Rat maternal diabetes impairs pancreatic β-cell function in the offspring

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Han J, Xu J, Long YS, Epstein PN, Liu YQ. Rat maternal diabetes impairs pancreatic β-cell function in the offspring. Am J Physiol Endocrinol Metab 293: E228–E236, 2007. First published March 27, 2007; doi:10.1152/ajpendo.00479.2006.—It has been shown that maternal diabetes increases the risk for obesity, glucose intolerance, and Type 2 diabetes mellitus in the adult life of the offspring. Mechanisms for these effects on the offspring are not well understood, and little information is available to reveal the mechanisms. We studied the effect of maternal diabetes on β-cell function in the offspring of streptozotocin (STZ)-induced diabetic rat mothers (STZ-offspring). STZ-offspring did not become glucose intolerant up to 15 wk of age. At this age, however, insulin secretion was significantly impaired, as measured by in vivo and in vitro studies. Consistent with these changes, islet glucose metabolism and some important glucose metabolic enzyme activities were reduced. No significant changes were found in islet morphological analysis. These data indicate that β-cell function is impaired in adult STZ-offspring; these changes may contribute to the development of type 2 diabetes mellitus in adulthood.

Intrauterine exposure to hyperglycemia is also associated with a higher prevalence of impaired glucose tolerance in adolescence (44) and with an excess of obesity, especially during the first 20 yr of life (10, 40, 41, 45). Systematic prevention of hyperglycemia and impaired glucose tolerance in pregnant women has significantly decreased the prevalence of diabetes mellitus in their children (12). Studies of Pima Indians provide further indication for a role of maternal diabetes in utero: NIDDM is present by age 20–24 yr in 45.5% of the offspring of diabetic mothers but only in 8.6% and 1.4% of respective offspring of prediabetic or nondiabetic mothers (39). Also in Pima Indians, offspring born after their mothers have developed NIDDM have a 3.7-fold higher risk of developing diabetes than those born before their mother have developed diabetes (11).

Animal studies suggest that an adverse influence of maternal diabetes on fetal pancreatic function (4) may contribute to development of NIDDM. Maternal diabetes alters the morphology, number, and size of offspring islets and the ability of the offspring islets to respond to the challenge of pregnancy (5). Insulin secretion has been shown to be abnormal in islets from offspring of diabetic mothers (3). Glucose metabolism is the primary regulator of β-cell function (33, 34). However, no studies have examined the changes produced by maternal diabetes on glucose metabolism of offspring islets. In the present study, we have examined β-cell insulin secretion and glucose metabolism in the offspring of streptozotocin (STZ)-diabetic mothers (STZ-offspring). Our results suggest that reduction in β-cell glucose metabolism contributes to the development of NIDDM in offspring during adulthood.

MATERIALS AND METHODS

Animals. The principles of animal laboratory care under the guidelines of both the National Institutes of Health and the University of Louisville’s Animal Care Committee were strictly followed. All animals were housed in the Animal Care Facility at the University of Louisville and maintained at 25°C with a 12:12-h light-dark cycle. Eight-week-old female Sprague-Dawley rats (Taconic, Germantown, NY) were divided into three groups: 1) STZ group, 2) control group, and 3) foster mother group. The STZ group was subjected to STZ (Sigma; 50 mg/kg body wt, made freshly in 10 mmol/l citrate buffer, pH 4.5, injected via jugular vein under anesthesia) to produce a maternal diabetic model. The control group and foster mother group were injected with citrate buffer only. Blood glucose levels and body weight were measured daily after injection until the STZ group became hyperglycemic (blood glucose of 400–450 mg/dl). On day 5 of being diabetic, the diabetic animals and the other two groups were mated with normal 8-wk-old Sprague-Dawley males. If the presence of vaginal plug was found in the morning, this was defined as day 1 of pregnancy. Fed blood glucose levels and body weight of mothers were measured weekly (last days of pregnancy week 1 and week 2; in pregnancy week 3, we measured these parameters at day 5 of the last pregnant week) during pregnancy and after delivery (within 12 h after delivery). If blood levels during pregnancy were lower than 350 mg/dl or higher than 550 mg/dl, these females were excluded from the STZ group. Numbers of littermate and birth weights of the offspring were recorded. The offspring from groups 1 and 2 were used for this project and are referred to as STZ-offspring and controls. After birth, all the STZ-offspring were fostered to group 3 females who delivered pups at the same day to exclude the effect from diabetic breast-feeding during lactation; then the diabetic mothers and the offspring of foster mothers were killed. Offspring body weight and blood glucose were measured weekly. Blood was obtained via tail snipping, and blood glucose was measured with a portable glucose meter (Johnson & Johnson). All offspring were killed at 15 wk of age for islet isolation and islet morphological analysis. Male db/+ (control) and db/db Type 2 diabetic mice were purchased from Jackson Laboratory (Bar Harbor, ME); they did not receive any treatment. All animals in this project were fed a basal diet.

