Glucokinase is required for high-starch diet-induced β-cell mass expansion in mice

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INTRODUCTION

Impaired insulin secretion, caused by pancreatic β-cell insufficiency, and insulin resistance in target organs are key aspects of the pathophysiology of type 2 diabetes1. In particular, the former plays a pivotal role in the natural history of type 2 diabetes2-5. Pancreatic β-cell failure is not only induced by a functional reduction in each β-cell, but also by a reduction of β-cell mass6-8. Therefore, preserving β-cell mass would be essential for the success in the treatment of type 2 diabetes, and it is important to understand the mechanisms of β-cell mass regulation.

ABSTRACT

Aims/Introduction: We aimed to determine whether glucokinase is required for β-cell mass expansion induced by high-starch diet (HSTD)-feeding, as has been shown in its high-fat diet-induced expansion.

Materials and Methods: Eight-week-old male wild-type (Gck+/+) or glucokinase haploinsufficient (Gck−/−) mice were fed either a normal chow (NC) or an HSTD for 15 weeks. The bodyweight, glucose tolerance, insulin sensitivity, insulin secretion and β-cell mass were assessed.

Results: Both HSTD-fed Gck+/+ and Gck−/− mice had significantly higher bodyweight than NC-fed mice. Insulin and oral glucose tolerance tests revealed that HSTD feeding did not affect insulin sensitivity nor glucose tolerance in either the Gck+/+ or Gck−/− mice. However, during the oral glucose tolerance test, the 15-min plasma insulin concentration after glucose loading was significantly higher in the HSTD group than that in the NC group for Gck+/+, but not for Gck−/− mice. β-Cell mass was significantly larger in HSTD-fed Gck+/+ mice than that in NC-fed Gck+/+ mice. In contrast, the β-cell mass of the HSTD-fed Gck−/− mice was not different from that of the NC-fed Gck+/+ mice.

Conclusions: The results showed that HSTD feeding would increase pancreatic β-cell mass and insulin secretion in Gck+/+, but not Gck−/− mice. This observation implies that glucokinase in β-cells would be required for the increase in β-cell mass induced by HSTD feeding.

Keywords

β-Cell mass, Glucokinase, High-starch diet

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Glucokinase, which is the predominant glucose-phosphorylating enzyme, has a central role as a glucose sensor in β-cells and elicits glucose-stimulated insulin secretion9,10. Indeed, the haploinsufficiency of glucokinase (Gck+/+) mice causes glucose intolerance as a result of impaired glucose-stimulated insulin secretion11,12, and glucokinase–maturity-onset diabetes of the young, which is caused by inactivating heterozygous mutations in the glucokinase gene, usually manifests as mild fasting hyperglycemia13. In contrast, glucokinase activation (by the administration of pharmacological activators or the presence of a heterozygous activating mutation) augments insulin secretion and reduces blood glucose concentration14-16.

It has been proven that glucokinase is involved in the regulation of β-cell mass17-19. Previously, we reported that Gck+/+...
mice showed an increase in β-cell mass after consuming a high-fat diet (HFD) for 20 weeks, but that Gck<sup>++/+</sup> mice did not respond with the same dietary load, despite having a similar degree of insulin resistance<sup>17</sup>. Therefore, glucokinase is required for the increase in β-cell mass induced by HFD feeding.

As well as mice fed a HFD, mice fed a high-starch diet (HSTD), generating a large amount of glucose, show exaggerated bodyweight gain<sup>20,21</sup>. Furthermore, it has been reported that HSTD feeding increases β-cell mass in wild-type mice<sup>22,23</sup>. However, it is unclear whether glucokinase is required for the increase of β-cell mass induced by HSTD feeding. In the current study, we aimed to determine the role of glucokinase in the HSTD-induced increase in β-cell mass using Gck<sup>++/+</sup> mice.

**MATERIALS AND METHODS**

**Animals**

Gck<sup>++/+</sup> mice and Gck<sup>+/−</sup> mice were generated as described elsewhere<sup>11</sup>. Male littermates derived from intercrosses were fed a normal chow (NC) until 8 weeks-of-age, and then given free access to either the NC diet or an HSTD for 15 weeks (Figure 1). The mice were housed on a 12-h light–dark cycle. The study was approved by the Animal Use Committee of Hokkaido University Graduate School of Medicine and carried out in compliance with the Animal Use Guidelines of Hokkaido University.

