Insulin-Resistant Male LEW.1WR1 Rats Do Not Develop β-Cell Mass Expansion in Response to a Moderate Sucrose Diet

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Abstract: Characterizing changes in beta cell function during prolonged hyperinsulinemia and dietary stress is important to study to prevent diseases like metabolic dysfunction-associated steatotic liver disease and insulin resistance. This research investigates how a moderate sucrose (MS) diet affects insulin resistance and β-cell mass in two rat strains: LEW.1WR1 and Wistar Furth (WF). LEW.1WR1 rats seem to be sensitive to beta cell disruptions as weanlings. Twenty-one male LEW.1WR1 rats and sixteen male WF rats were studied over 18 weeks. The rats were divided into groups and given either the control or MS diet. Their body weight was monitored twice a week. Insulin tolerance tests (ITTs) and fasting blood glucose measurements were taken at intervals. Urine samples were analyzed to assess metabolic shifts, and pancreas tissue was examined to evaluate changes in β-cell mass. The LEW.1WR1 rats became overweight and showed higher insulin resistance than the WF rats. Both strains of rats on the MS diet displayed changes in urine metabolite profiles in terms of levels of lactic acid and alanine. This study highlights the impact or lack thereof of a moderate sucrose diet on body mass, insulin resistance, and β-cell mass, with notable effects observed specifically in LEW.1WR1 rats. These findings contribute to our understanding of how dietary sugar intake can affect metabolism when observed in models sensitive to metabolic defects.

Keywords: insulin resistance; β-cell mass; LEW.1WR1 rats; Wistar Furth rats; moderate sucrose diet; metabolomics

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) [1], formerly known as non-alcoholic fatty liver disease, is one manifestation of ectopic lipid deposition often correlated with insulin resistance [2,3]. MASLD is characterized by excess fat buildup in the liver, not due to excessive alcohol use [4]. MASLD is part of a continuum that goes from steatosis and metabolic dysfunction-associated steatohepatitis (MASH) (steatosis, inflammation, ballooning of hepatocytes, and fibrosis) to the terminal stages of hepatocellular carcinoma and cirrhosis [5].

Insulin resistance is associated with MASLD and is considered an early marker of metabolic dysfunction [1,6]. Hyperinsulinemia and insulin resistance are often studied to understand the pathophysiological changes that precede MASLD and other comorbid conditions [7]. Insulin resistance occurs when insulin receptors are no longer sensitive to circulating insulin. According to the National Health and Nutrition Examination Survey, in 2021, 40% of adults aged 18 to 40 were insulin-resistant in the United States [8]. Insulin resistance can cause the pancreatic islets to produce more insulin, known as hyperinsulinemia, and can eventually lead to β-cell death [9]. In animal models, obesity and
insulin resistance can increase insulin secretion from β-cells in the islets of the pancreas, increase β-cell proliferation, and increase β-cell mass, a condition known as β-cell expansion [10]. Sometimes, during the progression of insulin resistance to more severe metabolic disease, mature β-cells can lose transcription factors that identify the β-cell as a β-cell, the general characteristics of an endocrine cell, or revert to a progenitor cell, known as dedifferentiation [10]. Dedifferentiation can also protect the cells from cell death [11].

The LEW.1WR1 rat is a unique animal model to quantify changes in weight, insulin resistance, and β-cell mass. LEW.1WR1 (1WR1) rats were created when a spontaneous mutation occurred in the major histocompatibility complex of congenic Lewis rats, which makes approximately 2% of 1WR1 rats susceptible to spontaneously developing type 1 diabetes before they are 59 days (8.4 weeks) old [12]. Type 1 diabetes can be induced in 1WR1 rats through TLR3 ligand poly I:C and viral infection [13]. Wistar Furth (WF) rats were our control rats because they were not susceptible to type 1 diabetes [14] and were also used as control rats to study type 1 diabetes in 1WR1 rats [15]. The 1WR1 rats showed a brief increase in insulin gene transcript a week after poly I:C induction before the expected decrease, which may indicate a compensatory mechanism in response to the treatment [16].

Our previous research indicated that weanling 1WR1 rats had higher insulin levels but were not insulin resistant compared to LEW/SsNHsd (SsNHsd) rats [17]. A limitation of that study was that the SsNHsd rats had increased circulating insulin, making them less-than-ideal control rats [17]. Our previous study also determined that 1WR1 rats were glucose intolerant compared to SsNHsd rats at 14 weeks old but did not show insulin resistance based on an insulin tolerance test at 10 weeks old [17]. A surprising finding from our previous study showed that 1WR1 rats had a decreased normalized β-cell area compared to SsNHsd rats despite having hyperinsulinemia [17]. Due to a limitation of the study design, we could not determine if these differences were due to a moderate sucrose diet. In this extended study, we will evaluate if moderate sucrose in the diet will induce 1WR1 rats to develop insulin resistance. Excessive sucrose is converted into glucose and fructose and can contribute to insulin resistance [18]. The moderate sucrose (MS) diet has 6.9% sucrose, which may play a role in the rats developing insulin resistance and beta cell expansion.

