Changes in Pancreatic Islet Glucokinase and Hexokinase Activities With Increasing Age, Obesity, and the Onset of Diabetes

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We examined changes in high- and low-K_m glucose phosphorylating activity in pancreatic islet extracts from the prediabetic Zucker diabetic fatty (ZDF) rat between 5–6 weeks and 12 weeks of age (after the onset of diabetes). Comparisons were made between the activity observed in the ZDF rat and that seen in the ZDF lean control (ZLC) rat and the obese nondiabetic Zucker fatty (ZF) rat. At 5–6 weeks of age, insulin resistant ZDF and ZF rats were hyperinsulinemic, compared with the ZLC rat, but had normal plasma glucose levels. Kinetic parameters (V_{max} and K_m for glucose) of hexokinase (HK) and K_m of glucokinase (GCK) did not differ between groups. Islet GCK activity for ZDF and ZF rats was 1.7-fold greater than in ZLC rats ($P < 0.02$ and $P < 0.001$, respectively). By 12 weeks of age, hypersecretion of insulin at 5.0 mmol/l glucose was observed in perifused islets from both obese groups relative to the ZLC rat. Islets from ZDF rats failed to increase insulin secretion in response to increased glucose concentration. Group differences in the kinetic parameters for GCK or in the K_m values for HK were not significant. Islet HK activity for ZDF and ZF rats was 1.9-fold ($P < 0.05$) and 1.7-fold ($P < 0.05$) greater, respectively, than for ZLC rats. Compared with the 5- to 6-week-old animals, HK activity increased 3.1-fold ($P < 0.001$), 2.5-fold ($P < 0.002$), and 2.0-fold ($P < 0.05$) for ZDF, ZF, and ZLC rats, respectively. Differences in GCK activity between 5- to 6- and 12-week-old rats were not significant for any of the groups. We conclude: *1*) increased islet glucose phosphorylating activity is present in insulin resistant and hyperinsulinemic ZF and ZDF rats, relative to the ZLC rat; *2*) at 12 weeks of age, hyperinsulinemic ZDF and ZF rats demonstrated significant increases in HK activity, compared with lean controls; and *3*) deficiency in GCK activity does not explain failure of diabetic ZDF islets to respond to glucose, since differences between diabetic ZDF and nondiabetic ZF rats were not statistically significant. Increases in pancreatic islet phosphorylating activity seem to be important in maintaining basal hyperinsulinemia in insulin-resistant animals, but do not appear to play a role in the progression to glucose intolerance and diabetes. *Diabetes* 46:1434–1439, 1997

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FFA, free fatty acid; GCK, glucokinase; HK, hexokinase; ISR, insulin secretion rate; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-PCR; TG, triglyceride; ZDF, Zucker diabetic fatty; ZF, Zucker fatty; ZLC, Zucker lean control.

Insulin secretion is coupled with glucose metabolism (1,2), and the phosphorylation of glucose by glucokinase (GCK) in the pancreatic β-cell represents a key regulatory site in insulin secretion (3–6). The K_m of GCK for glucose is within the normal physiological glucose concentration range, thereby facilitating the coupling of glucose metabolism and insulin secretion. Variations in nutritional state and the physiological characteristics associated with obesity and pregnancy affect islet GCK activity (7–9). GCK levels can also be manipulated in vitro by culturing pancreatic islets at different glucose concentrations over a period of several days (10). It is not currently known whether the onset of diabetes is associated with changes in islet GCK activity. The present study was undertaken primarily to examine whether the progression from the prediabetic state to overt diabetes in the ZDF rat is associated with changes in islet GCK activity.

In addition to GCK, the β-cell also contains type I hexokinase (HK), which catalyzes the same reaction as GCK but with a much lower glucose K_m. The low-K_m glucose activity is greater in islets from obese nondiabetic Zucker fatty (ZF) rats, compared with islets from lean Wistar rats (11). It was suggested that persistently high free fatty acid (FFA) concentrations led to increased low-K_m glucose usage, which in turn was associated with fasting hyperinsulinemia. A subsequent report suggested that increased low-K_m glucose activity might represent a compensatory mechanism set in motion to mitigate against the onset of diabetes (12). Here, we show increased islet HK activity concomitant with increased plasma FFAs in three animal models with increasing age and greater islet HK activity associated with obese models, relative to lean models.