**Diet protocol**

The NC was from the Oriental Yeast Co. Ltd (Tokyo, Japan) and the HSTD was as described previously<sup>20–23</sup>. The energy derived from each component of the diets is shown in Table 1.

![Figure 1](http://wileyonlinelibrary.com/journal/jdi)

**Table 1** | Energy contents of the diets

|        | NC   | HSTD |
|--------|------|------|
| Protein | 25.70 | 13.98 |
| Fat    | 12.77 | 14.74 |
| Carbohydrate | 61.53 | 71.29 |

Values are the percentage of total energy. HSTD, high-starch diet; NC, normal chow.

**Measurement of biochemical parameters**

Blood glucose was determined with a Glutestmint portable glucose meter (Sanwa Chemical Co., Nagoya, Japan). Insulin concentration was measured using a Morinaga Ultra-sensitive Mouse/Rat Insulin ELISA Kit (Moringa Institute of Biological Science, Yokohama, Japan).

**Insulin and oral glucose tolerance tests**

Insulin tolerance test was carried out under non-fasting conditions after 14 weeks on the NC or HSTD (Figure 1). Human regular insulin (0.75 mU/g bodyweight) was injected intraperitoneally, and blood samples were collected before, and at 30, 60, 90, and 120 min after the injection. Oral glucose tolerance test was carried out under 16 h of fasting conditions after 14 weeks on a NC or HSTD (Figure 1). Glucose (1.5 mg/g bodyweight) was orally given, and blood samples were collected before, and at 15, 30, 60, and 120 min after glucose loading.

**β-cell morphology and immunohistochemistry**

Isolated pancreata were immersion-fixed in 4% paraformaldehyde. The tissue was then routinely processed for paraffin embedding, and 5-μm sections mounted on glass slides were immunostained with rabbit anti-insulin polyclonal antibody (15848-1-AP, diluted 1:1000; Proteintech, Rosemont, IL, USA). The β-cell area was calculated using a BZ-II analyzer (Keyence Co., Osaka, Japan). The β-cell mass of each mouse was estimated by calculating the β-cell area as a proportion of the total pancreatic area for each mouse and multiplying this proportion by the pancreatic weight. β-Cell proliferation was evaluated by staining sections with 5-bromo-2-deoxyuridine and Ki67 antibody, as described previously<sup>24,25</sup>. The number of 5-bromo-2-deoxyuridine- and Ki67-positive pancreatic β-cells was quantitatively assessed as a ratio of the total number of β-cells.

**Islet isolation**

Islets were isolated using collagenase from Clostridium histolyticum (Sigma Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions, as described previously<sup>24</sup>.

**Real-time quantitative polymerase chain reaction**

Ribonucleic acid was isolated from islets using an RNeasy mini kit (Qiagen, Hilden, Germany), and was used as the starting material for complementary deoxyribonucleic acid preparation.
Real-time polymerase chain reaction (PCR) assays were carried out in duplicate using a 7500 Fast Real Time PCR system with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Primers were as follows: K67 forward, CTGCTTCGGAAGAGACATC; K67 reverse, AGCTC CACTTCGCTTTTGG; insulin receptor substrate 2 (Irs2) forward, AACCTGAAACCTAAGGGACTGG; Irs2 reverse, CGG CGAATTTCTCAAGAGCGT; β-actin (Actb) forward, ATAT CGCTGCGCCTGTTCTGTC; Actb reverse, AGCACACGCTG GATGGCTAC.

Microarray analysis

Samples containing >120 isolated islets were frozen in −196 and stored at −80 °C. Samples were subjected to Clariom S Assay on GeneChip Arrays (Thermo Fisher Scientific, Waltham, MA, USA) if they had a ribonucleic acid integrity number of ≥8. Three samples per group were evaluated. Gene expression profiles were determined using Transcriptome Analysis Console software (version 4.0.2.15; Thermo Fisher Scientific).

Statistical analysis

The results are presented as the mean ± standard deviation. Individual comparisons between more than two groups were carried out using analysis of variance, and the Bonferroni correction was used post-hoc, as appropriate. Statistical significance was set at P < 0.05.

