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

**NMR sample preparation**

Perchloric acid (PCA) or acetonitrile:isopropanol:water (3:3:2) extractions were performed for all samples to isolate metabolites. The latter method was more efficient in sample recovery due to the reduced number of steps in the procedure but did not affect the proportion of metabolites. For PCA extraction, isolated muscle samples were homogenized with a FASTPREP-24 (MP Biomedicals, Solon, Ohio, USA) with 6% (v/v) ice cold PCA and centrifuged with 13.2 K rpm at 4 oC. The solid muscle portion was washed again with the 6% (v/v) ice cold PCA followed by centrifugation (13.2 K rpm) at 4 oC. The supernatant (combined) obtained was further neutralized with 5M potassium hydroxide and centrifuged again maintaining 13.2 K rpm speed at 4 oC. The resulting supernatants were then lyophilized (Thermo-Scientific, Dallas, USA). The pH of the dried powder was adjusted to 7.2 after dissolving it in 200 μL of ultra-pure water using 1M sodium hydroxide and 1 M hydrochloric acid. The pH-adjusted solution was further centrifuged, the resulting supernatant was dried and the powder was used to prepare the NMR sample.

For acetonitrile:isopropanol:water extraction, homogenization of isolated muscle samples was carried out in 1 mL acetonitrile:isopropanol:water (3:3:2, v:v:v) ice cold mixture with a FASTPREP-24 (MP Biomedicals, Solon, Ohio, USA) and centrifuged at 4 oC in separate vials. Resultant supernatants were further lyophilized till dryness (Thermo-Scientific, Dallas, USA). The dried powder was further dissolved in 1 mL of Acetonitrile:Water (1:1, v:v) mixture, vortexed well for ~5 minutes. The resultant solution was further centrifuged, the supernatant obtained was further dried and the powder was used to prepare the NMR sample. The centrifugation speed for each step used was 13.2K rpm.

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.

**NMR spectra processing**

Proton spectra were zero filled to 64K, whereas carbon spectra were zero filled to 128k data points before Fourier transformation, with exponential line broadening of 0.5 Hz using MestReNova 11.0.0-17609 (Mestrelab Research, S.L., and Santiago de Compostela, Spain). DSS singlet resonance at 0 ppm was used to calibrate proton spectra. The carbon spectra were calibrated to a taurine singlet resonance at 48.4 ppm. Both proton and carbon spectra were baseline corrected with Whittaker-Smoother or spline as required. For carbon spectra, glutamate resonances were fitted with a mixed Gaussian/Lorentzian lineshape and integrals were measured. Integrals values were utilized to calculate peak multiplet ratios of isotopomers. Metabolite concentrations were determined using Chenomx NMR Suite 8.2 (Chenomx, Inc., Edmonton, Alberta, Canada), using 1H 13C decoupling ON spectra with respect to DSS peak at 0.00 ppm (0.5 mM added concentration). The concentration values thus obtained were further normalized to tissue mass while maintaining 35 µL of NMR sample (for both soleus and EDL muscles) to enable comparisons across each replicate of pooled samples.

**Determination of [1,2 13C2] enriched acetyl-CoA and anaplerotic flux**

A non-steady-state analysis was performed to determine the enrichment in acetyl-CoA (Fc3) and anaplerotic flux (Y), using total 13C enrichment in C3 and C4 carbon atoms of glutamate. The following equations were used:

*Fc3* = (C4Q)(C4/C3) (1)

*Y* = C4/C3 (2)

Here, C4Q represents the total area of the quartet (doublet of doublets) resonances for glutamate carbon C4 resulting due to *J345*. C4 is the total area of the 4-carbon resonance and C3 is the total area of the 3-carbon resonance of glutamate. Carbons C4 and C3 have essentially idenitical signal dependencies on the nuclear Overhauser effect and the same T1, therefore accurate C4/C3 ratios are easily collected.

**Statistical analysis**

Un-paired Student’s *t*-test (for two groups), ordinary one-way ANOVA with Tukey’s post hoc analysis (for comparison of more than two groups), and two way ANOVA with Sidak’s multiple comparisons test were used to determine the significant levels in concentration differences for metabolites. A *p*-value < 0.05 was considered statistically significant.