RESEARCH DESIGN AND METHODS

Animals. Studies were performed using the male Zucker diabetic fatty rat (ZDF/Gmi-*fa/fa*), a model of NIDDM, and lean male controls (ZDF/Gmi-+/+ or +/*fa*), both from Gmi (Indianapolis, IN) and obese nondiabetic male Zucker fatty (*fa/fa*) rats from Harlan Sprague Dawley (Indianapolis, IN). The latter animals served as controls for the presence of hyperlipidemia and insulin resistance in the absence of diabetes. The rats were studied at 5–6 and 12 weeks of age. In our experience, diabetes develops in >95% of animals by 12 weeks of age in the ZDF rat. The experimental design, therefore, allowed results to be compared in prediabetic and diabetic animals and their age-matched controls. Animals were fed a standard rodent chow diet (Purina 5008) ad libitum and had free access to water.

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Pancreatic islet isolation. Pancreatic islets were isolated from fed rats using a modification of the method of Lacy and Kostianovsky (13). The pancreas was distended with a 0.75-mg/ml solution of collagenase (Boehringer Mannheim, Indianapolis, IN) in Hanks' buffered saline solution (Biowhittaker, Walkersville, MA) containing 5.5 mmol/l glucose. Islets were separated from acinar tissue on a discontinuous Ficoll gradient, washed in Hanks' buffered saline solution, and hand picked. The islets were incubated for 90 min in RPMI 1640 medium supplemented with 11.6 mmol/l glucose, 10% (vol/vol) fetal calf serum, 2 mmol/l glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin before perifusion or enzyme assay.

Insulin secretory responses to glucose in perifused islets. Perifusion experiments were performed using the Accusyst-S small volume perifusion culture system (Cellex Biosciences, Minneapolis, MN), as described previously (14). Briefly, groups of 25 hand-picked islets were placed in a 500 μl chamber, surrounded by Biogel P-2 beads (Bio-Rad, Hercules, CA), and kept in place with a filter on the bottom of the chamber. Oxygenated Krebs Ringer solution supplemented with 5 mmol/l glucose was administered for 30 min, after which the glucose was increased to 20 mmol/l. The flow rate was constant at 1 ml/min, and the effluent was collected over the final 10 min at 5 mmol/l glucose and continued for 20 min after the introduction of 20 mmol/l glucose. Insulin was assayed using a micro enzyme-linked immunosorbent assay and a rat insulin standard, as described previously (15). Insulin secretory responses were evaluated by comparing the average insulin secretion rate (ISR) at 5 mmol/l glucose (basal secretion) with the secretion rate after the increase in glucose to 20 mmol/l.

DNA and metabolite measurements. DNA concentrations were determined in triplicate for each sample using the method of Lubarca and Paigen (16). Plasma glucose and FFA concentrations were measured by the glucose oxidase method using a glucose analyzer (Beckman, Fullerton, CA). Blood was taken from the tail vein for analysis of FFAs and triglycerides (TGs) by enzymatic calorimetric methods (NEFA C, Wako Chemicals, Richmond, VA, and Triglycerides, Boehringer Mannheim, respectively). Rats were allowed a 2-day recovery period after an overnight fast before killing for islet isolation.

