Inhibition of Progressive Reduction of Islet β-Cell Mass in Spontaneously Diabetic Goto-Kakizaki Rats by α-Glucosidase Inhibitor

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The Goto-Kakizaki (GK) rat, an animal model of type 2 diabetes, exhibits mild hyperglycemia with a reduction of β-cell mass. The mechanism for islet structural changes in this model and whether the changes are affected by metabolic control are not known. In the present study, we examined the process of islet changes in male GK rats aged 6, 8, 12, 24, and 36 weeks. Treatment effects with an α-glucosidase inhibitor (Voglibose; Takeda, Osaka, Japan) for 24 weeks (12 to 36 weeks of age) were also evaluated. The β-cell mass increased until 8 weeks of age in both GK and control rats, but the increase was significantly ($P < .01$) smaller in GK rats versus at 8 weeks of age. Thereafter, the β-cell mass decreased in GK rats, whereas it remained constant in controls. Voglibose treatment significantly ($P < .01$) inhibited the reduction of β-cell mass in GK rats. Proliferative activity of β cells as measured by bromodeoxyuridine (BrdU) uptake was significantly ($P < .05$) lower in GK rats versus control rats at 6 and 8 weeks, but the difference disappeared after 12 weeks of age, regardless of Voglibose treatment. The present study thus demonstrates a progressive loss of β cells in GK rats that was mitigated by Voglibose treatment. We consider that the β-cell loss in GK rats was due to an early impairment in proliferative activity and reduced survival. Voglibose did not appear to stimulate β-cell proliferation, but exerted its effect via a reduction of hyperglycemia.

DEVELOPMENT of the diabetic syndrome in non–insulin-dependent diabetes mellitus is associated with impaired insulin secretion and insulin resistance in peripheral tissues.[1-3] Such metabolic abnormalities in type 2 diabetes have been accounted for, in part, by a reduction of β-cell mass or an islet pathology such as amyloid deposition.[4,5] The longitudinal process of islet pathology in type 2 diabetes is not fully understood, and it is not clear if the β-cell loss can be prevented by metabolic control.

The Goto-Kakizaki (GK) rat is an animal model of non-obese non–insulin-dependent diabetes characterized by low insulin secretion.[6,7] This model also exhibits functional and structural features of diabetic complications and is therefore considered suitable for studying type 2 diabetes.[8-10] It has been shown that the β cell in this model has a defect in glucose-stimulated insulin secretion, whereas insulin synthesis is not affected.[11-13] In addition to functional defects of β cells, the loss of β cells has been demonstrated in this model in the fetal stage in the Paris colony[14,15] and at 8 weeks of age in the Stockholm colony[16] and 12 weeks of age in the Texas colony.[17] In our colony, we previously found a significant reduction of islet β cells in the GK rat at 12 weeks of age.[18] It was further found that the β-cell loss was enhanced by sucrose-feeding via an increase in the rate of apoptosis.[18] These findings suggest that differences in genetic and nutritional states between the colonies may influence pancreatic pathology and β-cell mass in the GK rat. Although GK rats were originally established in northern Japan, the pancreatic pathology was only briefly summarized in the Japanese literature as fibrotic changes with a starfish contour and β-cell depletion after several months of diabetes duration.[6-8] Temporal changes of islet β-cell mass associated with pancreatic growth and aging were never addressed by quantitative analysis. It also remains unknown as to whether the changes can be affected by metabolic control.

In the present study, we examined β-cell reduction from puberty to adulthood longitudinally in the GK rat in the original Japanese colony. We also determined if treatment with an α-glucosidase inhibitor halts or prevents the pathological progression.

MATERIALS AND METHODS

Animals

The male GK rats used for the current study were derived from F28 of the original colony established by Goto et al.[8] The normal male Wistar rats without glucose intolerance that served as controls were obtained from Jcl:Wistar rats maintained in the closed colony of Takeda Pharmaceutical Research Laboratories (Takeda Chemical Industries, Osaka, Japan). The animals were reared in individual plastic cages and received water and standard powdered diet (CRF-1; Oriental East, Tokyo, Japan) ad libitum.

