**NMR Metabolomics Methods for Neonatal AKI Urine samples**

Frozen urine study samples were thawed on ice and vortexed for 30 seconds. Aliquots of 400 µL were transferred into BSI-labeled eppendorf tubes. Aliquots of 60 µL per study sample were also transferred into a 10 mL tube to generate pooled samples for QC during analysis, and 400 µL was transferred into BSI-labeled eppendorf tubes. D2O (230 µL) was added to each tube. Chenomx Internal Standard solution (Chenomx ISTD, Edmonton, Alberta, Canada) contains 5mM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS, Chemical Shift Indicator), 100 mM Imidazole (pH indicator), and 0.2% NaN3 (to inhibit bacterial growth) was added (70 µl) to the tubes. Tubes were vortexed for 30 seconds and centrifuged at 12000 rcf for 5min. A 200 µL aliquot of the supernatant was transferred into 3mm NMR tubes (Bruker-Biospin, Switzerland), which were kept on ice until data acquisition.

1H NMR spectra of urine samples were acquired on a Bruker Avance 950 MHz NMR spectrometer (located at the David H. Murdock Research Institute at Kannapolis, NC, USA) using a 3 mm cryogenically cooled ATMA inverse probe and ambient temperature of 25℃. A 1D NOESY presaturation pulse sequence (noesypr1d, [recycle delay (RD)-90°-t1-90°-tm-90°-acquire free induction decay (FID) was used for data acquisition. For each sample 64 transients were collected into 65k data points using a spectral width of 14.01 kHz (20.14 ppm), 2 s relaxation delay, 100 ms mixing time, and an acquisition time of 2.324 s per FID. The water resonance was suppressed using resonance irradiation during the relaxation delay and mixing time. NMR spectra were processed using Chenomx NMR Suite 7.51 Professional (Chenomx, Edmonton, Alberta, Canada) software. Spectra were zero filled, and Fourier transformed after exponential multiplication with line broadening factor of 0.5. Phase and baseline of the spectra were manually corrected for each spectrum. Spectra were referenced internally to the DSS signal. The quality of each NMR spectrum was assessed for the level of noise and alignment of identified markers. Spectra were assessed for missing data and underwent quality checks. NMR bins (0.50-9.0 ppm) were made after excluding DSS, water (4.68-4.88 ppm), and Imidazole (7.20-7.28 ppm) using bucket Integration with a 0.04 ppm bucket width. Integrals of each of the bins were normalized to total integral of each of the spectrum.