

Quantifying Whole Pancreas Area in a Type 1 Diabetes Susceptible Rodent Model

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Overview/Introduction

Overexpression of the ubiquitin-like protein *fat10* has been implicated in diseases such as colorectal, gastric, and liver cancers, kidney disease, and type 1 diabetes [1, 2]. *Fat10* has also been implicated as a metabolic regulator with a role in aging, but it is unclear how *fat10* effects processes such as insulin sensitivity and lipid metabolism [1]. Weanling LEW.1WR1 (1WR1) rats overexpress *fat10* and have increased blood insulin concentrations. 1WR1 rats have increased body mass but show no significant difference in abdominal fat mass compared to control rats (unpublished data), suggesting ectopic lipid deposition, but the location of increased adiposity is unknown. By measuring differences in pancreas area, mass, and pancreatic beta cell mass, we will determine if *fat10* and a moderate sucrose diet promote ectopic lipid deposition in the pancreas of 1WR1 rats.

Materials and Methods

Experiment

LEW.1WR1 rats and Wistar Furth rats were separated into 4 different experimental groups based on strain and diet (control or moderate sucrose). The pancreas was carefully dissected out without disrupting the integrity of the organ — the organs were placed inside a cassette. The cassettes were put into a beaker of 10% neutral buffered formalin (Fisher Scientific) to fix for approximately 24 hours at room temperature. After fixing, the organ was placed in 70% Ethanol (Thermo Scientific™) inside and mailed to Histowiz (Brooklyn, New York) for paraffin embedding, sectioning, staining, and digitizing of slides. The samples were sectioned (4 μm) and stained against insulin (beta cells).