Glucose phosphorylating rates. Glucose phosphorylating activity was measured in fresh islet extracts prepared by homogenizing 300 islets in 300 μl of homogenization buffer (20 mmol/l K_2HPO_4 [pH 7.0] containing 1 mmol/l EDTA, 110 mmol/l KCl, and 5 mmol/l dithiothreitol). Tissue homogenates were prepared on ice by 10 strokes of a Kontes size 18 Teflon in glass homogenizer with a clearance of 0.004–0.006 in. The pestle was driven by a Wheaton overhead stirrer at a speed setting of 2.7. Gentle homogenization is important to avoid solubilization of HK bound to mitochondria. An aliquot of the homogenate was removed for DNA determination on the same day. The homogenate was spun at 12,400 rpm in a microcentrifuge at 4°C for 10 min, and the supernatant was carefully removed and kept on ice. Glucose phosphorylation was measured by the method of Liang et al. (17). This procedure couples the glucose phosphorylating reaction with the oxidation of glucose-6-phosphate by glucose-6-phosphate dehydrogenase. NADH generated in the latter reaction is then measured fluorometrically. The reaction mixture consisted of 50 mmol/l HEPES (pH 7.6), 5 mmol/l ATP, 100 mmol/l KCl, 7.4 mmol/l $MgCl_2$, 15 mmol/l 2-mercaptoethanol, 0.5 mmol/l NAD^+, 0.5% (wt/vol) bovine serum albumin (fraction V), glucose (0.01–100 mmol/l), and 0.7 U/ml glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroids* (Boehringer Mannheim). Final reaction volume was 100 μl. A calibration curve was set up in duplicate using glucose-6-phosphate standards (0.1, 0.2, 0.3, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, and 3.0 nmol) in a reaction buffer that contained 100 mmol/l glucose. The reaction was performed in 1.5-ml microcentrifuge tubes and was started by the addition of a 5-μl aliquot of the islet extract or by the addition of 80 μl of assay buffer for the calibration curve, followed by the incubation of the tubes at 30°C for 90 min. The reaction was stopped by the addition of 1 ml of 500 mmol/l $NaHCO_3$ (pH 9.4).

Duplicate sample measurements (F_1) were taken at each of six glucose concentrations in the HK range (0.01, 0.03, 0.05, 0.075, 0.25, and 0.50 mmol/l) and six concentrations in the GCK range (6, 10, 15, 20, 50, and 100 mmol/l). Two series of measurements were made for both glucose concentration ranges, where no tissue extract was added (reagent blanks) in the presence (F_2) and absence (F_4) of ATP, respectively. Finally, tissue blanks (F_3) were determined in buffer lacking ATP. The corrected fluorescence (FC) was then calculated using the formula:

$$FC = F_1 - F_2 - (F_3 - F_4)$$

as described previously (17). Duplicates were averaged to provide a single data point. GCK and HK V_{max} and K_m values were determined from an Eadie-Scatchard plot, using the iterative linear regression method of Spears et al. (18). Convergence of kinetic parameters was typically seen within six cycles, and the data was then extrapolated to 37°C, assuming a Q_{10} of 2 (19).

RNA isolation and cDNA synthesis. Total RNA was isolated from pancreatic islets using TRIZOL Reagent (GIBCO BRL/Life Technologies, Gaithersburg, MD), as suggested by the manufacturer and incubated with 1 U of RQ1 RNase-free DNase (Promega, Madison, WI) for 15 min at 37°C. cDNA was prepared from 3 μg of total RNA, using a kit (SuperScript Preamplification System for First Strand cDNA Synthesis, GIBCO BRL/Life Technologies). The 20-μl reaction volume was incubated at 37°C for 1 h and then at 65°C for 5 min to inactivate the reverse transcriptase. The cDNA was diluted to 1:100 with distilled water, and 1 or 10 μl of this dilution for β-actin or GCK, respectively, was used for polymerase chain reaction (PCR) amplification.

Quantitation of mRNA. Specific mRNA levels were determined using a PCR-based approach as previously described (20). Briefly, a semiquantitative reverse transcriptase (RT)-PCR assay, in which a constant amount of competitor template was added to each reaction, was used to compare the levels of given mRNAs in islets from different animals. The PCR products were subsequently separated by electrophoresis on a 5% nondenaturing polyacrylamide gel, and the radioactivity incorporated into each fragment was determined directly by use of a PhosphorImager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Radioactivity incorporated into each cDNA fragment was expressed relative to that incorporated into the competitor present in the same reaction. This was done for both GCK and β-actin. Subsequently, GCK values were normalized using β-actin values. Finally, the mean value for the ratio of GCK to β-actin for ZLC rats at 5–6 weeks of age was arbitrarily set at 100, and all other values were expressed with respect to this.

