

LEW.1WR1 rats have increased beta cell area and decreased pancreas area during their T1D susceptibility window

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Introduction

By the time the symptoms of Type I Diabetes (T1D) are noticed, irreparable damage to the patient's pancreas has been done. Therefore, the development of a test to identify people in the earliest stages of T1D development would be highly beneficial. LEW.1WR1 rats tend to develop T1D due to a defect in the FAT10 gene; this sensitivity allows T1D to be induced in these rats by treatment of polyinosinic:cytidylic acid (poly I:C) within 21-35 days of age.¹ In contrast, LEW.SsNHsd rats are diabetes resistant. In an earlier study, we identified through semiquantitative analysis that the total number of islets per section appeared to be significantly different between the two strains of rat (Figure 1). The purpose of this project was to calculate total pancreas area and islet of Langerhans area from these rodents and analyze this data to verify that the changes in islet number correlated with differences in beta cell or pancreas area.

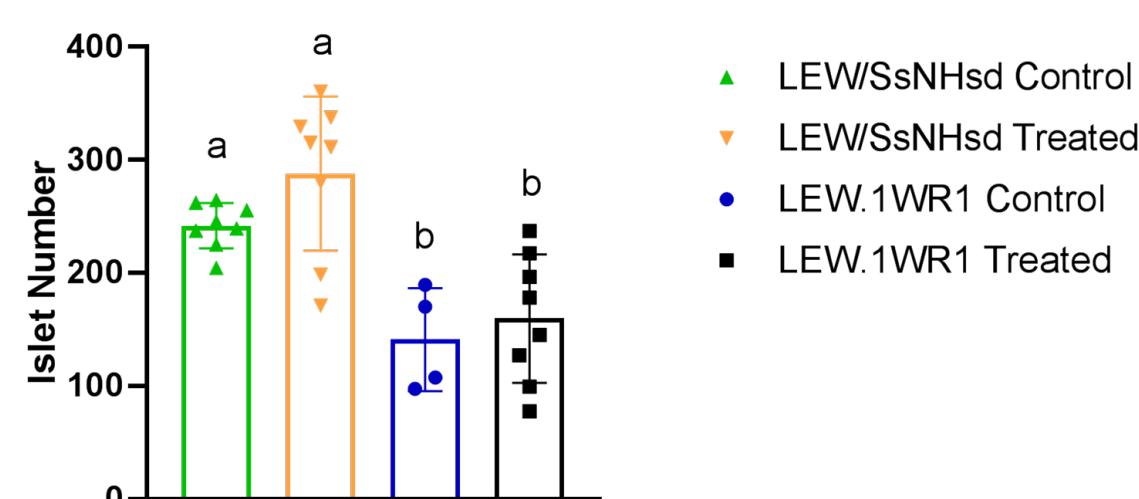


Figure 1. Total Islets. In this previous study, islets for each group of rats were counted. The LEW.1WR1 rats were found to have a significantly lower number of islets compared to SsNHsd rats.²

