LC-MS Metabolomics Methods

**Sample Preparation:**

Pooled samples were created by combining aliquots from the appropriate study samples to form all-pooled, prudent-pooled, and Western-pooled samples and were prepared using the same methods as the study samples. 50uL of L-tryptophan-d5 (used as an internal standard) and 400uL of methanol were then added to 100uL aliquots of serum or pooled samples. Samples were vortexed on a multi-tube vortexer for 2 min. at 5000rpm and centrifuged for 4 min. at 16000rcf. 400uL of the supernatant was then transferred to new tubes and dried on a SpeedVac for 3 hours at 30C. Samples were reconstituted in 100uL of 95:5 water: methanol, vortexed on a multi-tube vortexer for 2 min. at 5000rpm, and centrifuged for 4 min. at 16000rcf. The supernatant was transferred to autosampler vials.

**UPLC-MS Methods:**

UPLC-MS spectra were collected for all samples. UPLC was performed on a Waters Acquity UPLC with an Acquity BEH HSS T3 column (2.1x 100mm x 1.8 um) at 50C using the reversed phase method. Water with 0.1% formic acid (mobile phase A) and methanol with 0.1% formic acid (mobile phase B) were injected following the Dunn 22 minute method (see the 3. HOUMARD MetaData and Analytical Metadata.xlsx file for the flow gradient). Mass spectroscopy analysis was performed using a Synapt G2 Q-TOF. 10uL of each sample was injected into the instrument, and MS data was collected between 70-1000m/z in both positive and negative modes.