We have developed a model of gestational diabetes in the rat to determine whether an altered metabolic intrauterine milieu is directly linked to the development of diabetes later in life. Uteroplacental insufficiency is induced in the pregnant rat on day 19 of gestation. Sham-operated animals serve as controls. Offspring are growth retarded at birth; however, they catch up by 5–7 weeks of age. At ~8 weeks of age, they are bred to normal males. During pregnancy, these animals develop progressive hyperglycemia and hyper-insulinemia accompanied by impaired glucose tolerance and insulin resistance. Offspring, designated as infants of a diabetic mother (IDMs), are heavier at birth and remain heavy throughout life. IDMs are insulin resistant very early in life, and glucose homeostasis is progressively impaired. Defects in insulin secretion are detectable as early as 5 weeks of age. By 26 weeks of age, IDMs are overtly diabetic. These data demonstrate that the altered metabolic milieu of the diabetic pregnancy causes permanent defects in glucose homeostasis in the offspring that lead to the development of diabetes later in life. Diabetes 51:1499–1506, 2002

In humans, diabetic pregnancy induces marked abnormalities in glucose homeostasis and insulin secretion in the fetus that result in aberrant fetal growth (1). Population-based studies have also demonstrated long-term consequences for the offspring of gestational diabetic mothers. These progeny have an increased risk for obesity, glucose intolerance, and type 2 diabetes in later childhood and as adults (2–5). A genetic contribution to the development of diabetes is commonly assumed, but it is difficult to know the extent to which intrauterine metabolic perturbations rather than inherited genotype contribute to diabetic transmission via the mother. Animal models of diabetes during pregnancy can help pinpoint the specific effects of exposure to an abnormal intrauterine metabolic milieu, independent of inherited traits. Three experimental approaches have been widely used to generate hyperglycemia during pregnancy. High-dose streptozotocin (STZ) given during gestation induces marked maternal and fetal hyperglycemia. However, unlike human infants of gestational diabetic mothers, these rat fetuses and newborns are growth retarded and have low pancreatic insulin content and low plasma levels of insulin (6–9). In contrast to high-dosage effects of STZ, low dosage of the drug induces fetal macrosomia, and birth weights are increased. However, the effects on fetal growth, pancreatic insulin content, and plasma levels of insulin are highly variable (8–11). The third method was introduced by Picon’s group in France. Glucose infusions were used to induce hyperglycemia during the last days of gestation in the rat (12–15). The offspring are macrosomic, and in adulthood, they have very mildly elevated levels of glucose and insulin in the fed state (12–15).

Despite the different techniques used to induce hyperglycemia during pregnancy and the variability in outcome of these models, the offspring all develop either defects in insulin secretion or insulin action later in life (8,9,11–19). These studies unequivocally demonstrate that there are long-term and persistent effects of gestational diabetes on glucose homeostasis in the offspring.

In previous studies, we developed a model of uteroplacental insufficiency (intrauterine growth retardation) in the rat that produces diabetes in mature offspring (20). Intrauterine growth-retarded (IUGR) rats develop diabetes in middle age (5–6 months), with a phenotype remarkably similar to that observed in humans with type 2 diabetes: progressive dysfunction in insulin secretion and insulin action. Interestingly, pregnancy induces diabetes at a much earlier age. This model of gestational diabetes (onset of diabetes during pregnancy) circumvents many of the difficulties with the animal models outlined above and produces overgrown offspring that develop β-cell secretory defects and insulin resistance early in life. We hypothesize that the abnormal milieu of diabetes in pregnancy, in the face of a normal genetic background, leads to the development of diabetes in the offspring. We propose that the use of this model will elucidate mechanisms involved in the pathogenesis of altered glucose homeostasis in individuals who were subjected to gestational diabetes as fetuses.