Pyruvate, lactic acid, taurine, and alanine have been extensively viewed as biomarkers for dysfunctional glucose metabolism leading to conditions like insulin resistance [19–21]. Elevated pyruvate and lactic acid could indicate enhanced glycolysis and subsequent anaerobic fermentation, which can be likened to the body’s compensatory attempt to offset impaired glucose uptake during insulin resistance [21]. Elevated urinary glucogenic amino acids like alanine have been attributed to enhanced amino acid catabolism and gluconeogenesis [22]. High urinary taurine levels show the body’s response to oxidative stress and the intentional reset mechanism of improving insulin sensitivity and glucose metabolism [23].

Diet has an impact on health [24]. Diets high in sugar and fat are often used as a model to induce cardiometabolic conditions based on the knowledge that persons who consume diets with excessive macronutrients lead to obesity. Scientists also acknowledge that some of these dietary models need to be more realistic regarding the diets people consume. In this study, we chose a moderate sucrose diet to assess the impact a small stimulus would have on the metabolic outcomes of the rats.

We studied if 1WR1 rats became overweight over the 18-week study. We examined if the 1WR1 rats become hyperglycemic or if the 1WR1 rats become insulin resistant as a symptom of other metabolic disorders such as MASLD. Post-harvest, we measured the β-cell mass of the rats to determine if β-cell mass expansion occurred in the 1WR1 rats on the MS diet. We also measured the terminal blood insulin to determine if the 1WR1 rats had increased insulin secretion. The two hypotheses for this study are that (A) the LEW.1WR1 rats would become overweight and insulin resistant, and (B) being on an MS diet for a prolonged time would cause them to develop β-cell expansion.
We measured the weight of the 1WR1 and WF rats twice weekly to determine if the 1WR1 rats became overweight after 16 weeks on a moderate sucrose diet. We periodically collected the rats’ fasting blood glucose to determine if the 1WR1 rats became hyperglycemic. We conducted insulin tolerance tests and urine metabolomics to determine if the 1WR1 rats became insulin resistant over the 18-week study. To evaluate insulin secretion, we measured their terminal blood insulin. Lastly, we measured islet area in pancreas micrographs to determine if the 1WR1 (MS) rats had $\beta$-cell mass expansion due to the MS diet.

2. Materials and Methods

2.1. Animal Care

Twenty-one male 1WR1 rats were obtained from Biomere (Worcester, MA, USA), and sixteen male Wistar Furth (WF/NHsd) rats were sourced from Envigo (Indianapolis, IN, USA). These rats, aged 2–3 weeks upon arrival, are descendants of the Wistar stock, with the WF variety displaying resistance to autoimmune diseases and maintaining normal insulin levels in contrast to the 1WR1 rats [12]. All animals were housed under controlled conditions with a 12 h light–dark cycle, randomized into groups of 3–4 rats per cage, and allowed a week for acclimatization. A standard Envigo chow diet (Teklad Global 18% Protein Rodent Diet) and water were provided ad libitum and enrichment access throughout the acclimatization period. To monitor their growth, each group’s weight was recorded twice weekly throughout the experimental period, allowing for tracking of weight gain. This study spanned 18 weeks, starting after the baseline acclimatization period. Both groups were transitioned to a control diet (LFD: D12450k Research Diets, New Brunswick, NJ, USA) at five weeks of age, marking the experiment’s commencement. At seven weeks of age, WF and 1WR1 rats were divided into two dietary groups: ten 1WR1 rats and eight WF rats on a control diet (Control) and eleven 1WR1 rats and eight WF rats on a moderate sucrose diet (MS). The University of Alabama in Huntsville Institutional Animal Care and Use Committee approved this study’s protocol.

2.2. Fasting Blood Glucose Testing

Fasting periods of 4 h (at 5 and 7 weeks), 6 h (at 11 and 15 weeks), and 8 h (at 17 weeks) were implemented before testing fasting blood glucose concentrations. Glucose readings were taken via tail prick.

2.3. Insulin Tolerance Tests (ITTs)

Insulin tolerance tests were conducted when the rats were 7 and 15 weeks old. Fasting periods of 4 h (at 7 weeks) and 6 h (at 15 weeks) were implemented before testing. Baseline blood glucose readings were taken via tail prick, followed by intraperitoneal (IP) insulin injection at 0.75 units/kg body mass. Post-injection, blood glucose measurements were taken at 30 min intervals for 90 min using the AlphaTRAK 2 m by Zoetis (Parsippany, NJ, USA). Humulin R Insulin (NDC: 0002-8215-01, Patterson Veterinary Supply, Saint Paul, FL, USA) diluted in PBS was used for the injections. The recorded blood glucose values were normalized to the initial fasting levels and expressed as a percentage for graphical representation.