At 6, 8, 12, 24, and 36 weeks of age, body weight, fasting and nonfasting blood glucose levels, and basal insulin levels were monitored in all animals. Glucose concentrations in blood obtained from the tail vessels were determined by a glucose oxidase method (Toecho Super II; Toecho, Kagawa, Japan). Fasting plasma insulin was determined by an enzyme-linked immunosorbent assay kit for rat insulin (Morinaga, Yokohama, Japan). At the end of the experiment after an overnight fast, all animals were injected intraperitoneally with 5-bromo-2-deoxyuridine ([BrudU] Boehringer, Mannheim, Germany) dissolved in phosphate-buffered saline at a dose of 100 mg/kg body weight. Four hours after the injection, the whole pancreas was excised under anesthesia with pentobarbital (Abbott, Chicago, IL). Thereafter, the volume of the pancreas was measured by Archimedes' principle following the removal of connective tissue and extraneous fat. The pancreata were then fixed in 10% buffered Formalin and processed for paraffin embedment. Glycated hemoglobin levels were measured in blood at the end of the experiment using the BioRad (Hercules, CA) column test. During the experimental period, all nutritional and environmental conditions were kept constant between the 2 groups.

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Treatment With α-Glucosidase Inhibitor (Voglibose)

At 12 weeks of age, both GK and control Wistar rats were randomly divided into 2 groups based on body weight. One group received a diet (CRF-1) containing an α-glucosidase inhibitor (Voglibose 50 ppm; Takeda). The untreated group received CRF-1 diet without Voglibose. The diet consisted of 23.1% protein, 5.9% fat, 53.5% carbohydrate, 3.3% fiber, 6.5% mineral, and 7.7% water, yielding 360 kcal/100 g.

Islet Pathology and Morphometry of Endocrine Cells

Serial 4-μm thick paraffin sections were stained with hematoxylin-eosin (HE) for islet pathology and doubly immunostained for insulin and glucagon to identify β and α cells, as described previously.[18] Quantitative evaluation of islet β cells and α cells was based on the method described by Klöppel et al[19] using a computer-assisted point-counting method on an Olympus AX80 microscope (Olympus, Tokyo, Japan) connected to a personal computer using NIH Image (version 1.56, Wayne Rasband, National Institutes of Health).[18] The images of pancreas sections stained with HE were obtained under a 4× objective (×57 on monitor) in a regular lattice. The test grid was overlaid on the images, and 3,600 to 10,000 (mean, 5,400) points of the pancreas were counted. The ratio of the number of points hit on the parenchymal pancreas to the number of points hit on the pancreas (excluding the area of vessels and connective and fatty tissues) was expressed as the Paren/Panc ratio. The parenchymal volume of the pancreas (Vp) was then calculated by multiplying the total volume of the pancreas (Vpanc) and the Paren/Panc ratio. For evaluation of the islet cell area, a high-magnification (×200) image of doubly immunostained sections was overlaid with a grid consisting of 875 points. In each animal, 25 to 80 fields (average, 45) were subjected to quantitation of endocrine cells. The ratio of the number of points hit on α and β cells to the number of points hit on parenchymal cells was presented as the volume density of β cells (VvB) and α cells (VvA), respectively. Then, the mass of β cells (VB) and α cells (VA) was obtained by multiplying Vp by VvA or VvB.

Proliferative Activity of Pancreatic β Cells

The proliferative activity of β cells was quantified on the sections double-stained with insulin and BrdU. Double-staining was performed by methods described previously.[18,20] In each section, the positive cells among approximately 4,000 insulin-positive cells (BrdU/insulin index) were counted at high magnification (×400) as an index of proliferative activity and expressed as a percentage. All quantitative analysis was performed in a double-blind manner.

