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ORIGINAL ARTICLE

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

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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.

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 diabetes[1]. In particular, the former plays a pivotal role in the natural history of type 2 diabetes[2–5]. Pancreatic β-cell failure is not only induced by a functional reduction in each β-cell, but also by a reduction of β-cell mass[6–8]. 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.

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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 secretion[9,10]. Indeed, the haploinsufficiency of glucokinase (*Gck*$^{+/-}$) mice causes glucose intolerance as a result of impaired glucose-stimulated insulin secretion[11,12], and glucokinase–maturity-onset diabetes of the young, which is caused by inactivating heterozygous mutations in the *glucokinase* gene, usually manifests as mild fasting hyperglycemia[13]. 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 concentration[14–16].

It has been proven that glucokinase is involved in the regulation of β-cell mass[17–19]. 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*$^{+/-}$ mice did not respond with the same dietary load, despite having a similar degree of insulin resistance[17]. 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[20,21]. Furthermore, it has been reported that HSTD feeding increases β-cell mass in wild-type mice[22,23]. 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*$^{+/-}$ mice.

MATERIALS AND METHODS

Animals

Gck$^{+/+}$ mice and *Gck*$^{+/-}$ mice were generated as described elsewhere[11]. 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[20–23]. The energy derived from each component of the diets is shown in Table 1.

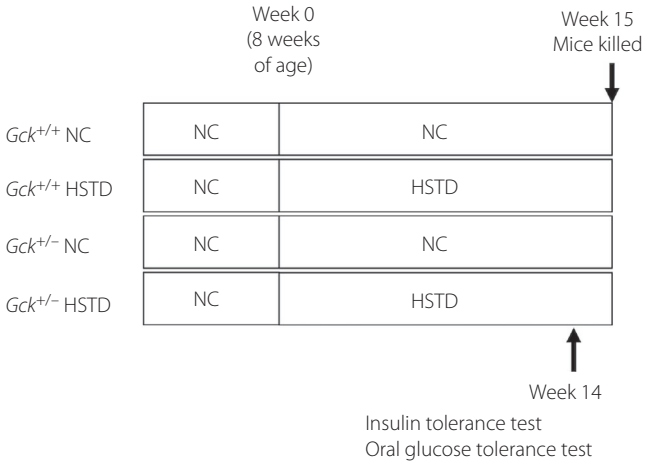

Figure 1 | Study protocol. *Gck*$^{+/+}$ NC: 8-week-old male wild-type (*Gck*$^{+/+}$) mice fed a normal diet (normal chow [NC]) for 15 weeks; *Gck*$^{+/+}$ HSTD, 8-week-old male *Gck*$^{+/+}$ mice fed a high-starch diet (HSTD) for 15 weeks; *Gck*$^{+/-}$ NC, 8-week-old male glucokinase haploinsufficient (*Gck*$^{+/-}$) mice fed an NC for 15 weeks; *Gck*$^{+/-}$ HSTD, 8-week-old male *Gck*$^{+/-}$ mice fed an HSTD for 15 weeks. Insulin tolerance test and oral glucose tolerance test were carried out during week 14 and the mice were killed after 15 weeks

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[24,25]. 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[24].

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: *Ki67* forward, CTGCCTGCGAAGAGAGCATC; *Ki67* reverse, AGCTC CACTTCGCCTTTTGG; *insulin receptor substrate 2 (Irs2)* forward, AACCTGAAACCTAAGGGACTGG; *Irs2* reverse, CGG CGAATGTTCATAAGCTGC; β-*actin (Actb)* forward, ATAT CGCTGCGCTGGTCTGTC; *Actb* reverse, AGCACAGCCTG GATGGCTAC.

Microarray analysis

Samples containing >120 isolated islets were frozen in −196 and stored at−80 . 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[17], 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 that 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*$^{+/-}$ NC group, as previously reported[17]. 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[17], 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[17].

