Characterizing Glucose Tolerance in Adult LEW.1WR1 Rats

Luis Mercado, Genoah Collins, Amelia Clopp, Dr. Sharifa Love-Rutledge - Department of Chemistry

Introduction
Diabetes prevention is an important topic of research that is focused on nutritional and exercise-based interventions with some genetic predisposition based interventions. FAT10 is a ubiquitin-like protein and Type 1 Diabetes (T1D) susceptibility gene that may play a role in age-related inflammation and adiposity. FAT10 knockout (KO) mice, which lack the FAT10 protein, appear to have smaller islets and increased beta-oxidation and insulin sensitivity. FAT10 KO mice are also protected from insulin resistance but not obesity in response to a high-fat diet. In comparison, the young T1D susceptible LEW.1WR1 rat overexpresses FAT10 and have higher fasting concentrations of blood insulin and triglyceride during the induction of T1D. The LEW.1WR1 rat has a susceptibility window of 21-40 days for T1D induction that is related to the genetic regulation of FAT10 (Mordes, J. P. et al., 2005). It is unclear how the overexpression of FAT10 directly affects insulin sensitivity. It is also unclear if the initial insulin sensitivity and glucose tolerance of this animal model plays a role in this disease susceptibility. This project aimed to analyze the effects of aging and high-fat diet on glucose metabolism in the LEW.1WR1 rat, an animal model shown to overexpress FAT10.

Materials and Methods
- Chemicals: Glucose (MP Biomedical), Humulin (Patterson Veterinary Supply)
- Food: High Fat Diet (HFD): D12451 45 kcal% and Control Diet: D12450J 10 kcal%
- Animals: LEW.ssNHSd [control] and LEW.1WR1 rats obtained from Envigo (Indianapolis IN) and Biomere (Worcester, MA) respectively. Animals were housed in the UAH Vivarium maintained with a 12 hour light dark cycle. Rats were allowed to acclimate for 1 week prior to beginning of study. The animals were approximately 6-7 weeks old at the beginning of the study. Protocol was approved by University of Alabama in Huntsville Institutional Animal Care and Use Committee.
- Glucose Tolerance Test: Alpha Traks 2 meter by Zoetis (Parsippany, NJ) used to measure a baseline glucose value as well as glucose values in 30 minute increments after initial glucose IP injection.
- Statistical Analysis: Area Under the curve, One and two way ANOVA were analyzed using GraphPad Prism 7.04 (La Jolla, California).

Results and Discussion

Figure 1. Body Mass. Body Mass was measured twice weekly during the duration of the experiment. Throughout the experiment the LEW.1WR1 Control, HFD, and LEW.ssNHSd HFD rat showed mirrored growth suggesting all three groups have increased adiposity. All groups of rats had no significant differences in food consumption.

Figure 2. Pre-diet (A) Glucose Tolerance Test and (B) Area Under the Curve. Before separating animals into diet specific groups, the rats were subjected to a glucose tolerance test. The experiment was performed at fasting state (4 hours). Error bars represent SD of n=14. There were no differences between the young LEW.1WR1 and LEW.ssNHSd rats.

Figure 3. Eight weeks Post-Diet (A) Glucose Tolerance Test and (B) Area Under the Curve. Glucose tolerance tests were performed at 8 weeks post diet change (14 weeks old). The experiment was conducted at fasting state (8 hours). Error bars represent SD of n=7 rats. After 8 weeks on new diets, we observed that both LEW.1WR1 groups responded similarly to the LEW.ssNHSd group fed a high-fat diet. High-fat diets are typically used to induce insulin resistance through diet-induced obesity. It appears that the LEW.1WR1 animal may develop increased glucose intolerance as it ages.

Figure 4. Six week and fourteen week old control rat (A) glucose tolerance test and (B) area under the curve. (A) Glucose tolerance tests were performed at six weeks and fourteen weeks of age in the LEW.1WR1 (blue) and LEW.ssNHSd (green) rats. Experiments were performed at fasting states (4 hour and 8 hours respectively). Error bars represent SD of n=14 (6 weeks) or n=7 (14 weeks). The aging and/or change in diet composition seem to exacerbate glucose intolerance in the LEW.1WR1 rat. At both 30 and 60 minutes the fourteen week old LEW.1WR1 has significantly higher blood glucose values when compared to all other groups suggesting that the rats are much more glucose intolerant. Aging is reported to have a baseline effect on glucose tolerance which is why in panel b, the older LEW.ssNHSd rat has a significantly increased AUC. However the LEW.1WR1 rat has a much higher AUC than all of the groups supporting an exacerbated glucose intolerance.

Conclusion
We observed that in the course of 10 weeks the control LEW.1WR1 rats became significantly more glucose intolerant. These animals also gained weight at a rate similar to the high-fat diet, a diet that is traditionally used to induce insulin resistance and glucose intolerance. This data suggests that FAT10 may be playing a role in age-related adiposity and glucose intolerance in the LEW.1WR1 rat. This study begins to lay the groundwork for understanding how alterations in FAT10 and metabolism increase T1D susceptibility.

References

Acknowledgements
Sincere gratitude to the RCEU Staff, UAH Office of the Provost, UAH Office of the Vice President for Research and Economic Development, The Alabama Space Grant Consortium, ALSAMP, and Dr. Emmanuel Waddell. The authors would also like to thank Helen Gibson, Hannah Underhill, Joshua Derbort, Victoria McConnell, Kayleigh Cantrell, and Madushika Wilmaranthne for their assistance with sample collection and animal care.

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