metabolite differences add to a growing body of literature that have characterized profound metabolic disturbance’s in the failing ischemic limb of CLTI patients.

**Sample Description:**

Gastrocnemius muscle specimens were obtained from ten older adult non-PAD controls (Control), ten patients with critical limb ischemia (CLI) undergoing surgical intervention (CLTI Pre-surgery), and ten CLI patients undergoing limb amputation (CLTI Amputation). Five pre-surgery patients underwent bypass interventions and five underwent endovascular procedures. Muscle specimens were collected within the confines of the operating rooms (CLI patients) or via percutaneous muscle biopsy using sterile procedures previously described. A portion of the muscle was quickly trimmed of fat/connective tissue and snap frozen in liquid nitrogen for metabolomics analysis. This study was approved by the institutional review board at the University of Florida and carried out according to the Declaration of Helsinki. All participants were fully informed about the research and informed consent was obtained.

For one set of samples, FOLCH extraction was performed and solution state 1H NMR spectra were collected on resulted aqueous and organic phase samples separately. For other set of intact samples, 1H HR-MAS spectra were collected.

The data obtained for the NMR metabolomics analysis can be found in the accompanying files:

Procedures: 1. PAD Experimental Section.docx

Study Design Tables: 2. PAD Study Design Table.xls

Metadata: 3. PAD METADATA.xlsm

Processed Data: 4. PAD processed data.xlsx

Raw Data: 5. PAD NMR Raw Data.zip

**Notes:**

Full NMR sample preparation and analysis procedures are available in the accompanying document entitled **1. PAD Experimental Section.**

The normalized data that was used in Metaboanalyst 4.0 analysis is available in the accompanying files: **4. PAD processed data.xlsx.**

The raw fid as well as 1r file can be found in **5. PAD NMR Raw Data.zip.**