**CNS and peripheral metabolomics of calorie restriction in a mouse model of Alzheimer’s disease**

Brain tissue samples were homogenized in 50:50 Acetonitrile:Water, and the final concentration of all of the extracts was 1 g/mL. Of the 71 samples, 48 had sufficient volume for study samples and for inclusion in an analytical quality control (QC) total pool. This total pool was created with 50 µL of each sample of sufficient volume which were divided into six replicates. The total pools were processed identically to the study samples. A 250 µL aliquot of each sample and pool homogenate was lyophilized and then reconstituted with 250 µL of Phosphate buffer with Chenomx ISTD and 6mM Imidazole. Samples were vortexed and centrifuged before transferring 225 µL of each sample into pre-labeled 3mm NMR tubes for data acquisition on a 700 MHz spectrometer.

1H NMR spectra of plasma samples were acquired on a Bruker Avance 700 MHz NMR spectrometer (located at the David H. Murdock Research Institute) 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 128 transients were collected into 65k data points using a spectral width of 8.417 kHz (12.0227ppm), 2 s relaxation delay, 100 ms mixing time, and an acquisition time of 3.893 s per FID. The water resonance was suppressed using resonance irradiation during the relaxation delay and mixing time. NMR spectra were processed using TopSpin 3.2 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. 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 0.75-9.2 ppm) were made after excluding water (4.78 – 4.80 ppm) and imidazole (7.340 – 7.385 ppm, 8.335 – 8.345 ppm) using intelligent bucket Integration with a 0.04 ppm bucket width using ACD NMR Processor (ACD Labs Inc, Toronto, Canada). Integrals of each of the bins were normalized to total integral of each of the spectrum.