**NEST**

Plasma aliquots were shipped the NIH RTI-RCMRC on dry ice and immediately stored at -80 °C after being logged in for metabolomics analysis. A total of 113 study samples were thawed on ice for sample preparation.

An aliquot (125 μl) of thawed plasma from each study sample was mixed with 375 μl of methanol vortexed and centrifuged. 400 uL of the supernatant was dried in vacuum overnight. Each sample was reconstituted with 250 μL of 0.2 mM phosphate buffer (pH 7.5) containing 10% Chenomx Internal Standard - 0.5 mM DSS-d6. Two random study pools were created by mixing 30 μl of plasma from a subset of study samples. Five replicates were created of each pool for a total of ten pooled samples, and these prepared identically to the study samples. The tubes were vortexed for 2 min on a multi-tube vortexer and centrifuged at 16,000 rcf for 10 min. A 200 µl aliquot of the supernatant was transferred into pre-labeled 3 mm (4”) NMR tubes for data acquisition on a 700 MHz spectrometer.

1H NMR spectra of plasma extract samples were acquired on a Bruker 700 MHz NMR spectrometer (located at David H. Murdock Research Institute in Kannapolis, NC, USA) 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 32 transients were collected into 65k data points using a spectral width of 12.09 kHz (17.17 ppm), 2 s relaxation delay, 100 ms mixing time, and an acquisition time of 2.726 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. 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 (0.50-8.70 ppm) were created excluding water (4.70-5.00 ppm) using intelligent bucket integration of 0.04 ppm bucket width with 50% looseness 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.