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Glucose tolerance test and insulin assay. After an overnight fast, rats were administered glucose (1.0 g/kg body wt) intraperitoneally. Blood samples, obtained by tail snipping using a scalpel, were obtained at various time points (0, 15, 30, 60, and 120 min), and duplicate glucose readings were taken with a FastTake glucose meter (Lifescan). Serum insulin levels at 0 and 30 min were determined with the Ultrasensitive rat insulin-ELISA test kit (Mercodia).

Islet isolation. Islets were isolated from offspring by an adaptation of the Gotot method (17): pancreatic duct infiltration with collagenase, histopaque gradient separation, and hand picking. Before measurements were made, islets were cultured for 1 h at 37°C in humidified air and 5% CO2 in RPMI 1640 supplemented with 5.5 mmol/l glucose and 10% newborn calf serum, 2 mmol/l glutamine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin (all from GIBCO, Grand Island, NY).

Insulin secretion. Ten isolated islets (duplicate) were separately cultured in a 5-ml vial with 1 ml KRB (Krebs-Ringer bicarbonate buffer supplemented with 10 mmol/l HEPES, pH 7.4, and 0.1% BSA, 2% rat serum, and 0.25% (vol/vol) Triton X-100. Supernatant was added to 1 ml of buffer that contained (in mmol/l) 50 Tris-HCl, pH 8.0, 1 EDTA, 2.5 DTT, 5 mmol/l ammonium sulfate, 2 MgCl2, 1 ATP, 1 fructose 6-phosphate, and 0.16 NADH, as well as 0.4 U/ml aldolase and 2.4 U/ml triose phosphate isomerase plus 0.8 U/ml glycerophosphate dehydrogenase mixture (Boehringer Mannheim, Indianapolis, IN) in a quartz cuvette. The NADH metabolized was assessed at 340 nm by spectrophotometry. PFK activity was calculated based on 1 μmol fructose 1,6-diphosphate = 2 μmol NADH consumed.

Pyruvate carboxylase activity assay. Pyruvate carboxylase (PC) activity was measured according to the method of MacDonald et al. (30). Ten microliters of islet homogenate (5 μg protein) were incubated in 40 μl of reaction buffer (in mmol/l: 2 Na3ATP, 2.5 NaHCO3, 0.15 ml of extraction buffer containing 15 mmol/l K2PO4, pH 7.0, 100 mmol/l KCl, 2 mmol/l EDTA, 2 mmol/l PMSF, 0.2 mg/ml leupeptin, and 50 μg/ml aprotinin). After centrifugation at 12,000 g for 15 min at 4°C, pyruvate-phosphokinase (PFK) activity was measured by previously described method (25). Supernatant was added to 1 ml of buffer that contained (in mmol/l) 50 Tris-HCl, pH 8.0, 1 EDTA, 2.5 DTT, 5 mmol/l ammonium sulfate, 2 MgCl2, 1 ATP, 1 fructose 6-phosphate, and 0.16 NADH, as well as 0.4 U/ml aldolase and 2.4 U/ml triose phosphate isomerase plus 0.8 U/ml glycerophosphate dehydrogenase mixture (Boehringer Mannheim, Indianapolis, IN) in a quartz cuvette. The NADH metabolized was assessed at 340 nm by spectrophotometry. PFK activity was calculated based on 1 μmol fructose 1,6-diphosphate = 2 μmol NADH consumed.