RESEARCH DESIGN AND METHODS

Animals. We have described our surgical methods previously (20,21–23). In brief, time-dated Sprague-Dawley pregnant rats (Charles River) were individually housed under standard conditions and allowed free access to standard rat chow and water. On day 19 of gestation (term is 21.5 days), the maternal rats were anesthetized with intraperitoneal Xylazine (8 mg/kg) and Ketamine (40 mg/kg) and both uterine arteries were ligated (IUGR rats). Control animals underwent the identical anesthetic and surgical procedure except for ligation (sham-operated rats). Rats recovered within a few hours and had ad libitum access to food and water. The pregnant rats were allowed to deliver spontaneously (n = 3 for each group), and the litter size was randomly.

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BrdU, 5-bromo-2′-deoxyuridine; 2-DG, 2-deoxyglucose; IDM, infant of a diabetic mother; IUGR, intrauterine growth-retarded; STZ, streptozotocin.
and analyzed at 1, 7, 15, and 26 weeks of age. Pancreas was harvested, weighed, and densitometry. Antibody was diluted 1:3,000 before use. Resulting signals were quantitated by -4; East Acres Biologicals, Southbridge, MA) was diluted 1:5,000. The second detection system (Arlington Heights, IL). The primary antibody (GLUT1 and antibody concentrations were determined using a Sigma kit (Sigma, St. Louis, MO). The within- and between-assay coefficients of variation for the insulin assay were 4 and 10%, respectively. For fetal samples, blood was pooled from one litter. Triglyceride concentrations were determined using a Sigma kit (Sigma, St. Louis, MO). The within- and between-assay coefficients of variation for the insulin assay were 4 and 10%, respectively.

**Glucose and insulin tolerance tests.** Glucose tolerance was serially investigated in IUGR and control pregnant rats, and their offspring (IDMs and controls). Glucose (2 g/kg) was injected intraperitoneally in awake fasted rats. Blood samples were collected via tail vein sequentially before and 60, 120, and 180 min after injection. Insulin tolerance tests were also performed serially in IUGR and control pregnant rats and their offspring. Next, 1 unit/kg insulin (Humulin R, Eli Lilly, Indianapolis, IN) was injected subcutaneously. Blood samples were collected via tail vein at 0, 20, 40, and 60 min.

**Glucose uptake.** Glucose uptake was measured in epitrochlearis muscles of IDM and control animals using the glucose analog 2-deoxyglucose (2-DG) and the procedure of Young et al. (24).

**Western blot analysis.** Membrane proteins were prepared from muscle tissue (25) of IDMs and controls. Protein concentration was determined by the method of Lowry et al. (26). Protein from muscle (50 μg) was loaded in duplicate onto a discontinuous 12% polyacrylamide gel and size fractionated. Membrane proteins were prepared from muscle tissue (25) of IDMs and controls. Protein concentration was determined by the method of Lowry et al. (26). Protein from muscle (50 μg) was loaded in duplicate onto a discontinuous 12% polyacrylamide gel and size fractionated. Colorimetric detection of GLUT was performed using a double-antibody system and the Immuno-Blot assay kit (Biorad) or the Amersham biotinylated detection system (Arlington Heights, IL). The primary antibody (GLUT1 and -4; East Acres Biologicals, Southbridge, MA) was diluted 1:5,000. The second antibody was diluted 1:3,000 before use. Resulting signals were quantitated by densitometry.

