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Investigating the Expression and Function of the Glucose Transporter GLUT6 in Obesity

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Abstract: Obesity-related insulin resistance is a highly prevalent and growing health concern, which places stress on the pancreatic islets of Langerhans by increasing insulin secretion to lower blood glucose levels. The glucose transporters GLUT1 and GLUT3 play a key role in glucose-stimulated insulin secretion in human islets, while GLUT2 is the key isoform in rodent islets. However, it is unclear whether other glucose transporters also contribute to insulin secretion by pancreatic islets. Herein, we show that SLC2A6 (GLUT6) is markedly upregulated in pancreatic islets from genetically obese leptin-mutant (ob/ob) and leptin receptor-mutant (db/db) mice, compared to lean controls. Furthermore, we observe that islet SLC2A6 expression positively correlates with body mass index in human patients with type 2 diabetes. To investigate whether GLUT6 plays a functional role in islets, we crossed GLUT6 knockout mice with C57BL/6 ob/ob mice. Pancreatic islets isolated from ob/ob mice lacking GLUT6 secreted more insulin in response to high-dose glucose, compared to ob/ob mice that were wild type for GLUT6. The loss of GLUT6 in ob/ob mice had no adverse impact on body mass, body composition, or glucose tolerance at a whole-body level. This study demonstrates that GLUT6 plays a role in pancreatic islet insulin secretion in vitro but is not a dominant glucose transporter that alters whole-body metabolic physiology in ob/ob mice.

Keywords: obesity; mouse model; glucose transporter; islet biology

1. Introduction

The high prevalence of obesity and associated metabolic disorders has become a major public health concern that is predicted to escalate in coming decades [1]. Obesity is closely related to type 2 diabetes, and the majority of patients with type 2 diabetes are overweight or obese [2]. Type 2 diabetes is characterized by chronic hyperglycemia due to an inability of the pancreatic beta cells to effectively compensate for insulin resistance. Pancreatic islets play a central role in the pathogenesis of diabetes, but their function and regulation remain incompletely understood. Islet biology is challenging to study in humans due to the low quantity of islets in the body, their heterogeneous and disperse distribution throughout the exocrine pancreas, and a lack of effective in vivo imaging techniques [3,4]. Therefore, mouse models that mimic obesity and type 2 diabetes are essential tools for gaining a better understanding of pancreatic islet function.

Ob/ob mice are a common model of obesity and insulin resistance and have been widely used to study islet function [5]. These mice lack the gene for functional leptin, a satiety hormone, and quickly develop severe obesity and insulin resistance [6]. However, their phenotype is strain-dependent. Ob/ob mice on a C57BL/6 background exhibit remarkable islet hypertrophy and compensatory insulin secretion to achieve relatively normal blood glucose levels, while mice on a BTBR background develop severe hyperglycemia [6].
Another frequently used mouse model is the \textit{db/db} mouse, which is commonly maintained on a BKS background and lacks the leptin receptor; this mouse also develops severe insulin resistance and hyperglycemia [6]. The \textit{ob/ob} and \textit{db/db} models have been important in demonstrating the efficacy of several pharmacotherapies, including exendin-4, liraglutide, and semaglutide, which have since been approved for clinical use, as well as the now-discontinued sibutramine [7,8].

The facilitative glucose transporter family (GLUTs) regulates the transport of glucose and other monosaccharides across lipid membranes in mammalian cells. There are 14 GLUT proteins in the family, several of which are exceptionally well-characterized, such as the constitutive transporters GLUT1 and GLUT2, and the insulin-sensitive GLUT4 [9]. In human islet beta cells, glucose influx is required for glucose-stimulated insulin secretion and is primarily controlled by GLUT1 and GLUT3, while GLUT2 performs the same role in rodents [10]. However, it is not known whether other GLUTs are also involved. In the publicly available Attie Lab Diabetes Database, GLUT6 mRNA is markedly upregulated in islets isolated from \textit{ob/ob} mice compared to islets from lean littersmates, on both C57BL/6 and BTBR backgrounds [11]. This suggests that GLUT6 may play a conserved role in the regulation of islet function in the obese, insulin-resistant state. However, no studies have investigated the functional role of GLUT6 in islets.