2.4. Urine Metabolomics

Urine was collected on ice from rats for four hours before the insulin tolerance tests (7 and 15 weeks old). Urine samples were prepared as previously described to determine the necessary volume of urine to normalize, and the sample was lyophilized for NMR analysis according to protocol [25]. Lyophilized urine was reconstituted into 160 $\mu$L of 100 mM potassium phosphate, and 40 $\mu$L of 5 mM sodium trimethylsilylpropanesulfonate (DSS) was added to obtain a final concentration of 1 mM of DSS. This solution was transferred to a 3 mm tube and run on a Varian Unity Inova with a $^1$H frequency of 500 MHz. As previously described, a 1D-NOESY experiment was used for acquisition [25].
2.5. Pancreas Histology

Twenty-three-week-old animals were anesthetized with isoflurane and exsanguinated via cardiac puncture. Each pancreas was placed in a cassette and fixed overnight in 10% neutral buffered formalin (Thermo Fisher Scientific, Waltham, MA, USA), put in 70% ethanol (Thermo Scientific, Waltham, MA, USA), and mailed to Histowiz (Brooklyn, New York, NY, USA), where they were paraffin-embedded, sectioned in 4 µm slices, stained with hematoxylin and eosin against insulin, mounted onto microscope slides, and digitized. The pancreas and β-cell areas were quantified using FIJI (version 2.3.0) [26]. Four blinded scorers analyzed one slide per rat to determine the different β-cell areas. A range of 118–367 β-cells per rat were measured. The total pancreas area was determined by converting the number of pixels² to mm² using the scale bar and pixels per unit of measure, turning the image of the pancreas into 8-bit format, applying an Otsu threshold, measuring the entire pancreas slice, freehand tracing the areas of the pancreas that were from different tissue types, measuring those areas in mm², and subtracting different tissue types from the region of interest. β-cell area was determined by converting the number of pixels² to µm², freehand tracing the β-cell, finding the edges, and measuring the area in µm². The β-cell area was then converted from µm² to mm². Normalized β-cell area was calculated as previously described [17]. β-cell mass was determined by dividing the β-cell area by the total area of the pancreas section and multiplying by the total pancreas mass [27].

2.6. Insulin ELISA

Blood serum samples were collected post-harvest. A Ray Biotech Rat Insulin ELISA kit (Cat.: ELR-Insulin-1) was used to measure 37 rat serum samples in duplicate. Samples were quantified on a ThermoScientific NanoDrop One at 450 nm absorbance. A standard curve was generated using the log–log method and then converting the interpolated concentrations to µU/mL using antilog (10^x).

2.7. qPCR

A 50 mg liver sample was ground in liquid nitrogen and then homogenized in 1 mL of Trizol reagent using a SuperFastPrep-2 homogenizer (MP Biomedicals, Pittsburgh, PA, USA) with MP Biomedicals Matrix D beads at setting 6 for 40 s. RNA extraction followed the Trizol manufacturer’s protocol with minor modifications. After adding 75% ethanol, the mixture was briefly vortexed and centrifuged at 10,000 × g for 5 min at 4 °C. The RNA pellet was resuspended in 25 µL of RNase-free water and incubated at 55–60 °C for 10 min, followed by cooling on ice for 15 s. The concentration and purity of the extracted RNA were measured using a NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA).

The RNA was then reverse transcribed into complementary DNA (cDNA) using the High-Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer’s instructions. Quantitative PCR (qPCR) reactions were conducted using PowerUp Sybr Green Master Mix (Applied Biosystems, Carlsbad, CA, USA). Relative gene expression levels were calculated using the delta–delta Ct method and reported as fold changes. The housekeeping gene Ribosomal protein L32 (Rpl32) was the reference for comparing the expression of Fibroblast Growth Factor 21 (Fgf21), Forkhead Box O1 (Foxo1), Insulin Receptor Substrate 2 (Irs-2), and Carbohydrate Response Element Binding Protein 1 (ChrebP-1) genes in the liver. Primers were designed with Primer Blast and synthesized by Invitrogen (Supplementary Table S1).

2.8. Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 9.4 and 10.2 software. A one-way analysis of variance (ANOVA) was used to compare the four rat groups with Tukey’s multiple comparisons post hoc testing. A t-test was used to compare significant differences between two rat groups. In some instances, outliers were identified using GraphPad Prism’s ROUT method with Q, the maximum false discovery rate, set to 1%.
Differences were determined to be significant if \( p < 0.05 \), and the letters above the columns indicate statistical differences unless otherwise indicated. Data were expressed as means +/- standard deviation (SD); \( n \) values reflect biological replicates and are listed as Wistar Furth (Control) rats, Wistar Furth (MS) rats, LEW.1WR1 (Control) rats, and LEW.1WR1 (MS) rats.