RESULTS

Effect of HSTD on bodyweight and blood glucose concentration in Gck+/− and Gck+/+ mice

We first measured bodyweight and casual blood glucose concentration in the Gck+/− NC, Gck+/+ HSTD, Gck+/− NC and Gck+/− HSTD groups. Similar amounts of bodyweight gain occurred in the Gck+/− and Gck+/+ mice fed the HSTD, and both sets of mice had significantly higher bodyweight after HSTD feeding than after NC feeding (Figure 2a,b). After 15 weeks of feeding of the NC or HSTD, the subcutaneous and visceral fat weights were significantly higher in mice fed the HSTD than in those fed the NC, both in Gck+/− and Gck+/+ mice, whereas there were no differences in liver or pancreas weights among the four groups (Figure 2c). The casual blood glucose concentrations in mice fed the NC or HSTD were higher in Gck+/− than Gck+/+ mice, as described previously for NC-fed mice, but the HSTD did not lead to an increase in blood glucose in either the Gck+/− or Gck+/+ mice (Figure 2d). These results show that HSTD feeding for 15 weeks increases bodyweight and fat mass, but does not affect blood glucose concentration in Gck+/− and Gck+/+ mice.

Effect of HSTD feeding on the glucose tolerance of Gck+/− and Gck+/+ mice

To determine the effect of HSTD on insulin sensitivity in Gck+/+ and Gck+/− mice, insulin tolerance test was carried out. There were no differences in the blood glucose concentrations after the insulin injection between the Gck+/+ NC and Gck+/− HSTD groups nor between the Gck+/− NC and Gck+/− HSTD groups (Figure 3a). Furthermore, there were no differences in the blood glucose concentrations among the four groups when the glucose concentrations were normalized to the baseline values (Figure 3b). To determine the effect of HSTD on glucose tolerance in Gck+/− and Gck+/+ mice, oral glucose tolerance test was carried out. There were no significant differences in the blood glucose concentrations or the areas under the curves of the blood glucose versus time curves between the Gck+/− NC and Gck+/− HSTD groups or between the Gck+/− NC and Gck+/− HSTD groups (Figure 3c,d). During this oral glucose tolerance test, the plasma insulin concentration at 15 min after glucose loading was significantly higher in the HSTD group than in the NC group for Gck+/+ mice. On the other hand, no increase in the plasma insulin concentration after glucose loading was observed in the Gck+/− mice fed the HSTD (Figure 3e). These results show that HSTD feeding for 15 weeks does not affect insulin sensitivity nor glucose tolerance in either mouse genotype. In contrast, HSTD feeding increased insulin secretion in the Gck+/− mice, but not in the Gck+/+ mice.

Effect of HSTD feeding on the β-cell mass of Gck+/− and Gck+/+ mice

To determine the effect of HSTD feeding on β-cell mass of these mice, we next carried out histological analysis of their pancreata after NC or HSTD feeding for 15 weeks. There was no difference in β-cell mass between the Gck+/− NC group and the Gck+/− HSTD group, as previously reported. The β-cell mass of the Gck+/− HSTD group was significantly higher than that of the Gck+/− NC group, but the β-cell mass of the Gck+/− HSTD group was not higher than that of the Gck+/− NC group (Figure 4a,b).

Effect of HSTD feeding on β-cell proliferation and Irs2 expression in the Gck+/− and Gck+/+ mice

Because the β-cell proliferation and Irs2 expression in Gck+/− mice, but not in Gck+/+ mice, were increased by HFD-feeding for 20 weeks, the β-cell proliferation and Irs2 expression of mice in each of the groups were also evaluated in the present study. As shown in Figure 5a,b, there were no differences in the ratios of 5-bromo-2-deoxyuridine - nor Ki67-positive pancreatic β-cells among the four groups. In addition, real-time quantitative PCR showed that there were no differences in Ki67 nor Irs2 expression among the groups (Figure 5c,d). Regarding apoptosis, there were very few fluorometric terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling-positive β-cells, which is consistent with our previous findings in mice fed an HFD.