Values are means ± SD; n = no. of animals. Weeks 1, 2, and 3 indicate that blood glucose levels were measured at the end of weeks 1, 2, and 3 of pregnancy (last day of 1st and 2nd pregnant weeks; in the 3rd week of pregnancy, we measured glucose levels at day 5 of the last pregnant week). Duration of pregnancy was 20–21 days in both control and streptozotocin-treated (STZ) groups. Females in the STZ-diabetic group, if their blood glucose levels during pregnancy were >550 mg/ml or <350 mg/dl, were excluded from this project.

Table 1. Fed blood glucose levels and body weight gain in Sprague-Dawley rat mothers during pregnancy

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Before Mating</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>After Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control mothers</td>
<td>130±10</td>
<td>134±11</td>
<td>111±6</td>
<td>109±10</td>
<td>122±18</td>
</tr>
<tr>
<td>STZ-diabetic</td>
<td>432±35</td>
<td>459±37</td>
<td>490±32</td>
<td>527±12</td>
<td>417±79</td>
</tr>
<tr>
<td>P value vs. controls</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body weight, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control mothers</td>
<td>168±7</td>
<td>183±5</td>
<td>238±10</td>
<td>341±17</td>
<td>254±10</td>
</tr>
<tr>
<td>STZ-diabetic</td>
<td>159±9</td>
<td>175±13</td>
<td>203±17</td>
<td>277±29</td>
<td>213±25</td>
</tr>
<tr>
<td>P value vs. controls</td>
<td>0.052</td>
<td>0.211</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
stopped by addition of 50 μl of 10% TCA followed by overnight air drying and liquid scintillation counting.

Islet morphology, immunohistochemistry, and β-cell mass. Immunohistochemistry was carried out as previously described (19, 24). Four 15-wk-old controls, four STZ-offspring, one db/+ (control) mouse, and one db/db mouse were killed for these experiments. Pancreas tissues were fixed in 4% formaldehyde and embedded in paraffin. Serial sections were cut at 5 μm. For immunostaining, slides were blotted, treated with 3% hydrogen peroxide, and then treated with Power Block (BioGenex). After three washes in PBS, sections were treated with primary antibody [rabbit anti-guinea pig insulin antibody (BioGenex), diluted 1:100; rabbit anti-glucagon (BioGenex), diluted 1:1,000]. After four washes in PBS, slides were incubated with the appropriate biotin-labeled secondary antibody. This was followed by peroxidase-labeled streptavidin (BioGenex). Slides were developed with 3-amino-ethylcarbazone as chromagen. Sections were counterstained with Mayer’s hematoxylin for 1 min. For each pancreas, three sections were spaced at least 40 and 160 μm apart, which were covered systematically by accumulating images from nonoverlapping fields captured with a digital camera (Nikon E600; Nikon, Melville, NY). The proportion of β-cell or α-cell surface area vs. surface area of the whole pancreas was determined by digitally imaging at least three nonoverlapping 3.89-mm² fields per section. β-Cell mass was calculated for each animal as the average proportional β-cell surface area multiplied by pancreas weight.

Data presentation and statistical methods. All data are expressed as means ± SD. Unless otherwise stated, the listed n values represent the number of experiments performed. Comparisons between control and STZ-offspring groups were performed by Student’s t-test. A value of P < 0.05 was considered significant.