3. Results

3.1. LEW.1WR1 Rats Had Significantly Increased Body Mass by 7 Weeks Old

This study investigated the impact of diet on body mass in 1WR1 and WF rats over time, from 5 weeks old until they turned 23 weeks old. Body mass measurements indicated divergence in growth trajectories between the strains at 7 weeks old (Figure 1B). Both 1WR1 rat groups showed a significant increase in body mass. This divergence persisted throughout the experiment, suggesting that diet had a lasting impact on body mass. Also, the 1WR1 rats on the moderate sucrose diet exhibited significantly higher body mass than their Wistar Furth counterparts, regardless of their dietary condition.

Figure 1. LEW.1WR1 rats have significantly increased body mass starting at 7 weeks old. This figure presents the longitudinal mass distribution for different animal groups throughout this study, beginning at 5 weeks old and continuing until the end of the experimental period. (A) Body mass trajectories for all strains. Significant differences between strains start at 7 weeks old, marked by an asterisk (*). (B) At week 7, LEW.1WR1 (Control) exhibits significant differences from Wistar Furth (Control) and MS (\( p = 0.0059 \) and \( p = 0.0019 \), respectively). The groups include LEW.1WR1 (Control) (\( n = 10 \)), LEW.1WR1 fed moderate sucrose (MS) (\( n = 11 \)), Wistar Furth (Control) (\( n = 8 \)), and Wistar Furth fed moderate sucrose (MS) (\( n = 8 \)). Statistical analysis was performed using a one-way ANOVA, with significance set at \( p < 0.05 \). Significant differences are marked with an asterisk (*). The data are expressed as the mean \( \pm \) SD. Wistar Furth (Control) are green up triangles. Wistar Furth (MS) are purple down triangles. LEW.1WR1 (Control) is blue circles. LEW.1WR1 (MS) is red rhombus. These groups are shown with Wistar Furth control on the left through LEW.1WR1 (MS) on the right.
3.2. LEW.1WR1 and Wistar Furth Rats Did Not Become Hyperglycemic

There was no significant difference in fasting blood glucose between 1WR1 rats and WF rats on chow diets at five weeks old or control and moderate sucrose diets at 11 and 17 weeks old, indicating they did not become hyperglycemic throughout this study (Figure 2).

![Figure 2. LEW.1WR1 and Wistar Furth rats did not become hyperglycemic. Fasting blood glucose of LEW.1WR1 and Wistar Furth rats on a chow diet at 5 weeks old and control and moderate sucrose diets at 11 and 17 weeks old. Data are presented as mean ± SD (n = 8, 8, 10, 11). Wistar Furth (Control) are green. Wistar Furth (MS) are purple. LEW.1WR1 (Control) is blue. LEW.1WR1 (MS) is red. These groups are shown with Wistar Furth control on the left through LEW.1WR1 (MS) on the right.](image)

3.3. LEW.1WR1 Rats Were Insulin Resistant at 7 Weeks Old, Which Worsened by 15 Weeks Old

Intraperitoneal insulin tolerance tests (ITTs) were conducted when the rats were 7 and 15 weeks old. The ITT at 7 weeks old was conducted before the 1WR1, and WF rats were placed on control and MS diets. During the ITT at 7 weeks old (Figure 3A), there was no significant difference in percent baseline blood glucose concentration for WF or 1WR1 rats at 0 and 30 min. At 60 min, WF (Control) and WF (MS) rats were not significantly different from each other, but WF (MS) rats had a significantly lower percent baseline glucose concentration than both 1WR1 groups. WF (Control) rats had a significantly lower percent baseline glucose concentration than 1WR1 (MS) rats but were not significantly different from 1WR1 (Control) rats. At 90 min, WF (Control) and WF (MS) rats had significantly lower percent baseline glucose concentrations than 1WR1 (Control) and 1WR1 (MS) rats. The area under the curve for the ITT at 7 weeks old showed that WF (Control) and WF (MS) were not significantly different from each other, and 1WR1 (Control) and 1WR1 (MS) were not significantly different from each other. However, both WF rat groups had significantly lower areas under the curve than both 1WR1 rat groups (Figure 3B). The 1WR1 and WF rats had no significant difference in fasting blood glucose at 7 weeks old (Figure 3C). The failure of both 1WR1 groups to respond to the insulin bolus indicates the rats were insulin resistant before starting the control and MS diets, though they were not hyperglycemic.

