PLASMA METABOLOMICS AND LIPIDOMICS REVEALS FED SAMPLING IS SUPERIOR TO FASTED FOR EARLY DETECTION OF DIABETES IN NILE RAT MODEL

COON LABORATORIES

Benton J. Anderson[1], Huishi Toh[3], Katherine A. Overmyer[2], Peng Jiang[4], Joshua J. Coon[1,2]

[1]University of Wisconsin-Madison, Madison, WI, USA; [2]Morgridge Institute for Research, Madison, WI, USA; [3]University of Santa Barbara, Santa Barbara, CA, USA; [4]Cleveland State University, Cleveland, OH, USA

BACKGROUND

Nile rat animal model for type 2 diabetes

Credit: *Nature Lab Animals*[1]

Nile rats are an emerging animal model of type 2 diabetes. Diabetic disease progression and risk indicators are more similar to humans than other rodent models, such as increased blood pressure, increased triacylglycerols, and hyper insulinemia. Also, onset of diabetes can be induced readily and at a young age with a high fat diet.

Question #1: Is collecting blood during random feeding a viable alternative to fasting?

Because diabetes onset occurs rapidly and at a young age in Nile rats, it is critical to sample blood plasma early and often. Sampling fasted rats causes high stress and therefore confounds analysis. We aim to assess the repeated-measures variability of sampling blood under random feeding via bulk phenotypes (random-fed blood glucose) and by using liquid chromatography-mass spectrometry (LCMS) metabolomics and lipidomics to measure plasma metabolites.

Question #2: Do plasma metabolites show different predictive ability of diabetes in Fed versus Fasted?

To understand differences in plasma metabolites that correlate to diabetes between fasted and fed sampling, we constructed statistical models that predict diabetic status of Nile rats, then used feature importance metrics from these models to propose metabolites that best correlate with diabetes.

FASTED VERSUS FED PLASMA METABOLITES

Figure 5 - Lipids volcano plot

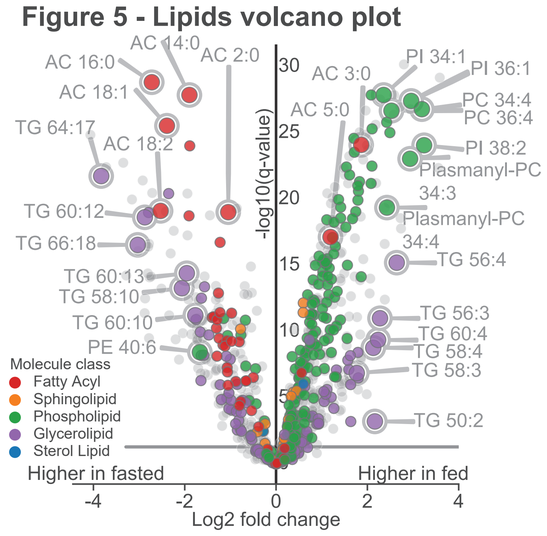


Figure 5 - Volcano plot of plasma lipids comparing mean quantitation between fasted and fed. High polunsaturated TG (triacylglycerol) lipids are enriched in fasted samples, whereas less unsaturated TGs are enriched in Fed. Phospholipids are generally upregulated in Fed samples.
Each point uses the average from all Nile rats, regardless of diabetic status, meaning that diabetes-level differences per animal (i.e. interaction effects) are masked.