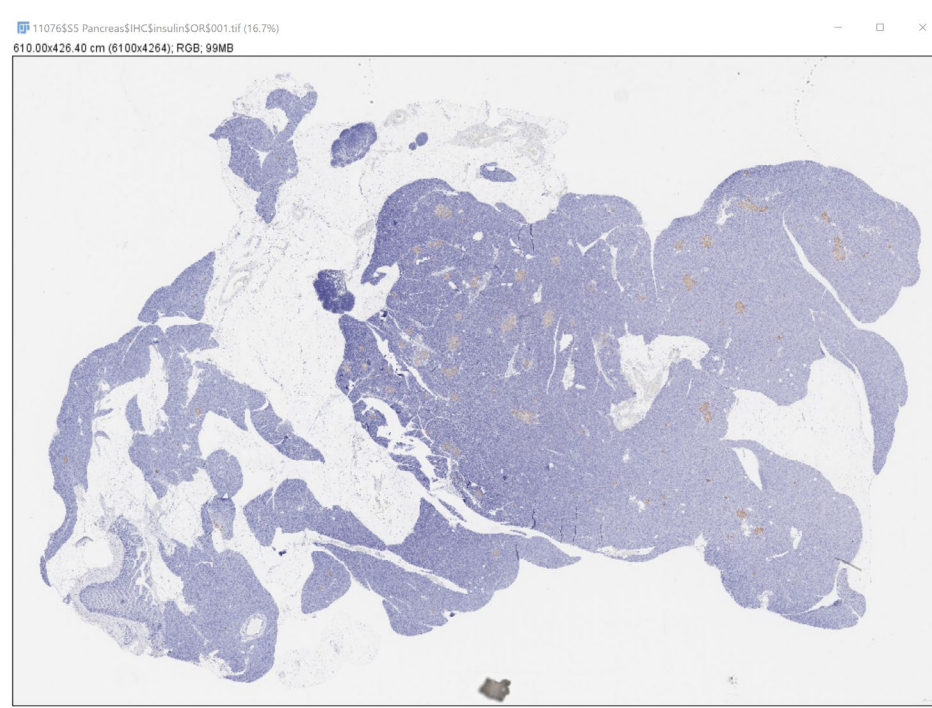


Figure 2. Pancreas Slide in Image J

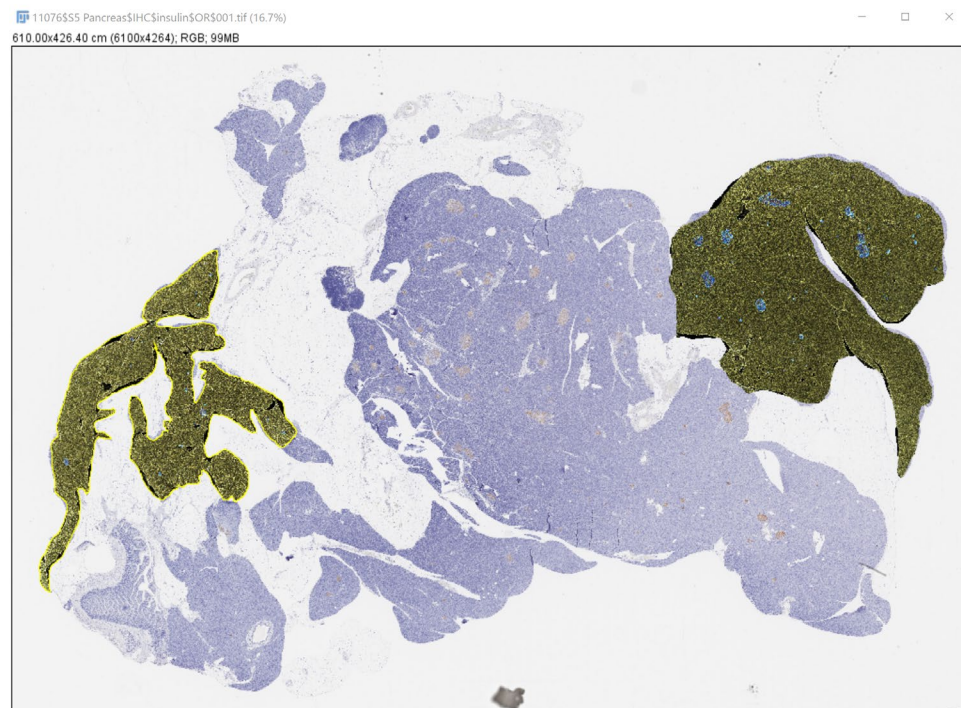


Figure 3. Pancreas Slide Measuring

Analysis

Using ImageJ, a public domain image processing software, the pancreas area and beta cell area were calculated. The surface area was measured using the number of pixels and a ratio of number of pixels of the selected image to square millimeters. Beta Cell Area was calculated by dividing the islet area by the total pancreas area. Beta Cell mass was calculated by multiplying beta cell area by the pancreas mass.

