Adaptation to Hyperglycemia Enhances Insulin Secretion in Glucokinase Mutant Mice

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The present study was undertaken to test the hypothesis that exposure to high glucose concentrations enhances insulin secretion in pancreatic islets from glucokinase-deficient mice. Insulin secretion and intracellular calcium ($[Ca^{2+}]_i$) were measured as the glucose concentration was increased from 2 to 26 mmol/l in islets from heterozygous glucokinase (GK)-deficient mice ($GK^{+/-}$) and their wild-type littermates ($GK^{+/+}$). Results obtained in islets incubated in 11.6 or 30 mmol/l glucose for 48–96 h were compared. $GK^{+/-}$ islets that had been incubated in 30 mmol/l glucose showed improved although not normal insulin secretory and $[Ca^{2+}]_i$ responses to the standard glucose challenge as well as an enhanced ability to sense small amplitude glucose oscillations. These effects were associated with increased glucokinase activity and protein. In contrast, exposure of $GK^{+/+}$ islets to 30 mmol/l glucose increased their basal insulin secretion but reduced their incremental secretory responses to glucose and their ability to detect small amplitude glucose oscillations. Thus exposure of $GK^{+/-}$ islets to 30 mmol/l glucose for 48–96 h enhanced their ability to sense and respond to a glucose stimulus, whereas similar exposure of $GK^{+/+}$ islets induced evidence of β-cell dysfunction. These findings provide a mechanistic framework for understanding why glucokinase diabetes results in mild hyperglycemia that tends not to increase over time. In addition, the absence of one allele of the glucokinase gene appears to protect against glucose-induced β-cell dysfunction (glucose toxicity). ***Diabetes*** **47:1881–1888, 1998**

Hyperglycemia in type 2 diabetes tends to become more severe with increasing duration of disease. Frequently, patients who initially responded to diet and exercise subsequently need to be treated with oral hypoglycemic agents, and a high proportion eventually need insulin treatment to maintain euglycemia. The progressive nature of type 2 diabetes is thought to be related at least in part to the adverse effects of hyperglycemia on insulin secretion and insulin action, a phenomenon that has been termed glucotoxicity (1–5).

In contrast, patients with maturity-onset diabetes of the young (MODY) due to mutations in the glycolytic enzyme glucokinase (glucokinase diabetes) tend to exhibit mild fasting and postprandial hyperglycemia that remains stable over time and is less severe than the hyperglycemia of type 2 diabetes (6–9). The relatively infrequent progression of mild hyperglycemia to overt diabetes in these subjects is surprising in view of the critical role played by glucokinase in the regulation of insulin secretion. Mathematical modeling of in vivo insulin secretory kinetics, in relation to the activity of different mutant glucokinase enzymes, has suggested that compensatory mechanisms operative in vivo increase insulin secretion, thereby limiting the progression of hyperglycemia (10).

The present study was undertaken to define the nature of these compensatory mechanisms. Islets were obtained from mice in which one allele of the glucokinase gene had been inactivated by gene targeting. Similar to human subjects with glucokinase mutations, these animals have mild hyperglycemia and demonstrate a reduction in insulin secretion, and thus represent a suitable animal model of human glucokinase diabetes (11). These experiments allowed us to test the hypothesis that exposure of islets from mutant mice to high glucose increases insulin secretion by increasing expression of the remaining glucokinase allele.

RESEARCH DESIGN AND METHODS

Animals. Experiments were performed on mice aged 10–16 weeks in which one allele of the glucokinase gene had been inactivated ($GK^{+/-}$) and their wild-type littermates ($GK^{+/+}$) (11). Because initial experiments showed no phenotypic difference between male and female $GK^{+/-}$ mice, subsequent experiments were performed on mice of both sexes, although the sex distribution of the $GK^{+/+}$ and $^{+/-}$ mice used was similar. On the morning of the experiments, mice in the nonfasted state (except in the case of glucose tolerance tests) were weighed and their blood was sampled from the tail for measurement of blood glucose (Hemocue AB, Angelholm, Sweden). Mice were killed using methods approved by the Animal Care and Use Committee of the University of Chicago.

Intraperitoneal glucose tolerance tests. Intraperitoneal glucose tolerance tests were performed after an overnight fast. Blood was sampled from the tail before and 15, 30, 60, 120, and 180 min after intraperitoneal injection of 2 g/kg of dextrose.

Isolation of islets of Langerhans. Islet isolation was accomplished by collagenase digestion using a modification of procedures previously described (12). Briefly, pancreases were inflated with a solution containing 0.3 mg/ml collagenase (Type XI; Sigma, St. Louis, MO) in Hank's balanced salt solution, injected via the common bile duct. The inflated pancreases were removed, incubated at 37°C for 15 min, and shaken vigorously to disrupt the tissues. The conditions used for isolation of islets from $GK^{+/+}$ and $^{+/-}$ mice, including concentration of collagenase and duration of digestion, were identical. Following differential centrifugation through a ficoll gradient to separate islets

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BSA, bovine serum albumin; $[Ca^{2+}]_i$, intracellular calcium; GK, glucokinase; KRBB, Krebs-Ringer bicarbonate buffer; PBS, phosphate-buffered saline; RIA, radioimmunoassay; TBS, Tris-buffered saline.

from acinar tissue, the islets were washed, handpicked, and placed in tissue culture plates containing RPMI 1640 supplemented with low (11.6 mmol/l) or high (30 mmol/l) glucose, 10% (vol/vol) fetal calf serum, 100 µU/ml penicillin, and 100 µg/ml streptomycin. During culture, islets were incubated free floating for subsequent harvest and measurement of insulin secretion or placed on 25-mm coverslips to facilitate adherence and measurement of intracellular calcium $[Ca^{2+}]_i$. All islets were incubated with 95% air/5% CO_2 at 37°C in a humidified incubator for periods between 48 and 96 h. The culture medium was changed once every 48 h.