Statistical analysis. Results are expressed as means ± SE. Differences between groups were analyzed by analysis of variance using Tukey's post hoc method for multiple comparisons, and statistical differences were determined at given values of α_T.

RESULTS

Physiological characteristics of animal models. The physiological characteristics of male ZLC, ZF, and ZDF rats at 5–6 and 12 weeks of age are summarized in Table 1. At 5–6

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TABLE 1
Physiological characteristics of animal models

	5- to 6-week-old rats			12-week-old rats		
	ZLC	ZF	ZDF	ZLC	ZF	ZDF
Body weight (g)	138 ± 11 (12)	263 ± 11 (8)‡‖	173 ± 15 (12)‡	340 ± 13 (8)	512 ± 27 (8)*‖	411 ± 9 (8)*
Nonfasting glucose (mmol/l)	6.1 ± 0.2 (12)	7.3 ± 0.3 (8)*‖	6.3 ± 0.3 (12)†	7.4 ± 0.2 (8)	7.7 ± 0.4 (8)¶	24.2 ± 0.9 (8)§
Fasting TG (mg/dl)	60 ± 10 (5)	186 ± 12 (4)§¶	116 ± 10 (5)*	26 ± 4 (3)	742 ± 54 (3)§	656 ± 26 (3)§
Nonfasting FFAs (g/l)	0.12 ± 0.02 (3)	0.86 ± 0.04 (3)‡	0.95 ± 0.03 (3)‡	0.18 ± 0.02 (3)	1.09 ± 0.15 (3)‡	1.30 ± 0.09 (3)‡
Fasting FFAs (g/l)	0.20 ± 0.01 (5)	0.30 ± 0.03 (4)*	0.32 ± 0.03 (5)*	0.25 ± 0.07 (3)	0.65 ± 0.02 (3)*	0.58 ± 0.08 (3)*
Basal ISR (pmol/min)	0.018 ± 0.004 (6)	0.050 ± 0.006 (4)*	0.058 ± 0.009 (6)†	0.066 ± 0.009 (6)	0.122 ± 0.015 (5)*	0.113 ± 0.017 (3)*
Stimulated ISR (pmol/min)	0.063 ± 0.008 (6)	0.183 ± 0.048 (4)*	0.182 ± 0.032 (6)*	0.237 ± 0.034 (6)	0.296 ± 0.031 (5)‖	0.141 ± 0.030 (3)

Data are means ± SE (*n*). Animals were weighed before killing, blood was taken from the tail vein, and plasma was separated for analysis of metabolites. Where indicated, rats were fasted overnight. Islets were isolated by collagenase digestion and perifused in groups of 25 islets per chamber in duplicate. Stimulated ISR refers to ISR at 20 mmol/l glucose. *$P < 0.05$ between ZF or ZDF and ZLC rats; †$P < 0.01$ between ZF or ZDF and ZLC rats; ‡$P < 0.001$ between ZF or ZDF and ZLC rats; §$P < 0.0001$ between ZF or ZDF and ZLC rats; ‖$P < 0.05$ between ZF and ZDF rats; ¶$P < 0.0001$ between ZF and ZDF rats.