After nine weeks on the control and MS diets, the rats were given an ITT at 15 weeks old. There was no significant difference in fasting percent baseline blood glucose concentration for WF or 1WR1 rats at 0 min (Figure 3D). At 30 min, WF (Control) rats were not significantly different from the WF (MS) rats, but WF (Control) rats had a significantly lower percent baseline glucose concentration than 1WR1 (Control) and 1WR1 (MS) rats. WF (MS) rats were not significantly different from all other rat groups. At 60 and 90 min, WF (Control) and WF (MS) rats were not significantly different from each other, and 1WR1 (Control) and 1WR1 (MS) rats were also not significantly different from each other. However, both WF rat groups had significantly lower percent baseline glucose concentrations than both 1WR1 rat groups. The area under the curve for the ITT at 15 weeks old showed that WF (Control) and WF (MS) were not significantly different from each other, and 1WR1 (Control) and 1WR1 (MS) were not significantly different from each other. However, data
for both WF rat groups showed significantly less area under the curve than for both 1WR1 rat groups (Figure 3E). Data for 1WR1 and WF rats on both diets showed no significant difference in fasting blood glucose at 15 weeks old (Figure 3F). The percent baseline glucose concentration differences at 30 min instead of 60 min, as seen in the first ITT, along with the increased area under the curve at 15 weeks old, indicate that the 1WR1 rats on both diets had worse insulin resistance after being on the control and MS diets for nine weeks.

To examine the effect of insulin resistance on the development of MASLD in the LEW.1WR1 rats, we looked at genes and proteins in the insulin signaling pathway in the liver. Insulin receptor concentration in Wistar Furth and LEW.1WR1 control rats showed that LEW.1WR1 rats had a significantly higher concentration of insulin receptors (Figure S2A). This suggests that LEW.1WR1 rats may be more responsive to insulin, potentially as an adaptive mechanism to maintain glucose homeostasis. The qPCR measurements of insulin responsive gene expression reveal 1WR1 rats had significantly less \( Irs-2 \) gene expression and a trend of decreased \( Foxo1 \) gene expression. Wistar Furth rats' higher \( Irs-2 \) expression indicates enhanced insulin signaling, while LEW.1WR1 rats had decreased \( Foxo1 \) gene expression, which could imply a reduced insulin effect, possibly a compensatory response (Figure S2B).

![Figure 3.](image-url)
AKT pathway (Figure S2D). The AKT/pAKT/AKT ratio in LEW.1WR1 rats suggesting a more active insulin signaling through the (n = 8, 8, 10, 11). Wistar Furth (Control) are green up triangles. Wistar Furth (MS) are purple down triangles. LEW.1WR1 (Control) is blue circles. LEW.1WR1 (MS) is red rhombuses. These groups are defined as α-Tubulin ratio was slightly higher in LEW.1WR1 rats (Figure S2E). The western blot for mTOR indicates higher mTOR signaling in Wistar Furth rats (Figure S2F,G). There was no significant difference in IRS-2 protein concentration for Wistar Furth and LEW.1WR1 rats on both diets, indicating there may be a delay in Irs-2 gene translation to protein (Figure S2H,I).

Figure 3. LEW.1WR1 rats were insulin resistant at 7 weeks old, which worsened by 15 weeks old. Intraperitoneal insulin tolerance tests (ITTs) were performed when the WF and 1WR1 rats were 7 and 15 weeks old. The ITTs were conducted in a fasting state (4 and 6 h, respectively). (A) ITT at 7 weeks old. (B) ITT at 7 weeks old’s area under the curve. (C) 7 weeks old fasting blood glucose concentrations. (D) ITT at 15 weeks old. (E) ITT at 15 weeks old’s area under the curve. (F) 15 weeks old fasting blood glucose concentrations. A one-way ANOVA was used to determine significance, defined as p < 0.05. Different letters indicate significant differences. Data are presented as mean ± SD (n = 8, 8, 10, 11). Wistar Furth (Control) are green up triangles. Wistar Furth (MS) are purple down triangles. LEW.1WR1 (Control) is blue circles. LEW.1WR1 (MS) is red rhombuses. These groups are shown with Wistar Furth control on the left through LEW.1WR1 (MS) on the right.

Western blot results for phosphorylated AKT (pAKT) and total AKT confirm active insulin signaling in both groups (Figure S2C). The quantification graph shows a higher pAKT/AKT ratio in LEW.1WR1 rats suggesting a more active insulin signaling through the AKT pathway (Figure S2D). The AKT/α-Tubulin ratio was slightly higher in LEW.1WR1 rats (Figure S2E). The western blot for mTOR indicates higher mTOR signaling in Wistar Furth rats (Figure S2F,G). There was no significant difference in IRS-2 protein concentration for Wistar Furth and LEW.1WR1 rats on both diets, indicating there may be a delay in Irs-2 gene translation to protein (Figure S2H,I).

