Impaired Pancreatic β Cell Function in the Fetal GK Rat
Impact of Diabetic Inheritance
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Abstract
The Goto-Kakisaki (GK) rat is a genetic model of non–insulin-dependent diabetes. At 21.5 d of age we found that GK fetuses had an increased plasma glucose concentration, a decreased plasma insulin level, and a reduced pancreatic β cell mass. To investigate the β cell function during fetal life we used a hyperglycemic clamp protocol applied to the mothers, which allowed us to obtain a steady-state hyperglycemia in the corresponding fetuses. At variance, with Wistar (W) fetuses, plasma insulin concentration in GK fetuses did not rise in response to hyperglycemia. In contrast, GK fetal pancreas released insulin in response to glucose in vitro to the same extent as W fetal pancreas. Such a discrepancy between the in vivo and in vitro results suggests that the lack of pancreatic reactivity to glucose as seen in vivo is extrinsic to the fetal GK β cell. Finally, the importance of gestational hyperglycemia was investigated by performing crosses between GK and W rats. Fetuses issued from crosses between W mother and GK father or GK mother and W father had a β cell mass close to normal values and were still able to increase their plasma insulin levels in response to hyperglycemia in vivo. Our data suggest that hyperglycemia in utero does not influence the severity of the decrease of the β cell mass or the lack of the insulin secretory response to glucose in the fetal GK rat. Moreover they indicate that conjunction of GK genes originating from both parents is necessary in order for these defects to be fully expressed. (J. Clin. Invest. 1998. 101:899–904.)

Key words: β cell function • diabetic inheritance • fetal GK rat • pathogenesis • non–insulin-dependent diabetes

Introduction
The Goto-Kakisaki (GK)1 rat is a spontaneous model of non–insulin-dependent diabetes mellitus (NIDDM) (1). Adult GK rats, at least those of the Paris colony initiated in 1988 with progenitors issued from the original colony established by Goto et al. (1), displayed mild hyperglycemia, glucose intolerance, impaired glucose-induced insulin secretion (2), decreased β cell mass (3), hepatic glucose overproduction, and moderate peripheral insulin resistance in muscles and adipose tissues (4). Data obtained in 4-wk-old GK rats (5) or even younger GK pups (6) have highlighted the primacy of the β cell defects in the etiology of NIDDM in the GK model. Since we have shown in preliminary reports that GK fetuses had a basal glycemia higher than that of Wistar (W) control fetuses together with a drastically decreased total pancreatic β cell mass (7), it has become necessary to extensively investigate the β cell function at a very early developmental stage, i.e., during fetal life. This study was undertaken first to evaluate the fetal GK β cell function in conjunction with the diabetic pregnancy in the GK mother, on day 21.5 of gestation. For that purpose, we have determined the total pancreatic β cell mass and quantified the insulin secretion by the GK fetuses in vivo and in vitro. In vivo, β cell reactivity to glucose was studied in the GK fetuses using hyperglycemic clamp protocol applied to the mothers. In vitro, secretory response to glucose was studied using either GK fetal islets as obtained after 1-wk tissue culture or perfused GK pancreatic tissue fragments. Our second aim was to evaluate the impact of maternal hyperglycemia during gestation. For this purpose, two crosses were performed: W female with GK male and GK female with W male rats. Pancreatic β cell mass was determined in the corresponding 21.5-d-old fetuses, together with their glucose-induced insulin response in vivo.

Methods

Animals. Diabetic GK rats were obtained from our local colony initiated in Paris in 1988 (2) with progenitors issued from the original colony established by Goto et al. (1). W rats were used as control nondiabetic animals. All animals had free access to water and standard laboratory pelleted chow. Crosses were performed by caging a female (W or GK) with a male (W or GK) rat for one night (from 1700 to 900). The next morning, the presence of sperm in the vaginal smear was confirmed and this was taken as day 0.5 of pregnancy. On day 21.5 of gestation pregnant rats (W/W, GK/GK, W/GK, GK/W) and their corresponding fetuses (f-W/W, f-GK/GK, f-W/GK, f-GK/W) were used in this study. Experiments were performed without previous fasting of the mothers.