RESULTS

Blood glucose levels and body weight of the STZ-diabetic pregnant rats. Blood glucose levels and body weight gain of the pregnant rats are shown in Table 1. Fed blood glucose levels were not significantly changed during pregnancy in the

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Newborn Birth Weight, g</th>
<th>Newborn Number</th>
<th>Newborns That Died After Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no.</td>
<td>No. per litter</td>
<td>Total no.</td>
</tr>
<tr>
<td>Control mothers (5)</td>
<td>6.5±0.6</td>
<td>73</td>
<td>14.6±1.1</td>
</tr>
<tr>
<td>STZ-diabetic mothers† (5)</td>
<td>5.3±0.7*</td>
<td>37</td>
<td>7.4±3.6</td>
</tr>
<tr>
<td>P value</td>
<td>0.005</td>
<td></td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of animals. *Body weights of littermates that died were not included. †Four diabetic females in the STZ-diabetic mother group (total animal no. was 9) could not be pregnant, but all control females were pregnant. All newborns were spontaneously delivered.

Fig. 1. Body weight gain in male (A) and female (B) rat offspring. Values are means ± SD; n = 10 animals. P < 0.05 between control rats (○) and offspring of streptozotocin (STZ)-induced diabetic rat mothers (STZ-offspring) (●) at each time point.

Fig. 2. Blood glucose levels in male (A) and female (B) controls (○) and STZ-offspring (●). Values are mean ± SD; n = 10 animals. *P < 0.05 vs. controls.
control group; however, hyperglycemia in the STZ group reached the highest levels on the last day (day 20 or 21) of pregnancy; after delivery, blood glucose levels returned to levels shown before pregnancy. Body weight gain in the STZ group was significantly lower at weeks 2 and 3 during pregnancy and after delivery than that shown in the control group.

Newborns, birth weight, body weight gain, and blood glucose levels in the offspring. The numbers of newborns in each litter in the STZ group were significantly lower than those in the control group (Table 2). Although all neonates were spontaneously delivered, on average 1.6 pups/litter died soon after delivery. Birth weight and body weight gain in STZ-offspring were significantly lower ($P < 0.01$) than those in the control group (Table 2 and Fig. 1). The STZ-offspring were initially hypoglycemic but became normoglycemic by weaning and remained normal up to at least 15 wk of age (Fig. 2).

Insulin secretion in the STZ-offspring. To assess glucose tolerance and insulin secretion in these animals, we gave animals an intraperitoneal injection of 1 mg glucose/g body wt.

Neither fasting glucose nor glucose tolerance curves were significantly different in control and STZ-offspring up to 15 wk of age (Fig. 3). Fasting insulin levels (Fig. 4; at 0 min) in STZ-offspring were also identical to controls, suggesting that β-cell function was not markedly damaged in these offspring. However, when plasma insulin levels were measured 30 min after a glucose challenge in rats 10 or 15 wk old, it was found that circulating insulin levels were reduced (Fig. 4). This reduction for STZ-offspring was significant in 10-wk-old females and 15-wk-old males and females, indicating that the β-cell response to glucose was impaired. To confirm this change, we isolated islets from 15-wk-old control and STZ-offspring to perform insulin secretion assays in the presence of low (2.8 mM) and high (16.7 mM) glucose. As shown in Fig. 5, insulin secretion in both male and female offspring of diabetic mothers was not changed during low-glucose stimulation but was significantly reduced after high-glucose stimulation. This result is consistent with the reduced circulating insulin found in rats challenged by glucose injection (Fig. 4).

![Glucose tolerance tests](http://ajpendo.physiology.org/)

**Fig. 3.** Glucose tolerance tests in 5-wk-old (A and B), 10-wk-old (C and D), and 15-wk-old (E and F) male (A, C, and E) and female (B, D, and F) offspring. Values are means ± SD; n = 6–9 animals.
Glucose utilization and glucose oxidation in islets isolated from the STZ-offspring. Because glucose metabolism regulates insulin secretion, we measured [5-3H]glucose utilization and [U-14C]glucose oxidation in isolated islets of 15-wk-old rats. As shown in Fig. 6, both glucose utilization and oxidation were significantly reduced in the islets of STZ-offspring. Basal metabolism was the same in both groups when measured at 2.8 mM glucose, but stimulated metabolism at 8 or 16.7 mM glucose was clearly and significantly reduced in the STZ-offspring. This result is consistent with insulin secretion data shown in Figs. 4 and 5.