Microarray analysis of ribonucleic acid from the islets of Gck+/− and Gck+/+ mice fed an NC or HSTD

To show differences in global gene expression, a microarray analysis was carried out of genes expressed by isolated islets of the four groups of mice. To identify genes that are related to
the differences in insulin secretion and β-cell mass, genes that were expressed at higher or lower levels in the Gck<sup>+/−</sup> HSTD group were identified. First, out of a total of 22,203 genes, 216 that were differentially expressed with \( P < 0.05 \) among the four groups were identified. Next, we narrowed these down to genes with expression levels in the Gck<sup>+/−</sup> HSTD group that were significantly different from, and >2- or <0.5-fold the expression levels in the other three groups. Eight genes fulfilled these
criteria (Figure 6). The expression of Aldh1a3, Slc17a9, Cthrc1 and Pde10a in the Gck\textsuperscript{+/+} HSTD group was significantly higher than that in the other three groups, and the expression of Sult1c2, visinin-like protein 1 (Vsnl1), Mt2 and Mt1 was significantly lower.

**DISCUSSION**

In the present study, we showed that HSTD feeding augmented insulin secretion and increased pancreatic β-cell mass in Gck\textsuperscript{+/+} mice, but those effects were not seen in Gck\textsuperscript{−/−} mice. We previously reported that glucokinase in β-cells played an important...
role in HFD-induced β-cell mass expansion\textsuperscript{17}, and the present findings showed that glucokinase was also required for the increase in β-cell mass induced by HSTD feeding.

Murase \textit{et al.}\textsuperscript{22} showed that β-cell mass is increased by HSTD feeding in wild-type mice, but there is no difference in the mass of β-cells between Kir6.2, an adenosine triphosphate-sensitive potassium channel, -deficient mice fed an NC and those fed an HSTD. These results suggest that an increase in the glucose signal in pancreatic β-cells induced by HSTD could lead to an increase in β-cell mass in a Kir6.2-dependent manner. The increase in the glucose signal would increase glycolysis through glucokinase in the β-cells, increasing the intracellular adenosine triphosphate concentration, which would trigger closure of adenosine triphosphate-sensitive potassium channels\textsuperscript{26}. This mechanism\textsuperscript{19,26} might be involved in the increase in pancreatic β-cell mass induced by HSTD feeding.

In wild-type mice fed an HFD for 20 weeks, there was significantly higher β-cell proliferation and Irs2 expression than in

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**Figure 4** | Histology of pancreatic tissue of mice in each group. (a) Pancreatic section stained with hematoxylin (purple). β-cells are immunostained using an anti-insulin antibody (brown). Scale bars, 100 μm. (b) β-cell mass, calculated from the pancreatic weight and the ratio of the β-cell area to the pancreatic area in the wild-type (Gck\textsuperscript{+/+}) normal Chow (white bar), Gck\textsuperscript{+/+} high-starch diet (HSTD; pale gray bar), glucokinase haploinsufficient (Gck\textsuperscript{+/−}) NC (dark gray bar) and Gck\textsuperscript{+/−} HSTD (black bar) groups (n = 11–14). Data are presented as the mean ± standard deviation. *\textit{p} < 0.05. NS, not significant.
those fed an NC, whereas no increases in β-cell proliferation or Irs2 expression were identified in Gck+/− mice fed a HFD17. Furthermore, Irs2 heterozygous mice fed an HFD do not have significantly higher β-cell mass than wild-type mice, and Irs2 overexpression in β-cells of Gck+/− mice fed a HFD restores their proliferation17. Considering that Irs2 is one of the major substrates for the insulin receptor tyrosine kinase and insulin-like growth factor receptor kinase, and is critically required for β-cell growth and survival27,28, these results show that IRS2, as well as glucokinase, plays an important role in high-fat-induced β-cell proliferation. However, in the present study, there were no differences in β-cell proliferation or Irs2 expression among the four mouse groups (Figure 5d). These data are consistent with those of a previous study that showed that β-cell proliferation and Irs2 expression in wild-type mice fed an HSTD for 22 weeks were not significantly higher than those in wild-type mice fed an NC22.

