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

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 as animal model for diabetes

Credit: *Nature Lab Animals*[1]

Nile rats are an emerging alternative to mouse models of type 2 diabetes due to diabetes disease progression occuring earlier in life, and a disease etiology that more closely resembles diabetes in humans[1].

Research Question #1: Is collecting blood during normal 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 confounds analysis. We aim to assess the viability of sampling blood while rats are normally feeding using liquid chromatography-mass spectrometry (LCMS) metabolomics and lipidomics.

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

With plasma metabolites measured in a cohort of rats with both fasted and fed data, two competing machine learning models can be trained to determine which plasma sampling method provides better predictions for diabetic status.

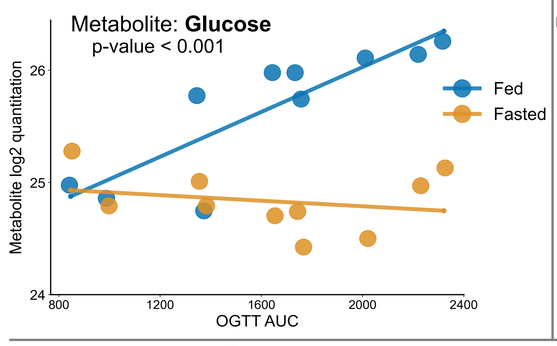
PREDICTING DIABETES WITH MACHINE LEARNING

"Flipping the model"

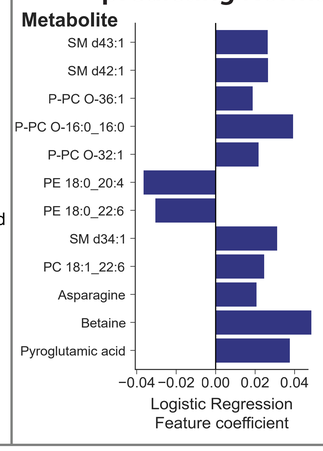
Nile rat phenotypes predict metabolite quant.

Classic Linear Model:
Diabetes ~ Metabolite1 + Metabolite2 + ... + MetaboliteN

Alternative "Flipped" Model plus Likelihood Ratio Test:
Metabolite ~ Diabetes + Fasting + Diabetes : Fasting

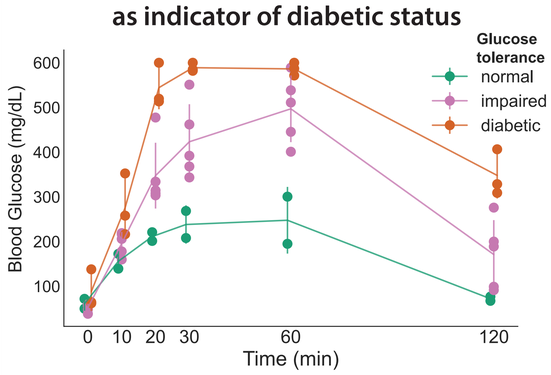


Regularized Logistic Regression predicting Normal vs. Diabetic



Because the number of features is much greater than the number of animals (P>>N), regularization with L1, L2 or both (Elastic Net, used here) limits the metabolites and lipids that contribute to predicting whether a sample comes from a normal or diabetic rat. L1/L2 ratio = 0.2.

Using Oral Glucose Tolerance Test (OGTT) as indicator of diabetic status

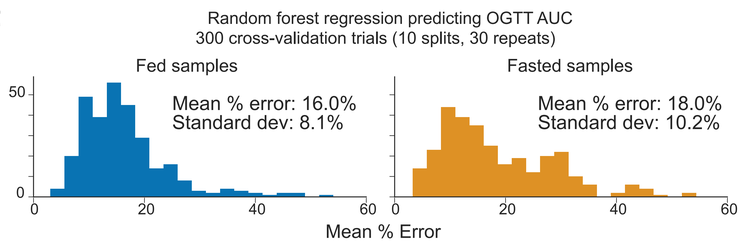


All rats underwent oral glucose tolerance test (OGTT) at age 12 weeks. Rats are fasted, then fed glucose. Blood glucose levels are measured at set intervals (x-axis). The area under the curve (AUC) for each rat yields an OGTT AUC value. Values above 1,000 have impaired glucose tolerance and values above 2,000 are diabetic.

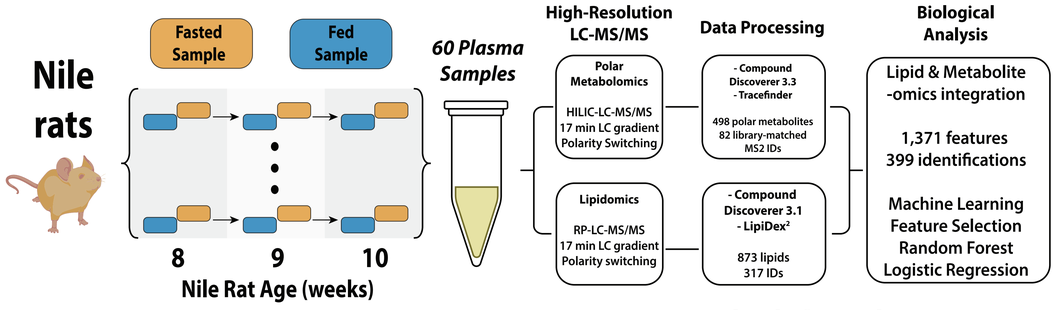
As a proxy for diabetes progression, machine learning models can predict OGTT AUC (Regression) or multinomial classification (Normal, Impaired, or Diabetic labels)

Comparing Random Forest models trained on Fasted data versus Fed data

Under repeated cross-validation, Random Forest regressors trained with only Fed data minimizes error and improves prediction of OGTT AUC.



