COLLEGE OF SCIENCE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

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LEW.1WR1 rat shows an increased risk of developing malignant forms of NAFLD

Luis Mercado, Madushika Wilmaranthne,
Dr. Sharifa Love-Rutledge - Department of Chemistry

Introduction

Non Alcoholic Fatty Liver Disease(NAFLD) is a blanket term for a variety of conditions that affect a liver containing an overaccumulation of fat. The first and most common condition seen is steatosis which is fat accumulation in the form of lipid droplets and is benign and stable. This benign form however can progress to more serious conditions like steatohepatitis, fibrosis, cirrhosis, liver cancer, and liver failure. NAFLD Activity score(NAS) is a scoring system grading liver ballooning, steatosis, and inflammatory foci to help draw conclusions in the likelihood of progression of NAFLD severity(Kleiner, David E., et al. , 2005). NAFLD is also a disease commonly found along with diabetes in patients. The LEW.1WR1 rat model overexpresses FAT10, a type 1 diabetes susceptibility gene that may play a role in age-related inflammation, adiposity, cancer, and kidney disease. This rat model when exposed to a moderate sucrose diet will also develop glucose intolerance. Past research done in our lab has shown increased FAT10 concentration in the liver. It is unclear how FAT10 affects the liver in addition to its effects in the pancreas and glucose intolerance. This project analyzed the conditions and calculated NAS of LEW.1WR1 and WF/NHsd rat livers subjected to a 7% sucrose and normal diet type.

Materials and Methods

- Animals: WF/NHsd [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 5-7 weeks old at the beginning of the 18 week study. Protocol was approved by University of Alabama in Huntsville Institutional Animal Care and Use Committee.
- Food: Moderate Sucrose Diet(MS): D12450K 10 kcal% and 7% Sucrose, Control Diet: D12450J 10 kcal% by Research Diets (New Brunswick, NJ)
- Histopathology: Formalin fixed Liver sections sent to Histowiz (Brooklyn,New York). Liver Tissues from each animal were sectioned(5μm) and Hematoxylin and Eosin stained (H and E). Resulting digital slides were graded in liver ballooning, steatosis, and inflammation following guidelines in “Design and Validation of a Histological Scoring System for Nonalcoholic Fatty Liver Disease”(Kleiner, David E. et al. , 2005). Circled features indicate NAFLD condition.
- Statistical Analysis: One and two way ANOVA were analyzed using GraphPad Prism 7.04 (La Jolla, California). Different letters represent $p<0.05$ differences.

Results and Discussion

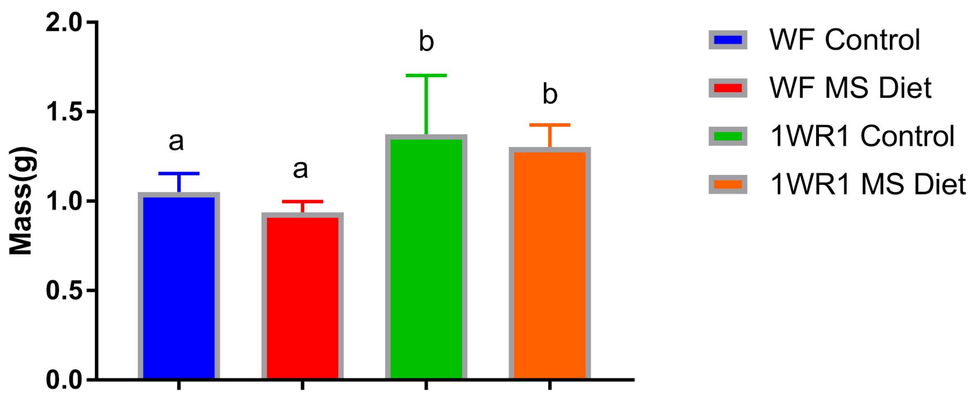
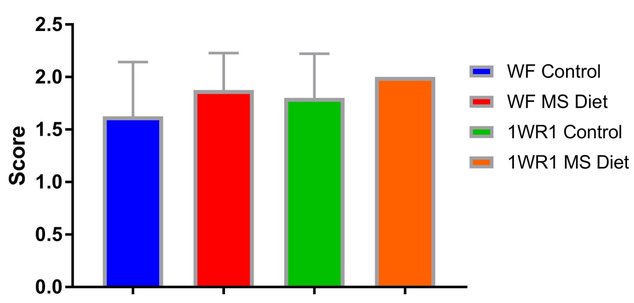