In vivo β cell reactivity to glucose under hyperglycemic clamp experiments. The hyperglycemic clamp technique used in this study was based on that developed by Kervran et al. (8). The pregnant rats were anesthetized with pentobarbital sodium (1 ml/kg body wt intraperitoneally; Sanofi Santé Animale, Sanofi, France). Body temperature was maintained at 37°C with heating lamps. A glucose infusion (Precidor, Bottmingen, Switzerland) was started 20 min later by administration of a 30% glucose solution into a maternal saphenous vein. A loading

Footnotes
1. Abbreviations used in this paper: GK, Goto-Kakisaki; NIDDM, non–insulin-dependent diabetes mellitus; W, Wistar.


1 Portions of this work were presented at the 32nd annual meeting of the European Association for the Study of Diabetes, 1–5 September 1996, in Vienna, Austria, and have appeared in abstract form (1996. Diabetologia. 38[Suppl. A]:A45.).

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infusion of 250–500 µl/min for 2 min was followed by a sustained infusion of 7–20 µl/min. Maternal blood samples were collected sequentially from the tail vein initially (time 0 min) and then every 5 min throughout the glucose infusion period. Plasma glucose concentration was immediately determined in order to adjust the flow rate of glucose infusion and obtain a maternal hyperglycemia nearly 2.5–3 g/litre. 20 min after the beginning of the glucose infusion, the plasma glucose levels of the mothers were stabilized and no more adjustment of the flow rate of glucose infusion was needed. This technique allowed us to obtain a steady-state hyperglycemia in mothers and their corresponding fetuses. On a same mother only four fetuses were taken at a given time point (0, 5, or 60 min after the beginning of the glucose infusion). Fetuses connected to the mother by their placenta and umbilical cord were successively exteriorized from the uterus, and blood samples were collected after section of the axillary vessels. Blood samples from mothers and fetuses were immediately centrifuged and the plasma was removed and stored at −20°C until assayed for insulin using the RIA described below.

**Fetal pancreases sampling.** Pancreases from f-W/W, f-GK/GK, and hybrids f-W/GK and f-GK/W were used for fetal islet preparation, perfusion experiments, insulin content determination, and/or pancreatic β cell mass evaluation. Pancreases were rapidly removed from the fetus and weighed. For insulin content determination fetal pancreases were homogenized, centrifuged at 4°C in an acid-alcohol solution, and the supernatant was stored at −20°C until insulin assay.

**Fetal islets preparation.** Fetal islets from f-W/W and f-GK/GK rats were prepared according to Helléström et al. (9) as described previously (10). At the end of the culture period (6–7 d), fetal islets were collected under a stereomicroscope and further processed for insulin release experiments.

**In vitro insulin release evaluation under static incubation.** In vitro insulin release in response to 2.8 mM glucose, 16.7 mM glucose, 10.0 mM leucine, 10.0 mM leucine + 10.0 mM glutamine, and 2.0 mM BaCl2 + 1.4 mM theophylline was evaluated in fetal islets as obtained after culture from f-W/W and f-GK/GK rats under static incubation as described in detail elsewhere (11).

**In vitro insulin release evaluation under perifusion experiments.** The perifusion technique used in this study was based on that first developed by Kikuchi et al. (12). Three minced pancreases were placed into one perifusion column (Pharmacia, Saint-Quentin en Yvelines, France) through which KRB, pH 7.4 supplemented with 5 mg/ml of BSA (fatty free acid, fraction V) (Sigma Chemical, St. Louis, MO), was driven by a peristaltic pump (minipuls 2; Gilson, Villiers-le-Bel, France). The KRB buffer was continuously gassed with 95% O2 and 5% CO2. After a 60 min prestimulatory period with KRB containing 2.8 mM glucose, the pattern of insulin release showed a stabilized baseline. The effluent of the last 30 min of this period was collected every minute and the samples obtained during this period were used for the determination of the basal rate of insulin release for each preparation. The basal period was followed by a 30 min stimulatory period with 16.7 mM glucose or the mixture 16.7 mM glucose + 5 µM forskolin. The effluent was collected with a fraction collector (Gilson) at a flow rate of 1 ml/min and frozen for storage at −20°C until assayed for insulin.