Glucose metabolic enzyme activities in islets isolated from the STZ-offspring. To better understand the significant reduction in glucose utilization and oxidation and insulin secretion, we measured metabolic enzyme activities in the islets of 15-wk-old control and STZ-offspring for enzymes that may

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**Fig. 4.** Insulin levels in 5-wk-old (A and D), 10-wk-old (B and E), and 15-wk-old (C and F) male (A–C) and female (D–F) offspring. 0 min, Overnight fasted blood insulin levels; 30 min, insulin levels 30 min after 1 mg/g body wt glucose ip injection. C, control; M, male; F, female. Values are means ± SD; n = 6–9 animals. *P < 0.05 vs. controls.

**Fig. 5.** Insulin secretion in islets isolated from 15-wk-old male (A) and female (B) offspring. Values are means ± SD; n = 4 animals. *P < 0.01 vs. controls.
regulate glucose metabolism and insulin secretion in the β-cell. PFK activity in β-cells is related to oscillatory insulin secretion (49), and it is reduced in the islets of Type 2 diabetic mice (21). PC (28, 30) and PDH (52, 53) activities are suppressed during the development of NIDDM. As shown in Fig. 7, islet PFK, PC, and PDH activities were significantly reduced in STZ-offspring. These changes are consistent with and may underlie the impaired insulin secretion (Figs. 4 and 5) and islet glucose metabolism (Fig. 6) in the STZ-offspring.

Islet characteristics in 15-wk-old STZ-offspring. Because β-cell dysfunction was demonstrated in 15-wk-old STZ-offspring, to know whether islet β-cell characteristics were affected by maternal diabetes, we measured protein, DNA, and insulin contents in isolated islets and performed islet morphological analysis in pancreas sections in 15-wk-old male STZ-offspring. As shown in Table 3, islet protein, DNA, and insulin contents showed no significant changes. In pancreas sections, there were also no significant differences between controls and STZ-offspring in islet insulin and glucagon staining (Fig. 8), islet size, β-cell or α-cell percentage, and β-cell mass (Table 3). We used Type 2 diabetic db/db mouse as a positive control to show islet abnormalities and stained pancreas sections from age-matched db/+ (control) and db/db mice. The density of insulin staining in db/db mouse islet (Fig. 8I) was much lower than that shown in control db/+ mouse islet (Fig. 8E), and this reduction is consistent with insulin contents measured in isolated db/db (24 ± 7 ng/islet, n = 4) mouse islets (control db/+ islet: 47 ± 9 ng/islet, n = 4). Staining and distribution of glucagon in db/db mouse islets were also markedly different from results shown in control islets, albeit the same as results in control rat offspring. When we compared islet micrographs of STZ-offspring with those of db/+ mouse and control offspring, it is clear that islet insulin and glucagon staining and islet features in STZ-offspring were not significantly different from results shown in the control groups. These data indicate that maternal diabetes mainly affected β-cell function but not islet morphological features and insulin synthesis in 15-wk-old offspring.

**DISCUSSION**

Numerous previous reports have demonstrated that maternal diabetes increases the risk for glucose intolerance and NIDDM in the later life of the offspring (10, 16, 32, 39, 40, 44, 50). This study focused on β-cell function of STZ-offspring. Our results from the pancreatic islets of STZ-offspring support the following physiological observations: 1) glucose-stimulated insulin secretion was reduced during glucose tolerance tests and in

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**Fig. 6.** Glucose utilization (A) and oxidation (B) in islets isolated from 15-wk-old male offspring. Data are means ± SD; n = 4. *P < 0.05 vs. controls.