GLUT6 was previously known as GLUT9 and is most similar to GLUT8, sharing 44.8% amino acid identity [12]. GLUT6 and GLUT8 are distinct from other GLUTs, as they have a glycosylation site on loop 9 rather than loop 1 [12] and an N-terminal dileucine motif. Dileucine motifs are important for intracellular targeting [13]. GLUT6 also has the notable presence of two arginine residues in helices 7 and 8, and lacks the QLS motif in helix 7 [12].

GLUT6 is encoded for by the \textit{SLC2A6} gene. In human tissues, \textit{SLC2A6} mRNA is predominantly expressed in the brain, spleen, and peripheral leukocytes, but has also been detected in the pancreas and heart [12]. In mice, \textit{Slc2a6} mRNA, but not GLUT6 protein, was detected in the brain and spleen [14]. However, GLUT6 protein was detected in mouse macrophages when they were stimulated with inflammatory molecules such as lipopolysaccharide [15]. When expressed in COS-7 cells and reconstituted into liposomes, GLUT6 exhibited significant glucose transport activity [12]. The authors predicted that GLUT6 was a high \(K_m\) transporter because its transport activity was detectable against background constitutive GLUT1 in 5 mM glucose, much more so than at 1 mM glucose, and its \(K_d\) was almost two times that of GLUT4 [12]. Furthermore, unlike GLUT4, the plasma membrane expression of GLUT6 was not sensitive to insulin, phorbol ester, or hyperosmolarity when expressed in rat adipocytes [13]. Interestingly, GLUT6 has been shown to be localized intracellularly in the lysosome in mouse macrophages [16]. Although insensitive to insulin, GLUT6 is a target gene of RelA, a transcription factor of the NF\(\kappa\)B family [15,16]. GLUT6 may play a role in disease-related metabolism as it is upregulated in several cancers, including endometrial cancer [17]. In endometrial cancer cells, siRNA knockdown of GLUT6 has been shown to inhibit cell survival and reduced glucose uptake and glycolysis [17].

Mice lacking whole-body GLUT6 have normal body composition and glucose metabolism compared to wild-type mice when fed chow and a high-fat diet, except for a 20% decrease in adiposity in females fed a high-fat diet [14]. However, the high-fat diet-induced obesity model is distinct from the \textit{ob/ob} model, as it exhibits only a mild disease state. In contrast, \textit{ob/ob} mice on a C57BL/6 background demonstrate severe islet hypertrophy and extremely high plasma insulin concentrations [18]. Therefore, it is possible that the marked upregulation of GLUT6 in \textit{ob/ob} mouse islets may have a role in islet function or hypertrophy.

To investigate the functional role of GLUT6 in islets, we created GLUT6 knockout (GLUT6KO) \textit{ob/ob} mice and assessed body composition, glucose homeostasis, and islet function. To our knowledge, this is the first time that a glucose transporter (GLUT) has been knocked out in \textit{ob/ob} mice. Herein, we show that \textit{ob/ob} islets lacking GLUT6 had increased insulin secretion when stimulated with glucose but not KCl, compared to \textit{ob/ob}.
islets expressing normal GLUT6. In contrast, GLUT6 deletion in islets from lean mice (+/+ for the leptin gene) did not impact insulin secretion. Despite the changes in ex vivo glucose-stimulated insulin secretion, the GLUT6KO ob/ob mice had no metabolic phenotype related to body weight, body composition, or glucose tolerance.