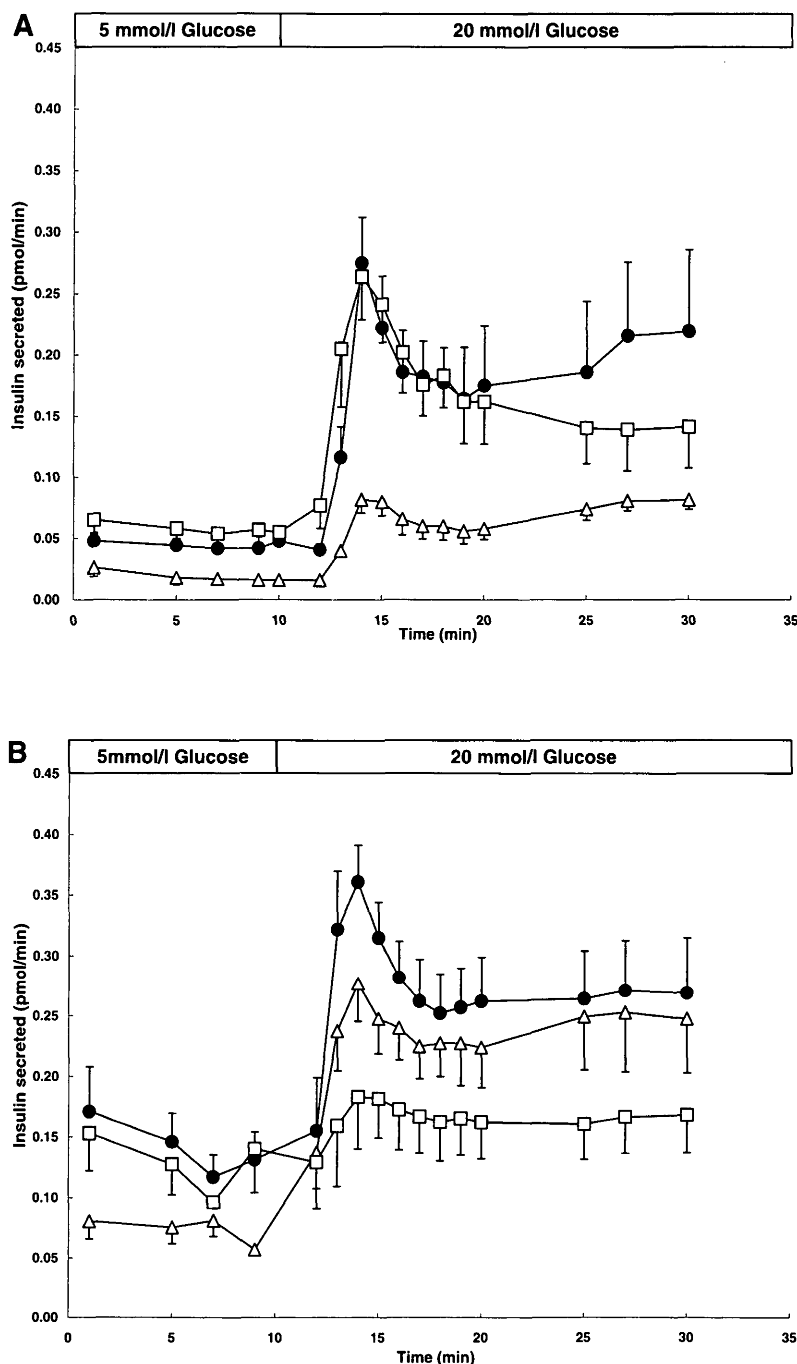

FIG. 1. Insulin secretion from perifused islets. Islets from 5- to 6-week-old rats (*A*) or from 12-week-old rats (*B*) were incubated in 11.6 mmol/l glucose at 37°C for 90 min. Twenty-five islets were hand picked into a perifusion chamber and equilibrated at 5 mmol/l glucose for 30 min with a flow rate of 1 ml/min. The effluent was collected (1 ml/min) over the last 10 min after which the glucose was increased to 20 mmol/l and collection continued another 20 min. Data are means ± SE. The number of animals is indicated in parentheses. △, ZLC rats (6); ●, ZF rats (5); □, ZDF rats (6).

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weeks of age, the ZF rats were heavier than the ZLC and ZDF rats ($P < 0.001$). Differences in weight between the ZDF and ZLC rats were not significant. By 12 weeks of age, ZF and ZDF rats were heavier than ZLC rats ($P < 0.03$), and ZF rats were heavier than ZDF rats ($P < 0.05$).

At 5–6 weeks of age, nonfasting plasma glucose concentrations in the ZF rat were significantly greater ($P < 0.05$) than for both ZDF and ZLC rats, but all were within the euglycemic range. By 12 weeks of age, the ZF and ZLC rats were still euglycemic, but the ZDF rats had developed significant hyperglycemia.