Results/ Discussion

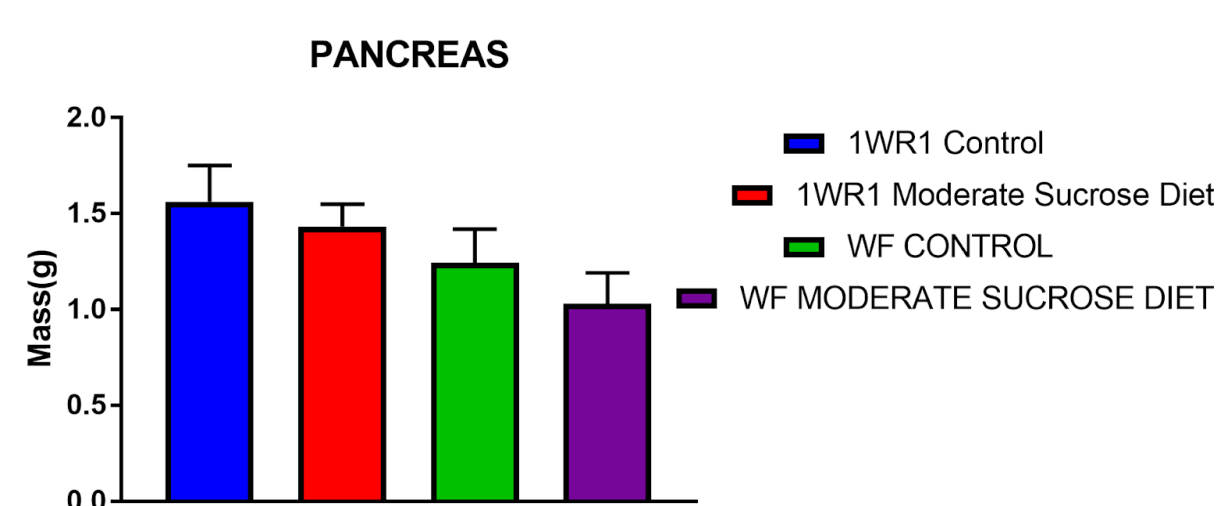


Figure 4. LEW.1WR1 rats have larger pancreases than the Wistar Furth rats. LEW.1WR1 control rats pancreas masses are significantly larger than both Wistar Furth Groups ($p = 0.0011$ and <0.0001). LEW.1WR1 moderate sucrose rats have significantly larger pancreases than Wistar Furth moderate sucrose rats ($p < 0.0001$)

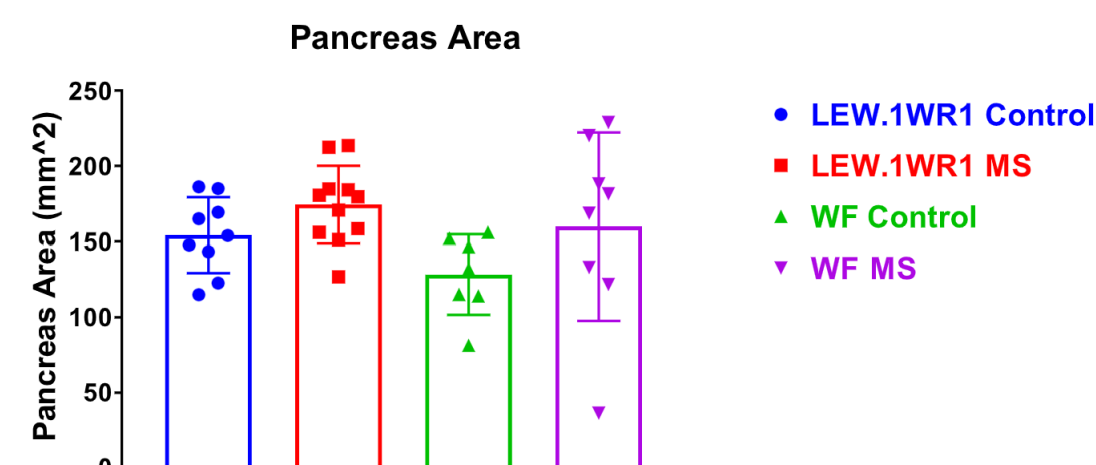


Figure 5. Pancreas areas are similar for all groups. This validates the normalcy of the calculations of normalized beta cell mass and area.

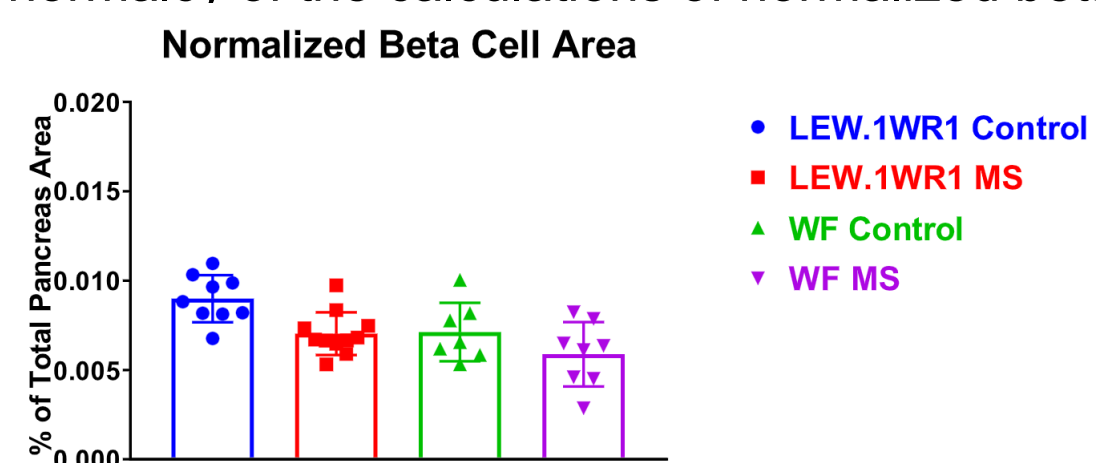


Figure 6. There is not a significant difference in beta cell mass between the two groups. There was no difference in the normalized beta cell area for any of the four groups.