METHODS AND STUDY DESIGN

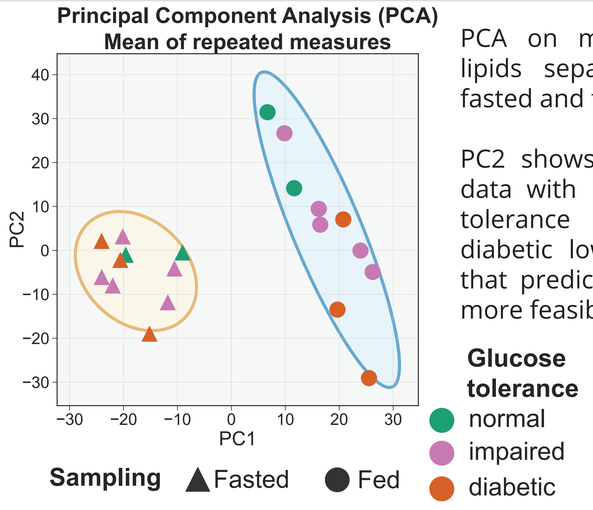


Nile rats (n=10) undergo repeated sampling of blood over 3 weeks (age 8-10 weeks), with the feeding method alternating between Fasted and Fed states

Separate LC-MS methods for polar metabolites and lipids expands LCMS analysis of small molecules in plasma

Small molecule -omics provides insight into plasma where proteomics and RNAseq can struggle due to sparsity of analytes or other confounders.

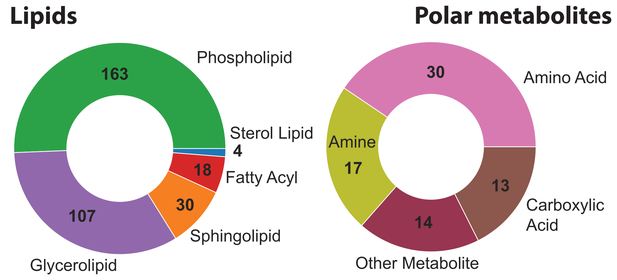
MASS SPECTROMETRY -OMICS



PCA on metabolites and lipids separates between fasted and fed data on PC1.

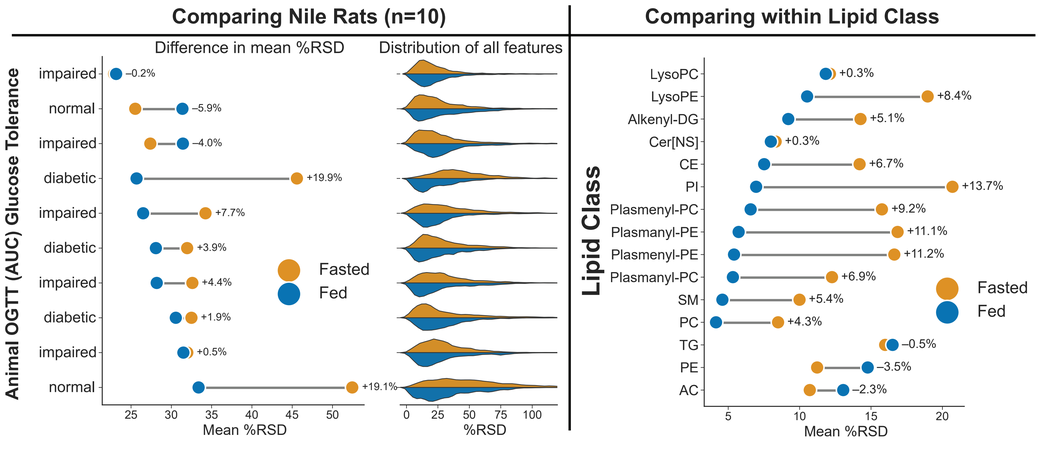
PC2 shows trends in Fed data with Normal glucose tolerance higher and diabetic lower, suggesting that predicting diabetes is more feasible in Fed data.

Identifications breakdown by molecule class



FASTED VERSUS FED VARIANCE

Relative Standard Deviation (%RSD) Difference between Fed and Fasted



Low variance in repeated measurements is ideal. How does variance compare in fed versus fasted?

Comparison of fasted vs. fed when broken down by each Nile rat (left plot) and within lipid class (right plot).

When fed is to the left of fasted, this suggests that using Fed data is a superior analytical method for repeated measures on Nile rats. **For 7/10 rats and 12/15 lipid classes, fed shows lower variance.**

The extreme percentage difference (+19.9% and +19.1%) in two rats is likely due to the fasted rats finding food remnants, which is relatively common and confounds fasted data.

CONCLUSIONS

Fed sampling shows less variance than Fasted

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

Fed sampling reveals different metabolites to predict diabetic status

- 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.

ACKNOWLEDGEMENTS

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.