

Intrauterine Growth Retardation Leads to the Development of Type 2 Diabetes in the Rat

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Intrauterine growth retardation has been linked to the development of type 2 diabetes in later life. The mechanisms underlying this phenomenon are unknown. We have developed a model of uteroplacental insufficiency, a common cause of intrauterine growth retardation, in the rat. Bilateral uterine artery ligation was performed on day 19 of gestation (term is 22 days) in the pregnant rat; sham-operated pregnant rats served as controls. Birth weights of intrauterine growth-retarded (IUGR) animals were significantly lower than those of controls until ~7 weeks of age, when IUGR rats caught up to controls. Between 7 and 10 weeks of age, the growth of IUGR rats accelerated and surpassed that of controls, and by 26 weeks of age, IUGR rats were obese ($P < 0.05$ vs. controls). No significant differences were observed in blood glucose and plasma insulin levels at 1 week of age. However, between 7 and 10 weeks of age, IUGR rats developed mild fasting hyperglycemia and hyperinsulinemia ($P < 0.05$ vs. controls). At age 26 weeks, IUGR animals had markedly elevated levels of glucose ($P < 0.05$ vs. controls). IUGR animals were glucose-intolerant and insulin-resistant at an early age. First-phase insulin secretion in response to glucose was also impaired early in life in IUGR rats, before the onset of hyperglycemia. There were no significant differences in β -cell mass, islet size, or pancreatic weight between IUGR and control animals at 1 and 7 weeks of age. However, in 15-week-old IUGR rats, the relative β -cell mass was 50% that of controls, and by 26 weeks of age, β -cell mass was less than one-third that of controls ($P < 0.05$). The data presented here support the hypothesis that an abnormal intrauterine milieu can induce permanent changes in glucose homeostasis after birth and lead to type 2 diabetes in adulthood. *Diabetes* 50:2279–2286, 2001

Intrauterine growth retardation is a common complication of pregnancy and a significant cause of perinatal morbidity and mortality. Barker et al. (1,2) first introduced the hypothesis that an adverse intrauterine environment could induce disease in later life. He proposed that low-birth weight infants were at increased risk for developing obesity, hypertension, and type 2 diabetes. Several subsequent studies have lent support to Barker's hypothesis (3–6). However, multiple problems associated with these population-based studies, such as the lack of association of gestational age with birth weight, inadequate recording of confounding variables, and loss to follow-up, have dampened enthusiasm for the universal acceptance of Barker's hypothesis. Experiments using animal models of intrauterine growth retardation can circumvent some of these difficulties and elucidate the underlying cellular and molecular mechanisms. To that end, we have developed a model of intrauterine growth retardation in the rat induced by bilateral uterine artery ligation (7,8). Blood flow to the fetus is not ablated, but reduced to a similar degree to that observed in human pregnancies complicated by uteroplacental insufficiency. In our model, the resulting uteroplacental insufficiency leads to growth-retarded fetuses with critical features of a metabolic profile very similar to that of intrauterine growth-retarded (IUGR) human fetuses: decreased levels of glucose, insulin, IGF-I, amino acid, and oxygen (7–9). Because of the model's similarity to human growth retardation, the IUGR rat is a uniquely suited experimental tool for studying the impact of uteroplacental insufficiency on the evolution of type 2 diabetes. These animals have a normal genetic background upon which environmental effects can be tested for their role in inducing diabetes. Therefore, this study was undertaken to 1) determine whether uteroplacental insufficiency does indeed lead to the development of diabetes in adulthood, 2) characterize the evolution of the disease state, and 3) begin elucidating some of the underlying mechanisms responsible.

RESEARCH DESIGN AND METHODS

Animal model. We have described our surgical methods previously (7–9). In brief, time-dated Sprague-Dawley pregnant rats 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). Control animals underwent the identical anesthetic and surgical procedure except for ligation. Rats recovered within a few hours and had ad libitum access to food and water. The pregnant rats were allowed to deliver spontaneously, and the litter size was randomly reduced to eight at birth to assure uniformity of litter size between IUGR and

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IUGR, intrauterine growth-retarded.