Statistical Methods

All experimental values are expressed as the mean ± SD of the group. Statistical comparisons between GK and control Wistar groups and between Voglibose-treated and untreated groups were performed by 1-way ANOVA with Bonferroni's post hoc test using StatView 4.0 (Abacus Concepts, Berkeley, CA). Statistical significance was set at a P level less than .05.

RESULTS

Laboratory Data for Wistar and GK Rats

Both GK rats and control Wistar rats gained weight with increasing age, but the mean body weight was always about 30 g less in the former versus the latter, except at 36 weeks of age (Table 1). Blood glucose was only modestly elevated in GK rats compared with control rats in both the fasting and nonfasting states during the whole experimental period. Plasma insulin was significantly increased in GK rats compared with control rats at 6 and 12 weeks of age, but it was similar to the values in control rats at 24 and 36 weeks of age.

Islet Pathology and Morphometry

Pancreatic islets appeared normal, and there was no fibrosis in 6-week-old GK rats. An irregular contour and fibrosis of the islets were apparent in GK rats at 8 weeks of age and became more severe thereafter. There was disruption of the endocrine cells in fibrotic islets. Swollen β cells with degranulated cytoplasm were often encountered in GK rats after 8 weeks of age.

The mean β-cell mass was similar in GK rats and control Wistar rats at 6 weeks of age, occupying 0.57% of the pancreatic parenchyma (VvB; Table 2). The mass of β cells rapidly expanded to 4.1-fold from 6 to 8 weeks of age in control rats, whereas the increase was only 2.6-fold in GK rats. The mass further increased to 4.7-fold in 12-week-old control rats compared with 6-week-old rats, and then remained at the same level at 24 and 36 weeks of age. By contrast, β-cell mass decreased gradually in GK rats after 8 weeks of age, and it was only 59%, 48%, 30%, and 25% of the level in control rats at 8, 12, 24, and 36 weeks of age, respectively ($P < .05$ at 8 and 12

Table 1. Laboratory Data for the Experimental Animals

Parameter	Age (wk)				
	6	8	12	24	36
Body weight (g)					
Wistar	173 ± 4	236 ± 11	307 ± 15	414 ± 21	472 ± 13
GK	146 ± 10*	208 ± 6*	279 ± 13*	386 ± 16*	418 ± 19*
FBG (mmol/L)					
Wistar	2.9 ± 0.3	3.9 ± 0.4	3.9 ± 0.2	4.3 ± 1.0	4.9 ± 0.7
GK	4.6 ± 0.8*	5.8 ± 0.9*	6.0 ± 0.9*	6.2 ± 1.3†	9.1 ± 1.5*
Non-FBG (mmol/L)					
Wistar	5.4 ± 0.3	5.0 ± 0.5	4.4 ± 0.5	4.6 ± 0.7	4.7 ± 0.3
GK	7.9 ± 0.9*	6.5 ± 0.7†	7.7 ± 0.7*	6.8 ± 1.0*	9.5 ± 2.4*
Basal insulin (ng/mL)					
Wistar	0.42 ± 0.28	0.41 ± 0.27	0.39 ± 0.13	0.78 ± 0.47	0.75 ± 0.21
GK	0.81 ± 0.17*	0.67 ± 0.20	0.74 ± 0.15†	0.88 ± 0.26	0.73 ± 0.09

NOTE. Values are the mean ± SD (n = 5 per group).

Abbreviations: FBG, fasting blood glucose; non-FBG, nonfasting blood glucose.

*$P < .01$, †$P < .05$ *v* control Wistar rat.