2. Results
2.1. GLUT6 Gene Expression in Mouse and Human Islets

Data mining the publicly available Attie Lab Diabetes Database [11] revealed that Slc2a6 is highly overexpressed in the pancreatic islets of obese mice. Specifically, islets from obese mice that lack the functional leptin gene (ob/ob) expressed significantly higher Slc2a6 compared with lean mice (ob/+ and +/+ genotypes) at both four and ten weeks of age, on both C57BL/6 and BTBR backgrounds (Figure 1A,B). To investigate whether GLUT6 expression was also upregulated in obese and insulin-resistant human islets, we analyzed SLC2A6 mRNA levels in pancreatic islets from a cohort of human patients with and without type 2 diabetes with a body mass index (BMI) between 18 and 53. Donor information is as previously published [19] and given in Table S1. These data showed that human islet SLC2A6 mRNA was significantly correlated with body mass index ($p = 0.005$) (Figure 1C).

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** Expression of Slc2a6 mRNA is increased in islets from obese (ob/ob) mice and correlates with body mass index in human islets. (A,B) Slc2a6 expression in isolated islets obtained from lean (ob/+ and +/+ , grey circles) or obese (ob/ob, black squares) male mice (Attie Lab Diabetes Database in the form of “mlratio”, which is the log10 of the ratio of gene expression for the experimental sample to a strain-specific reference pool for either C57BL/6 (A) or BTBR (B) mice). (C) Human islet SLC2A6 expression positively correlated with body mass index (BMI). Islets were isolated from non-diabetic humans (black circles) and humans with type 2 diabetes (grey squares). * indicates $p < 0.05$ by two-way ANOVA. n = 5 mice per group (A,B), n = 12 human samples (C).

We then examined the expression of Slc2a6 mRNA in islets from genetically obese mice in our laboratory. To do this, islets were isolated from genetically obese and lean mice of both the ob/ob line and the db/db line. Mice with two copies of the non-functional mutant
leptin gene (ob/ob) or mutant leptin receptor gene (db/db) develop hyperphagia and obesity, despite consuming a normal chow diet. Only one functional copy of the leptin gene or leptin receptor gene is needed to maintain normal physiology, so the controls included littermate heterozygous (e.g., ob/+ or db/+ ) and wild-type (+/+ ) mice. The expression of Slc2a6 was measured by qPCR and confirmed to be highly upregulated in obese ob/ob and db/db islets from male mice, with relative mRNA levels that were 6.6–12 times higher compared to the lean controls (Figure 2). Slc2a6 mRNA was also increased in islets from obese female mice, but to a lesser extent than in the males (Figure 2). However, GLUT6 protein expression was too low for detection in islets from male mice (Figure S4). GLUT6 knockout was not compensated for by increased GLUT2 expression (Figures S5B and S6). As the upregulation of Slc2a6 was more evident in males, only male mice were studied in subsequent experiments.

![Figure 2. Slc2a6 mRNA expression was increased in islets obtained from obese ob/ob and db/db mice, compared to lean control mice. Slc2a6 expression data from isolated mouse islets of ob/ob (A) and db/db (B) mice, including males (black circles) and females (red squares). * indicates p < 0.05 by two-way ANOVA. n = 3–4 mice per group.](image)

2.2. Effect of GLUT6 Knockout on Insulin Secretion in Isolated ob/ob Mouse Islets

To determine whether GLUT6 played a functional role in mouse islet glucose metabolism, we crossed the ob/ob line with GLUT6/Slc2a6 knockout (GLUT6KO) mice. We have previously reported that GLUT6 is successfully knocked out in this line [14], and confirmed this by qPCR analysis of isolated islets (Figure S5A). Glucose-stimulated insulin secretion (GSIS) was determined in islets from male mice of the following genotypes: wild type (WT, WT), obese (ob/ob, WT), Slc2a6 knockout (WT, KO), and obese with Slc2a6 knockout (ob/ob, KO) (Figure 3). The GSIS assay is an ex vivo measurement of the islets’ insulin secretory function [20]. Pancreatic islets were isolated from 16-week-old mice of the previously mentioned genotypes and incubated in low (2 mM) or high (20 mM) glucose, or 30 mM KCl, to determine glucose- and potassium-stimulated insulin secretion, respectively. Potassium bypasses the glucose-sensing pathway to directly cause the depolarization of beta cells, thus inducing insulin release.

