**Title: Metabolomics in the New Hampshire Birth Cohort Study**

**NMR Metabolomics Sample Preparation**

Aliquots of each de-identified sample were received by the NIH ERCMRC on dry ice and immediately stored at -80 °C after being logged in for metabolomics analysis. The samples were randomized into NMR batches for processing and data acquisition. Samples of each NMR batch was prepared as follows. The study plasma samples were thawed at 4°C overnight. An aliquot (350 µL) of thawed plasma was transferred into labeled 2.0 mL LoBind Eppendorf tubes. Low volume samples that were below 350 µL were made up to 350 µL with water. A volume of 1050 µL of Methanol was added to each sample, vortexed for 2 min on multi-tube vortexer at speed 5, centrifuged at 16,000 rcf for 20 min. A 1000 µL aliquot of clean supernatant was transferred into new pre-labeled LoBind Eppendorf tubes, dried on the SpeedVac. The dried extracts of samples were reconstituted in 700 µL of D2O Phosphate buffer NMR Master Mix (containing 0.5 mM DSS-d6 and 0.2% NaN3 (w/v) in D2O). The sample was vortexed for 2 min on multi-tube vortexer at speed 5, centrifuged at 12,000 rcf for 4 min., and a 600 µL aliquot of the supernatant was transferred into numbered 5 mm NMR tubes. Aliquots (350 of µL) CHEAR Plasma Reference (QC) samples were included in each NMR batch, processed exactly similar to the study samples described above, and used for QC purposes.

1H NMR spectra of plasma samples were acquired on a Bruker Avance 700 MHz NMR spectrometer using a 5 mm cryogenically cooled ATMA inverse probe and ambient temperature of 25 °C. A 1D NOESY pre-saturation pulse sequence (noesygppr1d, [recycle delay (RD)-90°-t1-90°-tm-90°-acquire free induction decay (FID) was used for data acquisition. For each sample 64 transients were collected into 64k data points using a spectral width of 12ppm), 2 s relaxation delay, 10 ms mixing time, and an acquisition time of 3.9 s per FID. The water resonance was suppressed using resonance irradiation during the relaxation delay and mixing time. NMR spectra were processed using TopSpin 3.5 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 (δ 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 bins (0.50-8.50 ppm) were made after excluding water (4.70 – 4.90 ppm), EDTA (2.53 – 2.62 ppm, 2.68 – 2.72 ppm, 3.57 - 3.66 ppm) and methanol regions (3.03 - 3.37 ppm), using intelligent bucket Integration with a 0.04 ppm bucket width and 50% looseness using ACD Spectrus Processor 2019 (ACD Labs Inc, Toronto, Canada). Integrals of each of the bins were normalized to total integral of each of the spectrum.