Figure 1. ***Liver Masses.*** Livers were harvested then weighed. The LEW.1WR1 rat had significantly heavier livers than the WF/NHsd. Increased liver mass correlates to the higher steatosis levels found in the LEW.1WR1 rats, as well as their already known characteristic of glucose intolerance.

A.



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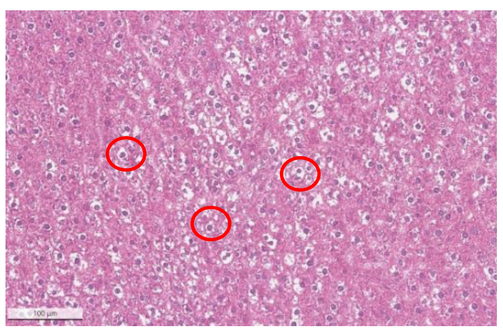
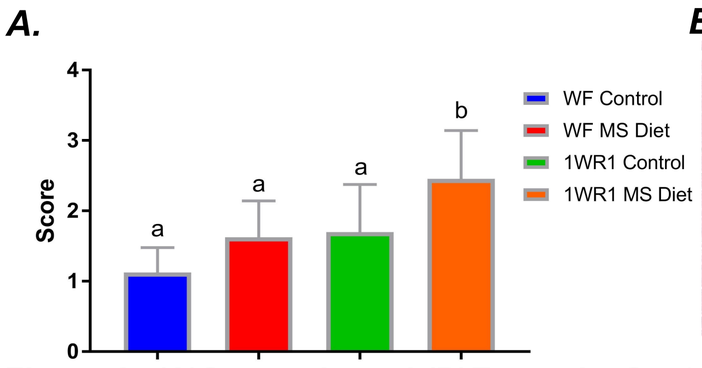


Figure 2. ***(A)Liver Ballooning and (B)Example.*** Graded on a scale from 0-3 with 0 : none, 1 : few, and 2 : many. No significant difference seen between the rat groups. Liver Ballooning is used in describing steatohepatitis as an early indicator of cell degeneration. Differentiation between early liver ballooning characteristics and later characteristics not apparent in scoring.

A.



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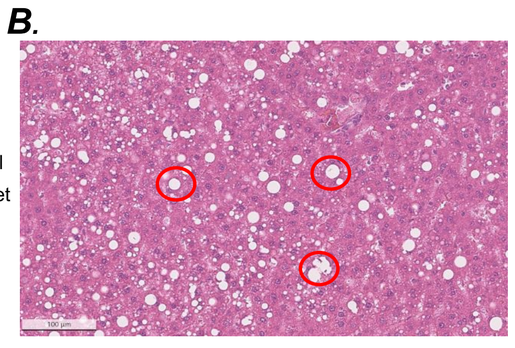
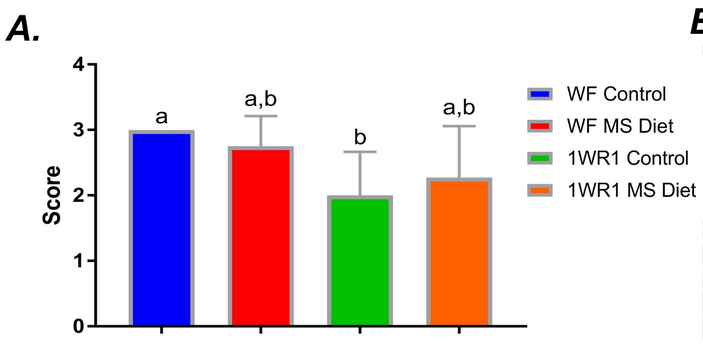