Determination of insulin release from perifused islets. Secretion of insulin from perifused islets was measured using a temperature-controlled multichamber perifusion system (ACCUSYST-S; Cellex Biosciences, Minneapolis, MN). Groups of 75 islets were suspended in Bio-Gel P-2 beads (Bio-Rad, Hercules, CA) and modified Krebs-Ringer bicarbonate buffer (KRBB) containing 2 mmol/l glucose and 5 mg/ml bovine serum albumin (BSA) and placed in four parallel 500-µl perifusion chambers. The perifusion system used two peristaltic pumps: pump 1 (Gilson Minipuls 2; Gilson, Middleton, WI) administered KRBB containing no glucose and pump 2 administered KRBB containing 26 mmol/l glucose. The two pumps were computer controlled to increase the perifusate glucose concentration in a gradual ramp fashion from 2 to 26 mmol/l over 48 min while maintaining a constant total flow rate. At the beginning of each experiment, KRBB containing 2 mmol/l glucose was perifused for a 30-min baseline period to allow equilibration of experimental conditions before initiation of the ramp increase in glucose concentration. Once the ramp protocol commenced, the effluent perifusate was collected at 1-min intervals. Insulin concentrations were measured in the effluent perifusate in the first and second minutes and every second minute thereafter. The insulin concentration of the effluent perifusate was measured by radioimmunoassay (RIA) (13) and expressed as picomoles per liter per 75 islets.

Measurements of islet $[Ca^{2+}]_i$. Islets from $GK^{+/+}$ and $^{+/-}$ mice, established in primary culture, were loaded with fura-2 acetoxymethylester (Molecular Probes, Eugene, OR), as described previously (14). Islets were perifused at a flow rate of 2.5 ml/min (37°C) with KRBB (without BSA) in a temperature-controlled microperifusion chamber (Medical Systems, Greenvale, NY) and mounted onto an inverted microscope (Nikon, Japan) equipped for epifluorescence. The glucose concentration of the perifusate was altered as a ramp increase from 2 to 26 mmol/l, as described above, or as a series of sine wave oscillations. To produce regular oscillations in the glucose concentration, two peristaltic pumps were used, one administering KRBB with no added glucose and the other administering KRBB containing 14 mmol/l glucose. The infusion rates were computer controlled so that the total flow rate was constant, but the glucose concentration was varied as a sine wave pattern with an amplitude of 10% (6.3–7.7 mmol/l) or 50% (3.5–10.5 mmol/l) around a mean of 7 mmol/l. The period of the sine waves was 6 min and each experiment was continued for 30 min (five sine waves). This protocol was designed to determine the ability of islets from the two groups of animals to detect small changes in glucose concentration. Fura-2 dual-wavelength excitation photometry was used to measure individual islet $[Ca^{2+}]_i$ using a fluorescence imaging system. Results are expressed as the ratio of the emitted light intensity (detected at 510 nm) after excitation at 340 and 380 nm (ratio 340/380).

Measurement of islet insulin content and β-cell mass. Insulin content of $GK^{+/+}$ and $GK^{+/-}$ islets incubated for 48 h in 11.6 or 30 mmol/l glucose was measured. After culture, triplicate groups of five islets were handpicked, washed twice in phosphate-buffered saline (PBS), and resuspended in 200 µl acid ethanol. Islets were sonicated and incubated overnight at 4°C. An aliquot of the islet extract was then assayed for insulin concentration by RIA; the results were expressed as pmol insulin per islet.

The β-cell mass of pancreases from $GK^{+/+}$ and $^{+/-}$ mice was determined as previously described (15).

Measurement of glucokinase enzyme activity. Glucose phosphorylating activity was measured as previously described (16). Briefly, groups of 300 islets isolated from 8–10 $GK^{+/+}$ and $GK^{+/-}$ mice and cultured for 48 h at 11.6 or 30 mmol/l glucose were homogenized and an aliquot removed for DNA determination. After centrifugation, the supernatant was recovered and kept on ice. A fluorometric assay was performed in duplicate at each of five glucose concentrations in the range of hexokinase activity (0.01, 0.03, 0.05, 0.25, and 0.5 mmol/l glucose) and six concentrations in the glucokinase range (6, 10, 15, 20, 50, and 100 mmol/l); duplicates were averaged. Glucokinase and hexokinase V_{max} and K_m were determined from an Eadie-Scatchard plot using iterative linear regression, as described by Spears et al. (17). The data were extrapolated to 37°C assuming a Q_{10} of 2 (18). DNA concentration was measured in triplicate for each sample using the method of Labarca and Paigen (19). Glucokinase and hexokinase V_{max} were expressed as micromoles of glucose phosphorylated per milligram of DNA per hour.

Western blot analysis. Islets isolated from $GK^{+/+}$ and $GK^{+/-}$ mice were incubated in supplemented RPMI 1640 medium and 11.6 or 30 mmol/l glucose, as described above, for 48 h. After culture, islets from each condition were handpicked, washed three times with PBS, and resuspended in 100 µl of distilled H_2O containing 1 µmol/l leupeptin and 0.25 mmol/l phenylmethylsulfonyl fluoride. The islets were sonicated for three 15-s periods on ice. Protein concentration was determined using a commercially available kit (Bio-Rad DC Protein Assay Kit #500-0116) using BSA as the protein standard. Then 12 µg of total protein from each mouse type and culture condition were separated on a 10% SDS gel and then transferred onto Immobilon-P polyvinyl difluoride sheets (Millipore, Bedford, MA). The blot was blocked in Tris-buffered saline (TBS) containing 5% casein and 0.01% Tween-20. The primary antibody—a polyclonal rabbit anti-rat glucokinase (gift from the late Dr. Simon Pilkis)—was used at 1:4,000 dilution and incubated overnight at 4°C. The secondary antibody, a goat anti-rabbit IgG (Bio-Rad), diluted 1:4,000 in TBS, was applied and incubated for 1 h at room temperature. Bound antibody was detected by chemiluminescence using SuperSignal (Pierce, Rockford, IL). The amount of bound antibody was determined directly by scanning the exposed X-ray film on a computing densitometer (model 325E; Molecular Dynamics, Sunnyvale, CA) and using ImageQuant software (Molecular Dynamics). Results in $GK^{+/-}$ and $^{+/+}$ islets cultured at 30 mmol/l glucose were expressed as a percentage of the glucokinase protein level in $GK^{+/+}$ islets cultured at 11.6 mmol/l glucose.

