Research and Creative Experience for Undergraduates Program (RCEU)
Summer 2018

COLLEGE OF SCIENCE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

HONORS COLLEGE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

Metabolomic Analysis of Urine and Tissue in Rats Prone to Type 1 Diabetes Using NMR

Emily Hunt, Sumedha Bobba, James Wolfsberger, and Dr. Bernhard Vogler,
Department of Chemistry

Introduction

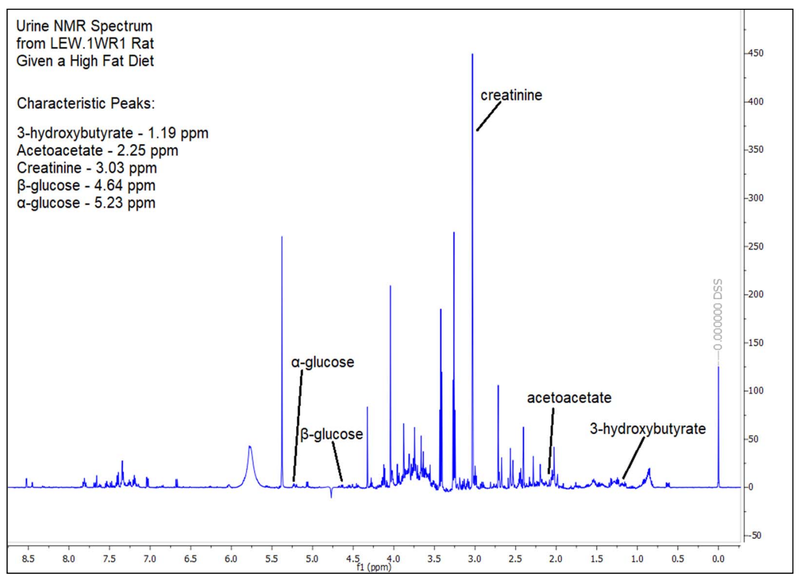
Type 1 Diabetes (T1D) is an autoimmune disease, in which insulin-producing cells are destroyed[1]. Insulin is an important transport and signal molecule in glycolysis[2]. While T1D is treatable with insulin therapy, little is known about how glycolysis is effected by the progression of T1D. Metabolomics is a data-based approach for identifying and quantifying metabolic compounds in biological samples[3]. One of the most common tools in metabolomics is nuclear magnetic resonance (NMR) spectroscopy, because it is non-destructive and quantitative[4]. NMR measures the emission frequency of nuclei excited by an applied electromagnetic field, producing a spectrum that can be used for the metabolomic fingerprinting of biological samples. This experiment aimed to identify and quantify key biomarkers in urine samples from T1D-prone rats and to develop a protocol for processing tissue samples that can be used in later experiments using NMR spectroscopy.

Methods

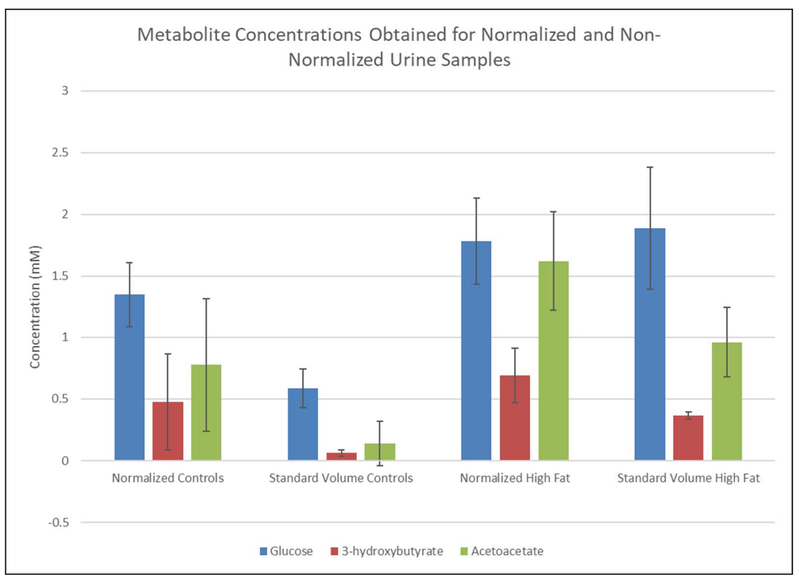
- NMR spectra of standard metabolite solutions were used to create a reference library with MestReNova software.
- Urine samples were obtained from LEW.1WR1 rats given either a high-fat diet or a normal diet and were normalized using the fluorescence emission of urochrome.
- Muscle tissue samples were obtained from control rats and processed with 70% ethanol to remove proteins and lipids.
- Urine and tissue samples were analyzed on a Varian Unity INOVA^{TM} 500 MHz NMR spectrometer, using a PROTON experiment with 128 scans, 6s acquisition, 25s relaxation delay and receiver gain of 30.
- Metabolites in the samples were identified and quantified by analyzing the sample spectra against the reference library.

Results

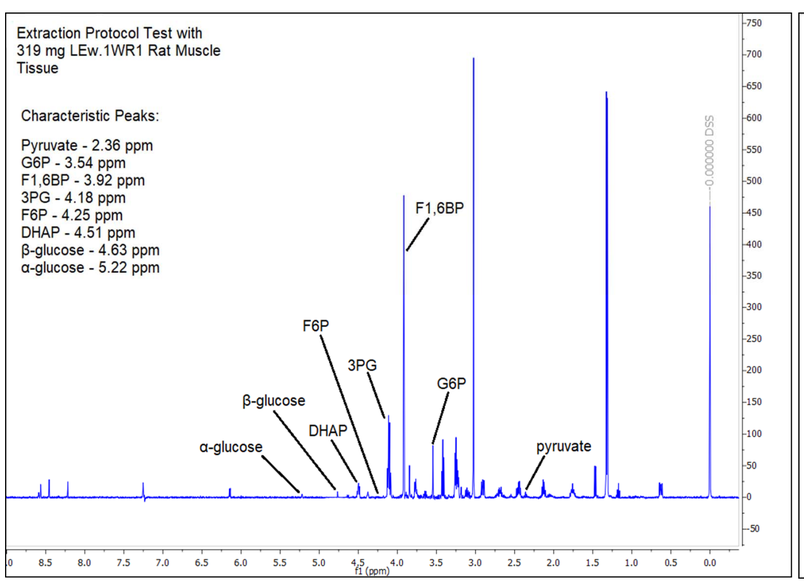
a.