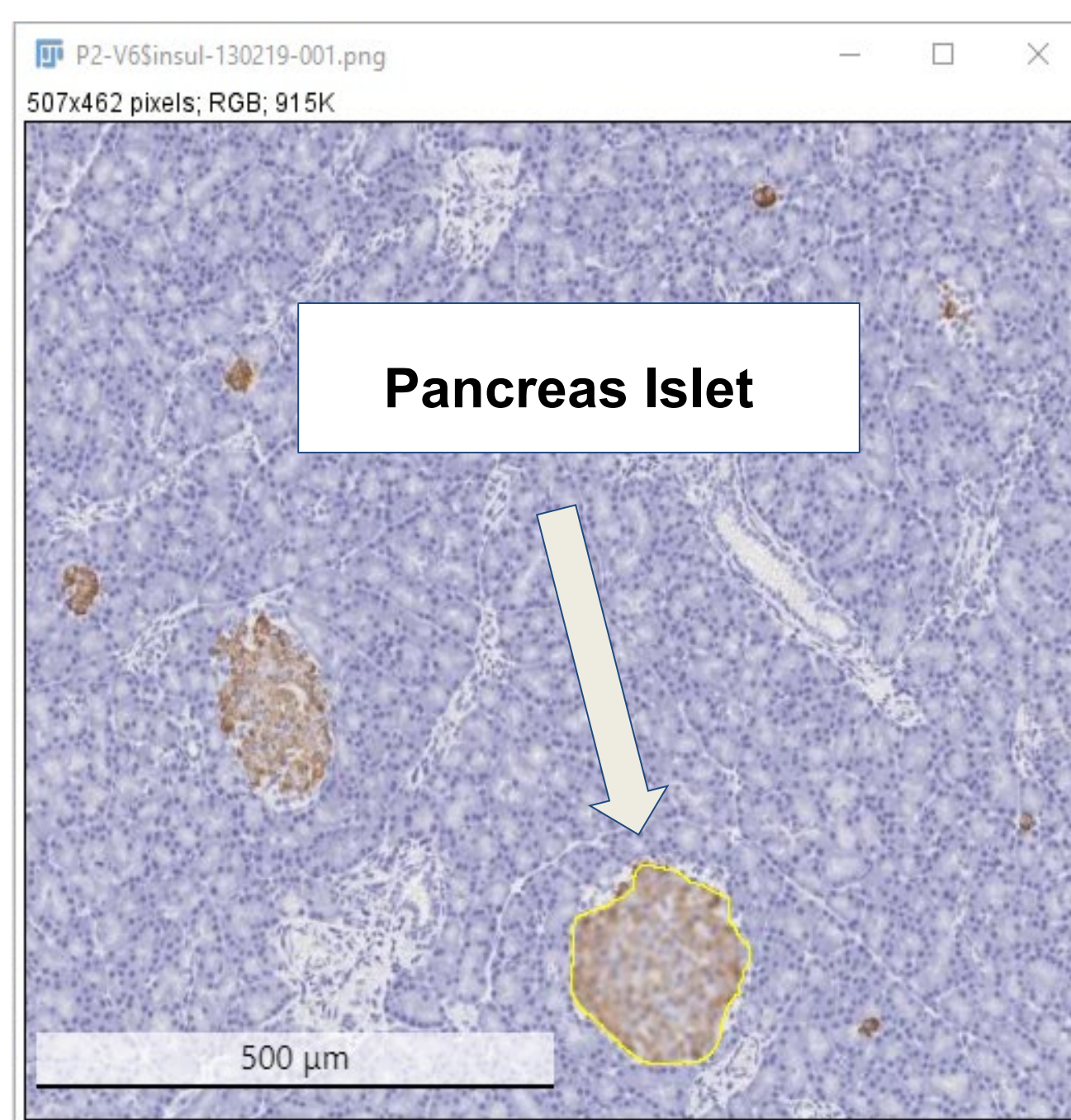
Materials and Methods

Experiment

Both models were split into a poly I:C treated group and a control group. Each group received either injections of poly I:C or phosphate buffered saline every other day for 6 days. After the day 6 injections, rats were placed in metabolic cages. Pancreases were harvested after 7 hours. The formalin fixed rats' pancreases were sent to Histowiz (New York, NY) for immunohistochemical staining and digitization. All procedures were approved by UAH IACUC.

Area Calculation

Images from Histowiz were opened in ImageJ, a public domain image processing program. Islets and pancreases were traced, and area was then automatically calculated in pixels (Figure 2).



Statistical Analysis
Total pancreas area and islet area for each rodent were analyzed using GraphPad Prism 7.04 (La Jolla, CA). One-way ANOVA followed by a Tukey test for comparison of individual means was carried out for each dataset. Different lowercase letters represent a statistically significant difference where $p < 0.05$.