Statistical analysis. The statistical significance of group differences between the $GK^{+/+}$ and $^{+/-}$ mice was determined using the paired or nonpaired t test or analysis of variance, where appropriate. For insulin secretion experiments, the average insulin concentration of the effluent perifusate was calculated for each experiment; because the data were not normally distributed, groups were compared using the nonparametric Wilcoxon's test with post hoc analysis by the Student-Newman-Keuls test. To compare the responses of $[Ca^{2+}]_i$ to ramp increases in glucose, the concentration of glucose required to produce a twofold increase over baseline in the 340/380 ratio was calculated and compared between groups. The relationship between oscillations in glucose and $[Ca^{2+}]_i$ was analyzed by calculating the normalized spectral power using a modification of a previously described procedure (9) and comparing the mean spectral power between groups. Differences were taken to be statistically significant at $P < 0.05$.

RESULTS

Ages, weights, and glucose tolerance levels. The $GK^{+/+}$ and $^{+/-}$ mice were matched for age (87 ± 3 vs. 85 ± 3 days) and weight (21 ± 1 vs. 22 ± 1 g). $GK^{+/-}$ mice demonstrated fasting hyperglycemia compared with $GK^{+/+}$ mice (11.8 ± 0.5 vs. 8.7 ± 0.3 mmol/l; $P = 0.0001$). After intraperitoneal administration of glucose, the mean blood glucose was 21.8 ± 1.6 vs. 14.0 ± 1.0 mmol/l in the $GK^{+/-}$ and $^{+/+}$ mice, respectively ($P < 0.000001$).

Effect of exposure to normal and high glucose on insulin release from perifused islets. These experiments examined the effects of incubating islets in culture medium containing 11.6 or 30 mmol/l glucose for 48–96 h on subsequent islet insulin secretory responses (Fig. 1*A* and *B*). There was no difference between the responses of islets that had been cultured for 48 h and those that had been cultured for 96 h; therefore, the data were pooled. In response to an increase in perifusate glucose concentration from 2 to 26 mmol/l over 48 min, $GK^{+/-}$ islets that had been incubated in 11.6 mmol/l glucose secreted considerably less insulin than $GK^{+/+}$ islets (16 ± 3 vs. 109 ± 16 pmol · l^{-1} · 75 islets^{-1}, respectively; $P < 0.01$). Incubation of $GK^{+/+}$ islets in 30 mmol/l glucose resulted in a slight but not significant increase in average insulin secreted (142 ± 32 pmol · l^{-1} · 75 islets^{-1}; $P > 0.05$) compared with islets incubated in 11.6 mmol/l glucose. This increase in secretion resulted from hypersecretion at low glucose (average insulin secreted between 2 and 4 mmol/l glucose was 82 ± 30 vs. 4.2 ± 0.7 pmol · l^{-1} · 75 islets^{-1}; $P < 0.05$) without a significant increase in the peak insulin secretory response to high glucose (136 ± 27 vs. 167 ± 20 pmol · l^{-1} · 75 islets^{-1} between 24 and 26 mmol/l in $GK^{+/+}$ islets cultured in 11.6 and 30 mmol/l glucose, respectively; $P > 0.05$). To document the glucose responsiveness of the islets, the increase in insulin secretion in response to the ramp glucose challenge

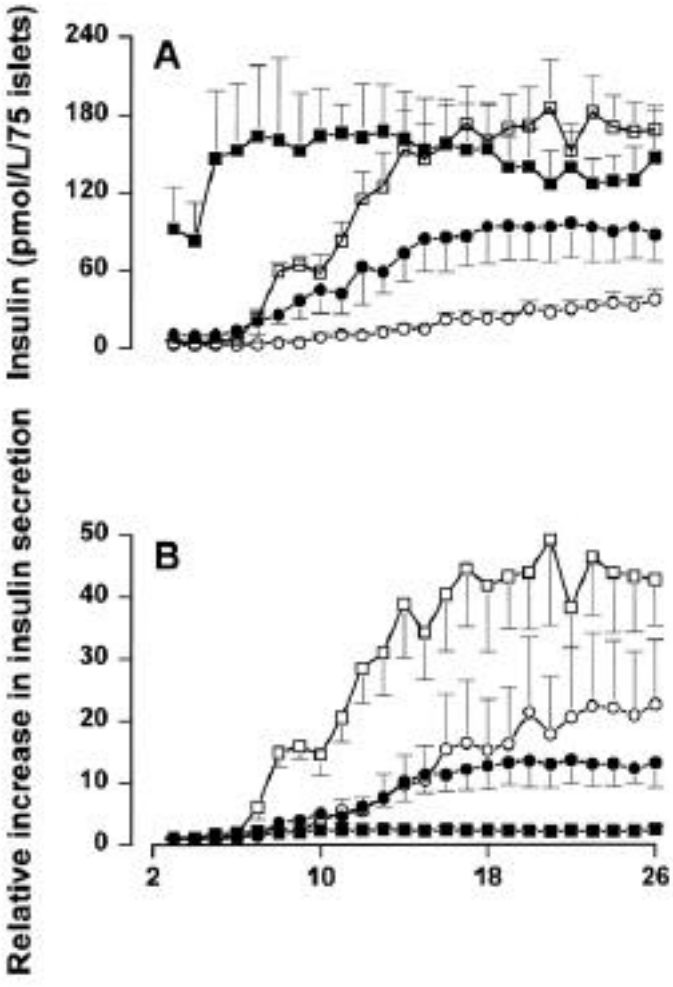

FIG. 1. Insulin secretory responses to glucose. *A*: Insulin secretory responses to glucose perifusion in islets from GK$^{+/+}$ (■, □) and GK$^{+/-}$ (●, ○) mice cultured for 48–96 h at 11.6 (□, ○) or 30 mmol/l (■, ●) glucose (n = 5 in each group). After a 30-min equilibration period during which the perifusate contained 2 mmol/l glucose, the concentration of glucose was increased progressively from 2 to 26 mmol/l over 48 min. *B:* The relative increase in insulin secretion was calculated by dividing each point in the dosage-response curve by the average insulin secreted between 2 and 4 mmol/l glucose. Data are means ± SE.

was expressed as a function of basal insulin secretion. For each experiment, the perifusate insulin concentration measured at each point in the ramp was divided by the average insulin secreted between 2 and 4 mmol/l glucose and the mean for each group was calculated (Fig. 1*B*). The mean relative insulin secretory response seen in GK$^{+/+}$ islets incubated in 30 mmol/l glucose was 2.0 ± 0.3 compared with 27.6 ± 5.4 in GK$^{+/+}$ islets cultured at 11.6 mmol/l (P < 0.05), indicative of a reduced incremental insulin secretory response to glucose.

