NMR Metabolomics Methods

Fifty (50) µl of 0.9% NaCl solution containing 4mM sodium formate (Chemical Shift Indicator) and 0.2% NaN3 (to inhibit bacterial growth) in D2O was pipetted into each of the 50 serum samples (150 µl aliquots). Each 200 µl sample was vortexed for 30 seconds and transferred into 3mm NMR tubes (Bruker Bio-Spin, Germany). Pooled serum samples for each phenotype were made by combining 12 µl aliquots from each of the study samples in the phenotypic group (low and high) into two separate eppendorf tubes. A combined total pooled sample was also prepared by combining 6 µl aliquots from all samples into an eppendorf tube. The pooled samples were prepared as described above and were used as quality check (QC) samples.

1H NMR spectra of serum samples were acquired on a Bruker Avance III 950 MHz NMR spectrometer (located at the David H. Murdock Research Institute at Kannapolis, NC, USA) using a 5 mm cryogenically cooled ATMA inverse probe and ambient temperature of 25℃. A CPMG pulse sequence with presaturation (cpmgpr1d) was used for data acquisition. For each sample 256 transients were collected into 32k data points using a spectral width of 19.5 kHz (20.5 ppm), 2 s relaxation delay, 400 µs fixed echo time, loop for T2 filter (l4)=80, and an acquisition time of 1.678 s per FID. The water resonance was suppressed using resonance irradiation during the relaxation delay. 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 Formate 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 spectra were processed using ACD NMR software (Advanced Chemistry Development, Toronto, ON, Canada). NMR bins (0.50-9.50 ppm) were made after excluding water (4.55-5.00 ppm) and formate (8.40-8.50 ppm) using Intelligent Bucketing Integration with a 0.04 ppm bucket width and a 50% looseness factor. Each of the NMR bins was normalized to total integral of each of the spectrum. In addition, 28 metabolites were selected for targeted profiling using the metabolite library in Chenomx NMR Suite 7.64 Professional software.