Table 2. Pancreatic Weight and Islet Morphometry in the Experimental Animals

Parameter	Age (wk)				
	6	8	12	24	36
Pancreas weight (g)					
Wistar	0.62 ± 0.08	0.93 ± 0.12	1.01 ± 0.15	0.95 ± 0.14	1.09 ± 0.09
GK	0.68 ± 0.19	0.97 ± 0.09	0.95 ± 0.13	1.00 ± 0.13	0.98 ± 0.10
VvB (%)					
Wistar	0.54 ± 0.17	1.14 ± 0.29	1.14 ± 0.43	1.18 ± 0.32	1.27 ± 0.57
GK	0.57 ± 0.33	0.92 ± 0.49	0.58 ± 0.26	0.34 ± 0.20*	0.41 ± 0.10*
β-Cell mass (mL)					
Wistar	1.93 ± 0.78	7.86 ± 1.76	9.10 ± 6.77	8.02 ± 2.50	10.14 ± 6.23
GK	1.78 ± 1.12	4.62 ± 2.49†	4.39 ± 3.85†	2.43 ± 1.23†	2.50 ± 1.22*
BrdU LI (%)					
Wistar	2.86 ± 0.25	0.69 ± 0.04	0.10 ± 0.05	—	0.07 ± 0.01
GK	1.67 ± 0.31*	0.42 ± 0.05*	0.11 ± 0.07	—	0.08 ± 0.02
VvA (%)					
Wistar	0.19 ± 0.01	0.30 ± 0.09	0.23 ± 0.13	0.28 ± 0.09	0.27 ± 0.07
GK	0.16 ± 0.10	0.28 ± 0.04	0.19 ± 0.11	0.17 ± 0.09	0.48 ± 0.16
α-Cell mass					
Wistar	0.67 ± 0.42	2.02 ± 0.46	1.98 ± 1.25	1.85 ± 1.25	2.11 ± 0.88
GK	0.49 ± 0.36	1.33 ± 1.09	1.58 ± 1.57	1.16 ± 0.49	2.93 ± 1.23

NOTE. Values are the mean ± SD. All groups consist of 5 animals, except for 12- and 36-week-old rats (n = 3-4).
Abbreviations: VvB, volume density of β cells; VvA, volume density of α cells.
*$P < .01$, †$P < .05$ *v* Wistar rat.

weeks and $P < .01$ at 24 and 36 weeks). The volume density of β cells was significantly smaller in GK rats versus control rats after 12 weeks of age ($P < .01$ at 24 and 36 weeks of age).

The mass of α cells also increased in both GK rats and control rats from 6 weeks until 8 weeks of age and then remained constant thereafter. There was no significant difference in the mass and volume density of α cells between GK rats and control rats throughout the observation period.

Proliferative Activity of β Cells

The BrdU labeling index (LI) of β cells was 2.86% in control rats at 6 weeks of age and then decreased to 0.69%, 0.10%, and 0.07% at 8, 12, and 36 weeks of age, respectively (Table 2). The LI in GK rats at 6 and 8 weeks of age was about half the LI in control rats ($P < .01$ for both), but the difference became negligible by 12 weeks of age.

Treatment With α-Glucosidase Inhibitor

Daily food consumption in GK rats (21 ± 2 g/d per rat) was similar to that of control Wistar rats (20 ± 3 g/d) and was not affected by Voglibose treatment in either GK (21 ± 2 g/d) or control rats (20 ± 2 g/d). Voglibose treatment for 24 weeks significantly reduced body weight in both GK and control rats by 12% and 22%, respectively (Table 3). The feces of Voglibose-treated animals became wet and soft compared with that of untreated animals. Fasting and nonfasting blood glucose and glycated hemoglobin were significantly higher in GK rats versus control rats ($P < .01$ for all). Voglibose treatment significantly decreased the levels in GK rats ($P < .01$ for all) but did not affect the values in control rats. Plasma insulin levels were significantly lower in Voglibose-treated GK rats compared with untreated GK rats ($P < .01$).

The degree of islet fibrosis was milder in Voglibose-treated GK rats versus untreated GK rats, and the islet contour was well preserved in the treated group (Fig 1A to C). Swollen β cells with degranulation were infrequent in treated GK rats. Voglibose treatment did not influence the volume density and mass of β cells in control Wistar rats. By contrast, the volume density and mass of β cells in treated GK rats were 2.0-fold (67% of control Wistar) and 2.5-fold (75% of control Wistar) the values in untreated GK rats, respectively ($P < .01$ for both; Table 4). Voglibose treatment did not affect the LI of BrdU in either GK or control rats, although there was an insignificant increase in the mean value in control rats.