Incubation of GK$^{+/-}$ islets in 30 mmol/l glucose resulted in an approximate fourfold increase (from 16 ± 3 to 60 ± 18 pmol · l^{-1} · 75 islets^{-1}; P < 0.01) in the average amount of insulin secreted as the perifusate glucose concentration was increased from 2 to 26 mmol/l. Despite this improvement in β-cell responsiveness, the GK$^{+/-}$ islets that had been incubated in 30 mmol/l glucose still secreted less insulin than the GK$^{+/+}$ islets incubated at 11.6 mmol/l glucose (60 ± 18 vs. 109 ± 16 pmol · l^{-1} · 75 islets^{-1}; P < 0.01). Insulin secretory responses increased in the GK$^{+/-}$ islets across a broad range of glucose concentrations after incubation in high glucose, but unlike in GK$^{+/+}$ islets, there was no significant difference in the glucose responsiveness of GK$^{+/-}$ islets after culture at 30 mmol/l glucose (mean relative insulin secretory response 8.0 ± 2.0 vs. 10.6 ± 5.3 in GK$^{+/-}$ islets cultured at 11.6 mmol/l glucose) (Fig. 1*B*).

Glucose thresholds for stimulation of insulin secretion and [Ca^{2+}]$_i$. As a measure of β-cell responsiveness to glucose, the glucose level at which the perifusate insulin concentration exceeded the detection limit of the assay (12 pmol/l) was calculated (Table 1). This threshold for glucose stimulation of insulin secretion was 1.9-fold higher in the GK$^{+/-}$ islets cultured at 11.6 mmol/l glucose than in the GK$^{+/+}$ islets (P < 0.02). Insulin secretion exceeded the threshold at significantly lower glucose concentrations in islets incubated in 30 mmol/l glucose in each group (Table 1). The threshold for glucose-induced insulin secretion in GK$^{+/-}$ islets cultured at 30 mmol/l glucose was not significantly different from that in GK$^{+/+}$ islets cultured at 11.6 mmol/l.

To determine whether the above changes in insulin secretion in the GK$^{+/+}$ and $^{+/-}$ islets were associated with parallel changes in [Ca^{2+}]$_i$, the glucose concentration required to produce a twofold rise in [Ca^{2+}]$_i$, as reflected by the change in the 340/380 ratio, was measured in GK$^{+/+}$ and $^{+/-}$ islets as the glucose concentration in the perifusate was increased from 2 to 26 mmol/l glucose (representative examples shown in Fig. 2). The glucose concentration required to produce a twofold rise in the mean baseline 340/380 ratio was compared among groups (Table 2). The dosage-response curve was shifted to the right in GK$^{+/-}$ islets cultured at low glucose, as indicated by the fact that the glucose concentration required to cause a doubling of [Ca^{2+}]$_i$ was 1.6-fold higher than in GK$^{+/+}$ controls (P < 0.0001). Culture of islets at high glucose reduced the threshold glucose concentration by 51% in GK$^{+/+}$ islets (P < 0.0005) and by 50% in GK$^{+/-}$ islets (P < 0.0001) to a level not significantly

TABLE 1
Threshold glucose concentration required to exceed the lower limit of detection of the insulin assay during ramp increase in glucose from 2 to 26 mmol/l

	GK$^{+/+}$		GK$^{+/-}$	
	11.6 mmol/l	30 mmol/l	11.6 mmol/l	30 mmol/l
Glucose threshold (mmol/l)	7.4 ± 0.2 (5)	<2.0 ± 0.3* (5)	14.0 ± 1.8† (5)	6.4 ± 2.1‡ (5)

Data are means ± SE (n of mice). *P < 0.03 compared with GK$^{+/+}$ cultured in 11.6 mmol/l; †P < 0.02 compared with GK$^{+/+}$ cultured in 11.6 mmol/l; ‡P < 0.01 compared with GK$^{+/-}$ cultured in 11.6 mmol/l.