Fasting plasma TG at 5–6 weeks of age for ZF and ZDF rats were greater than for ZLC rats ($P < 0.0001$ and $P < 0.02$, respectively). By 12 weeks of age, fasting plasma TG for ZF and ZDF rats were greater than FFA levels for ZLC rats ($P < 0.0001$). Typically, fasting plasma FFAs followed a similar trend, and these differences were more pronounced for nonfasting rats.

Insulin secretion from perifused islets. Using islets from the 5- to 6-week-old ZDF and ZF rats, ISRs at basal glucose (5 mmol/l) were 3.2-fold ($P < 0.004$) and 2.7-fold ($P < 0.02$) greater, respectively, than basal ISRs for islets from the ZLC rat (Fig. 1*A*; Table 1). In response to the glucose challenge (20 mmol/l glucose), mean ISRs increased 3.5-, 3.7-, and 3.1-fold for ZLC, ZF, and ZDF islets, respectively, relative to the rates observed at basal glucose.

In the 12-week-old age-group, mean basal ISRs from islets from both ZDF and ZF rats were 1.8-fold greater than from

age-matched ZLC rats ($P < 0.05$). In response to the glucose challenge, mean ISRs increased 3.6-, 2.4-, and 1.2-fold for ZLC, ZF, and ZDF islets, respectively, relative to the rates observed at basal glucose.

Glucose phosphorylating activity. Islet glucose phosphorylating rates for 5- to 6-week-old ZLC, ZF, and ZDF rats are illustrated in Fig. 2*A*. There were no significant differences in HK V_{max} and K_m values or in GCK K_m values among the three rat models in this age-group (Table 2). However, GCK V_{max} values were higher in islets of both the ZF rat (1.7-fold, $P < 0.001$) and the ZDF rat (1.7-fold, $P < 0.011$), relative to the ZLC rat.

Figure 2*B* shows the rates of islet glucose phosphorylation for the 12-week-old age-group. There were no significant differences in the kinetic parameters for GCK between any of the models (Table 2). Both the V_{max} and K_m values for GCK showed no significant change over the respective values in the 5- to 6-week-old age-group. Further, there were no significant differences in HK K_m values among the three rat models in this age group or between age-groups within the same model. Within the 12-week-old age-group, islet HK activity for the ZDF and ZF rat was 1.9- and 1.7-fold greater, respectively, than for the ZLC rat ($P < 0.012$ and $P < 0.05$, respectively). Further, islet HK activity for the ZDF rat increased 3.1-fold in the 12-week-old age-group, relative to the 5- to 6-week-old ZDF rat ($P < 0.001$). The changes between age groups for the other models were a 2.5-fold increase for the ZF rat ($P < 0.002$) and a 2.0-fold increase for the ZLC rat ($P < 0.03$).

Relationship between glucose phosphorylating activity and basal insulin secretion. In an effort to determine whether there is a relationship between enzyme velocities determined in islet extracts and insulin secretion measured from intact islets, basal ISRs were plotted against HK V_{max} values. A strong correlation was observed between these two parameters, the correlation coefficient was $r = 0.97$. A similar analysis, where GCK velocity at 5 mmol/l glucose was plotted against basal ISRs, had a much poorer correlation ($r = 0.37$).

Comparison of islet mRNA levels by competitive RT-PCR. GCK mRNA levels were normalized to their respective mRNA levels for the structural protein β-actin to take into account any differences in efficiency of RNA extraction and/or cDNA synthesis. We found no significant differences in the levels of GCK mRNA between 5- to 6-week-old ZLC, ZF, and ZDF rats or 12-week-old ZLC rats. However, there was a 50% reduction in GCK mRNA in 12-week-old ZF and ZDF rats (Table 2).