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

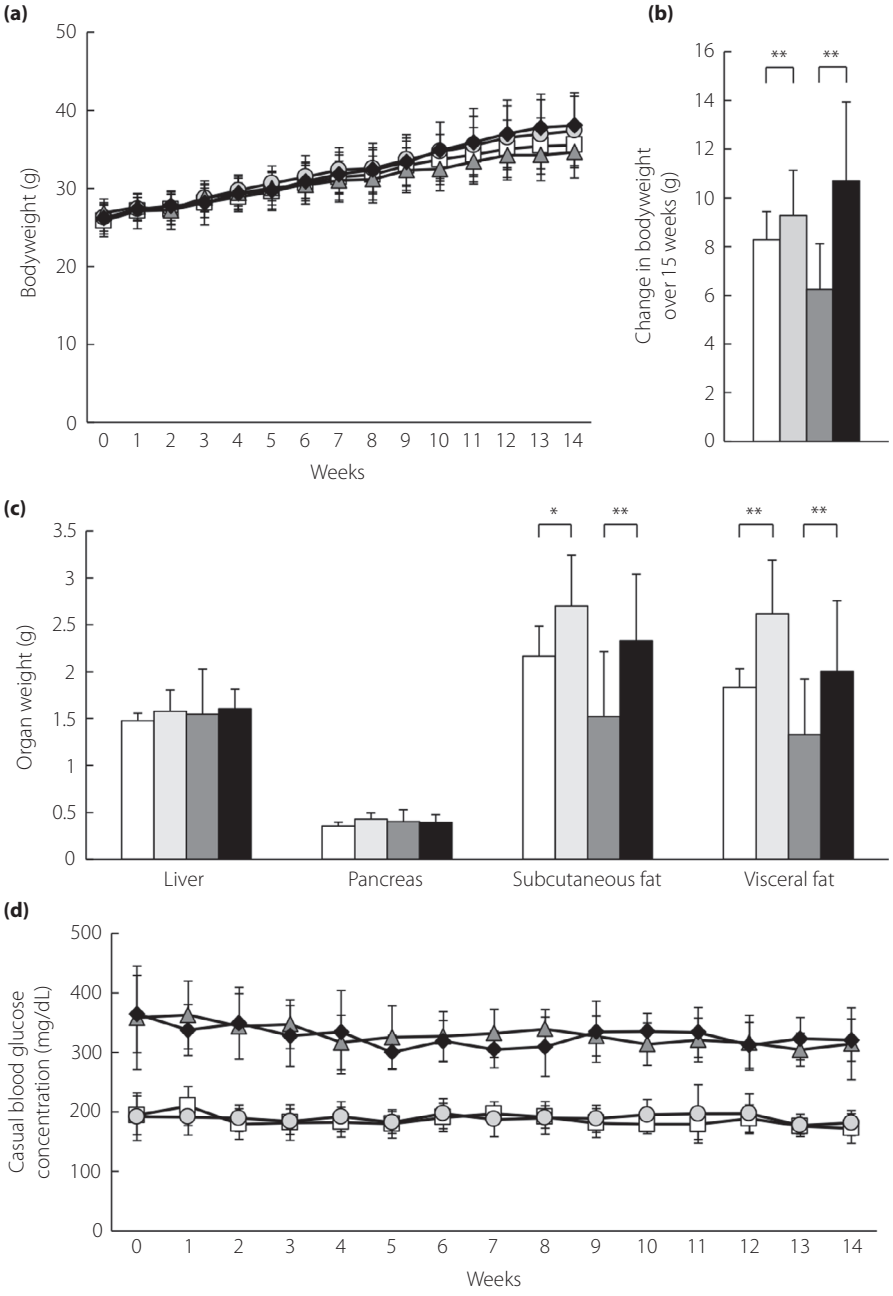

Figure 2 | Bodyweight and blood glucose concentration of mice in each group. (a) Bodyweight of the wild-type ($Gck^{+/+}$) normal chow (NC; white square), $Gck^{+/+}$ high-starch diet (HSTD; pale gray circle), glucokinase haploinsufficient ($Gck^{+/-}$) NC (dark gray triangle) and $Gck^{+/-}$ HSTD (black diamond) groups (n = 11–14). (b) Changes in bodyweight over 15 weeks in the $Gck^{+/+}$ NC group (white bar), $Gck^{+/+}$ HSTD group (pale gray bar), $Gck^{+/-}$ NC (dark gray bar) and $Gck^{+/-}$ HSTD group (black bar) (n = 11–14). (c) Liver, pancreas, subcutaneous and visceral fat weights in the $Gck^{+/+}$ NC group (white bar), $Gck^{+/+}$ HSTD group (pale gray bar), $Gck^{+/-}$ NC (dark gray bar) and $Gck^{+/-}$ HSTD (black bar) groups (n = 11–14). (d) Casual blood glucose concentration in the $Gck^{+/+}$ NC (white square), $Gck^{+/+}$ HSTD (pale gray circle), $Gck^{+/-}$ NC (dark gray triangle) and $Gck^{+/-}$ HSTD (black diamond) groups (n = 11–14). Data are presented as the mean ± standard deviation. *$P < 0.05$, **$P < 0.01$

the differences in insulin secretion and β-cell mass, genes that were expressed at higher or lower levels in the $Gck^{+/+}$ 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 $Gck^{+/+}$ 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