3.4. Analysis of Urinary Energy Marker Metabolites

An eight-hour urinary metabolomic study on samples obtained from all rat strains showed no differences in the excretion of energy marker metabolites before each insulin tolerance test between rats on a control diet and those on a moderate sucrose diet. We quantified seven metabolites (glucose, pyruvate, succinic acid, carnitine, lactic acid, taurine, and alanine) against a standard of DSS in each urine sample. It is worth noting that the
glucose values at 15 weeks were presented separately with a range to highlight observations (Figure 4A).

Throughout the study period, we evaluated metabolite levels at the rats’ ages. At 7 weeks old, our analysis revealed no differences in baseline urine metabolite percentages between WF and 1WR1 rats, regardless of their diet. This suggests an excretion pattern of energy metabolites among both groups at this stage.

However, by 15 weeks old, there were variations in metabolite concentrations. Specifically, we observed that the concentration of lactic acid in rats on the control diet was noticeably lower in WF rats compared to 1WR1 rats on a sucrose (MS) diet (Figure 4E). Similarly, when looking at alanine levels, WF control rats had levels higher than their 1WR1 MS counterparts (Figure 4G). Interestingly, although there were differences in comparisons, we did not find any significant difference between WF rats on both diets and 1WR1 rats on both diets regarding pyruvate concentrations at 7 weeks old. Pyruvate concentrations of WF (Control) and MS rats showed lower levels compared to 1WR1 (Control) and MS rats at 15 weeks old (Figure 4B).

Furthermore, taurine concentrations were significantly higher in 1WR1 rats on a moderate sucrose diet compared to WF rats on the same diet. This suggests that the sucrose-enriched regimen might impact taurine excretion (Figure 4F).

These findings indicate that dietary composition can have varying effects on the metabolic processing of energy markers in rats with a sucrose diet. The observed variations at 15 weeks shed light on metabolic adaptations or stresses induced by changes over time.

Figure 4. Targeted analysis of selected metabolites. The excretion of several energy marker metabolites preceding each insulin tolerance test: Urine samples were obtained from rats on both a control and a moderate sucrose diet, collected after an eight-hour fast in a metabolic cage. Measurements were taken at 7 (n = 8, 8, 10, 11) and 15 (n = 7, 5, 6, 6) weeks old. Samples quantified against an internal standard of DSS: (A) glucose (at 15 weeks, n = 6, 5, 6, 6), (B) pyruvate, (C) succinic acid, (D) malic acid, (E) citric acid, (F) fumaric acid, (G) alanine.

Figure 4. Cont.
Furthermore, taurine concentrations were significantly higher in 1WR1 rats on a moderate sucrose diet compared to WF rats on the same diet. This suggests that the sucrose-enriched regimen might impact taurine excretion (Figure 4F). These findings indicate that dietary composition can have varying effects on the metabolic processing of energy markers in rats with a sucrose diet. The observed variations at 15 weeks shed light on metabolic adaptations or stresses induced by changes over time.

Figure 4. Targeted analysis of selected metabolites. The excretion of several energy marker metabolites preceding each insulin tolerance test: Urine samples were obtained from rats on both a control and a moderate sucrose diet, collected after an eight-hour fast in a metabolic cage. Measurements were taken at 7 (n = 8, 8, 10, 11) and 15 (n = 7, 6, 6, 6) weeks old. Samples quantified against an internal standard of DSS: (A) glucose (at 15 weeks, n = 6, 5, 6, 6), (B) pyruvate, (C) succinic acid, (D) carnitine, (E) lactic acid, (F) taurine, and (G) alanine. A one-way ANOVA was used to determine significance, defined as \( p < 0.05 \). Different letters indicate significant differences. Data are presented as mean ± SD. Wistar Furth (Control) are green up triangles. Wistar Furth (MS) are purple down triangles. LEW.1WR1 (Control) is blue circles. LEW.1WR1 (MS) is red rhombuses. These groups are shown with Wistar Furth control on the left through LEW.1WR1 (MS) on the right.

3.5. No Rat Groups Had β-Cell Mass Expansion

The 1WR1 (Control) rats have significantly higher β-cell mass compared to the WF (Control) and WF (MS) rats but are not significantly different from the 1WR1 (MS) rats (Figure 5E). The β-cell mass of each rat was divided by its terminal body mass to determine the relative β-cell mass to body mass. The 1WR1 (Control) rats had significantly higher relative β-cell mass compared to WF (MS) and 1WR1 (MS) rats but not WF (Control) rats (Figure 5F). All rat groups had similar normalized β-cell areas, where the total area of the β-cells of each rat was divided by the total area of each pancreas section (Figure 5G). There was no significant difference in terminal blood insulin levels in WF and 1WR1 rats on both diets (Figure 5H).