Figure 6 - TG differences by FAs

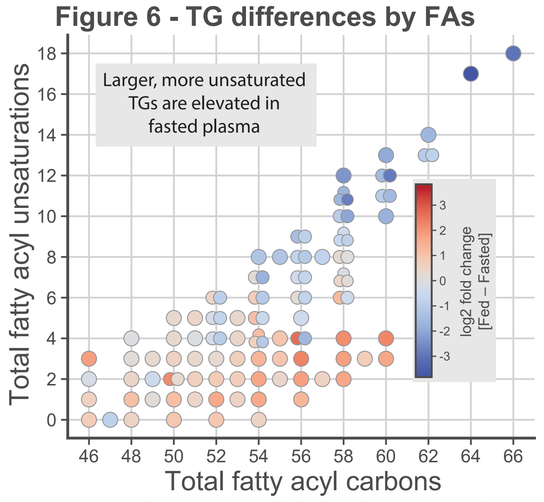


Figure 6 - Plasma triacylglycerols vary in their response to fasting versus feeding depending on number of unsaturations (y-axis) and number of carbons (x-axis) in their 3 fatty acyl tails.
In the fasted state, energy stores are depleted therefore metabolic activity shifts towards utilizing polyunsaturated fatty acyl groups typically found in structural phospholipids, leading to upregulation of longer, more unsaturated TGs (top right, enrichment of blue dots).

Figure 7 - Polar metabolites volcano plot

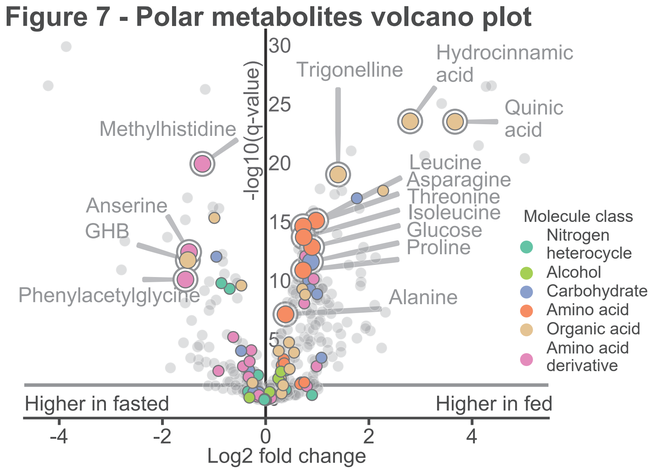
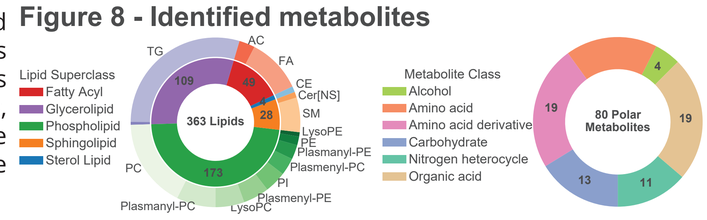


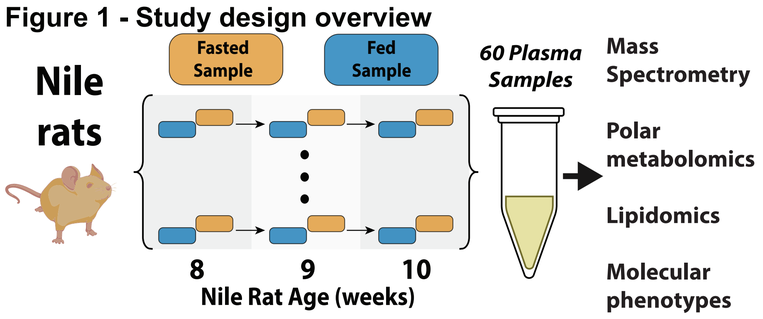
Figure 7 - Volcano plot of plasma polar metabolites comparing mean quantitation between fasted and fed. Fed samples enrich for amino acids that are present in diet (orange dots, right side), and fed samples also enrich for common metabolites of plant consumption (trigonelline, quinic acid).

Figure 8 - Identified metabolites



METHODS & STUDY DESIGN

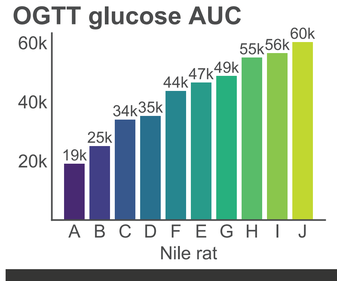
Figure 1 - Study design overview



Nile rats undergo weekly blood plasma sampling while fasted and fed over 3 weeks. The nested study design allows for assessing reproducibility between methods by comparing fasted versus fed measurements within each rat. Repeated sampling increases power of prediction of Nile rat blood phenotypes from molecular profiles.