Knockout of GLUT6 did not have any impact on glucose or potassium-stimulated insulin secretion in mice with normal leptin expression (Figure 3A). However, when compared to ob/ob WT mice, ob/ob mice lacking GLUT6 showed significantly greater glucose-stimulated insulin secretion and a trend towards increased KCl-stimulated insulin secretion (Figure 3B). These data indicate that the loss of GLUT6 increased islet secretion of insulin in response to high glucose concentrations only in obese ob/ob mice.
Figure 3. Ex vivo glucose-stimulated insulin secretion was increased in GLUT6-deficient ob/ob islets. Glucose- and potassium-stimulated insulin secretion was measured in isolated, cultured islets from wild-type (WT, A) or leptin-deficient ob/ob male mice (Lep genotype, B) with wild type (WT) or knockout (KO) of Slc2a6. Genotypes are WT, WT (light grey circles), WT, GLUT6KO (light orange squares), ob/ob, WT (dark grey triangles) and ob/ob, GLUT6KO (dark orange triangles). * indicates \( p < 0.05 \) by two-way ANOVA. \( n = 7–12 \) mice per group.

2.3. Effect of GLUT6 Knockout on Whole-Body Metabolic Phenotypes in ob/ob Mice

To investigate whether the loss of GLUT6 impacted metabolic physiology and glucose metabolism in genetically obese ob/ob mice, mouse body weights, body composition, and glucose tolerance were examined at 16 weeks of age (Figure 4). At 16 weeks of age, leptin-deficient ob/ob mice had significantly higher body weight and fat mass compared to their corresponding lean controls (Figure 4A–C), while fat-free mass was not significantly changed (Figure 4D). However, the loss of GLUT6 did not impact body weight or composition (Figure 4).

Figure 4. Body weight and composition were altered by leptin (Lep) but not by the Slc2a6 genotype at 16 weeks of age in male mice. Body weight (A), fat mass as percentage of total body mass (B), fat...
mass as a raw value (C), and fat-free mass (D) were measured when mice were 16 weeks of age for genotypes WT, WT (light grey circles), ob/ob, WT (dark grey triangles), WT, KO (light orange squares) and ob/ob, KO (dark orange triangles). * indicates \( p < 0.05 \) by one-way ANOVA. \( n = 10–13 \) mice per group.

Similarly, glucose tolerance was impaired in ob/ob mice, but GLUT6 knockout had no effect when compared to control mice expressing wild-type GLUT6 (Figure 5A,B). Blood glucose concentrations were elevated in fed, but not fasted, ob/ob mice, compared to lean controls (Figure 5C). Plasma insulin levels were also increased in ob/ob mice in both the fed and fasted states, but the Slc2a6 genotype did not affect plasma insulin concentrations (Figure 5D). Similar results were observed at 10 weeks of age, except that fasted blood glucose was significantly increased in obese mice that were WT for Slc2a6, but not in GLUT6 knockout mice (Figures S2 and S3).
Under in vitro conditions, pancreatic islets isolated from ob/ob mice lacking GLUT6 secreted more insulin in response to high glucose (20 mM) compared to ob/ob mice that expressed GLUT6. It is well established that islet immune cell infiltration is increased in islets from human patients with type 2 diabetes, as well as in diabetic mice, in several different models of obesity and diabetes [21]. This inflammation impairs beta cell function and contributes to blood glucose dysregulation. Since GLUT6 is known to be markedly upregulated in response to inflammatory stimuli [14,15] and may mediate some downstream effects, it is plausible that the absence of GLUT6 may have decreased these potential pro-inflammatory responses, thus minimizing stress on the beta cells. However, while the high concentration of glucose used in the GSIS assay is ideal for evaluating islet function, it is important to note that this condition may not be physiologically relevant. Therefore, in vivo analyses were performed to investigate the changes in physiology among mice that were lean or obese, with and without GLUT6. These whole-body studies showed that there was no observable phenotypic difference between these animals in terms of body mass, body composition, or glucose tolerance. Hence, the in vitro observation of increased GSIS in GLUT6KO obese islets did not translate to altered whole-body in vivo physiology in this model. One explanation for this finding is that islet GLUT6 confers a restraining effect on insulin secretion at hyperglycemic concentrations of glucose, which are not present in the ob/ob model, but may be relevant during obese type 2 diabetes. Future studies in ob/ob mice on a BTBR background or db/db mice on a BKS background, which demonstrate marked hyperglycemia even in a fasted state [6], may show a difference between WT and GLUT6KO mice. However, it is also possible that GLUT6 is a marker of the obese islet, rather than playing a key causal or reactive role. GLUT6 may also play a role independent of the functions evaluated in our study.