**Fig. 7.** Phosphofructokinase (PFK; A), pyruvate carboxylase (PC; B), and active pyruvate dehydrogenase (PDH; C) activities in islets isolated from 15-wk-old male offspring. Values are means ± SD; n = 4 animals. Enzyme activity assay in each sample was duplicated. *P < 0.01 vs. controls.
isolated islets, 2) β-cell glucose metabolism, which is essential for insulin secretion, was significantly reduced, and 3) activities of several enzymes of glucose metabolism were reduced. 4) No significant changes of islet characteristics were found in STZ-offspring islets. These data indicate that reduced β-cell glucose metabolism contributes to impaired insulin secretion in the STZ-offspring.

Insulin secretion is regulated by glucose metabolism (27, 29). A widely accepted hypothesis is that an increased ATP-to-ADP ratio leads to more insulin secretion (7, 43). Faster glucose metabolic rate elevates ATP production and increases the ATP-to-ADP ratio. Conversely, reduced glucose metabolism should suppress insulin secretion. Our present data showed that glucose utilization and oxidation were significantly decreased in adult STZ-offspring. Glucose metabolic enzyme activities, including PFK, PC, and PDH, which govern glucose metabolism and fluxes, were also significantly reduced. Lower activities of these enzymes would contribute to impaired β-cell glucose metabolism. The reduction in glucose metabolism may explain the decline in insulin secretion observed in this and prior studies (3, 5). In addition, islet morphological data did not demonstrate significant changes, indicating that insulin synthesis and storage in β-cells in 15-wk-old STZ-offspring were not significantly affected by maternal diabetes. Therefore, a reduction in metabolic enzyme activities may be a major factor to cause β-cell dysfunction in STZ-offspring.

Previous reports have also demonstrated biological changes in β-cells from offspring of mothers with mild and severe diabetes. In offspring of mildly diabetic mothers, moderate hyperglycemia stimulates the development of fetal islets, resulting in an increase in the number of fetal β-cells and biosynthetic activity (36, 38). However, when maternal diabetes is severe, fetal β-cell number was shown to be reduced and β-cells were shown to be degranulated (1, 37). Another study (3) examined insulin secretion in islets isolated from adult offspring of STZ-diabetic mothers. That study reported that mild maternal diabetes reduced adult islet insulin secretion, but severe maternal diabetes produced an increase in adult islet insulin secretion. Our results for insulin secretion in offspring from mothers with severe diabetes were most similar to the results obtained with mild diabetic mothers. We can only speculate why the secretion results for severe diabetes obtained in the previous studies were different from our results. The previous studies gave STZ after pregnancy began. We chose to treat the animals with STZ before pregnancy to completely eliminate exposure of the fertilized embryo to the toxin STZ. This different timing of diabetes induction or different exposure to STZ may explain the divergent results. The deficit that we observed in isolated islet secretion was consistent with the results that we obtained in vivo. They were also concordant with the metabolic results obtained from isolated islets.

Maternal diabetes causes short- and long-term complications in the offspring. It has been shown that tight glycemic control before and during pregnancy can prevent, for example, congenital malformations, birth trauma, and neonatal respiratory distress syndrome (2, 8). Short-term neonatal complications, such as hypoglycemia, hypocalcemia, hypomagnesemia, hy-

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Table 3. General characteristics of islets in 15-wk-old STZ-offspring and controls

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Isolated Islet</th>
<th>Ilet Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein Content, µg/islet</td>
<td>DNA Content, ng/islet</td>
</tr>
<tr>
<td>Control (4)</td>
<td>0.69±0.25</td>
<td>17.5±2.3</td>
</tr>
<tr>
<td>STZ-offspring (4)</td>
<td>0.72±0.27</td>
<td>18.4±2.8</td>
</tr>
<tr>
<td>P value</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of animals.