**β cell immunohistochemistry and morphometry.** After excision, whole fetal pancreases of f-W/W (n = 4), f-GK/GK (n = 4), f-W/GK (n = 5), and f-GK/W (n = 4) were immediately weighed and then fixed in aqueous Bouin’s solution overnight and embedded in paraplast. Each fetal pancreas was serially sectioned (7 µm) throughout its length and then mounted on slides. β cell immunohistochemistry and morphometry were performed as described previously in detail (3).

**Analytical techniques, data presentation, and statistical analysis.** In each experiment at least 2 groups of 20 fetal islets from W and GK rats were collected and DNA content was determined as described previously (11). Plasma glucose was determined with a glucose analyzer (Beckman Instruments, Inc., Fullerton, CA). Immunoreactive insulin in plasma, pancreases, islets, and perfusion effluent was estimated as described previously (13). The method allows the determination of 0.25 ng/ml, with a coefficient of variation within and between assays of 10%.

All results are expressed as means±SEM with the number of observations and significance of differences between the groups evaluated by Student’s t test or ANOVA (Fisher test).

**Results**

**Evolution of plasma glucose during gestation.** During gestation basal plasma glucose decreased in females (W/W) and the difference became statistically significant from day 13.5 of gestation. GK/GK females exhibited a higher plasma glucose level throughout the gestation period as compared to that of the W/W pregnant controls, but their pattern of plasma glucose decrease according to gestational age was similar (Fig. 1).

**Biological characteristics of the 21.5 d-old fetuses (Table 1).** Whatever the type of cross, the number of fetuses per litter was similar. Body weight of f-GK/GK was similar to that in f-W/W rats. By contrast, f-GK/GK exhibited a higher plasma glucose concentration and a lower plasma insulin level (P < 0.001) as compared to values in f-W/W controls. Their pancreas weights were significantly lower, and their pancreatic insulin content and β cell mass represented 36 and 38%, respectively, of that in f-W/W controls. In f-W/GK hybrids, plasma glucose concentration was significantly lower (P < 0.001) than in f-GK/GK and it was even lower (P < 0.05) than that in f-W/W controls. Their plasma insulin levels and pancreas weights were identical to f-W/W controls, but their β cell mass together with their pancreatic insulin content were slightly but significantly (P < 0.05) reduced (by 24 and 35%, respectively). f-GK/W
Table I. Biological Characteristics of the 21.5 d Postcoitum f-W/W, f-GK/GK, f-W/GK, and f-GK/W Fetuses

<table>
<thead>
<tr>
<th>Fetuses per litter</th>
<th>Body weight</th>
<th>Plasma Glucose</th>
<th>Plasma Insulin</th>
<th>Pancreas Weight</th>
<th>Insulin content</th>
<th>β cell mass</th>
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<tr>
<td></td>
<td>g</td>
<td>g/liter</td>
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<tr>
<td>f-W/W</td>
<td>10.2±0.6</td>
<td>4.7±0.1</td>
<td>0.72±0.02</td>
<td>21.8±2.4</td>
<td>25.9±0.8</td>
<td>0.183±0.009</td>
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<td>[45]</td>
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<tr>
<td>f-GK/GK</td>
<td>9.3±0.6</td>
<td>4.6±0.1</td>
<td>1.15±0.04†</td>
<td>6.3±0.4†</td>
<td>17.0±0.6†</td>
<td>0.066±0.002†</td>
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<td>[24]</td>
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<tr>
<td>f-W/GK</td>
<td>8.7±0.9</td>
<td>ND</td>
<td>0.62±0.02‡</td>
<td>21.4±2.1†</td>
<td>24.0±0.8‡</td>
<td>0.119±0.009§</td>
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<td>[18]</td>
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<td>[14]</td>
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<tr>
<td>f-GK/W</td>
<td>8.5±0.6</td>
<td>ND</td>
<td>0.93±0.02‡†</td>
<td>24.7±1.9†</td>
<td>25.1±1.0†</td>
<td>0.108±0.007‡</td>
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</table>

f-W/W, f-GK/GK, f-W/GK, and f-GK/W are fetuses (f) from crosses between W mother and W father, GK mother and GK father, W mother and GK father, and GK mother and W father, respectively. Values are means±SEM. Number of determinations is shown in parentheses. Fetuses were obtained from 4 to 22 different litters. *P < 0.05, †P < 0.001 vs. f-W/W, ‡P < 0.01, ‡‡P < 0.001 vs. f-GK/GK, ‡‡‡P < 0.001 vs. f-W/GK.