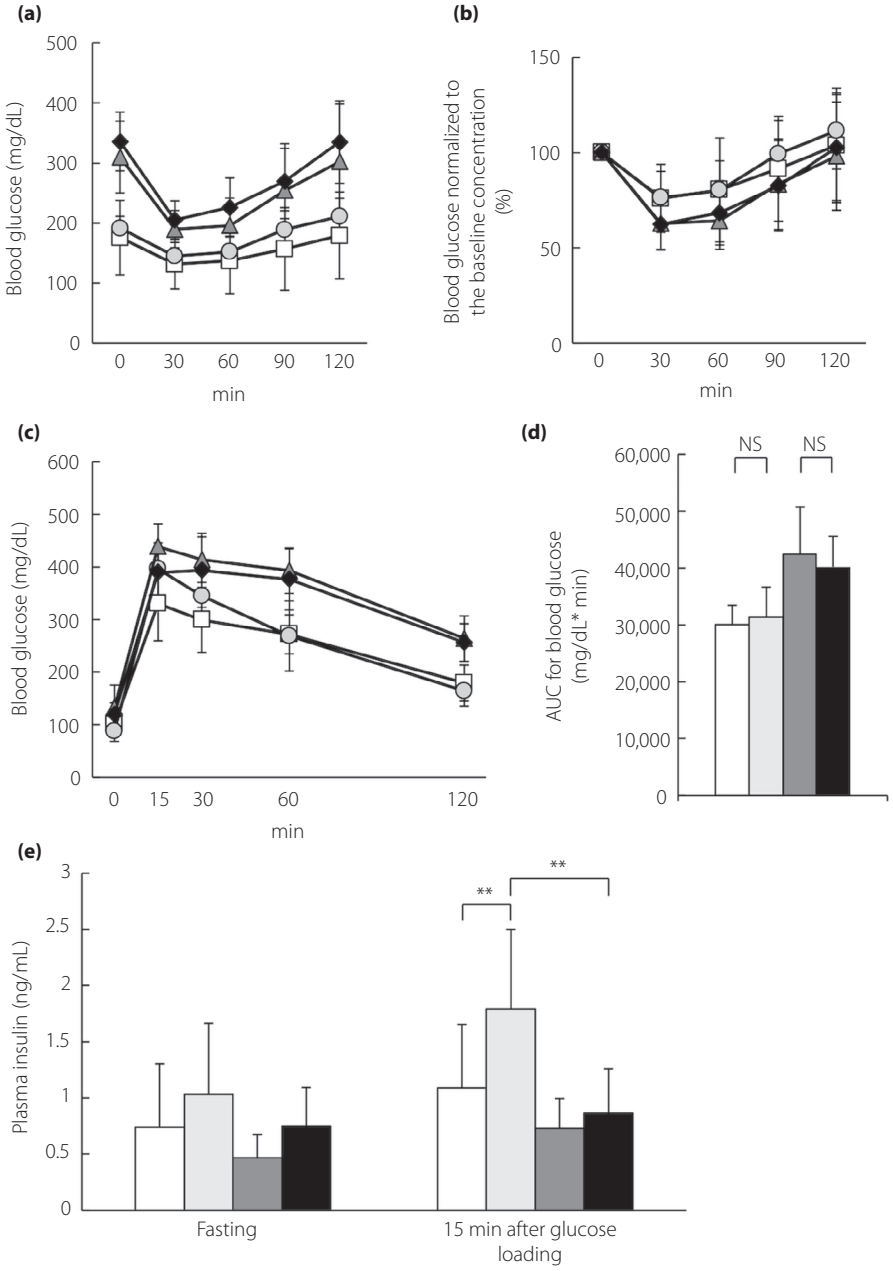

Figure 3 | Glucose tolerance of mice in each group. (a) Blood glucose concentrations and (b) blood glucose normalized to the baseline concentration during insulin tolerance test in the wild-type ($Gck^{+/+}$) normal chow (NC white square), $Gck^{+/+}$ high-starch diet (HSTD; pale gray circle), glucokinase haploinsufficient ($Gck^{+/-}$) NC (dark gray triangle) and $Gck^{+/-}$ HSTD (black diamond) groups (n = 11–14). (c,d) Blood glucose concentrations during oral glucose tolerance test and (d) the areas under the curves for the $Gck^{+/+}$ NC (white square and bar), $Gck^{+/+}$ HSTD (pale gray circle and bar), $Gck^{+/-}$ NC (dark gray triangle and bar) and $Gck^{+/-}$ HSTD (black diamond and bar) groups (n = 11–14). e: Plasma insulin concentration before and 15 min after glucose loading in the $Gck^{+/+}$ NC (white bar), $Gck^{+/+}$ HSTD (pale gray bar), $Gck^{+/-}$ NC (dark gray bar) and $Gck^{+/-}$ (black bar) groups (n = 11–14). Data are presented as the mean ± standard deviation. **$P < 0.01$. NS, not significant

criteria (Figure 6). The expression of *Aldh1a3*, *Slc17a9*, *Cthrc1* and *Pde10a* in the $Gck^{+/+}$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 $Gck^{+/+}$ mice, but those effects were not seen in $Gck^{+/-}$ mice. We previously reported that glucokinase in β-cells played an important