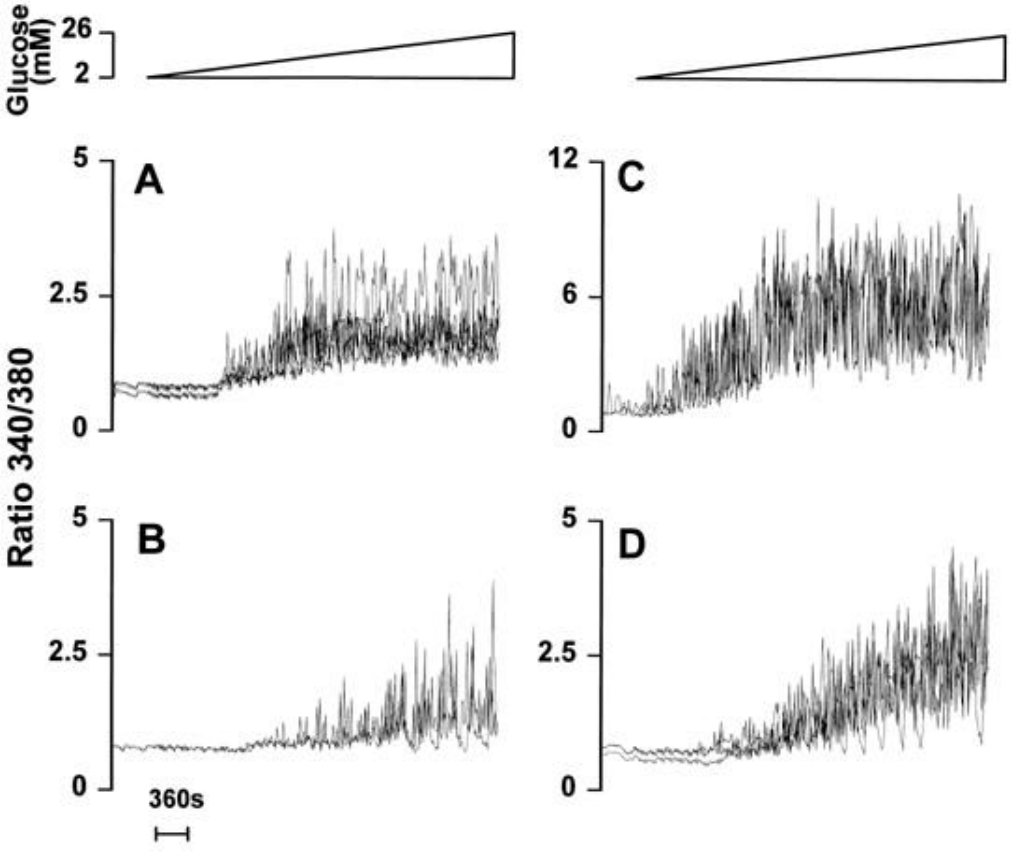

FIG. 2. Islet $[Ca^{2+}]_i$ responses to a ramp increase in glucose concentration. Intracellular Ca^{2+} responses to glucose perifusion were measured using fura-2 dual-excitation photometry. The 340/380 ratio was recorded as the perifusate glucose concentration was increased progressively from 2 to 26 mmol/l over 48 min, as indicated. Examples from $GK^{+/+}$ (*A* and *C*) and $GK^{+/-}$ (*B* and *D*) mice cultured at 11.6 mmol/l (*A* and *B*) and 30 mmol/l (*C* and *D*) are shown.

different from $GK^{+/+}$ control islets cultured at low glucose ($P > 0.05$).

Effects of oscillatory changes in glucose on islet $[Ca^{2+}]_i$. These experiments were designed to study the effect of incubating $GK^{+/+}$ and $^{+}/^{-}$ islets in 11.6 and 30 mmol/l glucose on their ability to detect small changes in the perifusate glucose concentration. Changes in $[Ca^{2+}]_i$ were measured in response to sine wave oscillations in glucose concentration during which the glucose level was varied by 10 or 50% around a mean of 7 mmol/l. The ability of these changes in glucose to induce parallel changes in $[Ca^{2+}]_i$ was quantified by calculating the mean normalized spectral power of oscillations in $[Ca^{2+}]_i$ at a period of 360 s corresponding to the interpulse interval of the administered glucose oscillations. The 10% oscillation changes in glucose elicited parallel changes in $[Ca^{2+}]_i$ in the $GK^{+/+}$, but not in the $GK^{+/-}$ islets (Fig. 3*A* and *B*). However, the $GK^{+/-}$ islets were more responsive when the relative amplitude of the oscillations was increased to 50% (Fig. 3*C*). However, the normalized spectral power of the $[Ca^{2+}]_i$ responses in the $GK^{+/-}$ islets remained lower than in the $GK^{+/+}$ islets with 10% glucose oscillations ($P < 0.000001$) (Table 3). Culture of the $GK^{+/-}$ islets in 30 mmol/l glucose improved their ability to detect 10 and 50% changes in oscillatory glucose, resulting in a significant increase in the normalized spectral power (Fig. 4*B* and *C*; Table 3). In contrast, incubation of $GK^{+/+}$ islets in 30 mmol/l glucose impaired the ability of the oscillatory glucose infusion to entrain $[Ca^{2+}]_i$; further, the spectral power in $GK^{+/+}$ islets was reduced by 75% for 10% amplitude oscillations ($P < 0.000001$) (Fig. 4*A*) and by 41% for 50% amplitude oscillations ~7 mmol/l glucose ($P < 0.0001$) compared with islets cultured at 11.6 mmol/l glucose.

Islet insulin content and β-cell mass. To determine whether the reduced insulin secretory response in $GK^{+/-}$

TABLE 2
Threshold glucose concentration required to produce a twofold rise in $[Ca^{2+}]_i$ in $GK^{+/-}$ and $GK^{+/+}$ islets cultured at 11.6 and 30 mmol/l glucose

	$GK^{+/+}$		$GK^{+/-}$	
	11.6 mmol/l	30 mmol/l	11.6 mmol/l	30 mmol/l
Glucose threshold (mmol/l)	7.4 ± 0.4 (34)	3.6 ± 0.3* (17)	11.7 ± 0.9† (25)	5.8 ± 0.8‡ (18)

Data are means ± SE (*n* of islets studied from four to six mice). *$P < 0.0005$ compared with $GK^{+/+}$ cultured at 11.6 mmol/l; †$P < 0.0001$ compared with $GK^{+/+}$ cultured at 11.6 mmol/l; ‡$P < 0.0001$ compared with $GK^{+/-}$ cultured at 11.6 mmol/l.