Thus, one of the mechanisms of increased β-cell mass in Gck+/+ mice fed an HSTD could be independent of the IRS2-induced β-cell proliferation. Microarray analysis showed that the expression levels of Mt1 and Mt2 in the Gck+/+ HSTD group was significantly lower than those in the other three groups (Figure 6). Although metallothioneins (MTs) are mainly involved in metal ion homeostasis, the roles of MT1 and MT2 in β-cell function are being clarified29. Mt1 and Mt2 expression is downregulated in the islets of β-cell compensation models, such as high-fat fed and ob/ob mice, and glucose causes the downregulation of Mt1 and Mt2 expression. In addition, a study of knockout and transgenic mice showed that MT1 is a negative regulator of insulin secretion by β-cells29. These
findings are consistent with the present data showing greater insulin secretion in Gck<sup>+/+</sup> mice fed an HSTD, but not in Gck<sup>−/−</sup> mice fed the same diet. However, histological analysis did not show any differences in pancreatic β-cell mass between wild-type and Mt1-Mt2 double-knockout mice<sup>29</sup>. With respect to the vesicular nucleotide transporter, which is an Scl17a9-encoded protein, it is expressed in β-cells and plays an important role in the regulation of insulin secretion<sup>30</sup>. Overexpression of vesicular nucleotide transporter in β-cells increases glucose-induced insulin secretion<sup>30</sup>, which is consistent with the present findings. Vsnl1 is also expressed in β-cells, and its downregulation increases insulin gene transcription<sup>31</sup>. However, it is unclear whether these genes affect β-cell mass.

Another possibility could be that proliferation occurs earlier in Gck<sup>+/+</sup> mice fed an HSTD, resulting in higher β-cell mass. Masuda et al.<sup>23</sup> showed that Irs2 expression in the isolated islets of mice fed an HSTD for a short period was significantly higher than that in mice fed an NC. The results of administering mice a glucokinase activator, which increases IRS2 expression, and the study of a mouse model of genetic activation of β-cell glucokinase suggest that an increase in glucose stimulation leads to an initial increase in β-cell proliferation, but this proliferation would not be sustained over the long term<sup>14,16,32,33</sup>. Therefore, β-cell proliferation might be caused by the initial glucose stimulation signal in Gck<sup>+/+</sup> mice fed an HSTD, but not in Gck<sup>−/−</sup> mice fed an HSTD, resulting in an increase in β-cell mass only in the former.

Microarray analysis carried out in the present study showed that Aldh1a3 expression was higher in Gck<sup>+/+</sup> mice fed the HSTD than in the other mouse groups (Figure 6). A similar result was obtained using real-time quantitative PCR (Figure S1). Because aldehyde dehydrogenase 1 family member A3, which is encoded by Aldh1a3, is considered to be a marker of β-cell dedifferentiation<sup>34,35</sup>, Gck<sup>+/+</sup> mice fed an HSTD might progress to β-cell failure, despite the higher β-cell mass in these mice. Thus, protective effects on pancreatic β-cells might not persist, although glucose stimulation of pancreatic β-cells by HSTD feeding initially increases pancreatic β-cell mass. From a clinical point of view, longer-term high-carbohydrate intake might have an adverse effect on β-cell function and mass, although there is no evidence regarding the ideal percentages of dietary energy that should be derived from carbohydrate, protein and fat for people with or at risk of diabetes<sup>36</sup>. Further investigations of the effects of longer-term feeding of the diet to these mice should be conducted in the future.

One of the limitations of the present study is that the mechanism underlying the expansion of β-cell mass in response to HSTD is unclear. To address this, the time course of β-cell proliferation in HSTD-fed mice should be characterized in a future study. Another limitation is that the phenotype of HSTD-fed mice might be ascribed to a difference in dietary protein load, rather than the difference in carbohydrate load. As shown in Table 1, the protein content of the HSTD was lower than that of the NC. A difference in protein load has previously been shown to have an effect on mouse phenotype<sup>37</sup>. Fortunately, the concentrations of substances that might be secreted in response to low-protein diet feeding, such as Fgf21<sup>37</sup>, were not measured in the present study.

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**Figure 6** | Gene expression in isolated islets from mice in each group. Out of a total of 22,203 genes in total, eight genes were identified that had expression levels in the Gck<sup>+/+</sup> HSTD group, which were significantly different and >2- or 0.5-fold the level of those in the other three groups. Expression levels of genes in the wild-type (Gck<sup>+/+</sup>) normal chow (NC; white bar), Gck<sup>−/−</sup> high-starch diet (HSTD; pale gray bar), glucokinase haploinsufficient (Gck<sup>+/−</sup>) NC (dark gray bar) and Gck<sup>−/−</sup> HSTD (black bar) groups (<i>n</i> = 3). Data are presented as the mean ± standard deviation. *<i>P</i> < 0.05, **<i>P</i> < 0.01.
Further studies are required to clarify the effects of this difference in dietary protein load.