**Figure 2.** In vivo β cell reactivity to glucose by GK mothers on day 21.5 of pregnancy. Plasma glucose and plasma insulin in pregnant female rats on day 21.5 of gestation were determined during an in vivo hyperglycemic clamp protocol applied to the females for 60 min. Pregnant females used were W females crossed with W males (W/W, open circles, solid line), GK females crossed with GK males, (GK/GK, closed circles, solid line), W females crossed with GK hybrids exhibited a plasma glucose concentration higher than that found in f-W/W and f-W/GK, but lower than that found in f-GK/GK. Their plasma insulin level, pancreas weight, and β cell mass were normal. Nevertheless, their pancreatic insulin content was significantly lower (P < 0.01) than corresponding values in f-W/W.

In vivo β cell reactivity to glucose by GK mothers and by their corresponding fetuses on day 21.5 of pregnancy. During the hyperglycemic clamp experiments, a rapid rise of the plasma glucose concentrations was observed within the first 5 min, both in the pregnant rats (Fig. 2) and their fetuses (Fig. 3). Thereafter the plasma glucose levels were maintained at relatively constant levels, ranging 2.5–2.4 g/liter in mothers (Fig. 2) and 2.0–2.4 g/liter in fetuses (Fig. 3), respectively, over a period of 60 min. Under these experimental conditions, the plasma insulin increased very quickly in response to hyperglycemia, to levels similar in f-W/W and W/GK mothers, reaching a peak at 15 min and thereafter remaining elevated for 60 min (Fig. 2). By contrast, the plasma insulin increased in GK/GK or W/GK mothers remained very weak despite hyperglycemia similar to that in W/W mothers (Fig. 2).

After only a 5-min exposure to hyperglycemia, f-W/W (P < 0.05) plasma insulin levels increased significantly and were still able to maintain a high level after 60 min (P < 0.05) (Fig. 3). By contrast, plasma insulin in the f-GK/GK fetuses remained at the basal level despite hyperglycemia similar to that in f-W/W (Fig. 3). Lack of reactivity to glucose in the f-GK/GK fetuses was observed after 5 min as well as after 60 min of hyperglycemic clamp experiments, a rapid rise of the plasma glucose concentrations was observed within the first 5 min, both in the pregnant rats (Fig. 2) and their fetuses (Fig. 3). Thereafter the plasma glucose levels were maintained at relatively constant levels, ranging 2.5–2.4 g/liter in mothers (Fig. 2) and 2.0–2.4 g/liter in fetuses (Fig. 3), respectively, over a period of 60 min. Under these experimental conditions, the plasma insulin increased very quickly in response to hyperglycemia, to levels similar in f-W/W and W/GK mothers, reaching a peak at 15 min and thereafter remaining elevated for 60 min (Fig. 2). By contrast, the plasma insulin increased in GK/GK or W/GK mothers remained very weak despite hyperglycemia similar to that in W/W mothers (Fig. 2).

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The incremental insulin response to glucose over the 60-min stimulation period was clearly lower ($P < 0.001$) in f-GK/GK group than in the f-W/W group (1.0 ± 0.3 ng/ml, $n = 20$ and 22.1 ± 3.8 ng/ml, $n = 32$ respectively). By contrast in the f-W/GK and f-GK/W hybrids (Fig. 3), the incremental insulin responses to glucose over the 60-min stimulation period were similar to that in the f-W/W group (18.4 ± 3.7 ng/ml, $n = 25$ and 17.2 ± 2.3 ng/ml, $n = 31$ in f-W/GK and f-GK/W groups, respectively).

In vitro insulin release in response to glucose and other secretagogues by isolated GK fetal islets (Fig. 4). No statistically significant difference between f-W/W and f-GK/GK fetal islets was observed with regard to their mean DNA content (5.5 ± 0.3 ng/islet, $n = 45$ and 5.1 ± 0.3 ng/islet, $n = 38$, respectively). Therefore, the measurements of the secretory function were expressed on a per islet basis.