DISCUSSION

In the present study, β-cell mass reached the maximum level in GK rats at about 8 weeks of age and then gradually decreased

Table 3. Laboratory Data for Voglibose-Treated Wistar and GK Rats and the Control Animals

Group	Body Weight (g)	Fasting Blood Glucose (mmol/L)	Non-Fasting Blood Glucose (mmol/L)	Basal Insulin (ng/mL)	Glycated Hemoglobin (%)
Wistar (n = 5)	472 ± 13	4.9 ± 0.7	4.7 ± 0.3	0.54 ± 0.21	2.9 ± 0.1
Wistar + Voglibose (n = 5)	420 ± 32*	3.8 ± 0.2	4.4 ± 0.6	0.67 ± 0.42	2.8 ± 0.1
GK (n = 7)	418 ± 19*	9.1 ± 1.5*	9.5 ± 2.4*	0.73 ± 0.08	4.1 ± 0.6*
GK + Voglibose (n = 7)	328 ± 19†	5.4 ± 0.5†	5.7 ± 0.5†	0.40 ± 0.13*	3.1 ± 0.2†

NOTE. Values are the mean ± SD.
*$P < .05$ *v* Wistar rats.
†$P < .01$ *v* untreated GK rats.

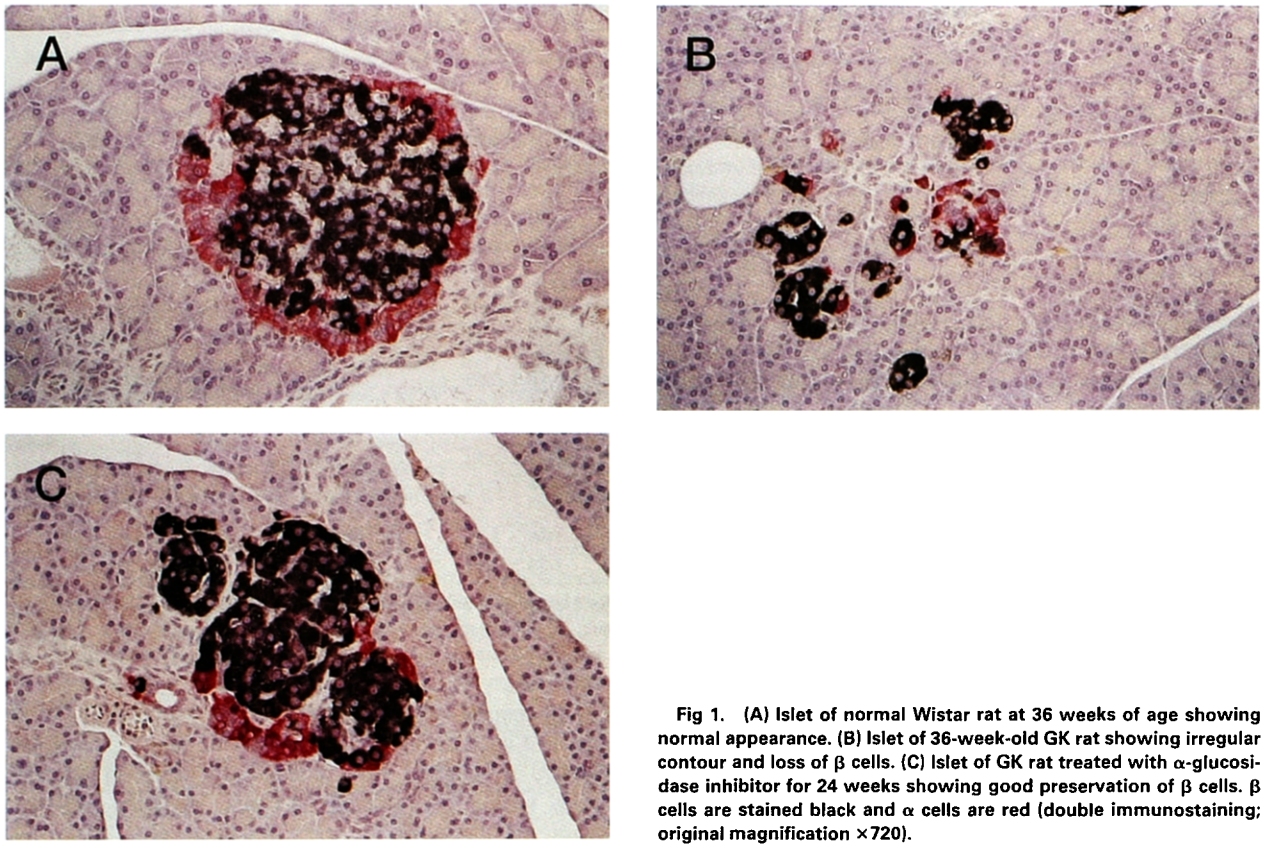

Fig 1. (A) Islet of normal Wistar rat at 36 weeks of age showing normal appearance. (B) Islet of 36-week-old GK rat showing irregular contour and loss of β cells. (C) Islet of GK rat treated with α-glucosidase inhibitor for 24 weeks showing good preservation of β cells. β cells are stained black and α cells are red (double immunostaining; original magnification ×720).

to approximately half of the maximum at 36 weeks of age (25% of control Wistar rats). The temporal changes of β-cell mass in GK rats are in contrast to those in control Wistar rats, which showed a constant age-related increase in β-cell mass. In the Japanese colony of GK rats, a significant reduction of β-cell mass to about 59% of the value in control Wistar rats was first demonstrated at 8 weeks of age. The loss of β cells was progressive until only 25% of the control value remained at 36 weeks of age. The appearance of significant β-cell loss is relatively late in our colony compared with the Paris colony (4 days),[14,15] but it is comparable to the colonies of Stockholm (8 weeks)[16,21] and Texas (12 weeks).