Insulin-secreting beta cells are the most abundant cell type in the pancreatic islet and comprise approximately 60–80% of the mouse islet [22]. The second most common cell type is the glucagon-secreting alpha cell. However, there are also many less abundant cell types found in islets, including other endocrine cell types such as delta cells, epsilon cells, and pancreatic polypeptide (gamma) cells, as well as immune cells. Given that Slc2a6 is known to be expressed in leukocytes [12] and is upregulated in response to inflammatory stimuli [15,16], it is plausible that the overexpression of Slc2a6 in obese islets is primarily due to the upregulation of Slc2a6 expression in islet immune cells and/or the higher immune cell content in obese or diabetic islets [21]. A single-cell RNA sequencing study of human islet cells revealed that SLC2A6 mRNA transcripts ranked within the top 1140 transcripts in mast cells and MHCII-positive cells. In contrast, SLC2A6 transcripts ranked below the top 4000 and 5000 transcripts in islet endocrine and exocrine cells, respectively [23]. In mouse islets, single-cell RNA sequencing showed that Slc2a6 was enriched in a cluster of cells primarily comprised of cells exposed to the pro-inflammatory cytokine IL-1β and highly enriched for Nos2 mRNA [24]. Nos2 is an inflammatory gene induced by NF-κB downstream of IL-1β stimulation. Since IL-1β levels are associated with obesity in humans and mice [25,26] and we have previously shown that GLUT6 expression is regulated by NF-κB [27], it is also possible that GLUT6 expression may be elevated in specific β-cells clusters within islets. Future studies using oligonucleotide in situ hybridization or cell sorting techniques may shed further light on the cell type in which GLUT6 is expressed in islets from obese mice.

This study has a number of limitations which should be acknowledged. The mouse model investigated involved the whole-body knockout of GLUT6, rather than islet-specific GLUT6 deletion. However, due to the diversity of cell types in the islet and their complex lineages, it would have been impractical to develop a cell-type-specific GLUT6 knockout mouse for this study. Moreover, as no change in phenotype was observed in this whole-body GLUT6 knockout model, it is unlikely that a tissue-specific knockout would yield significant further insight. Another limitation is that the GSIS results for islets from these mice were normalized to islet number. Knockout of GLUT6 did not change islet size; however, since ob/ob and WT islets typically differ in size and therefore beta cell
number [28,29], comparisons between the ob/ob and WT genotypes should be made with caution. Unfortunately, we were unable to normalize the data to DNA content, which is standard practice in the field.

This study was also limited by the small sample size (n = 12) in the human islet study, due to the scarcity of available islets from human donors. Future studies with larger sample sizes may provide further insights into the relevance of these findings to the human disease state.

In conclusion, despite a marked increase in Slc2a6 mRNA expression in obese mouse and human islets, whole-body GLUT6 knockout in leptin-deficient mice did not produce an observable change in body weight, body composition, or glucose tolerance. However, ob/ob GLUT6KO islets showed enhanced GSIS ex vivo, suggesting that GLUT6 may restrict insulin secretion in response to a hyperglycemic stimulus. Further investigation is required to elucidate the precise role of GLUT6 in pancreatic islet function.