Basal insulin release by the fetal islets was significantly lower ($P < 0.001$) in f-GK/GK islets as compared to f-W/W islets. The f-GK/GK islets also displayed a clear decrease ($P < 0.001$) of their secretory response to 16.7 mM glucose as compared to f-W/W islets. However, when the insulin response was expressed as percentage of basal release, the increment of the glucose-induced insulin output became similar in islets from

**Figure 3.** In vivo β cell reactivity to glucose by GK fetuses on day 21.5 of pregnancy. Plasma glucose and plasma insulin in day 21.5 p.c. fetuses were determined during an in vivo hyperglycemic clamp protocol applied to their mothers for 60 min. Fetuses used were issued from crosses between W mother and W father (f-W/W, open circles, solid line), GK mother and GK father (f-GK/GK, closed circles, solid line), W mother and GK father (f-W/GK, open circles, dotted line), GK mother and W father (f-GK/W, closed circles, dotted line). Values are means ± SEM of 10–46 individual observations in each group of fetuses. *$P < 0.05$ as compared to respective basal plasma insulin (0 min) in f-W/W. †$P < 0.05$ as compared to respective basal plasma insulin (0 min) in f-W/GK. §$P < 0.05$ as compared to respective basal plasma insulin (0 min) in f-GK/W.

**Figure 4.** In vitro insulin release in response to glucose and other secretagogues by isolated GK fetal islets as obtained after culture. Isolated fetal islets were obtained after a 6–7-d tissue culture period starting from pancreases from fetuses issued from crosses between W mother and W father (open bars, f-W/W) and GK mother and GK father (closed bars, f-GK/GK). Data were expressed as absolute values (top) or as percentage of basal value determined at 2.8 mM glucose in each group of islets, respectively (bottom). Each bar represents mean ± SEM for 32–42 batches of islets obtained from 6 distinct islet preparations. *$P < 0.01$, **$P < 0.001$ as compared to the respective values obtained at 2.8 mM D-glu or 10 mM L-leuc. Glu, Glucose; Leu, Leucine; and Theoph, Theophylline. The experiments related to the effect of leucine or glutamine were carried out in the absence of glucose. When the combination of Ba$^{2+}$ and theophylline was tested, both glucose and calcium were omitted in the incubation medium.
f-W/W and f-GK/GK (156±17, n = 42 and 130±10%, n = 32, respectively). The insulin release induced by 10 mM leucine or 10 mM leucine + 10 mM glutamine was similar in the two groups of fetal islets. In response to the combination of 2 mM Ba²⁺ and 1.4 mM theophylline in the absence of CaCl₂, the insulin release by f-GK/GK islets was significantly decreased as compared to f-W/W islets and again, when the results were expressed as the percentage of basal release, the stimulating mixture amplified the insulin release to the same extent as in control fetal islets.

In vitro kinetics of the insulin release by pieces of GK fetal pancreases (Fig. 5). The insulin release by pancreases from f-GK/GK under basal conditions (2.8 mM glucose) was decreased threefold as compared to that in f-W/W. After exposure to high (16.7 mM) glucose concentration, insulin levels in the f-W/W group rose rapidly and remained elevated. This pattern of the insulin release by fetal rat pancreases is in accordance with previous studies (14, 15). Absolute values of insulin release in response to 16.7 mM glucose were decreased in f-GK/GK as compared to fetal pancreas from f-W/W. However, when the insulin response was expressed as percent of basal release, the increment of glucose-induced insulin output was found similar in the two groups. When 5 μM forskolin was added to the medium containing 16.7 mM glucose, insulin release increased dramatically in f-W/W. Absolute values of insulin release were lower in f-GK/GK pancreases compared to f-W/W, but again when the results were expressed as the percent of basal value, forskolin amplified the insulin release to the same extent in the two groups.

Discussion

Previous studies have shown that pregnancy causes a decreased maternal basal plasma glucose concentration in normal rats (16, 17) as well as in rats with mild NIDDM (18). The present observation of a decreased plasma glucose level during gestation in W/W mothers and also in G/K/GK mothers supports these results. Nevertheless, as compared to W/W mothers, the basal plasma glucose level observed throughout pregnancy in G/K/GK mothers remained significantly more important.

