**Metabolomics and Childhood Obesity: A Pilot and Feasibility Study with Multiple Phenotypic Anchors**

Plasma samples were transferred to labeled tubes. A total of 219 study samples were thawed on ice for sample preparation200 uL of plasma sample were thawed and transferred to labeled tubes on ice where they were mixed with 50 uL Saline master mix (5mM Formate). Analytical quality control (QC) phenotypic pooled samples were generated by transferring a 25 µL of each sample of each respective phenotypical experimental sample into different 1.5 mL tubes. Whole study (total) pools were generated by transferring 200 uL of plasma from each Pool sample into a 2.0 mL tube. The tubes were vortexed for 4 min on a multi-tube vortexer and centrifuged at 16,000 rcf for 4 min. A 200 µl aliquot of the supernatant was transferred into pre-labeled 3mm NMR tubes for data acquisition on a 700 MHz spectrometer.

1H NMR spectra of plasma samples were acquired on a Bruker Avance 700 MHz NMR spectrometer (located at the David H. Murdock Research Institute) using a 5 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 128 transients were collected into 65k data points using a spectral width of 8.417 kHz (12.0227ppm), 2 s relaxation delay, 100 ms mixing time, and an acquisition time of 3.893 s per FID. The water resonance was suppressed using resonance irradiation during the relaxation delay and mixing time. NMR spectra were processed using TopSpin 3.2 software (Bruker-Biospin, Germany). Spectra were zero filled, and Fourier transformed after exponential multiplication with line broadening factor of 0.5 Hz. Phase and baseline of the spectra were manually corrected for each spectrum. Spectra were referenced internally to the DSS-d6 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 (0.65-7.85 ppm) were made after excluding DSS- d6, water (4.60 – 5.15 ppm), EDTA (2.53 – 2.59 ppm, 2.67 -2.72 ppm, 3.06 – 3.150 ppm, 3.22 – 3.28ppm, and 3.56 – 3.65ppm) and various noise regions (5.50-5.70 ppm, 5.90-6.80 ppm) using intelligent bucket Integration with a 0.04 ppm bucket width using ACD NMR Processor (ACD Labs Inc, Toronto, Canada). Integrals of each of the bins were normalized to total integral of each of the spectrum.