4. Materials and Methods

4.1. Mouse Colonies

The ob/ob mouse line (B6.Cg-Lepob/Aus) was established using breeders provided by the laboratory of Professor Greg Cooney from the University of Sydney, Australia. Heterozygous mice (ob/+), evaluated from the ob/ob line were crossed with homozygous GLUT6KO mice (C57BL/6-Slc2a6em1Ausb/Ausb) from our GLUT6KO line, as has been previously described and validated [14]. Offspring genotypes were determined by PCR amplification and gel electrophoresis. The genotype for Slc2a6, the gene encoding for GLUT6, was determined according to [14]. Genotyping for the Lep gene was performed as previously described [30].

Db/db mice (BKS.Cg-Dock7m+/- Leprdb/JAus) were obtained from breeders provided by Professor Kerry-Anne Rye’s laboratory at the University of New South Wales, Australia. Offspring genotypes were determined by PCR amplification and gel electrophoresis according to [31].

4.2. Mouse Studies

All mouse experiments were approved by the University of New South Wales (UNSW) Animal Care and Ethics Committee (approval number 20/67A). Mouse breeding was conducted at Australian BioResources (Moss Vale, NSW, Australia) before the mice were issued to the Wallace Wurth animal facility at UNSW. Mice were housed at 22 °C in a 12 h light/dark cycle and provided with ad libitum access to water and a standard chow diet (Gordons Speciality Feeds, Yanderra, NSW, Australia). Mice were monitored according to ethical guidelines. Body composition was measured by quantitative magnetic resonance imaging using an EchoMRI Body Composition Analyzer (EchoMRI™). At the experimental endpoint, mice were culled by cervical dislocation.

4.3. Glucose Tolerance Tests

Intraperitoneal glucose tolerance tests were conducted following a 6 h daytime fast. Mice were administered 33.3% D-glucose in saline by intraperitoneal injection at a dose of 2 g/kg lean mass. Blood glucose was measured at the specified time points using an Accu-Check Performa II glucometer (Roche, North Ryde, NSW, Australia).

4.4. Plasma Insulin Measurements

Approximately 40 µL of blood from the tail tip was collected in heparinized capillary tubes (Sarstedt 16.443, Mawson Lakes, SA, Australia) in the random-fed state and after a 6 h daytime fast. Samples were centrifuged at 2000 x g and 4 °C for 10 min to extract the plasma, which was stored at −80 °C. Plasma insulin was measured using the Crystal Chem Ultra-Sensitive Mouse Insulin ELISA Kit (Crystal Chem 90080, Elk Grove Village, IL, USA) according to the manufacturer’s instructions, except that samples were incubated overnight at 4 °C.
4.5. Mouse Islet Isolation

Islet isolation and culture was based on our published protocol [20], involving collagenase digestion of the pancreas and the Ficoll gradient separation of islets. Detailed methods are available in the supplementary materials. Islets were cultured overnight in a RPMI-1640 medium (Thermofisher 11875-093, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS), 15 mM HEPES, 100 U/mL penicillin, and 0.1 mg/mL streptomycin at 37 °C and 5% CO2 before GSIS assays, or washed in PBS and stored at −80 °C prior to RNA extraction.

4.6. Ex Vivo Glucose-Stimulated Insulin Secretion

Insulin secretion assays were conducted as previously described [20]. Mouse islets were incubated for 1 h at 37 °C in HEPES-buffered Kreb’s Ringer Buffer (KRBH) containing 0.1% bovine serum albumin (BSA) and 2 mM D-glucose. Batches of size-matched islets were handpicked and stimulated by incubation at 37 °C in KRBH containing glucose or KCl for 1 h. The reaction was terminated by placing the samples on ice and centrifuging at 1000 rpm for 1 min to pellet the islets. Supernatants containing secreted insulin were collected for analysis using an in-house ELISA assay, which is described in the supplementary materials (validation of standards shown in Figure S1).