[17] The absence of islet pathology and β-cell loss in 6-week-old GK rats in the presence of a slight elevation of blood glucose and plasma insulin indicates that impaired insulin action (insulin resistance) may precede a significant loss of β cells. The reason for the difference in the onset of significant β-cell loss among colonies is not clear, but perhaps it can be ascribed to differences in the technical problems of islet morphometry or to the characteristics acquired after transfer arising from different nutritional states and environments. It should also be taken into account that the use of different control animals may have an influence on the interpretation of morphometric results, because the 10% to 15% difference in body weight between GK and control rats used in this study was much smaller than the difference reported in studies on the Paris colony, which was as much as 30%.[14,15]

Table 4. Morphometric Data for Pancreatic β Cells in Voglibose-Treated Rats and Control Animals

Group	Pancreatic Weight (g)	VvB (%)	β-Cell Mass (μL)	BrdU LI (%)
Wistar (n = 5)	1.09 ± 0.09	1.27 ± 0.57	10.14 ± 6.23	0.08 ± 0.04
Wistar + Voglibose (n = 5)	1.09 ± 0.08	1.17 ± 0.39	11.63 ± 4.56	0.13 ± 0.08
GK (n = 7)	0.99 ± 0.09	0.42 ± 0.09*	2.76 ± 1.09*	0.07 ± 0.03
GK+Voglibose (n = 7)	0.98 ± 0.08	0.84 ± 0.29†	6.93 ± 2.34†	0.04 ± 0.03

NOTE. Values are the mean ± SD.
Abbreviations: VvB, volume density of β cells.
*$P < .01$ *v* Wistar and Voglibose-treated Wistar rats.
†$P < .05$ *v* untreated GK rats.