Results and Discussion

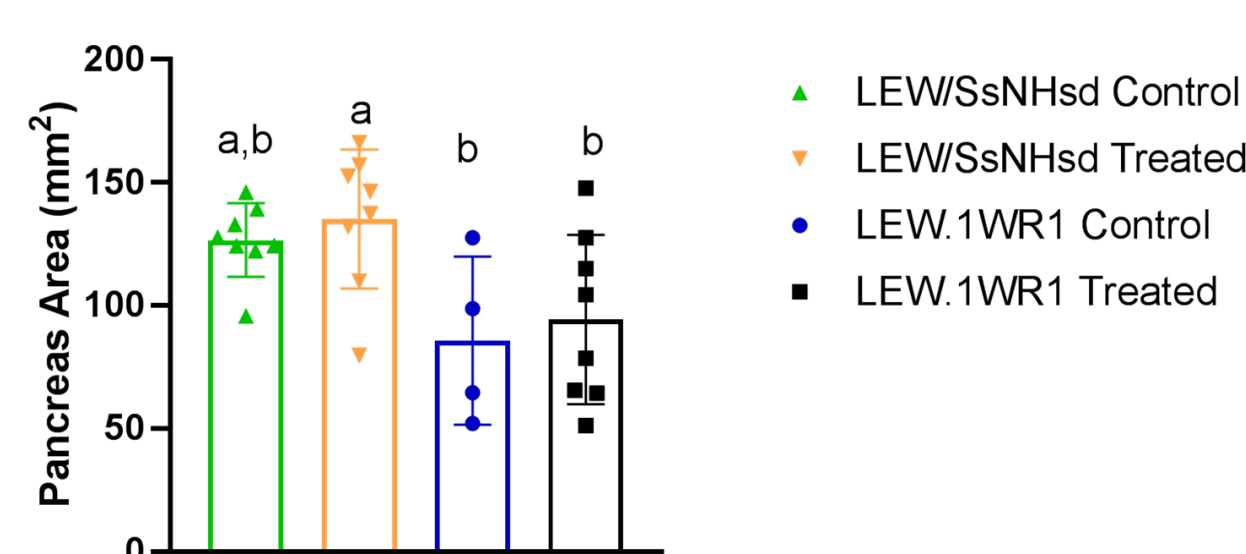


Figure 3. Total Pancreas Area. Data was converted from pixels to mm² using the scale bar included with each image. Each 1WR1 group was significantly smaller

than the SsNHsd Treated group. This could point to decreased pancreas size as a marker for T1D susceptibility.

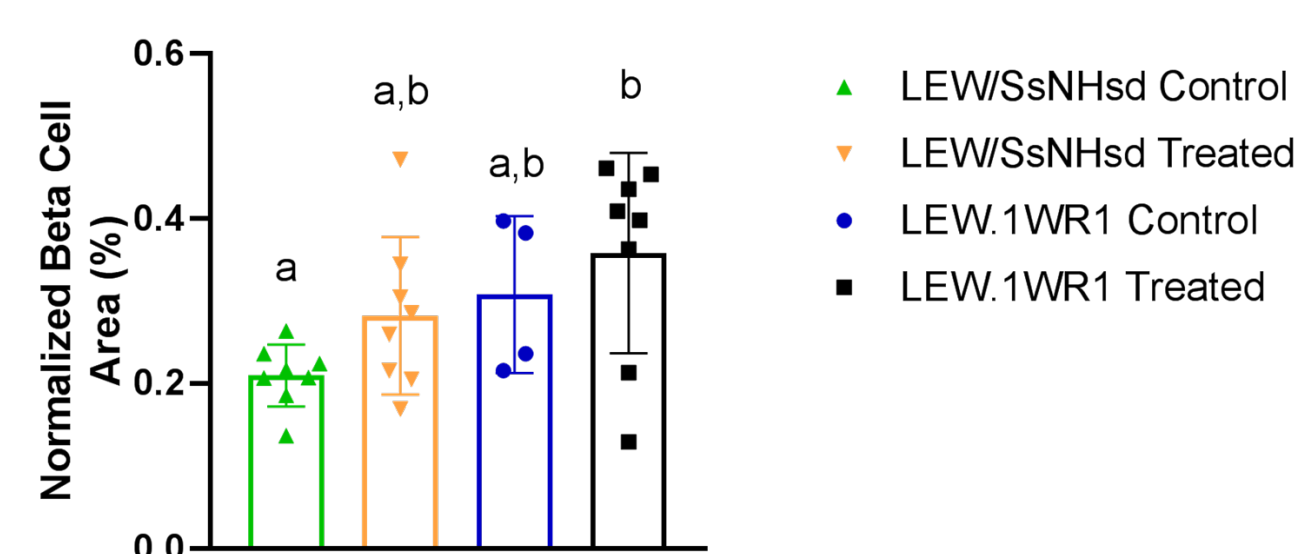


Figure 4. Normalized Beta Cell Area. Beta cell area was converted to μm² using a ratio found common to all pancreases. It was then normalized by

dividing by total pancreas area. The LEW.1WR1Treated rats had significantly larger normalized beta cell area than the LEW/SsNHsd Control rats, which suggests higher beta cell area as a possible marker for T1D development.

Conclusion

- The LEW.1WR1 Treated rats had a significantly higher beta cell area than the Control LEW/SsNHsd rats.
- Pancreas area was significantly smaller for T1D pre-disposed rats (both LEW.1WR1 groups) compared to T1D resistant rats (the LEW.SsNHsd Treated group).
- LEW.1WR1 rats had increased beta cell area and decreased pancreas area during their diabetes susceptibility window. These will need to undergo further testing and be evaluated as biomarkers for T1D susceptibility.

References

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Figure 2. Measuring Islet Area in ImageJ.