**β-Cell mass.** For immunohistochemistry, five animals from each group were analyzed at 1, 7, 15, and 26 weeks of age. Pancreas was harvested, weighed, and fixed in 4% paraformaldehyde before embedding in paraffin. Histological sections of pancreas (5 μm) were cut from paraffin blocks and mounted on glass microscope slides. Each block was serially sectioned throughout its length to avoid any bias due to regional change in islet distribution and cell composition. Immunohistochemistry was performed on insulin within islets by a modified avidin-biotin peroxidase method. Sections were incubated with a primary antibody (polyclonal guinea pig anti-porcine insulin antibody) at 1:300 dilution for 1 h at room temperature. Then, sections were immunoperoxidase labeled with the Vectastain system (Vector Labs, Burlingame, CA), developed with 3,3′ diaminobenzidine (DAB; Amersham, Arlington Heights, IL), and counterstained with hematoxylin. Additional sections were also stained with a cocktail of antibodies to non-β-cell hormones, somatostatin, glucagon, and pancreatic polypeptide (Vector Labs). At least 200 fields were counted per animal. Using point-counting morphometrics (27), the relative volumes of both islets and β-cells were quantitated. The islet and β-cell masses were then calculated by multiplying the relative volume times the pancreatic weight and expressed in milligrams.

**β-Cell proliferation.** Rats (n = 7 per age, per treatment) at 1, 7, and 15 weeks of age were injected with 100 mg/kg body wt i.p. 5-bromo-2′-deoxyuridine (BrdU; Sigma) 6 h before killing. BrdU is a thymidine analog that is incorporated in newly synthesized DNA. After killing, the pancreas was excised, fixed in Bouin’s solution, and embedded in paraffin. Sections (5 μm) were double stained with immunoperoxidase for BrdU (Amersham cell-proliferation kit; Amersham, U.K.) and for the endocrine non-β-cells of the islets. BrdU-positive and -negative cells were counted using an Olympus BH-2 microscope. In each section, 1,200 β-cells were counted, and the results were expressed as a percentage of BrdU-positive β-cells. A total of 20 sections were counted per age (27).

---

**TABLE 1**

Weights (in grams) of IUGR and control animals

<table>
<thead>
<tr>
<th></th>
<th>IUGR</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Means</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>5.26 ± 0.17*</td>
<td>6 liters</td>
</tr>
<tr>
<td>Birthweight</td>
<td>250 ± 10</td>
<td>16</td>
</tr>
<tr>
<td>Weight at breeding</td>
<td>350 ± 21*</td>
<td>16</td>
</tr>
<tr>
<td>Midgestation weight</td>
<td>370 ± 19*</td>
<td>16</td>
</tr>
<tr>
<td>End gestation weight</td>
<td>150 ± 15*</td>
<td>16</td>
</tr>
</tbody>
</table>

Data are means ± SD or n. *P < 0.05 vs. control.

---

**TABLE 2**

Glucose, insulin, and triglyceride concentrations in pregnant IUGR (n = 16) and control (n = 16) animals

<table>
<thead>
<tr>
<th>Glucose (mg/dL)</th>
<th>IUGR</th>
<th>Control</th>
<th>Insulin (μU/ml)</th>
<th>IUGR</th>
<th>Control</th>
<th>Triglyceride mg/dL</th>
<th>IUGR</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 14</td>
<td>Day 19</td>
<td>Day 0</td>
<td>Day 14</td>
<td>Day 19</td>
<td>Day 0</td>
<td>Day 14</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>90.2 ± 8.4</td>
<td>149.1 ± 10.2*</td>
<td>250.2 ± 16.6*</td>
<td>89.3 ± 9.3</td>
<td>100.5 ± 9.6</td>
<td>103.7 ± 8.7</td>
<td>50.1 ± 4.8</td>
<td>87.4 ± 8.9*</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>45.2 ± 8.3</td>
<td>44.5 ± 8.6</td>
<td>50.2 ± 7.3</td>
<td>45.2 ± 8.3</td>
<td>44.5 ± 8.6</td>
<td>50.2 ± 7.3</td>
<td>501.3 ± 6.9</td>
<td>104.5 ± 8.6</td>
</tr>
</tbody>
</table>

Data are means ± SD. *P < 0.05 vs. control.