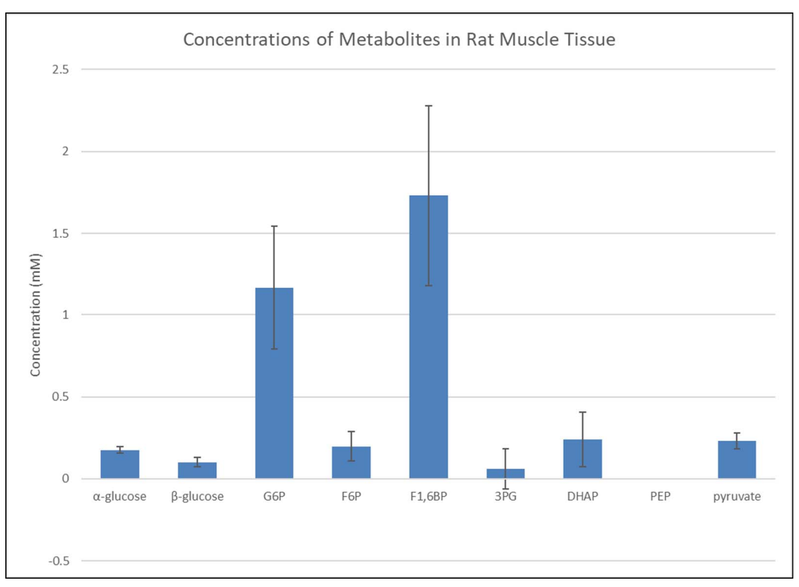
b.



c.



d.



A single characteristic peak was used to quantify each metabolite (see figures a and c). In rats given a high fat diet, elevated levels of glucose, 3-hydroxybutyrate, and acetoacetate were noted in urine. Glycolytic intermediates were not found in the urine samples, but glucose 6-phosphate (G6P), fructose 6-phosphate (F6P), fructose 1,6-bisphosphate (F1,6BP), 3-phosphoglycerate (3PG), dihydroxyacetone phosphate (DHAP), and pyruvate were found in muscle tissue samples (see figure c). Phosphoenolpyruvate (PEP) was not detected. After precipitating proteins in 70% ethanol, peaks that indicate the presence of protein (~5.5ppm) were no longer present in the NMR spectra. When the tissue extraction protocol was carried out several times, similar quantities of all metabolites but PEP were obtained once the concentration was normalized to the weight of the tissue (see figure d).

Figure a: *Typical NMR spectrum obtained from rat urine with characteristic peaks used for identification labeled.* ***Figure b:*** *Graph comparing the average metabolite concentrations in the normalized and non-normalized urine of high-fat and control rats.* ***Figure c:*** *Typical NMR spectrum obtained from rat muscle tissue.* ***Figure d:*** *Graph showing average metabolite concentrations in different samples taken from the same rat muscle.*

Discussion and Conclusions

The reference library created for the quantification of metabolites via NMR allows for single-peak identification of numerous molecules associated with T1D in rat urine. This technique has proven sensitive enough to differentiate between α-glucose and β-glucose. Additionally, the urine of rats given a high-fat diet showed increased amounts of acetoacetate and 3-hydroxybutyrate, indicators of ketoacidosis, which is a common risk for those with T1D. The normalization of urine samples using urochrome improved variation among the high-fat samples and control samples. Additional work will improve this normalization technique. The findings of the tissue analysis show that proteins can be removed from tissue to yield a clearer NMR spectra and reduce peak overlaps. PEP was not identified in tissue NMR spectra, perhaps indicating that the reaction converting PEP to pyruvate may occur too quickly for PEP to be detected. Furthermore, the large standard deviation for 3PG could be the result of peak overlaps with F6P or F1,6BP. Further work is needed using other metabolomics techniques, such as LC-MS, to confirm the findings of this study.

References

1. Atkinson, M. A.; Eisenbarth, G. S.; Michels, A. W. Type 1 Diabetes. *Lancet* 2014, *383* (9911), 69–82.
2. Wu, C.; Khan, S. A.; Lange, A. J. Regulation of Glycolysis - Role of Insulin. *Exp. Gerontol*. 2005, *40* (11), 894–899.
3. Zhang, G. F.; Sadhukhan, S.; Tochtrop, G. P.; Brunengraber, H. Metabolomics, Pathway Regulation, and Pathway Discovery. *J. Biol. Chem.* 2011, *286* (27), 23631–23635.
4. Wishart, D.S. Quantitative Metabolomics Using NMR. *TrAC – Trends Anal. Chem*. 2008, *27* (3), 228-237.

Acknowledgements

David Cook, Director of Undergraduate Research, and RCEU staff. UAH Office of the Provost, UAH Office of the Vice President for Research and Economic Development and the Alabama Space Grant Consortium for funding. Dr. Sharifa Love-Rutledge for providing samples and assistance.

ALABAMA SPACE GRANT CONSORTIUM
NASA
INDUSTRY
UNIVERSITY
COMMUNITY
1989