In conclusion, HSTD feeding increases insulin secretion and pancreatic β-cell mass in Gck+/− mice, but not in Gck−/− mice. These findings show that glucokinase is required for the increase of β-cell mass induced by HSTD feeding.

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DISCLOSURE
The authors declare no conflict of interest.

REFERENCES
1. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet 2017; 389: 2239–2251.
2. Pretkii M, Nolan CJ. Islet beta cell failure in type 2 diabetes. J Clin Invest 2006; 116: 1802–1812.
3. Kendall DM, Cuddihy RM, Bergenstal RM. Clinical application of incretin-based therapy: therapeutic potential, patient selection and clinical use. Am J Med 2009; 122: S37–S50.
4. Morimoto A, Tatsumi Y, Deura K, et al. Impact of impaired insulin secretion and insulin resistance on the incidence of type 2 diabetes mellitus in a Japanese population: the Saku study. Diabetologia 2013; 56: 1671–1679.
5. Ohn JH, Kwak SH, Cho YM, et al. 10-year trajectory of β-cell function and insulin sensitivity in the development of type 2 diabetes: a community-based prospective cohort study. Lancet Diabetes Endocrinol 2016; 4: 27–34.
6. Rhodes CJ. Type 2 diabetes—what is the matter of β-cell life and death? Science 2005; 307: 380–384.
7. Meier JJ, Bonadonna RC. Role of reduced β-cell mass versus impaired β-cell function in the pathogenesis of type 2 diabetes. Diabetes Care 2013; 36(Suppl 2): S113–S119.
8. Yagihashi S, Inaba W, Mizukami H. Dynamic pathology of islet endocrine cells in type 2 diabetes: β-Cell growth, death, regeneration and their clinical implications. J Diabetes Investig 2016; 7: 155–165.
9. Matschinsky FM, Magnuson MA, Zelent D, et al. The network of glucokinase-expressing cells in glucose homeostasis and the potential of glucokinase activators for diabetes therapy. Diabetes 2006; 55: 1–12.
10. Matschinsky FM, Wilson DF. The Central Role of Glucokinase in Glucose Homeostasis: A Perspective 50 Years After Demonstrating the Presence of the Enzyme in Islets of Langerhans. Front Physiol 2019; 10: 148.
11. Terauchi Y, Sakura H, Yasuda K, et al. Pancreatic beta-cell-specific targeted disruption of glucokinase gene. Diabetes mellitus due to defective insulin secretion to glucose. J Biol Chem 1995; 270: 30253–30256.
12. Terauchi Y, Iwamoto K, Tamemoto H, et al. Development of non-insulin-dependent diabetes mellitus in the double knockout mice with disruption of insulin receptor substrate-1 and beta cell glucokinase genes. Genetic reconstitution of diabetes as a polygenic disease. J Clin Invest 1997; 99: 861–866.
13. Chakera AJ, Steele AM, Glyn AL, et al. Recognition and management of individuals with hyperglycemia because of a heterozygous glucokinase mutation. Diabetes Care 2015; 38: 1383–1392.
14. Nakamura A, Terauchi Y, Ohyama S, et al. Impact of small molecule glucokinase activator on glucose metabolism, beta cell function and mass. Endocrinology 2009; 150: 1147–1154.
15. Bonadonna RC, Heise T, Arbet-Engels C, et al. Piragliti (RO4389620), a novel glucokinase activator, lowers plasma glucose both in the postabsorptive state and after a glucose challenge in patients with type 2 diabetes mellitus: a mechanistic study. J Clin Endocrinol Metab 2010; 95: 5028–5036.
16. Nakamura A, Terauchi Y. Present status of clinical deployment of glucokinase activators. J Diabetes Investig 2015; 6: 124–132.
17. Terauchi Y, Takamoto I, Kubota N, et al. Glucokinase and IRS-2 are required for compensatory beta cell hyperplasia in response to high-fat diet-induced insulin resistance. J Clin Invest 2007; 117: 246–257.
18. Weir GC, Bonner-Weir S. A dominant role for glucose in beta cell compensation of insulin resistance. J Clin Invest 2007; 117: 81–83.
19. Porat S, Weinberg-Corem N, Tornovsky-Babaey S, et al. Control of pancreatic beta cell regeneration by glucose metabolism. Cell Metab 2011; 13: 440–449.
20. Maekawa R, Seino Y, Ogata H, et al. Chronic high-sucrose diet increases fibroblast growth factor 21 production and energy expenditure in mice. J Nutr Biochem 2017; 49: 71–79.
21. Maekawa R, Ogata H, Murase M, et al. Glucose-dependent insulinotropic polypeptide is required for moderate high-fat diet- but not high-carbohydrate diet-induced weight gain. Am J Physiol Endocrinol Metab 2018; 314: E572–E583.
22. Murase M, Seino Y, Maekawa R, et al. Functional adenosine triphosphate-sensitive potassium channel is required in high-carbohydrate diet-induced increase in beta-cell mass. J Diabetes Investig 2019; 10: 238–250.
23. Masuda A, Seino Y, Murase M, et al. Short-term high-starch, low-protein diet induces reversible increase in beta-cell mass independent of body weight gain in mice. *Nutrients* 2019; 11: 1045.

