**NMR Metabolomics Methods for Blaser Liver tissue samples**

Frozen liver samples were weighed (50-100 mg) into labeled homogenizer bead tubes. Cold acetonitrile:water (50%) was added to tissue based on weight to make 1 mg/mL homogenates. The samples were extracted and homogenized on a Spex Geno/Grinder for two 45 second pulses at 1750 rpm. Samples were centrifuged at 12000 rcf for 5 min. Liver supernatants were transferred into BSI-labeled tubes. A 500 μL aliquot/sample was transferred into a second set of BSI-labeled tubes for further processing. To make pooled samples, a 300 μL aliquot of selected TranSTAT liver supernatants was combined in a 2 mL tube and used for QC samples in DuraSTAT, TranSTAT, EstroSTAT and NOD sub-studies. Additionally, a 200 μL aliquot of selected VG STAT liver supernatants was combined in a separate 2 mL tube and used as QC samples in the VG STAT sub-study. Three 500 μL aliquots were transferred into BSI-labeled tubes for each set of Pooled QC samples. All samples were then dried on an Eppendorf rotaVap (V-AL setting) at 30°C until dry and stored at -80 °C. On the second day of processing, 630 μL of D2O was added into each dried liver extract tube. Chenomx Internal Standard solution (Chenomx ISTD, Edmonton, Alberta, Canada) contains 5mM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS, Chemical Shift Indicator), 100 mM Imidazole (pH indicator), and 0.2% NaN3 (to inhibit bacterial growth) was added (70 µl), and the samples were vortexed on a multi-tube vortexer for 10 minutes at speed 5. Tubes were then centrifuged at 12000 rcf for 5 minutes and a 600 μL aliquot of the supernatant was transferred into 5mm NMR tubes (Bruker-BioSpin, Switzerland) which were kept on ice until data acquisition.

1H NMR spectra of liver samples were acquired on a Bruker Avance 700 MHz NMR spectrometer (located at the David H. Murdock Research Institute at Kannapolis, 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 64 transients were collected into 64k data points using a spectral width of 14.01 kHz (20.14 ppm), 2 s relaxation delay, 100 ms mixing time, and an acquisition time of 2.324 s per FID. The water resonance was suppressed using resonance irradiation during the relaxation delay and mixing time. 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 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 spectra were processed using ACD NMR software (Advanced Chemistry Development, Toronto, ON, Canada). NMR bins (0.50-9.00 ppm) were made after excluding water (4.70-4.85 ppm) and imidazole (7.32-7.39 ppm) using Intelligent Bucketing Integration with a 0.04 ppm bucket width and a 50% looseness factor. Integrals of each of the bins were normalized to total integral of each of the spectrum.