**Smoking Status**

Vials of pooled urine (smoker and non-smoker) were shipped the NIH RTI-RCMRC on dry ice and immediately stored at -80 °C after being logged in for metabolomics analysis. Five 1 mL aliquots of each pool were taken for NMR analysis. A total of 10 study samples were thawed on ice for sample preparation.

Two aliquots (400 μl) of thawed urine from each sample was mixed with 230 μl of 0.5 mM phosphate buffer (pH 7.5) and 70 uL of Chenomx ISTD solution. A total study pool was created by mixing 150 μl of urine from each sample. Additionally, two random QC pools were created. The tubes were vortexed for 2 min on a multi-tube vortexer. A 600 µl aliquot of the supernatant was transferred into pre-labeled 5 mm (7”) NMR tubes for data acquisition on a 700 MHz spectrometer.

1H NMR spectra of urine samples were acquired on a Bruker 700 MHz NMR spectrometer (located at North Carolina State University, Raleigh, 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 16 transients were collected into 65k data points using a spectral width of 12.02 ppm, 2 s relaxation delay, 100 ms mixing time, and an acquisition time of 3.89 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 (0.7-9.50 ppm) were created excluding water and urea (4.25- 6.5 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.