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Fig. 8. Insulin and glucagon staining in the islets of control (A–D), STZ-offspring (F–I), db/+ control (E), and db/db Type 2 diabetic mice (J). db/db mouse islet (J) served as a positive control. Micrographs (magnification of ×40) are typical of appearance in the islets. Each micrograph shows a representative islet that was from one section of separate mouse pancreas. Green color indicates insulin staining; red color indicates glucagon staining.
peribilirubinemia, and polycythemia, are mainly related to fetal hyperinsulinemia, hyperoxemia, and prematurity (2, 13). In mothers with hyperglycemia, high glucose can be transferred to the fetus, inducing fetal hyperglycemia; this leads to fetal hyperinsulinemia (6). Chronic fetal hyperinsulinemia causes elevated metabolic rates and oxygen consumption, leading to fetal hypoxemia (2). Fetal hyperinsulinemia promotes glucose usage, leading to hypoglycemia (2, 9). Short-term complications were observed in STZ-offspring. Figure 2 shows that neonatal hypoglycemia appeared in these offspring, and the neonatal hypoglycemia lasted up to age 3 (male) or 4 (female) wk. Other short-term complications shown in STZ-offspring include low birth weight (Table 1) and neonate death and reduced littermate numbers (Table 2). Long-term complications include increased rate of obesity, impaired glucose tolerance or NIDDM in adulthood. β-Cell dysfunction in STZ-offspring in this report is one of the long-term complications, and it may contribute to the development of NIDDM in adulthood. Other long-term complications, such as impaired glucose tolerance or clinical diabetes and obesity, were not present in the STZ-offspring at 15 wk of age. We tested insulin tolerance in these STZ-offspring, and no significant changes were found (data not shown). We also tested glucose tolerance in some STZ-offspring up to 30 wk of age, and these offspring still did not become glucose intolerant (data not shown). Despite the failure to induce glucose intolerance, our insulin secretion results suggest that these STZ-offspring are at greater risk for developing NIDDM. In fact, Thamotharan et al. (46) and Aerts et al. (5), using slightly different models of maternal diabetes, were able to show glucose intolerance in adult STZ-offspring. Obesity was also not shown in STZ-offspring; in contrast, body weight gain was lower than that shown in controls. Because we used a severe maternal diabetic model, our data might suggest that severe maternal diabetes may affect offspring growth up to 15 wk of age or for a longer time.

The most widely proposed hypothesis for β-cell damage during diabetic pregnancy is that increased free radical reactive oxygen species (ROS) induce damage to the developing fetus. Evidence of ROS involvement in hyperglycemia-induced embryopathy was first obtained by Eriksson et al. (14, 15); in their studies, antioxidant enzymes were proved to be protective in vitro. In neonates of diabetic mothers, ROS levels are higher in several tissues (42). Rat embryos cultivated with medium containing a high concentration of ROS show growth retardation and severe malformation (14, 15). Also, pancreatic β-cells are more sensitive than other cell types to ROS. Compared with other tissue culture cell lines, pancreatic β-cell lines were 10 times more sensitive to hydrogen peroxide than macrophage-like cell lines (51). Rat islets are 2.5 to 25 times more sensitive to peroxide radicals than muscle, exocrine pancreas, kidney, and liver cells (31). Total islet superoxide dismutase is <30% of that measured in the liver (18). Measured values for glutathione peroxidase and catalase are <2% of liver cells (18, 31) and among the lowest of all tissues assayed. The lack of these enzymes makes the islet particularly vulnerable to hydrogen peroxide. Another important characteristic of pancreatic β-cells is that the enzymes cannot be upregulated after exposure to high concentrations of glucose (23, 48). These characteristics make β-cells more sensitive than other cell types to ROS; thus fetal β-cells would be easily damaged by maternal diabetes.

In summary, our results demonstrated that maternal diabetes induces many metabolic and functional aberrations in adult offspring pancreatic islets that lead to impaired insulin secretion. These aberrations may contribute to the development of NIDDM in later life of the offspring of diabetic mothers.

REFERENCES


