**Isolated murine skeletal muscles utilize pyruvate over glucose for oxidation**

The goal of this study was to determine the differential utilization of substrates in isolated murine skeletal muscle, and to evalute how isopotomer anlaysis provided insight into skeletal muscle metabolism.

**Abstract**

*Introduction*

Fuel sources for skeletal muscle tissue include carbohydrates and fatty acids, and utilization depends upon fiber type, workload, and substrate availability. The use of isotopically labeled substrate tracers combined with nuclear magnetic resonance (NMR) enables a deeper examination of not only utilization of substrates by a given tissue, but also their contribution to tricarboxylic acid (TCA) cycle intermediates.

*Objectives*

The goal of this study was to determine the differential utilization of substrates in isolated murine skeletal muscle, and to evalute how isopotomer anlaysis provided insight into skeletal muscle metabolism.

*Methods*

Isolated C57BL/6 mouse hind limb muscles were incubated in oxygenated solution containing uniformly labeled 13C6 glucose, 13C3 pyruvate, or 13C2 acetate at room temperature. Isotopomer analysis of 13C labeled glutamate was performed on pooled extracts of isolated *soleus* and *extensor digitorum longus* (EDL) muscles.

*Results*

Pyruvate and acetate were more avidly consumed than glucose with resultant increases in glutamate labeling in both muscle groups. Glucose incubation resulted in glutamate labeling, but with high anaplerotic flux in contrast to the labeling by pyruvate. Muscle fiber type distinctions were evident by differences in lactate enrichment and extent of substrate oxidation.

*Conclusion*

Isotope tracing experiments in isolated muscles reveal that pyruvate and acetate are avidly oxidized by isolated soleus and EDL muscles, whereas glucose labeling of glutamate is accompanied by high anaplerotic flux. We believe our results may set the stage for future examination of metabolic signatures of skeletal muscles from pre-clinical models of aging, type-2 diabetes and neuromuscular disease.

**Sample Description:**

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Florida. Male C57BL/6 mice 16±3 weeks old were housed at 22oC with a 12-hour light/dark cycle and free access to ad libitum chow diet food and water.

Mice were anesthetized (using a combination of xylazine (80mg/kg) and ketamine (10mg/kg)) to allow removal of *soleus* and *extensor digitorum longus* (EDL) muscles. Upon removal, muscles were incubated at 22 oC in Ringer/MEM solution gas equilibrated with 95/5% O2/CO2 with appropriate 13C labeled substrates in a perfusion chamber routinely used for isolated muscle mechanics for 30 minutes. These included the following: 5.5 mM [U-13C6] glucose; 5.5 mM [U-13C3] pyruvate, or 16.5 mM [13C2] labeled Na-acetate.  Following incubation, muscles were quickly removed, blotted, and then rapidly frozen in liquid nitrogen for subsequent NMR analysis. N=4 muscles were pooled into a single biological replicate of 30-50 mg tissue to afford detectable levels of substrates in the NMR analysis.

To extract metabolites, either Perchloric acid extraction or acetonitrile:isopropanol:water (3:3:2) vol:vol:vol extraction was performed for all samples. Each NMR sample consisted of 50 mM phosphate buffer (pH 7), 2 mM EDTA, 0.02% of NaN3 with 0.5 mM of DSS as a standard internal reference in deuterated environment. 1H NMR spectra were taken at 25oC using a 600 MHz Bruker Avance II Console equipped with a TCI CryoProbe that utilized Bruker Topspin 4 software (Bruker BioSpin Corporation, Billerica, MA, USA). The first slice of a NOESY pulse sequence (noesypr1d) was used to acquire proton NMR. Fractional enrichment for glutamate, lactate and alanine were determined using 13C decoupling ON/OFF 1H proton spectra as well as 1D NOESY spectra. To determine enrichements, a standard zgig pulse sequence was adapted to allow 13C decoupling during the acquistion period (1.36 s) to remove the satellites. Total enrichment was measured by taking a ratio of the metabolite peak heights in the decoupling on/off experiments. NOESY spectra were collected with a 1 s relaxation delay (d1), and a 4 s acqusition time (at), in accordance with Chenomx recommendations for producing quantitative estimates of concentration. Using the Chenomx quantification and the fractional enrichments, a final concentration of the metabolites was calculated. Conventional 1H decoupled 13C spectra were acquired using a 600 MHz Agilent with a specially designed 1.5 mm superconducting (HTS) probe at 30oC.

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

Procedures: 1. Isolated muscle Procedures.docx

Study Design Tables: 2. Study Design Table isolated muscle.xlsx

Metadata: 3. METADATA isolated muscle.xlsx

Processed Data: 4. 13C NMR Data.xlsx

Raw Data: 5. isolated muscle Raw Data.zip

**Notes:**

Full NMR sample preparation and analysis procedures are available in the accompanying document entitled 1**. Isolated muscle Procedures.docx.**

The normalized data is available in the accompanying files: **4. 13C NMR Data.xlsx**

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