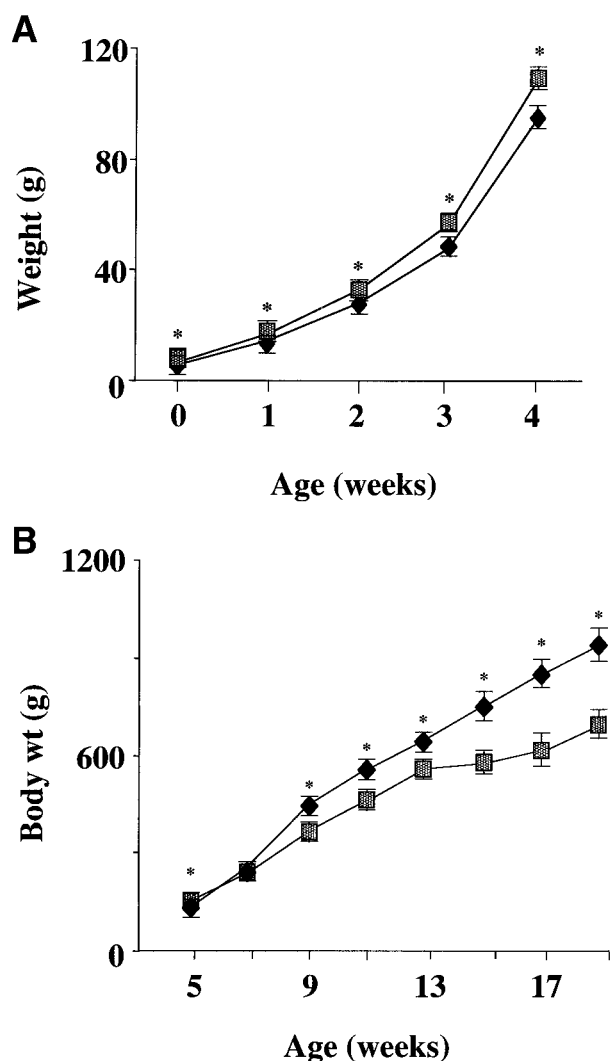


FIG. 1. Weights of IUGR (◆) and sham-operated control (□) rats from birth until 4 weeks of age (A) and from 5 to 26 weeks of age (B). Values are the means \pm SE from 55 animals from each group followed sequentially from birth until 26 weeks of age. * $P < 0.05$ vs. sham-operated control rats.

control litters. The pups were fostered to unoperated normal female rats and remained with their foster mothers until they were weaned.

Assays. Blood glucose concentrations were determined in duplicate using the HemoCue blood glucose analyzer (Angelholm, Sweden). Plasma insulin concentrations were measured in duplicate by radioimmunoassay using rat insulin as the standard (Linco, 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 rats. 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 rats; for this test, 1 unit/kg insulin was injected subcutaneously. Blood samples were collected via tail vein at 0, 20, 40, and 60 min.

Insulin release. Rats were fasted overnight for ~ 14 h. Glucose (3 g/kg) or L-arginine (10 mmol/l in 0.2 mol/l phosphate-buffered saline) was injected intraperitoneally, and blood was collected via tail vein at 0, 2, 5, 10, 20, and 30 min after injection. Whole-blood glucose was measured using an automatic glucometer (HemoCue, Mission Viejo, CA). Insulin levels were measured via radioimmunoassay.

Immunohistochemistry. For immunohistochemistry, 10 animals of each group were analyzed at 1, 7, and 26 weeks of age. Histological sections of pancreas (5 μ m) 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 to determine insulin within islets

by a modified avidin-biotin-peroxidase method. Sections were incubated with primary antibody (polyclonal guinea pig anti-porcine insulin antibody) at 1:300 dilution for 1 h at room temperature, and then they were immunoperoxidase-labeled with the Vectastain system (Vector Labs, Burlingame, CA), developed with 3,3' diaminobenzidine (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, Burlingame, CA). Morphometric analysis was performed using an Olympus BH2 microscope connected via a color video camera to a personal computer at a magnification of $\times 250$ or $\times 400$. At least 200 fields were counted per animal. Using Weibel's point-counting morphometrics (10), 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 expressed in milligrams.

Statistical analysis. Statistical analyses were performed using analysis of variance and Student's unpaired t test. These studies were approved by the Animal Care Committee of the Children's Hospital of Philadelphia and the University of Pennsylvania.

RESULTS

Animal weights and glucose homeostasis. Birth weights of IUGR animals were significantly lower than those of controls (5.96 ± 0.68 vs. 7.00 ± 0.89 g) until ~ 7 weeks of age, when IUGR rats caught up to controls. Between 7 and 10 weeks of age, the growth of IUGR rats accelerated and surpassed that of controls, and by 26 weeks of age, IUGR rats were obese (Fig. 1A and B). Epididymal, mesenteric, and perinephric fat pads were removed and weighed at 7, 15, and 26 weeks of age. By 7 weeks of age, fat pad mass was significantly increased in IUGR compared with control rats (Table 1).

Glucose and insulin levels were measured in the fasted and random-fed states at several time points as the animals matured. No significant differences were observed in blood glucose levels at 1 week. However, between 7 and 10 weeks of age, IUGR rats developed mild fasting hyperglycemia, which progressively worsened as the animals aged (Table 2). The fasting insulin levels were comparable between the two groups at 1 week, but at 7 and 15 weeks of age, fasting insulin levels were significantly higher in IUGR animals compared with controls ($P < 0.05$). However, as IUGR animals aged, insulin levels progressively declined and were no longer elevated.

