**Nile Rat Plasma Metabolomics – fasting vs. random**

**Objective**

**No. 0192**15 September 2021

**Project Lead:**Ben Anderson

**Project Contributors:**

Joshua J. Coon

Katherine A. Overmyer

Annie Jen

**Collaborators:**

Huishi Toh

Peng Jiang

James Thomson

**Acknowledgement:**

NIH P41 GM108538

Nile rats are a unique model system to explore incidence and biomarkers of diabetes because Nile rats have increased susceptibility to developing hyperglycemia when provided typical rodent chow diet during early development months (conception through weaning) vs. when provided a high-fiber rabbit chow diet in earlier development months. One of the challenges with Nile rats is that blood collections need to happen when animals are still quite young in order monitor diabetic progression and typical fasted-state blood collections could lead to increased stress in young animals. Random (non-fasted) blood sampling would be a better alternative; however, different postprandial states will likely drive higher metabolite variance in non-fasted samples.

This study aims to determine feasibility of random blood glucose-level sampling versus the standard fasted blood glucose sampling on diabetic Nile rats. The analysis presented here performed liquid chromatograph-mass spectrometry metabolomics on Nile rat plasma samples (same samples as report no. 0191, Nile Rat Plasma Lipidomics). Raw data were processed and subjected to preliminary data analysis.

**Results Summary**

, v/v) containing 0.1% ammonium hydroxide. Mobile phase B was initially held at 95% for 2 min and then decreased to 30% over 18 min. Mobile phase B was held for 6 min at 35%, then raised to 95% over 1 min. The column was re-equilibrated

Data are shared in Excel Document [insert name].

The **Targeted Metabolomics Raw** sheet contains the raw output from TraceFinder 4.0 (*vide infra*).

The **Targeted Metabolomics Imputed** sheet contains raw output post-imputation steps using

**Future Plans**

Ben will continue analyzing raw data and may share additional findings with collaborators.

**Sample Preparation**

**Metabolomics sample preparation (same as report no. 0191):**

Plasma samples were removed from freezer and thawed on ice. Each sample was extracted with 500 uL 6:2:2 n-butanol:acetonitrile:water. Samples were vortexed for 10 s and then centrifuged at 14,000 x g for 2 min at 4 °C to precipitate protein. 100 uL of extract was dried down in an amber autosampler vial with glass insert by SpeedVac evaporator. For metabolomics, each extract was resuspended in 25 uL 1:1 Acetonitrile:Water then analyzed on the mass spectrometer.

**LC-MS Analysis**

**HILIC-LC-MS Metabolomics:** Sample analysis was performed on a ZIC-pHILIC HPLC column held at 50 °C (100 mm x 2.1 mm x 1.7 μm particle size; Millipore) using a Vanquish Binary Pump (150 μL/min flow rate; Thermo Scientific). Mobile phase A consisted of 10 mM ammonium acetate in ACN:H2O (10:90, v/v) containing 0.1% ammonium hydroxide. Mobile phase B consisted of 10 mM ammonium acetate in ACN:H2O (95:5, v/v) containing 0.1% ammonium hydroxide. Mobile phase B was initially held at 95% for 2 min and then decreased to 30% over 18 min. Mobile phase B was held for 6 min at 35%, then raised to 95% over 1 min. The column was re-equilibrated at 95% mobile phase B for 8 min. 2 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 350 °C, sheath gas flow rate at 40 units, aux gas flow rate at 15 units, sweep gas flow rate at 1 units, spray voltage at |3.0 kV| for both positive and negative modes, and S-lens RF at 50.0 units. The MS was operated in a polarity switching mode acquiring positive and negative full MS and MS2 spectra (Top10) within the same injection. Acquisition parameters for full MS scans in both modes were 60,000 resolution, 1 × 10e6 automatic gain control (AGC) target, 100 ms ion accumulation time (max IT), and 70 to 900 m/z scan range. MS2 scans in both modes were then performed at 45,000 resolution, 1 × 10e5 AGC target, 100 ms max IT, 1.0 m/z isolation window, stepped normalized collision energy (NCE) at 20, 30, 40, and a 30.0 s dynamic exclusion.

