Sample preparation protocol

Samples were removed from -80°C storage and placed on ice for the duration of the experiment. Triple-distilled H2O (300 µL) was added to each sample tube containing a frozen pellet, and two Process Blanks (PB) were created by placing same volume of H20 into empty tubes. Samples were re-suspended, transferred to the wells of a 1mL deep-well plate containing 2-3 3mm glass beads and homogenized for 5 minutes at room temperature (RT) by a Hard Tissue homogenizer (VWR).

Protein concentration was determined by mixing a 2µL aliquot of homogenized sample with 198µL of Bradford reagent (Sigma B6916). Assay was performed at RT in a clear, flat bottom, shallow 96-well plate. The plate was vortexed and absorption data collected on BioTek uQuant with KC Junior software (v1.41.8) within 15 minutes after mixing sample and reagent. A 14 point calibration curve was created using bovine albumin (Sigma B4287). All measurements were performed in duplicate on the same plate.

Sample volumes for metabolomic analyses were determined by normalization to a protein content of 8 mg/mL using the formula: 8/x (mg/mL) *100= µL sample volume aliquoted. Pooled human plasma controls (HB, pooled human plasma from in-house reserve of American Red Cross supply stored at -80°C) were processed in parallel with other samples following this step.

For de-proteination the calculated volume of each sample was transferred to a 2mL deep-well plate and diluted with 8 volumes of 100% ethanol containing 13C algal amino acid mixture (Sigma-Aldrich 426199) as a recovery standard. The plate was then vortexed, centrifuged at 4750 RPM, 4°C for 10 min, and supernatant transferred to a new 2 mL deep-well plate. A pre-washed filter plate (Nunc 278010) was attached and the assembly centrifuged at 4750 RPM, 4°C for 10 min to further clarify the samples.

Aliquot of each sample was transferred into glass inserts placed in custom-made 96 well aluminum plate and dried down under the stream of nitrogen for 1.5 hr.