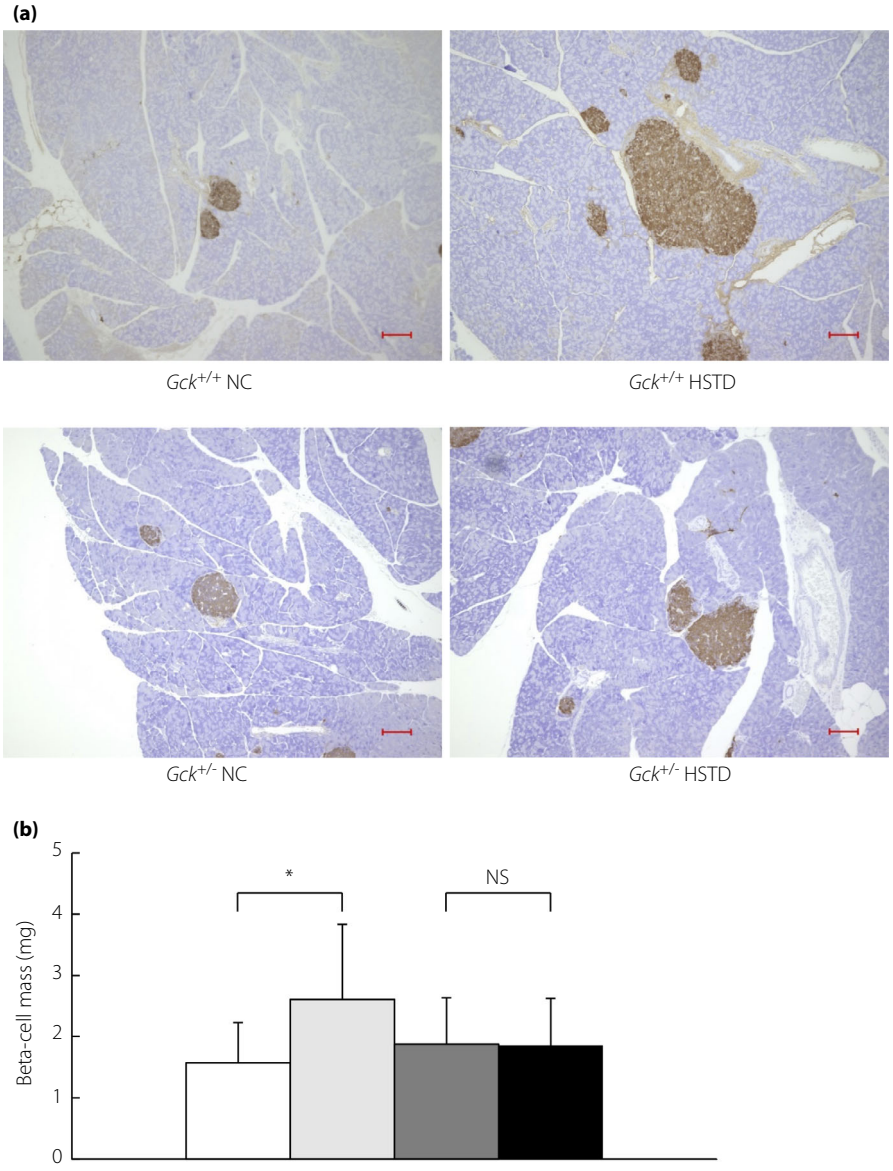

Figure 4 | Histology of pancreatic tissue of mice in each group. (a) Pancreatic section stained with hematoxylin (purple). β-cells are immunostained using an anti-insulin antibody (brown). Scale bars, 100 µm. (b) β-cell mass, calculated from the pancreatic weight and the ratio of the β-cell area to the pancreatic area in the wild-type (*Gck*$^{+/+}$) normal chow (white bar), *Gck*$^{+/+}$ high-starch diet (HSTD; pale gray bar), glucokinase haploinsufficient (*Gck*$^{+/-}$) NC (dark gray bar) and *Gck*$^{+/-}$ HSTD (black bar) groups (n = 11–14). Data are presented as the mean ± standard deviation. *$P < 0.05$. NS, not significant.

role in HFD-induced β-cell mass expansion[17], and the present findings showed that glucokinase was also required for the increase in β-cell mass induced by HSTD feeding.

Murase *et al.*[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[26]. This mechanism[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