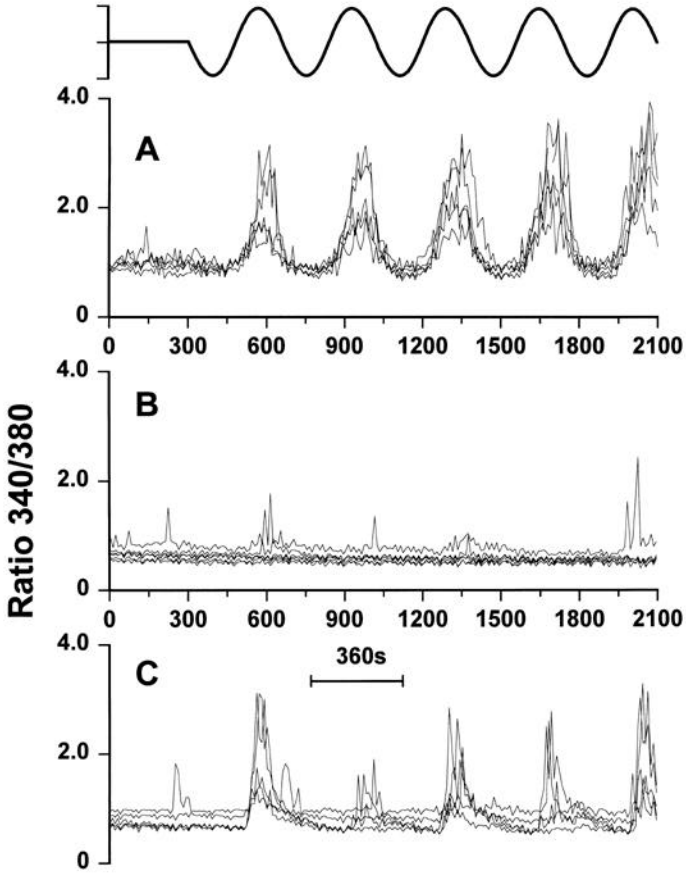

FIG. 3. Islet $[Ca^{2+}]_i$ responses to oscillatory glucose. $[Ca^{2+}]_i$ responses to oscillatory glucose perifusion were measured in islets after culture in 11.6 mmol/l glucose. A series of sine wave oscillations in glucose concentration was generated using computer-controlled peristaltic pumps (*A*). The average glucose concentration was 7 mmol/l and the concentration was varied by ± 10% (*A* and *B*) or ± 50% (*C*) every 360 s. Examples are from $GK^{+/+}$ (*A*, spectral power, 28.5) and $GK^{+/-}$ (*B*, spectral power, 3.6; *C*, spectral power, 13.9) islets.

islets was due to lower islet insulin content or smaller islet size, islet insulin content and β-cell mass were quantified in groups of $GK^{+/+}$ and $^{+/-}$ mice. Insulin content in $GK^{+/-}$ islets incubated for 48 h in 11.6 mmol/l glucose (3.8 ± 0.3 pmol/islet) was not significantly different from that in $GK^{+/+}$ islets cultured at 11.6 mmol/l glucose (4.3 ± 0.5 pmol/islet; n = 8; P >0.05) (Fig. 5). Insulin content was 26% lower in $GK^{+/-}$ islets cultured at 30 mmol/l glucose (2.8 ± 0.2 pmol/islet; n = 8; P < 0.05 vs. $GK^{+/-}$ islets incubated in 11.6 mmol/l) and 33% lower in $GK^{+/+}$ islets cultured at 30 mmol/l glucose (2.9 ± 0.5; n = 8; P = 0.05 vs. $GK^{+/+}$ islets incubated in 11.6 mmol/l).

There was no noticeable difference between the size of $GK^{+/+}$ and $^{+/-}$ islets on microscopy. However, because the size of isolated islets is difficult to quantify, sections of pancreases from a subgroup of $GK^{+/+}$ and $^{+/-}$ islets were stained for insulin and the β-cell mass was quantified as an indirect measure of islet size (Table 4). Differences in β-cell mass between $GK^{+/+}$ and $^{+/-}$ were not statistically significant, despite the tendency for the values to be increased in the $GK^{+/-}$ animals.

Glucokinase enzyme activity and protein levels. Glucokinase and hexokinase enzyme activities were measured in extracts from groups of 300 $GK^{+/+}$ and $^{+/-}$ islets cultured in 11.6 or 30 mmol/l glucose for 48 h; V_{max} and K_m measurements for glucokinase and hexokinase are shown in Table 5. Hexokinase V_{max} was slightly higher (28%) in the $GK^{+/-}$ islets than in $GK^{+/+}$ islets cultured at 11.6 mmol/l glucose and increased a further 32% after culture at high glucose. Glucokinase V_{max} in $GK^{+/-}$ islets represented 63% of the value in the $GK^{+/+}$ islets and was similar to the level previously reported (11). Culture in high glucose increased the glucokinase V_{max} in both groups. After culture in 30 mmol/l glucose, the V_{max} in $GK^{+/-}$ islets was 89% of the value in $GK^{+/+}$ islets cultured in 11.6 glucose and 74% of the value in $GK^{+/+}$ islets cultured in 30 mmol/l glucose. The glucokinase K_m was 45 and 19% higher in $GK^{+/-}$ islets cultured in 11.6 and 30 mmol/l glucose, respectively, compared with $GK^{+/+}$ islets cultured at the same glucose concentrations.

Glucokinase protein levels in $GK^{+/-}$ islets cultured in 11.6 mmol/l glucose were 40 ± 7% of the level in wild-type islets (P < 0.05) (Fig. 6). In $GK^{+/-}$ islets cultured in 30 mmol/l glucose, the glucokinase protein level was twofold higher than in $GK^{+/-}$ islets cultured at 11.6 mmol/l (P < 0.05), and represented 81 ± 17% of the level in $GK^{+/+}$ islets cultured in 11.6 mmol/l glucose. Glucokinase protein levels in islets from $GK^{+/+}$ mice cultured at 30 mmol/l glucose were 52 ± 12% higher than in $GK^{+/+}$ islets cultured at 11.6 mmol/l glucose (P < 0.05).

DISCUSSION

The severity of hyperglycemia tends to increase in subjects with type 2 diabetes with increasing duration of disease. In contrast, diabetes due to mutations in the enzyme glucokinase is characterized by mild fasting and postprandial hyperglycemia that remains stable for prolonged periods (6–9). Because glucokinase, which catalyzes the first rate-limiting step in glucose metabolism, plays a key role in β-cell glucose sensing (20,21), it is surprising that mutations in this enzyme are generally associated with only mild hypergly-

TABLE 3
Spectral power at 360 s in $GK^{+/+}$ and $GK^{+/-}$ islets cultured at 11.6 and 30 mmol/l glucose

	$GK^{+/+}$		$GK^{+/-}$	
	11.6 mmol/l	30 mmol/l	11.6 mmol/l	30 mmol/l
7 mmol/l ± 10%	22.6 ± 0.9 (36)	5.6 ± 1.1* (24)	5.4 ± 0.9* (26)	14.5 ± 1.3† (29)
7 mmol/l ± 50%	27.9 ± 1.8 (6)	13.8 ± 3.4* (12)	10.1 ± 1.6 (19)	22.7 ± 2.0‡ (16)

Data are means ± SE (n of islets studied from two to seven mice). *P < 0.0001 compared with $GK^{+/+}$ cultured at 11.6 mmol/l; †P < 0.005 compared with $GK^{+/-}$ cultured at 11.6 mmol/l; ‡P < 0.000001 compared with $GK^{+/+}$ cultured at 30 mmol/l.