24. Kitao N, Nakamura A, Miyoshi H, et al. The role of glucokinase and insulin receptor substrate-2 in the proliferation of pancreatic beta cells induced by short-term high-fat diet feeding in mice. *Metabolism* 2018; 85: 48–58.

25. Omori K, Nakamura A, Miyoshi H, et al. Effects of dapagliflozin and/or insulin glargine on beta cell mass and hepatic steatosis in db/db mice. *Metabolism* 2019; 98: 27–36.

26. Ferrer J. Glucose as a mitogenic hormone. *Cell Metab* 2011; 13: 357–358.

27. Withers DJ, Gutierrez JS, Towery H, et al. Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 1998; 391: 900–904.

28. Kubota N, Tobe K, Terauchi Y, et al. Disruption of insulin receptor substrate-2 causes type 2 diabetes due to liver insulin resistance and lack of compensatory beta-cell hyperplasia. *Diabetes* 2000; 49: 1880–1889.

29. Bensellam M, Shi YC, Chan JY, et al. Metallothionein 1 negatively regulates glucose-stimulated insulin secretion and is differentially expressed in conditions of beta cell compensation and failure in mice and humans. *Diabetologia* 2019; 62: 2273–2286.

30. Geisler JC, Corbin KL, Li Q, et al. Vesicular nucleotide transporter-mediated ATP release regulates insulin secretion. *Endocrinology* 2013; 154: 675–684.

31. Dai FF, Zhang Y, Kang Y, et al. The neuronal Ca²⁺ sensor protein visinin-like protein-1 is expressed in pancreatic islets and regulates insulin secretion. *J Biol Chem* 2006; 281: 21942–21953.

32. Nakamura A, Togashi Y, Orime K, et al. Control of beta cell function and proliferation in mice stimulated by small molecule glucokinase activator under various conditions. *Diabetologia* 2012; 55: 1745–1754.

33. Tomovsky-Babeay S, Dadon D, Ziv O, et al. Type 2 diabetes and congenital hyperinsulinism cause DNA double-strand breaks and p53 activity in beta cells. *Cell Metab* 2014; 19: 109–121.

34. Kim-Muller JY, Fan J, Kim YJ, et al. Aldehyde dehydrogenase 1A3 defines a subset of failing pancreatic beta cells in diabetic mice. *Nat Commun* 2016; 7: 12631.

35. Cinti F, Bouchi R, Kim-Muller JY, et al. Evidence of beta-cell dedifferentiation in human type 2 diabetes. *J Clin Endocrinol Metab* 2016; 101: 1044–1054.

36. Evert AB, Dennison M, Gardner CD, et al. Nutrition Therapy for Adults with Diabetes or Prediabetes: A Consensus Report. *Diabetes Care* 2019; 42: 731–754.

37. Laeger T, Henagan TM, Albarado DC, et al. FGF21 is an endocrine signal of protein restriction. *J Clin Invest* 2014; 124: 3913–3922.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1** | *Aldh1A3* expression in isolated islets from mice in each group.