Islet β-cell mass is determined by cell survival and proliferative activity. The proliferative activity of β cells was significantly ($P < .01$) less in GK rats versus control Wistar rats, being about 60% of the control level at 6 and 8 weeks of age. The decrease in β-cell mass (60% of controls) detected in 8-week-old GK rats may therefore be the result of poor proliferative activity of the β cells. The current results are in contrast to the finding by Ostenson et al,[21] who did not detect a significant reduction of the proliferative activity of β cells in GK rats at 4, 12, and 24 weeks of age compared with Wistar rats of corresponding age. Such discordant results may be attributable to the different methods used for detection of proliferative cells or to differences between the colonies used for these studies. For the detection of proliferative cells, we used immunodetection of BrdU uptake, whereas they performed measurements of autoradiographic grains of ^{3}H-thymidine

uptake on the β-cell nuclei.[21] They identified proliferative cells as cells containing more than 20 grains on the nuclei. The identification of positive cells is relatively easier using thin sections of double immunocytochemistry than autoradiographic sections. Movassat et al[15] reported an impaired development of β cells in fetuses of the GK rat. Such severely impaired proliferative activity of β cells in young GK rats may account, in part, for the lack of hyperplastic changes in the islets, which are characteristically found in other rat models of type 2 diabetes such as Otsuka-Long-Evans Tokushima diabetic rats,[20] db/db diabetic mice,[22] and Zucker diabetic fatty rats.[23]

Proliferative activity in β cells was drastically reduced after 12 weeks of age in both GK and control rats. A progressive reduction of the β-cell mass from 12 to 24 weeks in GK rats may also reflect a reduced survival of β cells. Voglibose treatment did not influence the proliferative activity of β cells in GK rats. It is therefore likely that the inhibition of β-cell depletion in treated GK rats is the consequence of improved β-cell survival achieved by Voglibose treatment. Hyperinsulinemia detected in 6- and 12-week-old GK rats suggests an early presence of insulin resistance, which may underlie the progressive damage to β cells in this model. In this setting, the decrease in plasma insulin in treated GK rats reflects an improvement of insulin resistance that was responsible for the reduction of the postprandial blood glucose elevation. It is thus possible that Voglibose in GK rats caused an improvement of insulin resistance, which then decreased blood glucose levels, which in turn resulted in preservation of islet β-cell mass. The significant loss of body weight in Voglibose-treated animals also may have contributed to the reduced insulin resistance. The loss of body weight in GK rats can be explained by malabsorption, because food intake was not altered in treated rats. The restriction of glucose uptake from the intestine by the inhibitor is mainly responsible for the malabsorption and increased excretion of feces or diarrhea.[24,25]

Prolonged hyperglycemia perturbs insulin secretion by changing the activity of glycolytic enzymes.[26,27] Nonenzymatic glycation together with free-radical formation suppresses the activity of insulin gene promoter and thereby impairs insulin synthesis.[28,29] Kaneto et al[30] have shown that reducing sugars generated under hyperglycemic conditions trigger oxidative stress through glycation and subsequent apoptotic cell death in cultured β cells. Hyperglycemia thus not only impairs the function of β cells but also leads to premature cell death. In fact, recent experimental studies provide evidence that an increased rate of apoptotic cell death contributes to the reduction of β-cell mass in other animal models of type 2 diabetes.[31,32] In the gerbil *Psammomys obesus*, when exposed to high glucose, β cells underwent apoptosis during development of diabetes.[31] An increased rate of apoptotic cell death, rather than a reduced proliferative activity, correlated more directly with the reduced β-cell mass in Zucker diabetic fatty rats.[32] Our recent studies on GK rats also demonstrate that augmented hyperglycemia further promotes β-cell reduction when animals are fed sucrose.[18]

The current finding that β-cell loss was mitigated in GK rats by Voglibose treatment is in keeping with previous preliminary findings of inhibition of islet fibrosis in treated GK rats.[33] Such beneficial effects of Voglibose on islet pathology may be mainly ascribed to a relief from the glucotoxicity-related oxidative stress that is likely enhanced in a sucrose-fed state.[34] Finally, the direct effects of Voglibose on β cells cannot be neglected. Further studies on an earlier effect of Voglibose on the proliferative activity of the β cell, weight loss, and lipid changes are required to address these issues.

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