Targeted Metabolomics Methods

Amino Acids and Biogenic Amines

Amino acids and biogenic amines were measured by liquid chromatography - tandem mass spectrometry (LC-MS/MS) using a method adapted from **Gray et al., PMID:28194962**. The samples were spiked with a mixture of stable isotope-labeled internal standards and deproteinized with methanol. The supernatants were derivatized with AccQTag reagent (Biosynth-Carbosynth) at 55 °C for 10 min. Chromatographic separation was performed using a Waters Acquity UPLC system (Milford, MA) equipped with a Waters Acquity UPLC HSS T3 column (1.8 µm, 2.1 × 100 mm). Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B was acetonitrile. The flow rate was set to 0.6 ml/min, and the column temperature was maintained at 40°C. The gradient program was as follows: 0–1.0 min, 0% B; 1.0–6.0 min, linear increase to 95% B; followed by a 1-min wash and a 1-min re-equilibration. Analytes were detected in the positive ion mode using multiple reaction monitoring (MRM) on a Waters Xevo TQ-XS mass spectrometer.

Organic Acids

Concentrations of lactate, pyruvate, succinate, fumarate, malate, α-ketoglutarate, citrate, isocitrate, and oxaloacetate were measured by LC-MS/MS. The samples spiked with the heavy isotope-labeled internal were centrifuged, and the supernatants were derivatized with 1M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 1M O-benzylhydroxylamine (O-BHA) in pyridine buffer for 1h at room temperature as described previously by **Tan et al., PMID: 25102203**. The organic acids were extracted with ethyl acetate; the extracts were dried under nitrogen, reconstituted in 50% methanol, and 1 µl was injected onto a Waters Acquity UPLC HSS T3 1.8 µm, 2.1x100 mm column. Chromatographic separations were performed using a Waters Acquity UPLC system (Milford, MA). Mobile phase A was composed of 0.1% formic acid and mobile phase B was acetonitrile. The flow rate was set to 0.4 ml/min and the column temperature was maintained at 50 °C. A 9.5-min gradient method (t=0, %B=25; t=6.0 min., %B=33; t=9.5 min., %B=95) was run followed by a 1-minute wash and a 1-minute equilibration at initial conditions. All metabolites were detected in the positive ion MRM mode based on a characteristic fragmentation reaction employing a Waters Xevo TQ-XS mass spectrometer (Milford, MA).

Acylcarnitines

Acylcarnitines were analyzed by flow injection electrospray ionization tandem mass spectrometry and quantified by isotope or pseudo-isotope dilution using methods described previously (**PMID: 14770177, PMID:18369453**). Samples were spiked with a cocktail of heavy-isotope internal standards (Cambridge Isotope Laboratories, MA, USA; CDN Isotopes, Canada) and deproteinized with methanol. The methanol supernatants were dried under nitrogen and derivatized with acidified methanol for 15 min. at 50°C. Mass spectra for acylcarnitines

were obtained using a precursor ion scan of m/z 99. The data were acquired in the positive ion mode using a Xevo TQD mass spectrometer equipped with AcquityTM UPLC system and a data system controlled by MassLynx 4.1 operating system (Waters, Milford, MA). 80% methanol was used as a mobile phase. Ion ratios of analytes to their respective internal standards, computed from centroided spectra, were converted to concentrations using calibrators constructed from authentic aliphatic acylcarnitines (Sigma, MO, USA; Larodan, Sweden) prepared in dialyzed fetal bovine serum (Sigma, MO, USA).