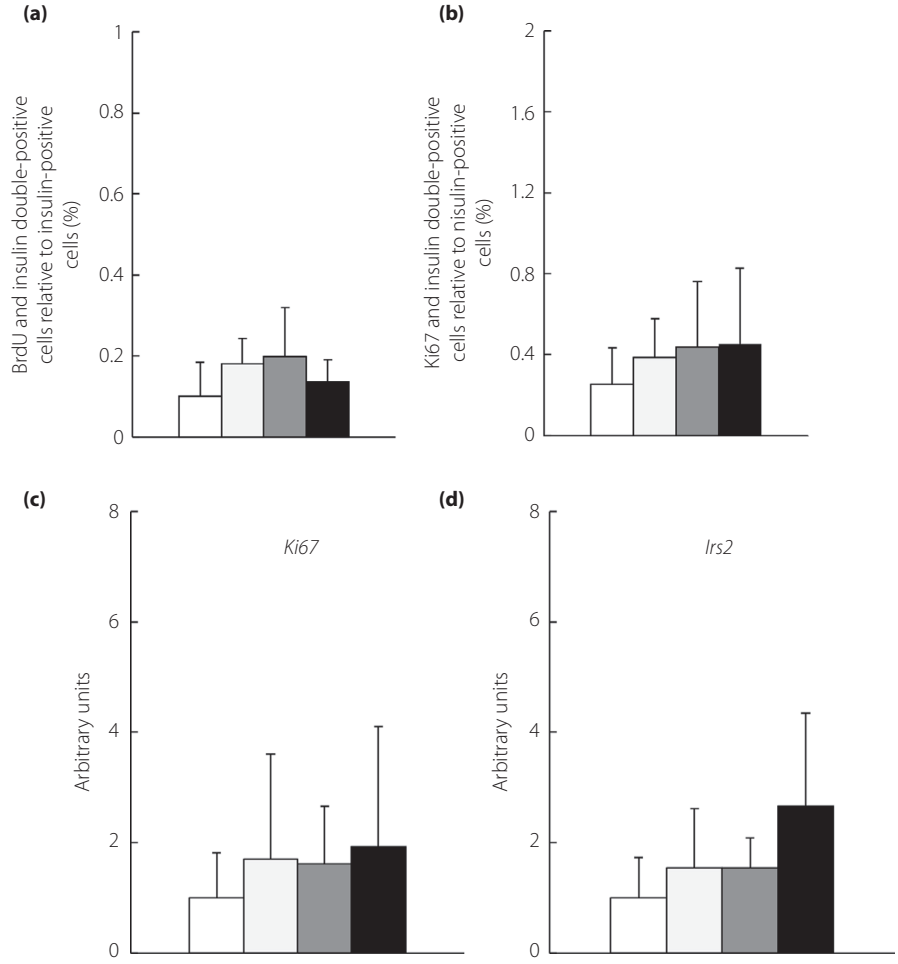

Figure 5 | β-cell proliferation and expression of related genes in mice in each group. (a,b) β-cell proliferation rate, assessed using (a) 5-bromo-2-deoxyuridine (BrdU) incorporation and (b) Ki67 staining in the wild-type ($Gck^{+/+}$) normal chow (NC; white bar), $Gck^{+/+}$ high-starch diet (HSTD; pale gray bar), glucokinase haploinsufficient ($Gck^{+/-}$) NC (dark gray bar) and $Gck^{+/-}$ HSTD (black bar) groups (n = 9–13). (c,d) Gene expression of (c) *Ki67* and (d)*Irs2* in isolated islets from the $Gck^{+/+}$ NC (white bar), $Gck^{+/+}$ HSTD (pale gray bar), $Gck^{+/-}$ NC (dark gray bar) and $Gck^{+/-}$ HSTD (black bar) groups, measured using real-time quantitative polymerase chain reaction (n = 6). Data were normalized to *Actb* expression and are presented as the mean ± standard deviation.

those fed an NC, whereas no increases in β-cell proliferation or *Irs2* expression were identified in $Gck^{+/-}$ mice fed a HFD[17]. 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 proliferation[17]. 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 survival[27,28], 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 NC[22].

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 clarified[29]. *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-cells[29]. These

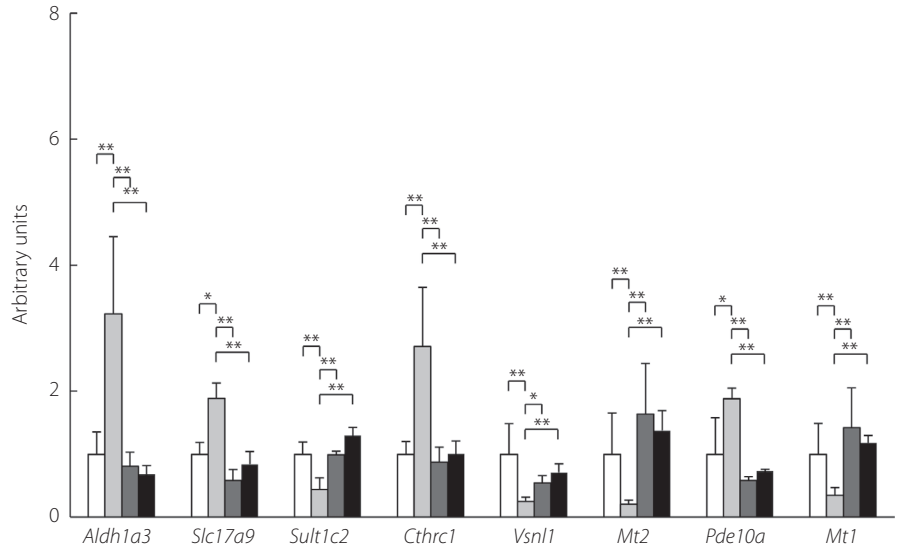

Figure 6 | Gene expression in isolated islets from mice in each group. Out of a total of 22,203 genes in total, eight genes were identified that had expression levels in the $Gck^{+/+}$ HSTD group, which were significantly different and >2- or<0.5-fold the level of those in the other three groups. Expression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

findings are consistent with the present data showing greater insulin secretion in $Gck^{+/+}$ mice fed an HSTD, but not in $Gck^{+/-}$ 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[29]. With respect to the vesicular nucleotide transporter , which is an *Slc17a9*-encoded protein, it is expressed in β-cells and plays an important role in the regulation of insulin secretion[30]. Overexpression of vesicular nucleotide transporter in β-cells increases glucose-induced insulin secretion[30], which is consistent with the present findings. *Vsnl1* is also expressed in β-cells, and its downregulation increases insulin gene transcription[31]. However, it is unclear whether these genes affect β-cell mass.

Another possibility could be that proliferation occurs earlier in $Gck^{+/+}$ mice fed an HSTD, resulting in higher β-cell mass. Masuda *et al.*[23] 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[14,16,32,33]. Therefore, β-cell proliferation might be caused by the initial glucose stimulation signal in $Gck^{+/+}$ mice fed an HSTD, but not in $Gck^{+/-}$ 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 $Gck^{+/+}$ 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[34,35], $Gck^{+/+}$ 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[36]. 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[37]. Unfortunately, the concentrations of substances that might be secreted in response to low-protein diet feeding, such as Fgf21[37], were not measured in the present study.

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.

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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.