4.7. Human Islet RNA Extraction and Quantitative Real-Time PCR (qPCR)

The source of the human islet samples from patients with or without type 2 diabetes used herein has been described previously [19]. Ethics approval was obtained from the University of Virginia Institutional Review Board (protocol #14904). Informed consent was obtained from the donors and their families before organ collection by the local organ procurement agency. RNA isolation and qPCR were performed according to [19]. In brief, RNA was extracted using an AllPrep DNA/RNA/Protein mini kit (Qiagen, Hilden, Germany). cDNA was obtained by reverse transcription using 0.25–1 µg RNA with a Life Technologies High-Capacity cDNA kit (Thermofisher 4368814, Waltham, MA, USA). Quantitative real-time PCR (qPCR) was conducted using the following primer sequences for SLC2A6 (forward primer: GCC CGG ACT ACG ACA CCT, reverse primer: AGC TGA AAT TGC CGA GCA C) and the housekeeping gene HPRT1 (forward primer: ATG GAC AGG ACT GAA CGTCT, reverse primer: TCC AGC AGG TCA GCA AAG AA). iQ SYBR green SuperMix (Bio-Rad, Hercules, CA, USA) was used to detect amplification products on an iCycler (MyiQ Optical Module) Bio-Rad System. Quantification was performed using the Pfaffl method [32].

4.8. Mouse Islet RNA Extraction and qPCR

RNA extraction was performed on isolated mouse islets using a TRI reagent according to the manufacturer’s protocol (Sigma T9424, Castle Hill, NSW, Australia). cDNA synthesis was performed using 225 to 350 ng RNA and an iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer’s protocol.

Quantitative real-time PCR (qPCR) was conducted using the following primer sequences for Slc2a6 (forward primer: GCC ACT CCT GGA GAG AGA GA, reverse primer: CAG GAT GCC TGG ATT TTG TC), Slc2a2 (forward primer: TGT GCT GCT GGA TAA ATT CGC CTG, reverse primer: AAC CAT GAA CCA AGG GAT TGG ACC) and the housekeeping gene Ppia (forward primer: CGA TGA CGA GCC CTT GG, reverse primer: TCT GCT GTC TTT GGA ACT TTG TC). PCR products were amplified in the following (15 µL) reaction: 700 nM primers, 7.5 µL iTaq Universal SYBR Green Supermix (Bio-Rad), 3.5 µL nuclease-free water, and 2 µL cDNA, using an Applied Biosystems ViiA7 Real-Time PCR System (Thermofisher, Waltham, MA, USA). PCR cycling conditions were as follows: 95 °C (20 s), 40 cycles (95 °C (1 s) and 60 °C (20 s)) and melt curve stage (95 °C (15 s), 60 °C (1 min), and 95 °C (15 s)). qPCR analysis was performed using the Pfaffl method [32].
4.9. Statistical Analysis

All statistical analyses were conducted using Graphpad Prism 9.3.1 (San Diego, CA, USA). Data show mean ± SEM, and data points indicate biological replicates unless otherwise specified. A p-value of less than 0.05 was considered statistically significant.

For gene expression data with more than two groups, significance was determined by two-way ANOVA with Sidak’s correction. When comparing only two groups, the Student’s T-test was performed. Pearson’s correlation was conducted on human SLC2A6 expression and BMI data. For body weight, body composition, and glucose tolerance data that were normally distributed with standard deviations that were not significantly different according to the Brown–Forsythe test, significance was assessed by one-way ANOVA with Sidak’s correction. For data that failed the Brown–Forsythe test, Welch’s one-way ANOVA with Dunnett’s correction was performed instead. The Kruskal–Wallis test with Dunn’s correction was utilized for non-parametric data. For fed and fasted glucose and insulin data, significance was assessed by two-way ANOVA with multiple comparisons using Graphpad Prism QuickCalcs, which performs Bonferroni’s correction and allows for the manual selection of relevant comparisons to avoid inappropriate comparison of groups where both Lep and Slc2a6 genotypes were different.

For GSIS data, data points indicate biological replicates, which are the average of 4 technical replicate wells per mouse. Any outlier wells were removed using Grubbs’ test [33]. Significance was assessed by two-way ANOVA with Sidak’s multiple comparisons test.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms23179798/s1.

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Informed Consent Statement: Informed consent was obtained from all donors and their families before organ collection by the local organ procurement agency.

Data Availability Statement: Data are available upon reasonable request to the corresponding author.

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