LC-MS Metabolomics Methods

**Sample Preparation:**

10uL of L-tryptophan-d5 (used as an internal standard) and 10uL of 95:5 water:methanol per 1mg of feces were added to each feces sample. Sample tubes were then placed on ice and allowed to soften for 1 hour, followed by vortexing on a multi-tube vortexer for 4 min. at 5000rpm. Any remaining large lumps of feces were broken up using a pipette tip, and samples were vortexed again for 2 min. at 5000rpm. Samples were then centrifuged at room temperature for 10min. at 16,000rcf. Pooled samples were created by combining aliquots of the supernatant from the appropriate study samples to form total-pooled, control-pooled, and high fat-pooled samples. Approximately 100uL of each study and pooled sample were transferred into autosampler vials.

**UPLC-MS Methods:**

UPLC-MS spectra were collected for all samples. All liquid chromatography was performed on a Waters Acquity UPLC. For reversed phase analysis, 10uL of each sample was injected into an Acquity BEH HSS T3 column (2.1x 100mm x 1.8 um) at 50C. Water with 0.1% formic acid (mobile phase A) and methanol with 0.1% formic acid (mobile phase B) were injected following the RCMRC-RF methods (see the 3. WILLIAMS 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.