DISCUSSION

The present study examined the relationships between glucose phosphorylating activity and ISRs in prediabetic and diabetic male ZDF rats. The results demonstrate that a deficiency of glucokinase does not explain the reduced insulin secretory responses to glucose that are a feature of diabetes in this rat model of NIDDM. The profiles of glucose phosphorylating activity were very different in the three animal models. First, there were no significant differences in V_{max} and K_m values for islet HK between any of the rat models at 5–6 weeks of age. However, islet GCK activities for 5- to 6-week-old ZDF and ZF rats were significantly greater than for the ZLC rat, despite there being no differences in GCK mRNA levels normalized to β-actin mRNA levels (Table 2). It seems likely

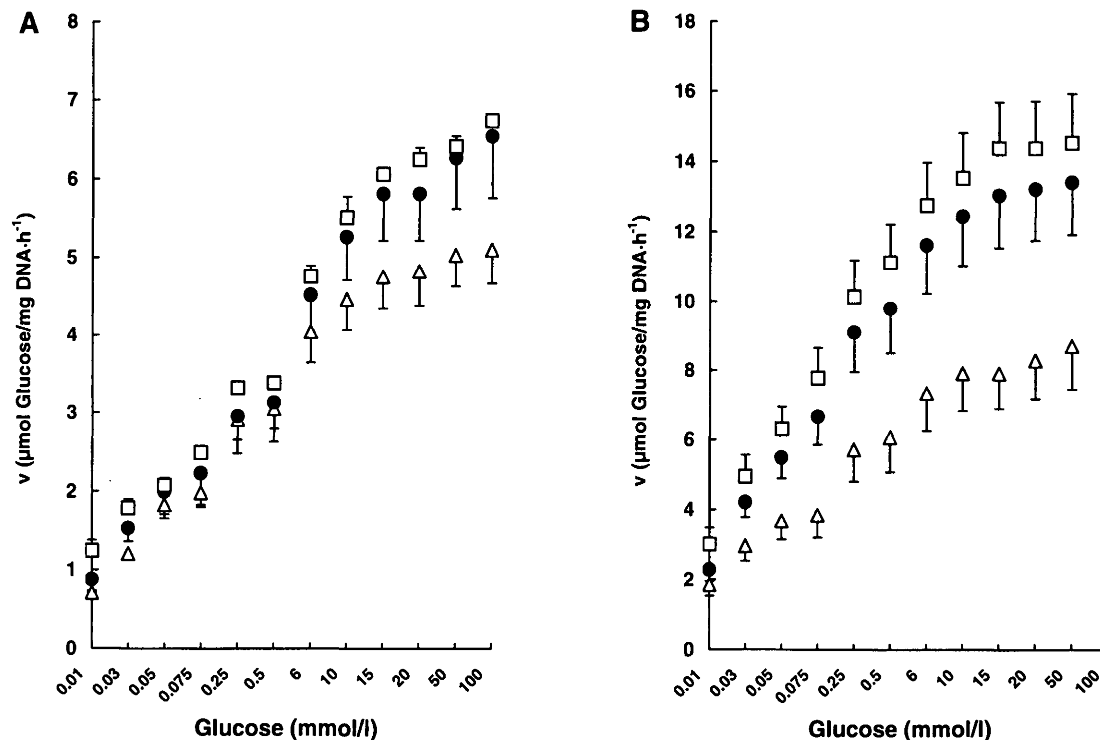

FIG. 2. Islet glucose phosphorylating activity. Activity was measured fluorometrically in fresh islet extracts from ~300 islets isolated from 5- to 6-week-old rats (*A*) or from 12-week-old rats (*B*). Data are means ± SE for islets isolated from seven to eight rats. △, ZLC rats; ●, ZF rats; □, ZDF rats.

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TABLE 2
Islet DNA and mRNA measurements and kinetic parameters for glucose phosphorylating activity