---

**FIG. 1.** A: Blood glucose levels during an intraperitoneal glucose tolerance test at 19 days of pregnancy in IUGR and control (sham) rats. Values are the means ± SE from five animals from each group. *P < 0.05 vs. control. B: Blood glucose levels during a subcutaneous injection of insulin at 19 days of pregnancy in IUGR and control (sham) rats. Values are the means ± SE from five animals from each group. *P < 0.05 vs. control.
Statistical analyses were performed using ANOVA and Statistical analysis.

Islet studies. Islets were isolated from IDMs and controls at 1, 7, and 15 weeks of age by collagenase digestion (26). A total of 200 islets were subjected to static incubation experiments under the following conditions: 3.3 mmol/l glucose, 3.3 mmol/l glucose + 10 mmol/l arginine, 16.7 mmol/l glucose, and 16.7 mmol/l glucose + 10 mmol/l arginine. After 90 min of incubation, insulin was measured in the incubation medium and the results expressed per DNA. Islet insulin content was determined by radioimmuno assay, and islet DNA content was determined by fluorometric assay (29).

Statistical analysis. Statistical analyses were performed using ANOVA and the Student’s unpaired t test (30). These studies were approved by the animal care committee of Children’s Hospital of Philadelphia and the University of Pennsylvania.

RESULTS

Induction of gestational diabetes. The body weights of IUGR animals were significantly lower than controls until ~7 weeks of age, when catch-up growth was attained (Table 1). At the time of breeding, there was no difference in weight between IUGR and control females. However, during pregnancy IUGR females gained significantly more weight than controls (Table 1).

Blood glucose and plasma insulin and triglyceride levels progressively increased during pregnancy in both IUGR and controls, but they were significantly higher in IUGR animals (Table 2). In contrast to Wistar rats, glucose levels in normal pregnant Sprague-Dawley rats increase with gestation. By 14 days of gestation, IUGR pregnant animals developed diabetes characterized by elevated fasting glucose and insulin levels and glucosuria (Table 2). On day 19 of gestation, intraperitoneal glucose tolerance tests demonstrated impaired glucose tolerance in IUGR pregnant rats. A 2-g/kg injection of glucose in IUGR pregnant females caused a rapid and sustained increase in blood glucose concentration, to a maximum of 350 mg/dl, decreasing slowly to 270 mg/dl at 120 min (Fig. 1A). In contrast, an intraperitoneal glucose bolus given to control pregnant rats elicited a rise in glucose levels to only 160 mg/dl, which decreased back to baseline by 120 min after injection (Fig. 1A).

To determine whether pregnant IUGR rats were insulin resistant, we measured fasting serum insulin levels and determined insulin sensitivity in vivo. At the time of breeding (day 0 of gestation), insulin levels in IUGR and control pregnant rats were not significantly different. However, by day 19 of gestation, fasting insulin levels were twofold higher in IUGR mothers compared with controls ($P < 0.05$) (Table 2) and insulin-tolerance tests showed a significantly blunted glycemic response to exogenous insulin (Fig. 1B).

**TABLE 3**

Glucose, insulin, and triglyceride concentrations in IDM and control animals

<table>
<thead>
<tr>
<th></th>
<th>Fetal (day 20)</th>
<th>1 week</th>
<th>15 weeks</th>
<th>26 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 litters</td>
<td>40</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDM</td>
<td>140.2 ± 5.8*</td>
<td>87 ± 7.2</td>
<td>199.1 ± 20.2*</td>
<td>310.2 ± 26.5*</td>
</tr>
<tr>
<td>Control</td>
<td>102 ± 6.5</td>
<td>91.1 ± 9.2</td>
<td>130.4 ± 11.6</td>
<td>143.6 ± 10.7</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDM</td>
<td>320.2 ± 29.1*</td>
<td>49.2 ± 8.2</td>
<td>77.4 ± 8.9*</td>
<td>87.7 ± 9.6*</td>
</tr>
<tr>
<td>Control</td>
<td>150.1 ± 12.4</td>
<td>37.9 ± 6.3</td>
<td>49.5 ± 8.9</td>
<td>55.2 ± 6.2</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDM</td>
<td>NA</td>
<td>NA</td>
<td>287.2 ± 28.4*</td>
<td>337.6 ± 29.5*</td>
</tr>
<tr>
<td>Control</td>
<td>NA</td>
<td>NA</td>
<td>142.5 ± 12.9</td>
<td>152.5 ± 16.4</td>
</tr>
</tbody>
</table>

