APPENDIX

Metabolite Measurement SOP:
For: Preparation of Biological Fluids
Type of Analysis: Targeted LC-MS with Absolute Conc. of 31 Metabolites

1. Thaw samples at 4°C.
2. Vortex the serum sample for 10 seconds
3. Transfer 20 µL serum sample in an 1.5 mL Eppendorf vial
4. Add 20 µL spiking solution containing 29 internal standards
5. Add 100 µL Methanol containing 13C6-Glucose and 13C2-Glutamic Acid
6. Vortex for 10 seconds
7. Store the sample at -20°C for 20 min
8. Centrifuge at 14000 rpm (@ 4°C) for 15 min
9. Collect 75 µL supernatant into a new 2 mL Eppendorf vial
10. Completely dry the samples using the Vacufuge at 30˚C (~1.5 hrs)
11. Reconstitute with 250 µL HILIC solvent containing 13C2-Tyrosine and 13C3-Lactate
12. Vortex for 10 seconds, then centrifuge for 5 mins at 14000 rpm (@ 4°C).
13. Transfer 200 µL supernatant into LC vials, ready for MS analysis.

Chemicals:

Acetonitrile, Methanol, Acetic Acid and ammonium acetate (all Optima LC-MS Grade) were obtained from Fisher Scientific. Milli-Q water was from an in house Ultrapure Water System from EMD Millipore (Billerica, MA)

LC System:
Shimadzu Nexera XR LC-20AD, CTC Analytics PAL HTC-xt autosampler 4 °C

Column	Waters XBridge BEH Amide XP, 130Å, 2.5 µm, 2.1 mm X 150 mm, PN: 186006724
Mobile phase A	10 mM ammonium acetate in 95% water, 3% acetonitrile, 2% methanol, and 0.2% acetic acid
Mobile phase B	10 mM ammonium acetate in 93% acetonitrile, 5% water, 2% methanol, and 0.2% acetic acid
Gradient	Time (min) %B 0 95 3 95 8 50 12 50 13 95 18 95
Flow rate	0.3 mL/min
Column temperature	40 °C
Injection volume	5 µL for positive ionization mode, 10 µL for negative ionization mode

Mass Spectrometer:
SCIEX Triple Quad 6500+

Targeted MRM Metabolomics:
Samples were injected into a chromatography system consisting of a dual injection valve setup allowing injections onto two different LC columns with each column dedicated to an ESI polarity. 5 µL were injected on the positive mode column and 10 µL on the negative side column. The columns were a matched pair from the same production lot number and were both a Waters XBridge BEH amide column (2.1 x 150 mm). Autosampler was maintained at 4 °C and column oven was set to 40 °C. 0.3 mL/min gradient was 0-3 min 95% B, 3-8 min 95 -> 50% B, 8-12 min 50% B, 12-13 min 50 – 95% B, 13-18 min 95% B. After completion of the 18 minute gradient, injection on the opposite column was initiated and the inactive column was allowed to equilibrate at starting gradient conditions. A set of QC injections for both instrument and sample QC were run at the beginning and end of the sample run.

Data Integration:
Data was integrated by Sciex-OS v1.5 software. Peaks were selected based on peak shape, a signal-to-noise of 10 or better and retention times consistent with previously run standards and sample sets.