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Metabolomic Analysis of Urine From a Rat Model Relevant to Diabetes Using NMR

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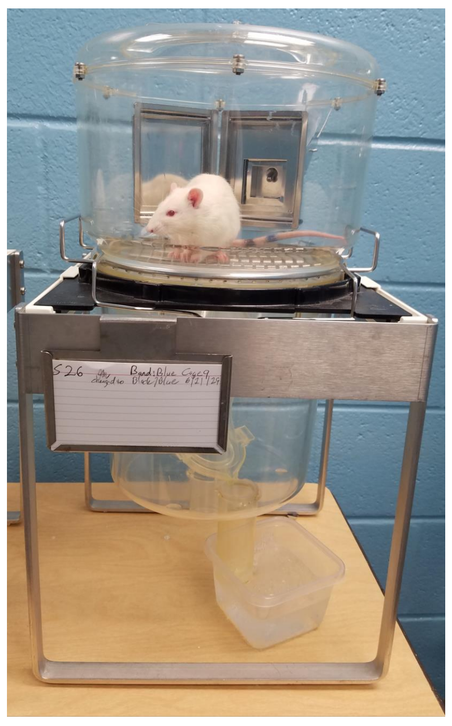
Overview

The metabolism is very complex with a numerous amount of pathways. In those pathways there are hundreds of metabolites with many present in urine which may be detected using NMR spectroscopy. Diabetes is an autoimmune metabolic disorder which causes certain metabolites to often be seen in higher concentrations, which are denoted as taller peaks on nuclear magnetic resonance (NMR) spectroscopy. Identifying these metabolites and their concentrations may give a better understanding of Diabetes and the early biomarkers of the disease.

Methods

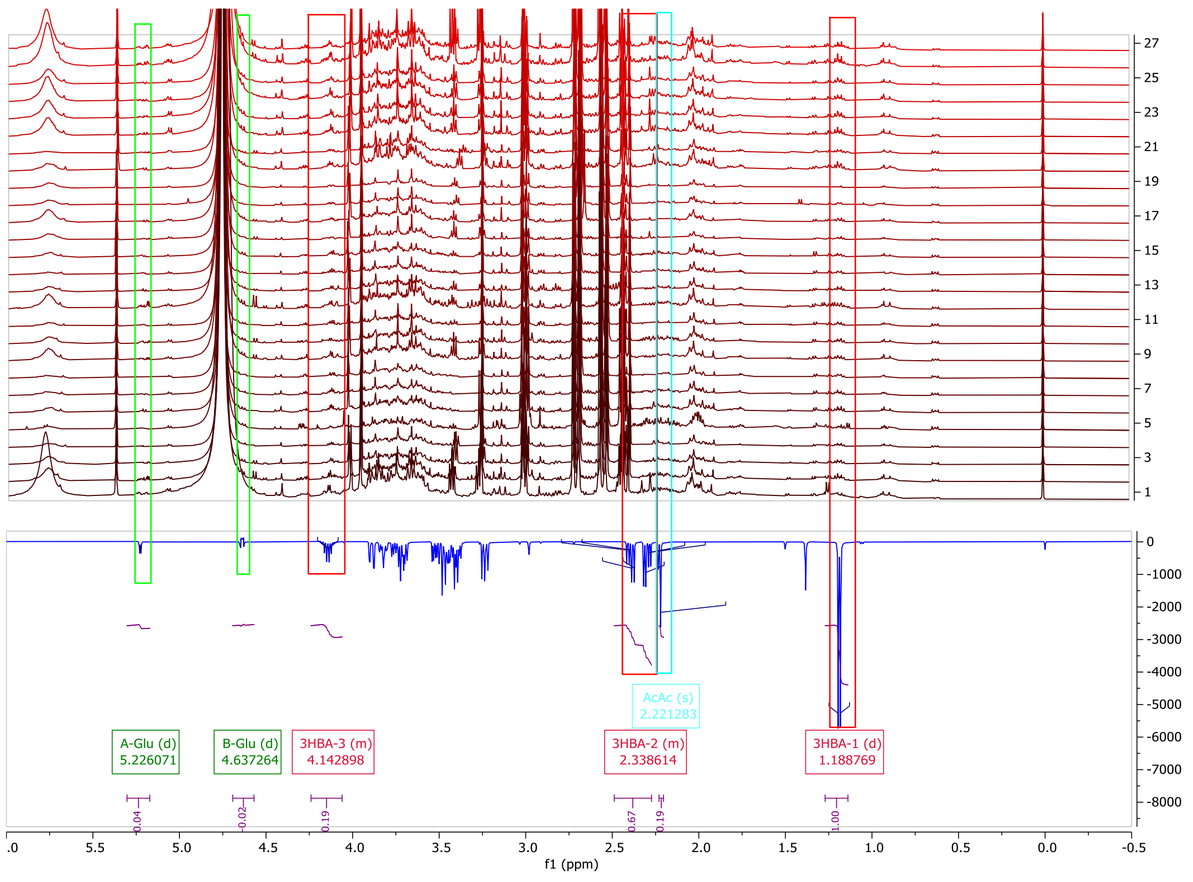
- Urine was obtained from LEW.1WR1 and Wistar Furth Rats. LEW.1WR1s are susceptible to Type 1 Diabetes (T1D). Urine was collected after 4 and 8 hour fasts in both dark and light cycles. It was normalized using the fluorescence and lyophilized and stored at -20 ° C.
- For urine only sample prep, it was reconstituted with 160 μL of 100 mM pH=7 H_2O K-phosphate buffer and mixed with 40 μL of DSS and placed in 3 mm NMR tubes.
- For spike study sample prep, urine was reconstituted with 600 μL of 100 mM, pH=7, H_2O K-phosphate buffer and mixed with 150 μL of DSS and placed in 5 mm NMR tubes. After baseline run, the urine was spiked with 50 μL of desired metabolite solution such as glucose.
- Samples were ran on NMR using the NOESY experiment. Analysis and quantification were done using MestReNova software (version 14.01).