	5 to 6-week-old rats			12-week-old rats		
	ZLC	ZF	ZDF	ZLC	ZF	ZDF
DNA (ng/islet)	22.9.1 ± 0.9	12.1 ± 1.6	13.3 ± 1.5	20.8 ± 2.2 ‡	54.5 ± 7.7 †§	51.7 ± 6.4 †‖
HK V_{max} (μmol glucose · mg^{-1} DNA.· h^{-1})	4.95 ± 0.87	6.53 ± 0.73	5.92 ± 0.85	9.75 ± 1.69 ‡	16.59 ± 2.40 *§	18.12 ± 1.65 *‖(7)
HK K_m (glucose mmol/l)	0.025 ± 0.005	0.025 ± 0.003	0.023 ± 0.003	0.027 ± 0.003	0.040 ± 0.006	0.036 ± 0.005 (7)
GCK V_{max} (μmol glucose · mg DNA.· h^{-1})	4.42 ± 0.49	7.59 ± 0.51*	7.30 ± 0.79*	5.32 ± 0.50	7.01 ± 1.00	7.14 ± 0.10 (7)
GCK K_m (glucose mmol/l)	11.11 ± 1.90	11.67 ± 1.36	10.49 ± 1.09	8.56 ± 1.00	10.23 ± 2.21	11.40 ± 3.03 (7)
Relative GCK mRNA	100 ± 5 (3)	101 ± 25.7 (3)	122 ± 16.8(4)	102 ± 17.9 (3)	54 ± 12.5 (3)	52 ± 19.4 (4)

Data are means ± SE (n = 8) except where indicated. Relative GCK mRNA levels were determined using competitive PCR, followed by the separation of products on a 5% polyacrylamide gel and direct quantitation as outlined in the text. For competitive PCR of GCK and β-actin, the respective competitors were used at final concentrations of 2.0×10^{-6} ng/μl and 5.0×10^{-8} ng/μl in a 50-μl reaction. *$P < 0.05$ between ZF or ZDF and ZLC rats; †$P < 0.004$ between ZF or ZDF and ZLC rats; ‡$P < 0.05$ between 12-week-old ZLC and 5- to 6-week-old ZLC; §$P < 0.002$ between 12-week-old ZF and 5- to 6-week-old ZF; ‖$P < 0.001$ between 12-week-old and 5- to 6-week-old ZDF rats.

that at 5–6 weeks of age, basal insulin hypersecretion in ZF and ZDF rats is facilitated by high levels of GCK activity. Basal insulin hypersecretion was still evident in islets from insulin-resistant 12-week-old ZF and ZDF rats. However, islets from the ZF rats were still responsive to glucose, whereas those from ZDF rats had a markedly attenuated response. The HK activity was higher in islets of 12-week-old ZF and ZDF rats, compared with the ZLC rat. Our results show that basal hyperinsulinemia is associated with greater islet glucose phosphorylating activity due to greater HK or GCK activity in islets.

There were no significant differences in islet GCK activity between 12-week-old ZLC, ZF, and ZDF rats, although there were differences in glucose-stimulated insulin secretion and in the presence or absence of diabetes. Thus, insulin resistance and diabetes are not associated with a reduction in islet GCK activity in ZF and ZDF rats. There was, however, a specific 50% reduction in GCK mRNA levels in the ZF and ZDF rats (Table 2), a result that indicates there are posttranscriptional mechanisms regulating GCK activity, as has been previously suggested (10,21,22). The nature of these mechanisms is unknown, but could include increased stability of GCK protein or higher rates of translation of GCK mRNA.

Previous studies and those reported here show that a proportional relationship exists between islet HK activity and FFA levels (Tables 1 and 2) (11,12,23). Additionally, our results show that there is an approximately twofold increase in fasting FFA levels for 12-week-old ZF and ZDF rats, compared with 5- to 6-week-old ZF and ZDF rats. Concomitantly, islet HK activity increased by ~2.5-fold for ZF and ZDF rats. Hence, although increased FFAs may fail to promote increased HK activity in ZDF islets in vitro (12), the increase in plasma FFAs maybe associated with increases in islet HK activity in vivo. The failure of HK activity and, more importantly, GCK activity to increase further in the presence of hyperglycemia could be interpreted as the failure of this mechanism to compensate sufficiently to prevent diabetes.

Our results show a strong correlation between HK activity and basal ISRs. The general view is that islet HK activity does not play an important role in the regulation of insulin secretion because of feedback inhibition by glucose-6-phosphate (24,25). However, there is growing evidence that increased islet HK activity may be involved in an increase in basal ISRs, based on results of diverse in vivo and in vitro systems (11,12,26–32).

In summary, the results show that the defect in glucose-stimulated insulin secretion seen in diabetic ZDF rats is not due to a change in GCK activity. Further studies are necessary to elucidate the molecular basis of the secretory defect.

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