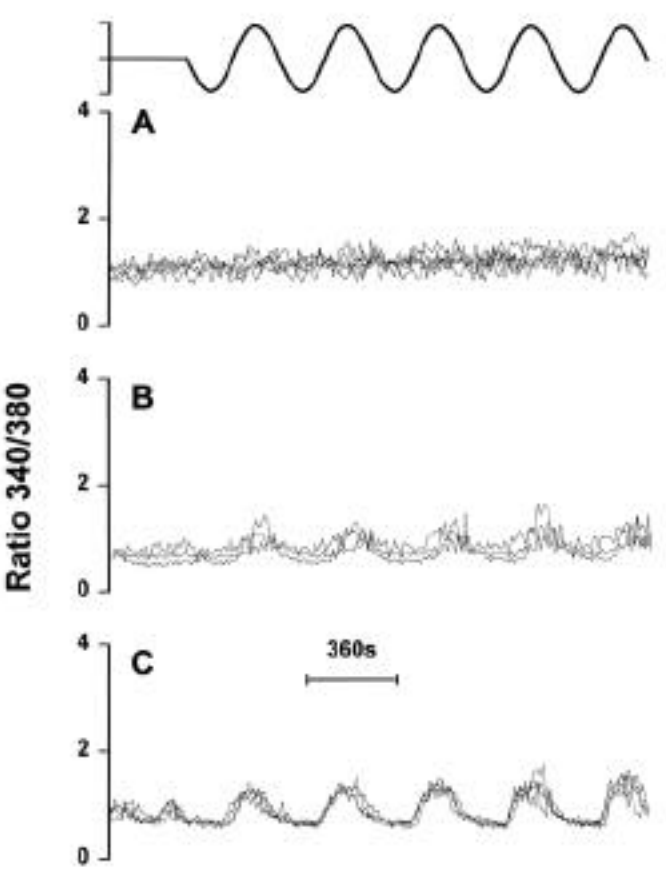

FIG. 4. Islet $[Ca^{2+}]_i$ responses to oscillatory glucose. $[Ca^{2+}]_i$ responses to oscillatory glucose perifusion were measured in islets after culture in 30 mmol/l glucose. Sine wave oscillations (*A*) with an average glucose concentration of 7 mmol/l ± 10% (*A* and *B*) or ± 50% (*C*) are shown. Examples are from $GK^{+/+}$ (*A*, spectral power, 3.8) and $GK^{+/-}$ (*B*, spectral power, 20.2; *C*, spectral power, 29.6) islets.

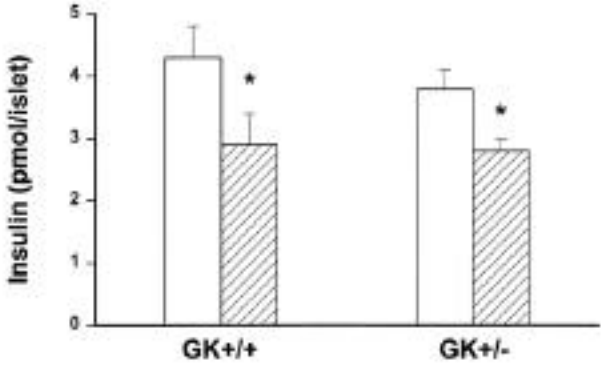

FIG. 5. Insulin content in islets from $GK^{+/+}$ and $GK^{+/-}$ islets. Insulin content was measured in triplicate groups of five islets from $GK^{+/+}$ and $GK^{+/-}$ islets after culture in 11.6 (□) or 30 (▨) mmol/l glucose for 48 h. Insulin was extracted by overnight incubation in acid ethanol at 4°C and quantified by RIA. Data are means ± SE of islets from n = 8 mice per group. *$P \leq 0.05$.

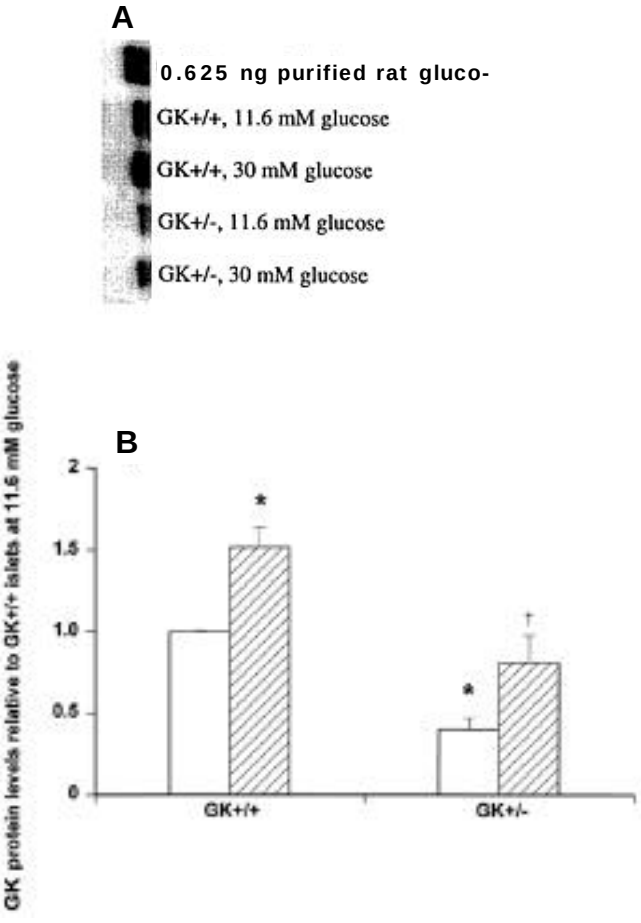

FIG. 6. Western blot analysis of glucokinase protein levels. *A*: Representative Western blot performed on extracts of islets from $GK^{+/+}$ and $^{+/-}$ islets after culture in 11.6 or 30 mmol/l glucose. The positive control in the left lane is 0.625 ng of purified rat glucokinase. The other lanes are as indicated. *B*: Glucokinase protein levels were measured in islets from $GK^{+/+}$ (n = 4) and $GK^{+/-}$ (n = 4) mice cultured in 11.6 (□) or 30 (▨) mmol/l glucose. The results are expressed relative to the GK protein levels in $GK^{+/+}$ islets cultured in 11.6 mmol/l glucose. *$P < 0.05$ vs. $GK^{+/+}$ islets cultured in 11.6 mmol/l glucose; †$P < 0.05$ vs. $GK^{+/-}$ islets cultured in 11.6 mmol/l glucose.

cemia. Our previous studies in humans suggested that, at least in subjects with glucokinase mutations causing severe impairment of enzyme function, compensatory mechanisms operate in vivo that enhance insulin secretion, thereby presumably limiting the progression of hyperglycemia (10). However, in those studies it was not possible to define the nature of the compensatory mechanisms. The current studies were undertaken to explore the nature of these compensatory mechanisms and, because glucose is the key regulator of the β-cell glucokinase gene (22), to test the hypothesis that insulin secretory function in islets lacking one allele of the glucokinase gene is enhanced by exposure to high glucose.