In agreement with previous reports (19) plasma glucose concentration on day 21.5 of gestation was lower in W/W fetuses than in their mothers. Such was also the case in G/K/GK fetuses. The relative hyperglycemia in G/K/GK mothers was responsible for the slightly but significantly enhanced plasma glucose level in the corresponding fetuses. It is known that manifest experimental diabetes in rat (hyperglycemia = 30 mM) induced before pregnancy causes severe growth retardation and decreased pancreatic insulin stores and β cell mass in the fetuses, while fetuses from subdiabetic mothers (hyperglycemia = 6 mM) have been reported to exhibit increased pancreatic insulin content and β cell mass and to be identical to fetuses from normoglycemic mothers in all other respects (16). In the G/K/GK fetuses the pattern is strikingly different since basal hypoinsulinemia together with very low pancreatic insulin stores and β cell mass are found.

The reduced β cell mass in the G/K/GK pancreas detectable as early as day 21.5 of gestation is known to persist at least until adult age (3). Therefore, one important conclusion of this study is that a reduction of total β cell mass has to be considered as a primary feature in the pathological sequence leading to diabetes in GK rats, at least in those originating from the Paris colony.

Although the GK rat is a genetic model of NIDDM (1), environmental factors such as gestational hyperglycemia may contribute to the transmission of diabetes. Studies in the lineage of pregnant rats injected with STZ or infused with glucose have demonstrated that mild hyperglycemia induced in the mother during pregnancy leads to persistent impairment of glucose tolerance and insulin secretion in the adult progeny regardless of genetic interference (20–22). In the GK model it has been proposed as an effect of maternal inheritance on the severity of diabetes in the adult (23). This is probably not due to a genetic defect in mitochondrial DNA (10, 23), but rather has been ascribed to intrauterine factors acting during the gestational period. However this conclusion has not been confirmed in another GK colony (6).
factors, such as gestational hyperglycemia, on β cell mass has been investigated in the this study by performing crossing experiments between diabetic GK and normoglycemic W rats. Results concerning fetuses issued from W/GK or GK/W mothers indicate that their pancreatic insulin content and β cell mass, albeit slightly decreased, are close to normal values (f-W/W). Concerning the β cell mass, the absence of significant differences when comparing the respective impact of maternal or paternal origin of the GK genes suggests that hyperglycemia in utero does not significantly influence the severity of the alteration of the β cell mass in the f-GK/GK rat. Moreover the conjunction of GK genes originating from the mother and the father is necessary in order for the defect related to β cell mass to be fully expressed.

We showed in this study that during hyperglycemic clamp conditions, f-W/W rats significantly increased their plasma insulin levels in response to the imposed hyperglycemia in vivo, which is consistent with earlier observations by Kervran et al. (8). The possibility that the rise of fetal plasma insulin concentration observed in f-W/W could be due to placental transfer of maternal hormone is unlikely since it is well documented that insulin does not cross the placenta in the rat (24, 25). Under the same hyperglycemic clamp conditions, a defective β cell reactivity to glucose was observed in vivo in f-GK/GK rats since fetal plasma insulin concentration did not rise in response to the imposed hyperglycemia. This is a main point in this study since it demonstrates for the first time that the impairment of the β cell function in vivo is a very early event in the diabetic GK rat.

We then attempted to identify the mechanism behind the in vivo decreased β cell response to glucose in f-GK/GK rats. At first glance, this defect might seem related to the decreased β cell mass observed in their pancreas. However this possibility can be eliminated by taking into account the ability of the fetal pancreas from f-GK/GK to release insulin in response to 16.7 mM glucose during in vitro perfusion experiments. Therefore glucose is indeed able to act directly on the fetal β cells to stimulate insulin secretion in the f-GK/GK rats. Such a discrepancy between the in vivo and in vitro results suggests that the lack of pancreatic reactivity to glucose as seen in vivo is extrinsic to the fetal GK β cell. The possibility that a maternal and/or placental factor released during hyperglycemia or that chronic mild fetal hyperglycemia per se inhibits the fetal insulin release seems unlikely since the β cell reactivity to glucose in vivo was found normal in GK/W fetuses.

In summary, the results of this study showed that: (a) a lack of pancreatic reactivity to glucose is detectable in vivo in the GK rat as early as fetal age. Such a defect together with a reduced β cell mass represent primary features of this rat model of NIDDM. (b) These features do not seem related to maternal hyperglycemia. Moreover, the conjunction of both maternal and paternal genes are necessary in order for these defects to appear in the GK model. (c) The lack of pancreatic reactivity to glucose is not intrinsic to the GK fetal β cell.

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References