Figure 3. ***(A)Steatosis and (B)Example.*** Graded on a scale of 0-3, 0 : <5%, 1 : 5-33%, 2 : 34-66%, 3 : >66%. LEW.1WR1 MS Diet showed significantly higher amounts of lipid content than all other rat groups. WF/NHsd MS Diet and LEW.1WR1 control showed mirrored scores higher but not significant over WF/NHsd control. LEW.1WR1 tended to have larger more aggressive amounts of steatosis than WF/NHsd, however when scored difference is not as apparent. The size of percentile groups does not differentiate a 99% and 66% steatosis grade though the amount is indicative of progression in NAFLD.

A.



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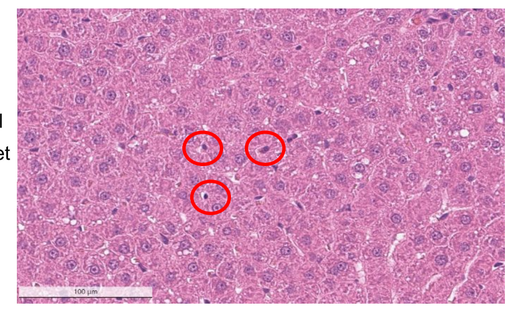

Figure 4.*(A) Inflammatory Foci and (B)Example.* Graded on a scale of 0-3, 0 : none, 1 : <2 foci/20x Field, 2 : 2-4 foci/ 20x field, 3 : >4 foci.20x field. Significant differences between WF/NHsd control and LEW.1WR1 control rats. Foci were congregated mainly on edges of livers and around blood veins. Uncertain fibrosis may have accounted for the lower LEW.1WR1 scores.

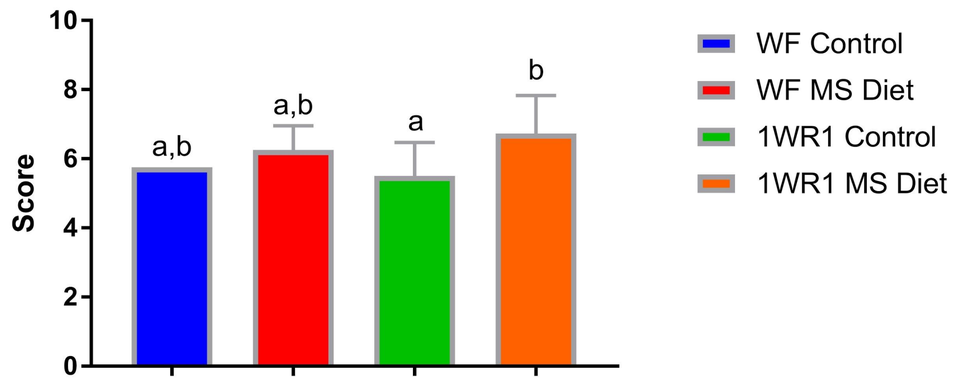

Figure 5. ***NAS.*** NAS calculated from addition of liver ballooning, steatosis, and inflammatory foci average values for each rat group. Significant difference between LEW.1WR1 MS Diet and LEW.1WR1 Control groups. NAS ≥4 is generally agreed to distinguish steatosis and steatohepatitis. Steatohepatitis being the gateway to fibrosis, cirrhosis, and liver failure or liver cancer.

Conclusions

- Diet of Moderate Sucrose affected liver fat development in LEW.1WR1 MS diet more than the WF/NHsd rats MS diet.
- LEW.1WR1 rats showed higher steatosis rate and liver weight than the WF/NHsd rats. FAT10s role in liver glucose intolerance may increase steatosis development shortening time for future steatohepatitis and liver failure risk.
- Predicted fibrosis occurred within some LEW.1WR1 rats. Qualification is a future path. Masson Trichrome staining can be done to see fibrosis level.

References

1. Kleiner, David E., et al. “Design and Validation of a Histological Scoring System for Nonalcoholic Fatty Liver Disease.” *Hepatology*, vol. 41, no. 6, 2005, pp. 1313–1321.

Acknowledgements

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