Figure 5. Cont.
Figure 5. Cont.
Our previous study suggested that LEW.1WR1 rats show early stages of metabolic syndrome, or prediabetes, so we were interested in following the rats’ responses to a carbohydrate-based challenge. This study compares mass to a model that has milder carbohydrate-based challenge. The results of this study confirm that LEW.1WR1 rats develop insulin resistance and visceral fat accumulation. These diet-induced changes in insulin resistance and visceral fat accumulation provide novel insight into mechanisms underlying type 2 diabetes.

Both LEW.1WR1 rat groups were significantly heavier than Wistar Furth rat groups. However, neither rat group developed insulinemia and insulin resistance without hypoglycemia or hyperglycemia. While the LEW.1WR1 rats did consume more food per rat than the Wistar Furth, this amount plateaued and was maintained across the time of this study. It was also a strain-specific characteristic, with LEW.1WR1 rats demonstrating increased accumulation under both conditions. The rats did not have hyperglycemia, a physiological state that is often associated with hyperplasia and/or hypertrophy, which may be why we did not observe the expansion that we predicted. When characterizing the condition of the islets based on the appearance of their periphery staining intensity, we did not notice apparent disruption in the LEW.1WR1 rats, similar to what is observed in the early stages of hyperplasia.

The β-cell mass was divided by its terminal body mass (n = 7, 8, 10, 11). A one-way ANOVA was used to determine significance, defined as p < 0.05. Different letters indicate significant differences. Data are presented as mean ± SD. Wistar Furth (Control) are green up triangles. Wistar Furth (MS) are purple up triangles. LEW.1WR1 (Control) is blue circles. LEW.1WR1 (MS) is red rhombuses. These groups are shown with Wistar Furth control on the left through LEW.1WR1 (MS) on the right.

Figure 5. LEW.1WR1 (Control) rats had the highest β-cell mass, relative β-cell mass, and normalized β-cell mass. Representative sections of (A) Wistar Furth (Control), (B) Wistar Furth (MS), (C) 1WR1 (Control), and (D) 1WR1 (MS) pancreas stained for insulin (red-brown stain, 1× magnification). (E) β-cell mass measurements for WF (Control), WF (MS), 1WR1 (Control), and 1WR1 (MS) rats in grams (n = 7, 8, 10, 11). (F) Each rat’s β-cell mass was divided by its terminal body mass (n = 7, 8, 10, 11). (G) Normalized β-cell area is reported as a percentage (n = 7, 8, 10, 11). (H) Terminal blood insulin concentration for WF and 1WR1 rats on both diets (n = 8, 8, 9, 11).
4. Discussion

The results of this study confirm that LEW.1WR1 rats develop insulin resistance and are overweight, independent of diet. However, neither rat group developed β-cell mass expansion on a moderate sucrose diet but may be trending toward reduced β-cell area in response to the diet. These differences indicate how strain and dietary conditions affect growth patterns. LEW.1WR1 rats demonstrate increased accumulation under both conditions. Future work studying the underlying genotype in the LEW.1WR1 rat that leads to insulin resistance can provide novel insight into β-cell-specific changes that lead to hyperinsulinemia and insulin resistance without hypoglycemia or hyperglycemia.

Our previous study suggested that LEW.1WR1 rats show early stages of metabolic syndrome, or prediabetes, so we were interested in following the rats’ responses to a milder carbohydrate-based challenge [17]. This study compares mass to a model that has normal circulating insulin levels and evaluates the impact of a diet with a moderate amount of sucrose on beta cell mass and area. Others have shown that high-carbohydrate diets have mild effects on weight gain, fasting blood glucose levels, and significant changes in insulin resistance and visceral fat accumulation [28]. These diet-induced changes are often driven by hyperphagia and/or leptin resistance. While the LEW.1WR1 rats did consume more food per rat than the Wistar Furth, this amount plateaued and was maintained across the time of this study. It was also a strain-specific difference, mostly with the occasional statistically significant point that resolved within two time points.

Both LEW.1WR1 rat groups were significantly heavier than Wistar Furth rat groups. Wistar Furth rats were only in the lower range of male adult rat masses, while the LEW.1WR1 rats were on the higher side of the range [29]. The LEW.1WR1 rats also had significantly reduced insulin sensitivity throughout the experiment. The LEW.1WR1 rats had increased fasting insulin and reduced sensitivity, which would have suggested differences in β-cell mass. However, the rats did not have hyperglycemia, a physiological state that is often associated with hyperplasia and/or hypertrophy, which may be why we did not observe the expansion that we predicted. When characterizing the condition of the islets based on the appearance of their periphery staining intensity, we did not notice apparent disruption in the LEW.1WR1 rats, similar to what is observed in the early stages of type 1 diabetes [12] or in Zucker fatty rats [30]. As observed, the islets do not appear to be going through hyperplasia or hypertrophy at this time point.