Lew.1WR1 Rat in Metabolic Cage for Urine Collection



Wistar Furth Rat in Metabolic Cage for Urine Collection

Results



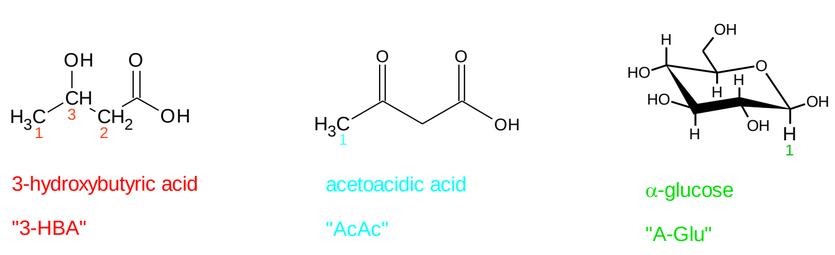


Figure 1: Diabetic LEW.1WR1 rat in comparison to a set of 27 non diabetic rats consisting of both LEW.1WR1 and SsHNsd.

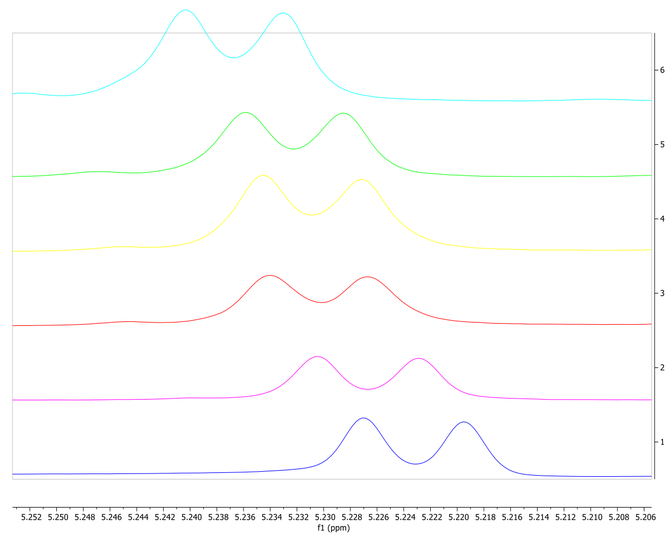


Figure 2: Spike study to evaluate the change in glucose shift relative to the concentration of urine. Shown is the anomeric proton of α-D-glucose.

Conclusions

- The spike studies performed helped determine the peak position of some key metabolites such as glucose and 3-hyroxybutyric acid, this knowledge assisted with quantification.
- Some key metabolites including glucose, 3-hyroxybutyric acid, and li-acetoacetate all showed relatively high concentrations in diabetic rat urine when compared to non-diabetic, this may be an early indicator of T1D.

References

1. Mordes, John P et al. *LEW.1WR1 Rats Develop Autoimmune Diabetes Spontaneously and in Response to Environmental Perturbation*, Diabetes; New York Vol. 54, Iss. 9, (Sep 2005): 2727-33.
2. Human Metabolome Database [Online], http://www.hmdb.ca/, Accessed August 2019.

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