The heterozygous glucokinase mutant mice used in this study demonstrated the hyperglycemia and reduced insulin secretory responses to glucose that are characteristic of glucokinase diabetes, and therefore represented an appropriate animal model of the human disorder (6–9). After incu-

TABLE 4
Body mass, pancreatic mass, and β-cell mass in $GK^{+/+}$ and $GK^{+/-}$ mice

	n	Body mass (g)	Pancreatic mass (g)	β-Cell mass (mg)
$GK^{+/+}$	3	28.0 ± 1.0	0.36 ± 0.05	0.12 ± 0.02
$GK^{+/-}$	3	30.3 ± 5.9	0.35 ± 0.10	0.19 ± 0.04

bation of pancreatic islets obtained from $GK^{+/-}$ mice at 11.6 mmol/l glucose, the insulin secreted in response to a ramp increase in glucose concentration was 85% lower than in their wild-type littermates ($GK^{+/+}$). Because the β-cell mass tended to be higher and the insulin content was similar in $GK^{+/-}$ mice compared with their littermate controls, the differences were not due to smaller size of or reduced insulin content in islets from the mutant mice. The dosage-response curve relating insulin secretion to glucose concentration was shifted to the right in $GK^{+/-}$ islets compared with in $GK^{+/+}$ islets. Previous clinical studies demonstrated a similar right shift in the dosage-response curve relating insulin secretion and glucose concentration in human subjects with glucokinase diabetes (9). After a 42-h glucose infusion in these subjects, this dosage-response curve shifted toward the left (9). In the current study, similar improvements in β-cell function were produced in $GK^{+/-}$ islets by prolonged exposure to elevated glucose concentrations. The threshold glucose concentration that caused a significant increase in insulin secretion was reduced and the amount of insulin secreted in response to the ramp increase in glucose was increased almost fourfold. Despite these improvements in β-cell function, insulin secretory responses remained lower than those exhibited by $GK^{+}/^{+}$ islets incubated in 11.6 mmol/l glucose.

Another defect that has been demonstrated in human subjects with glucokinase diabetes is the failure of insulin secretion to be entrained by an oscillatory glucose infusion (9). In this study, we showed that although glucose oscillations of small amplitude elicited parallel changes in $[Ca^{2+}]_i$ in $GK^{+/+}$ islets, the ability of the β-cell in $GK^{+/-}$ mice to detect these small changes in glucose concentration was impaired and was also enhanced by prolonged exposure to a high-glucose concentration. The enhancement of β-cell glucose responsiveness resulting from exposure of $GK^{+/-}$ islets to 30 mmol/l glucose appeared to result from increased expression of the remaining allele of the glucokinase gene, since there was an increase in glucokinase protein and activity levels.

$GK^{+/+}$ islets exposed to elevated glucose concentrations demonstrated enhanced basal insulin secretion rates and reduced glucose-stimulated insulin secretion. Furthermore, normalized spectral power during oscillatory glucose perifusion was reduced in $GK^{+/+}$ islets maintained in 30 mmol/l glucose, which was indicative of their reduced ability to detect and respond to small changes in glucose. This was in contrast to the improvements in β-cell function seen in the $GK^{+/-}$ islets incubated in 30 mmol/l glucose. Although the $GK^{+/-}$ islets tended to be slightly less responsive to glucose when incubated in 30 mmol/l glucose (Fig. 1*B*), it appears that $GK^{+/-}$ islets are relatively protected from the β-cell dysfunction induced in the normal islets by prolonged exposure to high-glucose concentrations. Because the insulin content was significantly reduced in both groups after incubation at 30 mmol/l glucose, the contrasting effects of high glucose on the islets from the two groups of mice could not be explained by preservation of insulin stores in the mutant islets compared with partially exhausted stores in the wild-type islets.

These results are consistent with those from previous studies that demonstrated that exposure of normal pancreatic islets or insulin-secreting cell lines (1–5) to high glucose induces defects in insulin secretory function. On the basis of these observations, the important role that high glucose plays in promoting the deterioration in β-cell function that occurs in type 2 diabetes is recognized. The present studies demonstrated that although exposure of normal islets to high glucose for prolonged periods might induce "toxic" effects on the β-cell, resulting in an impairment of insulin secretion, exposure of $GK^{+/-}$ islets to similarly elevated glucose concentrations enhanced β-cell function by inducing increased expression of the remaining allele of the glucokinase gene. It is likely that similar mechanisms operating in vivo would increase insulin secretion, thereby limiting the severity of hyperglycemia and its tendency for progression in subjects with glucokinase mutations.

TABLE 5
Glucokinase and hexokinase enzyme activities in $GK^{+/+}$ and $GK^{+/-}$ islets

	$GK^{+/+}$		$GK^{+/-}$	
	11.6 mmol/l	30 mmol/l	11.6 mmol/l	30 mmol/l
Hexokinase				
V_{max}*	3.48	3.46	4.47	5.92
K_m	0.0115	0.0225	0.0603	0.0364
Glucokinase				
V_{max}	7.43	8.94	4.7	6.59
K_m	8.95	9.39	12.99	11.21

Data are the results of one experiment performed using islets isolated from eight to ten mice per group. *μmol glucose-6-phosphate · mg DNA^{-1} · h^{-1}.

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Q2: Did you use KRBB (bicarbonate buffer) or KRPB (phosphate buffer)?
Q3: In sentence beginning “The two pumps,” should perfusate be perifusate?
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