**NMR Metabolomics Methods for ABO Blood Type Study**

**Serum Samples Samples**

Aliquots of serum samples (500 uL) were transferred to eppendorf tubes for each individual sample and an aliquot (200 uL) from each sample was pooled by phenotype. An aliquot of each phenotypic pool (400uL) was combined to prepare a total pool sample. Finally an aliquot (500uL ) of each of the pooled samples was transferred to an eppendorf tube for further sample processing. Add 1000uL of methanol to each of the samples. Centrifuge at 12000 rcf for 5 mins. Transfer a 900uL aliquot to a new tube. Dry the samples under nitrogen using a turbovap. Reconstitute each sample with 630 uL of D2O and add 70uL of Chenomx ISTD 5mM DSS and 6mM imidazole and 0.02% NaN3 to each sample. Transfer a 600 uL of the sample to a 5 mm sample jet tube NMR tube. 1H NMR spectra of serum samples were acquired on a Bruker Avance III 700 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 noesy pulse sequence with presaturation (noesypr1d) was used for data acquisition. For each sample 128 transients were collected into 64k data points using a spectral width of 14.1 kHz (20.1 ppm), 2 s relaxation delay, 400 µs fixed echo time, loop for T2 filter (l4)=80, and an acquisition time of 2.324s 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 DSS signal at 0.0 ppm. 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 pre-processed using ACD 1D NMR Processor 12.0 (ACD Labs, Toronto, CA) for metabolomics analysis. NMR bins (0.13 - 9.00 ppm) were made after excluding water (4.70 – 4.80 ppm) using intelligent binning width of 0.04 ppm and 50% looseness factor. Integrals of each of the bins were normalized to total integral of each of the spectrum.