Data are means ± SD. *$P < 0.05$ vs. control.
IDM rats had lower 30-min blood glucose levels than controls (Fig. 3); however, at 60 and 120 min, glucose levels were no different from controls. In contrast, 7-week-old IDM animals displayed mild glucose intolerance, which progressively worsened as the IDM animals aged. Even in the prediabetic stage (7–15 weeks), glucose levels remained elevated 180 min after injection of glucose.

β-Cell secretion of insulin. We assessed β-cell function by static islet studies in IDM and control rats at 1, 7, and 15 weeks of age. Insulin secretion at low and high glucose concentrations was significantly more robust in 1-week-old IDMs than in controls (Table 4). However, as the animals aged, insulin secretion in response to low and high glucose was markedly attenuated in IDMs compared with controls (Table 4).

Arginine stimulates insulin release by mechanisms independent of those used by glucose. This secretagogue induces an increase in the intracellular concentration of Ca$^{2+}$, which results in the depolarization of the β-cell membrane. To determine whether this pathway is adversely affected, we measured insulin release in the presence of arginine. There was no difference in insulin output in response to arginine between IDMs and controls at any age (data not shown).

Morphometry of the pancreas and β-cell proliferation. Immunohistochemical studies on the pancreas of IDM and control animals were carried out at sequential ages. At 1 week of age, β-cell mass was slightly increased in IDM newborns. However, by 15 weeks of age, β-cell mass was significantly lower in IDMs than in controls (Table 5).

Despite the increase in β-cell mass at 1 week of life, the proliferation rate of preexisting β-cells was not significantly different between IDM and controls at 1 week of life. However, by 7 weeks of age, the rate of β-cell proliferation was significantly reduced in IDMs compared with controls (Fig. 4).

Insulin resistance. To determine insulin sensitivity, we performed sequential insulin tolerance tests. Remarkably, even at 1 week of age, insulin tolerance tests showed a significantly blunted glycemic response to exogenous insulin in IDM rats (Fig. 5A). Insulin sensitivity further deteriorated with age, and by 15 weeks, there was only a small drop in glucose levels, compared with a 50% decrease in blood glucose in controls, after insulin was administered (Fig. 5B).

To gain some insight into the mechanisms underlying the blunted insulin sensitivity that we observed in IDM animals, we measured the insulin responsiveness of glucose transport in isolated epitrochlearis muscle of 5-week-old IDM and control rats. The optimal age in which to determine glucose uptake in isolated muscle strips is 5 weeks (24). Before this age it is difficult to isolate high-quality muscle strips, and after this age obesity complicates the accurate measurement of glucose uptake.

In IDM rats, basal 2-DG transport was similar to that of controls. However, maximal glucose transport in response to insulin (1,000 μU/ml) only increased 45% in IDMs compared with a more than fourfold increase in glucose transport in response to insulin in controls (Fig. 6).

Insulin resistance in some models of diabetes has been linked to decreased GLUT expression. To determine whether this situation occurs in IDM rats, we measured GLUT1 protein levels in muscle of newborn rats and GLUT4 protein levels in muscle of adult IDM and control rats. In the newborn period, GLUT1 protein levels were significantly lower in IDMs than in controls. Correspondingly, GLUT4 protein levels were significantly lower in IDM rats than in controls at 15 weeks of age (Fig. 7).
**TABLE 4**
Glucose-induced insulin release from noncultured islets