**Data Analysis**

For data analysis, selected m/z and retention times were used to quantify metabolites (see Supplemental table), these peak areas were quantified using Thermo’s Tracefinder 4.0 application.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Tracefinder compound table. | | | | | | |
| **Compound Name** | **Formula** | **MS Order** | **Precursor m/z** | **Peak Polarity** | **Adduct** | **Retention Time** |
| Nicotinamide | C6 H6 N2 O | ms1 | 123.0553 | Positive | M+H | 4.43 |
| O-Isovaleryl-L-carnitine | C12 H23 N O4 | ms1 | 246.17 | Positive | M+H | 8.55 |
| O-Butyryl-L-carnitine | C11 H21 N O4 | ms1 | 232.1543 | Positive | M+H | 9.06 |
| Propionylcarnitine | C10 H19 N O4 | ms1 | 218.1387 | Positive | M+H | 9.65 |
| Tryptophan | C11 H12 N2 O2 | ms1 | 205.0964 | Positive | M+H | 9.9 |
| Acetyl-L-carnitine | C9 H17 N O4 | ms1 | 204.123 | Positive | M+H | 10.43 |
| L-Phenylalanine | C9 H11 N O2 | ms1 | 164.0717 | Negative | M-H | 10.74 |
| DL-Leucine/Isoleucine | C6 H13 N O2 | ms1 | 130.0874 | Negative | M-H | 10.82 |
| Pantothenic acid | C9 H17 N O5 | ms1 | 218.1034 | Negative | M-H | 11 |
| Indole-3-acrylic acid | C11 H9 N O2 | ms1 | 188.0706 | Positive | M+H | 11.6 |
| DL-Proline | C5 H9 N O2 | ms1 | 116.0706 | Positive | M+H | 11.79 |
| L-Valine | C5 H11 N O2 | ms1 | 116.0717 | Negative | M-H | 11.85 |
| DL-Carnitine | C7 H15 N O3 | ms1 | 162.1125 | Positive | M+H | 12.05 |
| Guanosine | C10 H13 N5 O5 | ms1 | 282.0844 | Negative | M-H | 12.06 |
| L-Iditol to Six-carbon sugar alcohol | C6 H14 O6 | ms1 | 181.0718 | Negative | M-H | 12.48 |
| L-Tyrosine | C9 H11 N O3 | ms1 | 180.0666 | Negative | M-H | 12.76 |
| Glycine | C2 H5 N O2 | ms1 | 0 | Positive | M+H | 12.8 |
| L-Alanine | C3 H7 N O2 | ms1 | 88.0404 | Negative | M-H | 13.54 |
| Threonine | C4 H9 N O3 | ms1 | 118.051 | Negative | M-H | 13.6 |
| DL-Glutamine | C5 H10 N2 O3 | ms1 | 147.0764 | Positive | M+H | 14.11 |
| α-Lactose | C12 H22 O11 | ms1 | 360.15 | Positive | M+NH4 | 14.32 |
| Adenosine 5'-monophosphate | C10 H14 N5 O7 P | ms1 | 348.0704 | Positive | M+H | 14.39 |
| L-(+)-Citrulline | C6 H13 N3 O3 | ms1 | 176.103 | Positive | M+H | 14.49 |
| L-Serine | C3 H7 N O3 | ms1 | 104.0353 | Negative | M-H | 14.56 |
| Cytidine 5'-diphosphocholine | C14 H26 N4 O11 P2 | ms1 | 489.1146 | Positive | M+H | 14.88 |
| L-Glutamic acid | C5 H9 N O4 | ms1 | 146.0459 | Negative | M-H | 14.91 |
| L-Aspartic acid | C4 H7 N O4 | ms1 | 132.0302 | Negative | M-H | 15.14 |
| L(+)-Ornithine | C5 H12 N2 O2 | ms1 | 133.0972 | Positive | M+H | 19.29 |
| DL-Lysine | C6 H14 N2 O2 | ms1 | 147.1128 | Positive | M+H | 19.96 |
| L-(+)-Arginine | C6 H14 N4 O2 | ms1 | 175.119 | Positive | M+H | 20.67 |

Raw data table for all raw files were subjected to imputation using MetImp 1.2, with parameters MCAR/MAR, Imputation method Random Forest, Set Seed = 1234, filter variables by non-missing proportion = 0.8, Checked group-wise missing filtering (all default values) [citation <https://www.nature.com/articles/s41598-017-19120-0>]

Unlike with the Lipidomics output, there is no evidence for analytical run order affecting the median feature quantitation, therefore no normalization was performed on these data.