Mass spectrometry with two types of liquid chromatography enables quantification of molecular phenotypes both within and between Nile rats. Molecular abundances can then be correlated with Nile rat phenotypes.

Figure 2 - OGTT glucose AUC



Oral glucose tolerance test (OGTT) is a common method for assessing glucose tolerance in animals and humans. We use OGTT Area Under the Curve (AUC) calculated from blood glucose timepoints over 2 hours as a proxy for diabetic status. ***Higher is more diabetic.***

MOLECULAR CORRELATIONS TO DIABETES

Figure 9 - Fasted vs. Fed regression slopes

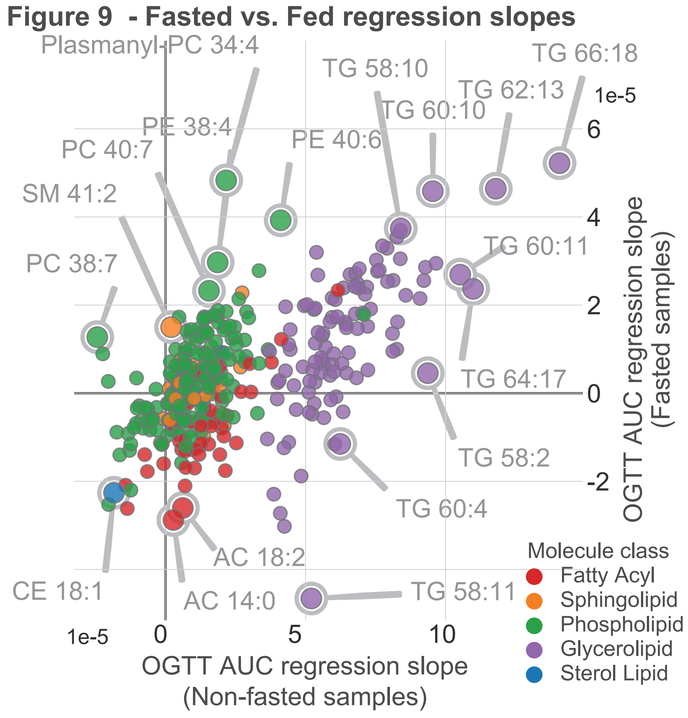


Figure 9 - Slopes of linear models to predict abundance based on OGTT AUC, with one model per metabolite. y-axis is slope of models trained on fasted data, x-axis slope of models trained on fed data. Greater distance from 0 indicates metabolite abundance shows stronger correlation with OGTT AUC and diabetes.

Figure 10 - Average of highly unsaturated TGs (>10 unsat.)

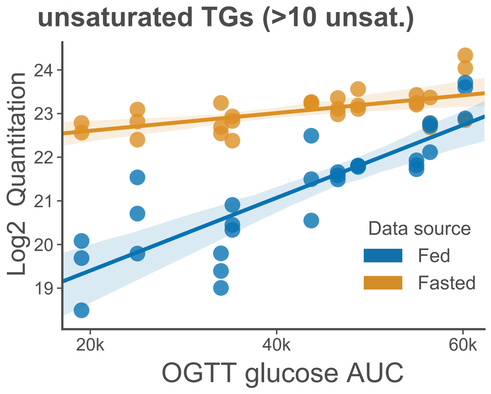


Figure 11 - OGTT glucose AUC prediction R^2

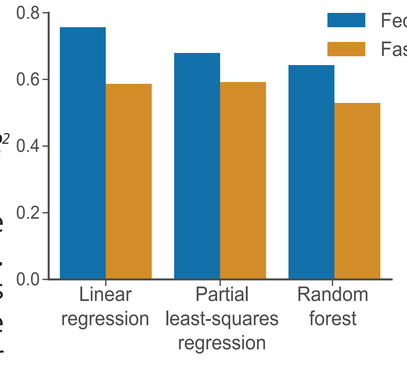


Figure 10 - Mean quantitation of highly polyunsaturated TGs when Nile rats are Fed show different average levels and different responses depending on Nile rat's OGTT AUC measurement. The difference in slopes between Fed (blue line) and Fasted (orange line) is an indication of an interaction between Fasted/Fed and diabetic status.