<table>
<thead>
<tr>
<th>Glucose (mmol/l)</th>
<th>1 Week</th>
<th>7 Weeks</th>
<th>15 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IDM</td>
<td>Control</td>
<td>IDM</td>
</tr>
<tr>
<td>3.3</td>
<td>0.85 ± 0.01*</td>
<td>0.25 ± 0.02</td>
<td>0.35 ± 0.02*</td>
</tr>
<tr>
<td>16.7</td>
<td>6.01 ± 0.24*</td>
<td>1.92 ± 0.32</td>
<td>0.71 ± 0.08*</td>
</tr>
</tbody>
</table>

Results are means ± SE and expressed as microunits of insulin released per nanogram of islet DNA per hour. *P < 0.05 vs. control.

**DISCUSSION**

The extent to which an abnormal intrauterine milieu contributes to the development of type 2 diabetes in offspring is difficult to determine because genetic factors play such an important role in the development of the disease. Animal models of gestational diabetes during pregnancy are vitally important in determining the specific metabolic effects of exposure to an abnormal intrauterine milieu against a homogeneous genetic background. A number of animal models have been used to determine the effects of hyperglycemia during pregnancy on the adult offspring (8-10,12-17,31,32). To extend these experimental studies of gestational diabetes, we have developed a new model of gestational diabetes that produces offspring that eventually develop diabetes later in life.

In previous studies, we have characterized a model of uteroplacental insufficiency in the pregnant rat that causes fetal and neonatal growth retardation (20). These animals eventually develop a phenotype that is consistent with type 2 diabetes in the human: progressive deterioration in maternal insulin secretion and insulin action. By age 6 months, the IUGR animals are overtly diabetic (20). The results of the current studies demonstrate that pregnancy prematurely precipitates the development of diabetes in the IUGR rat. By day 14 of gestation, pregnant IUGR rats demonstrated glucose intolerance and insulin resistance.

Normal pregnancy is associated with hyperinsulinemia and a progressive decline in insulin sensitivity (33,34). In response to the increased demand for insulin, the maternal pancreas adapts by increasing insulin synthesis, secretion, and β-cell proliferation (35-37). In the rat, these adaptations peak around day 14 of pregnancy, and by the end of gestation, the maternal β-cell mass has nearly doubled (38,39). In the IUGR rat, the β-cell does not function normally, and proliferation is impaired (20). The additional stress of pregnancy may result in a further deterioration of these processes in the IUGR rat, leading to the onset of diabetes in pregnancy.

The results of our studies show that the abnormal intrauterine metabolic milieu of the diabetic pregnancy in IUGR rats has profound effects on the offspring. It is well established that the altered metabolic parameters of maternal diabetes result in fetal hyperglycemia and hyperinsulinemia (1,40). This leads to the development of macrosomia. Similar to the human IDM, IDM rats were significantly heavier at birth than controls and remained so throughout life. Of note was our finding that even though IDM animals were fostered to normal mothers, they still remained heavy throughout life.

Early in life, insulin output of islets in IDM rats was increased in the presence of high glucose concentrations, indicating that β-cells of IDMs have increased responsiveness to glucose stimulation. However, as the IDM animal matures, β-cell dysfunction is manifested by decreased insulin secretion in response to glucose. Impaired insulin secretion was specific to glucose because arginine-stimulated insulin release was similar in IDM and control rats, indicating an intact secretory apparatus. The mechanisms responsible for β-cell malfunction are unknown. One possibility may be that fetal IDM β-cells are chronically overstimulated, which eventually leads to β-cell exhaustion. Alternatively, progressive hyperglycemia may exacerbate β-cell abnormalities through a process known as glucose toxicity (41-43). Chronic exposure to high glucose concentrations decreases insulin gene promoter activity and binding of PDX-1 (pancreatic/duodenal homeobox-1) to the insulin promoter, leading to defects in insulin secretion (43).