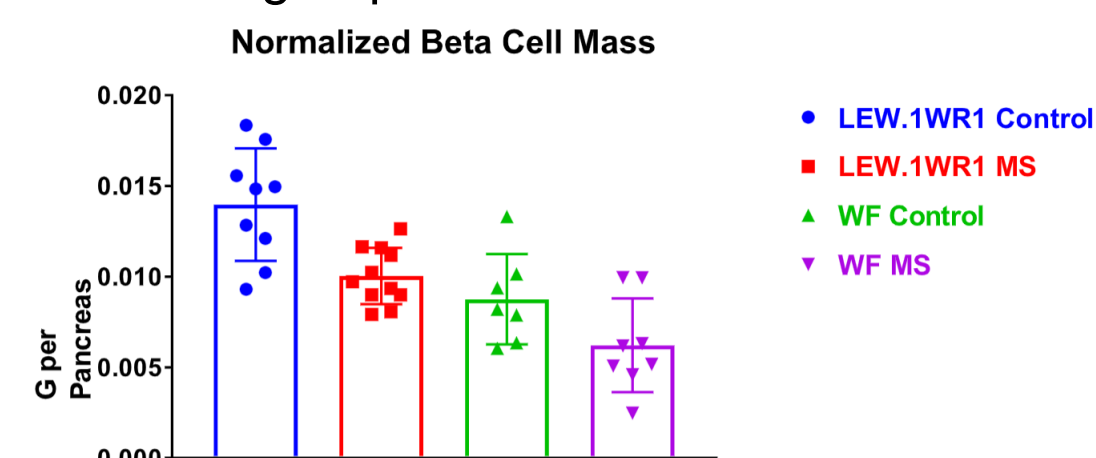
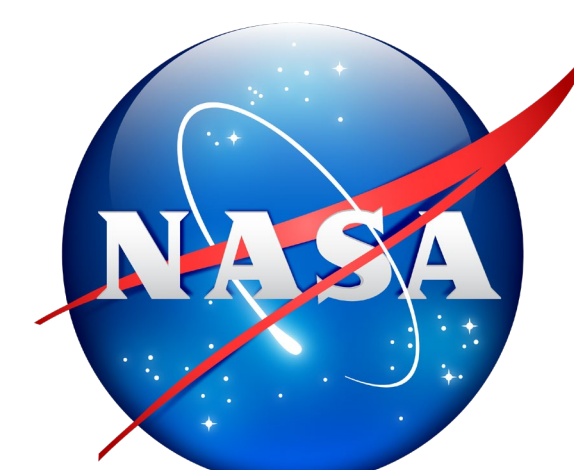


Figure 7. Normalized beta cell mass is down in the Moderate Sucrose groups and Wistar Furth control group compared to the 1WR1 control group. The 1WR1 control group has a p value of 0.0565 and 0.052 with the 1WR1 moderate sucrose and Wistar Furth moderate sucrose groups respectively. This suggests that the increased sucrose in the diet may have a negative impact on the total beta cell mass in the pancreases of these animals. This data also suggests that 1WR1 rats have enlarged pancreases.

Conclusion

- LEW.1WR1 rats have a tendency to have increased pancreas and beta cell masses.
- Increased sucrose in the diet has a negative impact on beta cell mass
- More work has to be done to assess the impact that these differences have on blood insulin levels and insulin sensitivity in the 1WR1 rat.



References

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Acknowledgements

The authors would like to thank the Office of the Provost, UAH College of Science, and UAH Department of Chemistry for funding support. We would also like to thank Mr. David Cook and Dr. Bernhard Vogler for their assistance with the RCEU program. We would also like to thank the Alabama Space Grant Consortium and NASA for RCEU program funding.