Figure 11 - 3 different types of statistical models were trained on Fed and Fasted data to determine which feeding state can provide a better estimate of OGTT AUC. In the parametric model (linear regression), latent space model (partial least squares) and non-parametric model (random forest), Fed gives higher R^2 values in cross validation.
Feature importance for predicting diabetes was calculated using Elastic Net linear regression, giving the top 10 most explanatory metabolites (below).

Top metabolites for predicting diabetes using Elastic Net regularization:

-PC 36:3	-FA 18:0
-PC 38:7	-SM d37:1
-TG 22:6_22-:6_22:6	-4-Guanidinobutyric acid
-TG 18:1-_22:6_22:6	-Plasmanyl-PC O-20:0_20:4
-TG 20:5_22:6_22:6	-CE 18:1

FASTED VERSUS FED REPRODUCIBILITY

Comparing Reproducibility using Relative Standard Deviation (%RSD)

Figure 3 - Within Nile Rats

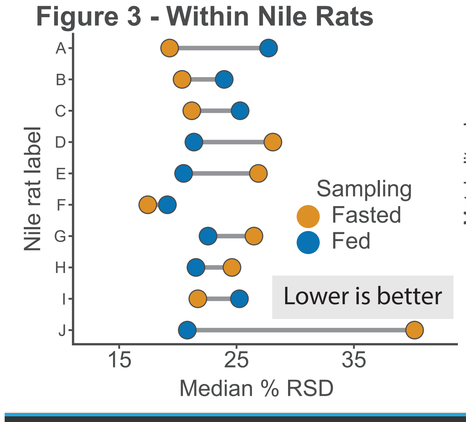
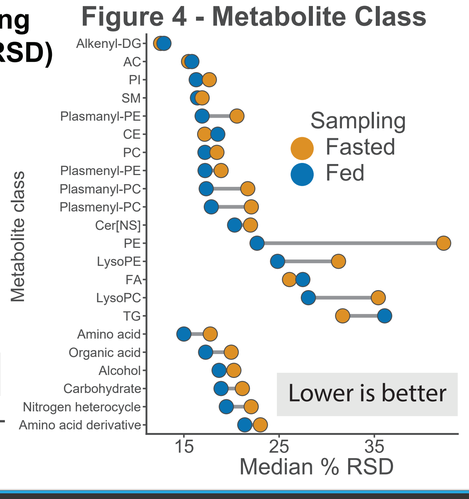


Figure 4 - Metabolite Class



In 50% of Nile rats, the median metabolite variance is lower in non-fasted plasma. This goes against conventional wisdom that eating induces high variability in plasma metabolites.

Key stat:

60%

of plasma metabolites show better reproducibility when Nile rats are fed

CONCLUSIONS

Fed sampling has superior repeatability versus fasted

- Lower variance from fed samples ensures better repeatability of plasma metabolite and lipid analysis
- Avoiding fasting will induce less stress on animals and minimize confounding effects

Fed sampling reveals more metabolites that show effects of diabetes

- Fasting minimizes the presence or differential expression of metabolites such as glucose or triacylglycerols in plasma, obscuring important metabolic changes that occur in diabetes

REFERENCES

(1) Refinetti, R. The Nile Grass Rat as a Laboratory Animal. Lab Anim 33, 54–57 (2004). https://doi.org/10.1038/laban1004-54
(2) Hutchins, P. D.; Russell, J. D.; Coon, J. J. LipiDex: An Integrated Software Package for High-Confidence Lipid Identification. Cell Systems, 2018, 6, 621-625.e5. https://doi.org/10.1016/j.cels.2018.03.011.

We thank the University of Wisconsin-Madison and the National Institutes of Health for support. Work in this project was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award P41GM108538. Benton Anderson acknowledges support of the NLM training grant 5T15LM007359.