Our previous work on LEW.1WR1 rats suggested that there was a reduction in β-cell mass due to the comparison to a rat model with slightly elevated insulin levels, but this work shows that our β-cell mass, while significantly increased relative to Wistar Furth rats, is not abnormally low. We also observed that, unlike our prediction of diet-induced increases in β-cell mass, we saw the opposite. This is more in line with the observations of others like Pashen et al., who saw a reversible loss of beta cell mass in response to a high-fat, high-sucrose diet in islets transplanted in the anterior chamber of the eye [31]. This beautiful study assesses the effects diet has on functional β-cell mass, showing increased body weight and impaired insulin sensitivity [31]. We hypothesize that although the diet in our study was only moderate in its sucrose content, 1WR1 rats are predisposed to fatty infiltrations in the liver, suggesting that the dietary stimulus may be more similar to the high-fat, high-sugar stimulus of the above study [12]. We see similar changes develop over time in the 1WR1 rat. We observed a non-significant reduction in β-cell mass for both groups of rats on the moderate sucrose diet. We believe the increased baseline circulating insulin levels drive the differences in insulin sensitivity. The increased insulin resistance does not correspond with reduced insulin receptor levels in tissues like the liver (Supplementary Figure S2A). To date, no studies have reported that LEW.1WR1 rats develop type 2 diabetes, although they are a model of virus-induced type 1 diabetes. The lack of a compensation response may be an important observation relative to the longevity of islets in these rats. Tersey et al. suggest that the inflexibility of β-cells and dedifferentiation may be a feature of the changes in glycemia during obesity [32]. They have been shown to develop glucose intolerance, insulin resistance, and a fatty liver, yet they were protected for 23 weeks of life.
from developing frank diabetes [17,33]. They maintained normoglycemia across the entire study. Studying these rats longer and following changes in the islet with Ki67 staining, cyclin D, or a marker of β-cell dedifferentiation may be useful in determining if these rats will go on to develop diabetes. It is unclear from this study if a difference in β-cell mass would reach significance at a future time point.

Due to the lack of commercial availability of the LEW.1WR1 rats, we were unable to pursue the β-cell function in isolated islets from this rodent model to better understand if there are differences in insulin content. The lack of validated methods to accurately evaluate insulin content using the micrographs also left us unable to evaluate the differences in insulin content of the islets measured in the pancreas sections. The Wistar Furth rat, however, allowed for the comparison of LEW.1WR1 rat insulin sensitivity to a more normal model of insulin sensitivity. In the future, we hope to pursue the question of how some of the underlying gene differences may play a role in regulating insulin content or insulin secretion. Lastly, some beta cell mass changes may have occurred prior to the start of this study when the rats were weaning. In future studies, researchers may find following the pancreas development earlier useful to determine if the pancreas starts out larger.

5. Conclusions

LEW.1WR1 rats are a rat model for induced type 1 diabetes. Our previous research has shown that 1WR1 rats developed glucose intolerance and hyperinsulinemia on a moderate sucrose diet [17]. This study showed that 1WR1 rats develop insulin resistance on chow and moderate diets but not hyperglycemia or β-cell expansion, suggesting they will not develop type 2 diabetes after 16 weeks on a moderate sucrose diet. In another recent study from our lab, 1WR1 rats developed MASLD on a chow diet [33], supported by their developing insulin resistance and decreased Irs-2 gene expression in their livers (Supplementary Figure S2). This study supports the claim that 1WR1 rats may be a more human-like model for people with a predisposition for metabolic disorders because they develop these disorders on a chow diet without hyperphagia (Supplementary Figure S3).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmp5030020/s1, Figure S1: Body mass graphs for 5-, 8-, 9-, 10-, 11-, 12-, and 13-week-old rats. Figure S2: Liver insulin resistance in WF and 1WR1 rats on a control diet. Figure S3: Food consumption graph for 5–23-week-old rats. Supplementary Table S1: qPCR primers. Supplemental Western blotting method.


Funding: This research was funded by the University of Alabama in Huntsville, the Louis Stokes Alliance for Minority Participation NSF Grant (1619659, NSF), the Research Publication Grant in Engineering, Medicine, and Science from the American Association of University Women, the UAH Adriel D. Johnson, Sr. Fellowship, and the Alabama Space Grant Consortium. W. Thomas Love donated funding for the insulin ELISA.

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board of the University of Alabama in Huntsville Institutional Animal Care and Use Committee (Approval Number: R004.FAT10).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data will be made available on request.
Acknowledgments: Thanks to Victoria McConnell, Hannah Underhill-Key, Jennifer Nix, Luis Mercado, Nathalie Jones, Zane Griffin, Gracie Knight, Anna Hart, Laura Catherine Wright, Riley Apperson, and Olivia Casimir. Special thanks to John Vincent for the use of the metabolic cages.

Conflicts of Interest: The authors declare no conflicts of interest.

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