Nile Rat Fasting vs. Random Blood glucose plasma lipidomics: data analysis results and preliminary data analysis

Objectives:

1. Perform liquid chromatography-tandem mass spectrometry (LC-MS) lipidomics method on nile rat plasma samples.
2. Perform MS file searching, execute data quality checks and show results of preliminary data analysis.

Results Summary

Data are shared as follows:

Lipidomics\_processed\_raw\_data.csv contains the results of output from the Compound Discoverer/LipiDex software data analysis. This table gives the quantitation of individual lipid features, as well as lipid identifications by molecule and class and other summary metrics. Quantitation values are given as peak area, defined as the integrated signal of the quantitative m/z ion over chromatographic elution time.

Lipidomics data checks and metrics in the **figures** folder. Figures are shared as .png images:

**all samples feature quant analytical order.png** shows boxplots of all 60 LC-MS sample runs, given in analytical order on the X axis. Y axis shows the log2 of all lipid features per LC-MS run. The boxplots do not show any large deviations from the median, nor any drift in the plots as a consistent up- or downward trend, suggesting that our LC-MS runs were of good quality.

**average feature quant rbg vs fbg.png** shows a comparison of all RBG and FBG feature quants averaged.

**lipid class feature quant comparison.png** gives a breakdown of 9 different lipid classes (one class per row) across the 3 different rat glucose tolerances (diabetic on left, impaired in middle, normal on right), with a comparison of FBG vs. RBG within each.

**PCA colored by BG method.png** shows clustering of samples based on glucose test type.

**PCA colored by animal.png** is the same PCA plot as above, except colored by animal (10 different colors). The BG method is now given by shape (square vs. circle).

**QC samples feature quant.png** shows our quality control (QC) samples run on LCMS. QC1 shows a concerning lower average feature quant, however this may be due to it being the first run (post-water blanks) on the instrument during this analysis cycle. Notably, no sample feature quant as given in the **all samples feature quant analytical order** is as low as QC1, suggesting that QC1 was a one-off event in our analysis.

Future plans

Parties will meet in September to discuss these results and other results shared in the interim.

Ben and Katie will perform additional analysis of the LC-MS data in combination with the phenotypic observations of the nile rats as given in the shared excel document that contains animal weights, FBG/RBG, and post-mortem tissue analysis.

Katie aims to complete MS metabolomics experiments prior to next meeting, with the understanding that it is proving difficult to get high-quality data right now.

Ben plans to continue analyzing the data presented here. Additional filtering steps, such as feature found in N files and relative standard deviation (RSD) of features will occur.

Goals of analysis

Under the hypothesis that random blood glucose testing matches the quality of fasted testing for nile rat diabetes models, data analysis will show evidence of this comparison.

More aspirational goals of the data analysis and publication will be discussed at the next meeting.

Sample Preparation

Plasma samples were removed from freezer and thawed on ice. Each sample was extracted with 500 uL 6:2:2 n-butanol:acetonitrile:water. 100 uL of extract was dried down in SpeedVac evaporator per sample in triplicate, in separate amber autosampler vials. For lipidomics, each extract was resuspended in 50 uL 9:1 MeOH:toluene then analyzed on mass spectrometer.

LC-MS Analysis method

Sample analysis was performed on an Acquity CSH C18 column held at 50 °C (100 mm x 2.1 mm x 1.7 μm particle size; Waters) using a Vanquish Binary Pump (400 μL/min flow rate; Thermo Scientific). Mobile phase A consisted of 10 mM ammonium acetate in ACN:H2O (70:30, v/v) containing 250 μL/L acetic acid. Mobile phase B consisted of 10 mM ammonium acetate in IPA:ACN (90:10, v/v) with the same additives. Mobile phase B was initially held at 2% for 2 min and then increased to 30% over 3 min. Mobile phase B was further increased to 50% over 1 min, then raised to 85% over 14 min, and finally raised to 99% over 1 min and held at 99 % for 7 min. The column was re-equilibrated with mobile phase B at 2% for 1.75 min before the next injection. 10 µL of extract was injected by a Vanquish Split Sampler HT autosampler (Thermo Scientific).

The LC system was coupled to a Q Exactive HF Orbitrap mass spectrometer through a heated electrospray ionization (HESI II) source (Thermo Scientific). Source conditions were as follow: HESI II and capillary temperature at 300 °C, sheath gas flow rate at 25 units, aux gas flow rate at 15 units, sweep gas flow rate at 5 units, spray voltage at |3.5 kV| for both positive and negative modes, and S-lens RF at 90.0 units. The MS was operated in a polarity switching mode acquiring positive and negative full MS and MS2 spectra (Top2) within the same injection. Acquisition parameters for full MS scans in both modes were 17,500 resolution, 1 × 106 automatic gain control (AGC) target, 100 ms ion accumulation time (max IT), and 200 to 1600 m/z scan range. MS2 scans in both modes were then performed at 17,500 resolution, 1 × 10 5 AGC target, 50 ms max IT, 1.0 m/z isolation window, stepped normalized collision energy (NCE) at 20, 30, 40, and a 10.0 s dynamic exclusion.

LC-MS Data Analysis method

The resulting LC–MS data were processed using Compound Discoverer 3.1 (Thermo Scientific) in conjunction with LipiDex (3). All peaks with a 0.4 min to 23 min retention time and 100 Da to 5000 Da MS 1 precursor mass were aggregated into distinct chromatographic profiles (i.e., compound groups) using a 10-ppm mass and 0.2 min retention time tolerance. Profiles not reaching a minimum peak intensity of 5x105, a maximum peak-width of 0.25 min, a signal-to-noise (S/N) ratio of 1.5, and a 3-fold intensity increase over blanks were excluded from further processing. MS/MS spectra were searched against an *in silico* generated lipid spectral library comprising 35,000 unique molecular compositions from 48 lipid classes. Spectral matches with a dot product score greater than 500 and a reverse dot product score greater than 700 were retained for further analysis. Lipid MS/MS spectra which contained no significant interference (<75 %) from co-eluting isobaric lipids, eluted within a 3.5 median absolute retention time deviation (M.A.D. RT) of each other, and found within at least 2 processed files were then identified at the individual fatty acid substituent level of structural resolution. If individual fatty acid substituents were unresolved, then identifications were made with the sum of the fatty acid substituents.