Like humans with type 2 diabetes and animal models of diabetes in pregnancy, the β-cell of the IDM fetus exposed to short-term elevations in glucose concentrations responds by proliferating (44). Once the fetus is removed from the hyperglycemic intrauterine milieu, β-cell mass

![FIG. 4. Rats (n = 7 per age, per group) were injected with BrdU 6 h before killing. After killing, the pancreas was excised, fixed in Bouin’s solution, and embedded in paraffin. Sections (5 μm) were double stained with immunoperoxidase for BrdU and for the endocrine non-β-cells of the islets. A total of 20 sections were counted per age. Using the methods of Scaglia et al. (27), we calculated the proliferation rate. Data are expressed as the percent control. *P < 0.05 vs. control.](chart)
very quickly involutes and total pancreatic β-cell mass progressively declines, resulting in a deterioration of glucose homeostasis. These defects result in chronic hyperglycemia, which stimulates a cascade of events culminating in the development of overt diabetes.

In humans and animals predestined to develop type 2 diabetes, after prolonged exposure to hyperglycemia, β-cell proliferation is inhibited and β-cell death is increased (45–47). In animals, this is associated with the loss of expression (43,48,49) of a number of β-cell/islet transcription factors that are important for the development of the endocrine pancreas (50–52), such as PDX-1, RIPE-3b1 activator, Nkx 6.1, β2/Neuro D, Pax 6, HNF1a, HNF4a1, and HNF3b. A decreased expression of any of these transcription factors will profoundly inhibit further proliferation and neogenesis of the β-cell, making it impossible for the β-cell to compensate for progressive worsening of defects in insulin secretion and insulin action. It remains to be determined whether any of these mechanisms are responsible for the decline in β-cell mass observed in IDM animals.

Insulin resistance was apparent at a very early age in IDMs. Insulin tolerance tests were abnormal at 1 week, the earliest age that we could reliably test the animals. In an attempt to determine the mechanisms underlying defects in insulin action, we measured glucose transport in isolated muscle of IDMs and controls at different ages. Glucose transport in skeletal muscle is widely considered the rate-limiting step for whole-body glucose disposal under most conditions (53,54). We found that insulin-stimulated glucose uptake was significantly attenuated in 5-week-old IDM rats compared with controls. We measured protein levels of GLUT1 and -4, the transporters responsible for insulin-stimulated glucose transport in newborns and adults, respectively. GLUT1 protein levels were significantly diminished in muscle of IDMs compared with controls, and, correspondingly, GLUT4 protein levels were decreased in adult IDM rats. Further studies will be necessary to determine whether other steps in the pathway of insulin-stimulated glucose transport are altered.

The mechanisms underlying the observed downregula-

FIG. 5. Blood glucose levels during an intraperitoneal injection of insulin performed sequentially at 1 week (A) and 15 weeks (B) of age in IDM and control rats. Values are the means ± SE of eight animals from each group at each age. *P < 0.05 vs. control.
tion of GLUT1 and -4 in the newborn and adult IDM rat may be related to high fetal glucose concentrations resulting from maternal hyperglycemia. High circulating levels of glucose induce a substantial downregulation of glucose transporters in muscle under chronic diabetic conditions (55–57). Downregulation of glucose transporters may be an adaptive mechanism by which the fetus protects itself against the deleterious effects of high cellular glucose concentrations. Of interest is our observation that once glucose levels are normalized after birth, glucose transporter expression remains permanently reduced in IDM animals.

In conclusion, we have developed a model of diabetes in pregnancy with a phenotype that is similar to that observed in humans with gestational diabetes. This model results in the onset of diabetes in the offspring once they reach adulthood. The development of diabetes appears to be related to defects in both insulin secretion and insulin action. The data presented here support the hypothesis that an abnormal intrauterine milieu can induce permanent changes in glucose homeostasis after birth. The fetus adapts to an altered environment in utero that may enhance its short-term survival probability at the expense of a long-term capacity for normal growth and development, resulting in the development of diabetes.

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