Glucose tolerance. To assess the impact of impaired insulin release on the ability of IUGR rats to dispose of a glucose load, glucose tolerance was measured by intraperitoneal injection of glucose (2 g/kg) after an overnight fast. After challenge with a glucose load, IUGR rats had significantly higher 30-min blood glucose levels compared with controls (Fig. 2A–C). Early in life, glucose intolerance was mild; however, as IUGR animals aged, they showed a

TABLE 1
Fat pad weights (in grams)

Fat pad	7 Weeks	15 Weeks	26 Weeks
Epididymal			
Sham-operated rats	4.78 ± 0.31	6.55 ± 0.42	8.97 ± 0.59
IUGR rats	$5.73 \pm 0.29^*$	$8.01 \pm 0.46^*$	$16.34 \pm 1.01^*$
Perinephric			
Sham-operated rats	4.12 ± 0.27	6.01 ± 0.51	8.21 ± 0.49
IUGR rats	$4.99 \pm 0.21^*$	$9.11 \pm 0.78^*$	$14.22 \pm 1.21^*$
Mesenteric			
Sham-operated rats	3.08 ± 0.11	5.22 ± 0.44	6.78 ± 0.42
IUGR rats	$4.11 \pm 0.21^*$	$8.01 \pm 0.51^*$	$11.79 \pm 1.01^*$

Data are means \pm SE. * $P < 0.05$ vs. shams.

TABLE 2
Glucose and insulin values

Age (weeks)	n	Glucose (mg/dl)		IRI* (ng/ml)	
		Sham-operated rats	IUGR rats	Sham-operated rats	IUGR rats
1	58	89.3 ± 8.4	87.2 ± 9.8	48.2 ± 6.1	47.6 ± 5.2
7	48	91.8 ± 15.1	135 ± 18.2†	40.9 ± 4.8	79.2 ± 7.1†
15	48	92 ± 13.2	159 ± 20.3†	35.3 ± 5.1	59.3 ± 8.9†
26	47	130 ± 18.9	280 ± 19.4†	42.6 ± 4.8	49.6 ± 5.8

Data are means ± SE. *Immunoreactive insulin; † $P < 0.05$ vs. shams.

progressive loss in the ability to handle a glucose load. Even in the prediabetic stage (7–15 weeks), glucose levels remained elevated 180 min after the injection of glucose. **β -cell secretion of insulin.** To evaluate the effects of uteroplacental insufficiency upon pancreatic β -cell function, insulin release was measured in response to stimulation with the two major nutrient secretagogues of insulin, glucose and arginine, at 1, 7, 15, and 26 weeks of age. In 1-week-old controls, a nearly twofold increase in insulin secretion was observed 2 min after intraperitoneal glucose injection, and the levels remained higher than baseline values for up to 30 min, indicating a second-phase response (Fig. 3A). At 7, 15, and 26 weeks of age, glucose elicited a threefold increase in insulin secretion and a normal second-phase response (Fig. 3B–D). In contrast, insulin secretory capacity was impaired in IUGR rats. At 1 week of age (Fig. 3A), the acute first-phase insulin secretory response to glucose was reduced by 50%, and by 26 weeks of age (Fig. 3D), it was virtually absent in IUGR rats.

Arginine stimulates insulin release by mechanisms independent of those used by glucose. This secretagogue induces an increase in intracellular concentration of Ca^{2+} , which results in the depolarization of the β -cell membrane. To determine whether this pathway is adversely affected by uteroplacental insufficiency, rats were also given an acute arginine challenge. In contrast to the response to glucose, arginine elicited a two- to threefold increase in insulin release 2 min after injection in both IUGR and control rats at all ages (Fig. 4A–D).

Morphometry of the pancreas. Blunted insulin secretion in IUGR rats could be caused by reduced numbers or function of β -cells, or both. Therefore, we carried out immunohistochemical studies on the pancreases of IUGR and control animals at sequential ages. There were no significant differences in β -cell mass, islet size, or pancreatic weight between IUGR and control animals at 1 and 7 weeks of age (Fig. 5A–B). However, in 15-week-old IUGR rats, the relative β -cell mass was 50% that of controls, and by 26 weeks of age, β -cell mass was less than one-third that of controls ($P < 0.05$) (Fig. 5A–B).

Insulin tolerance. At 7–10 weeks of age, IUGR rats developed hyperinsulinemia, which suggested that they were insulin-resistant. To determine whether this was indeed the case, 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 IUGR rats ($P < 0.05$) (Fig. 6A). Insulin sensitivity further deteriorated with age, and at 15 weeks, there was only a small drop in glucose levels compared

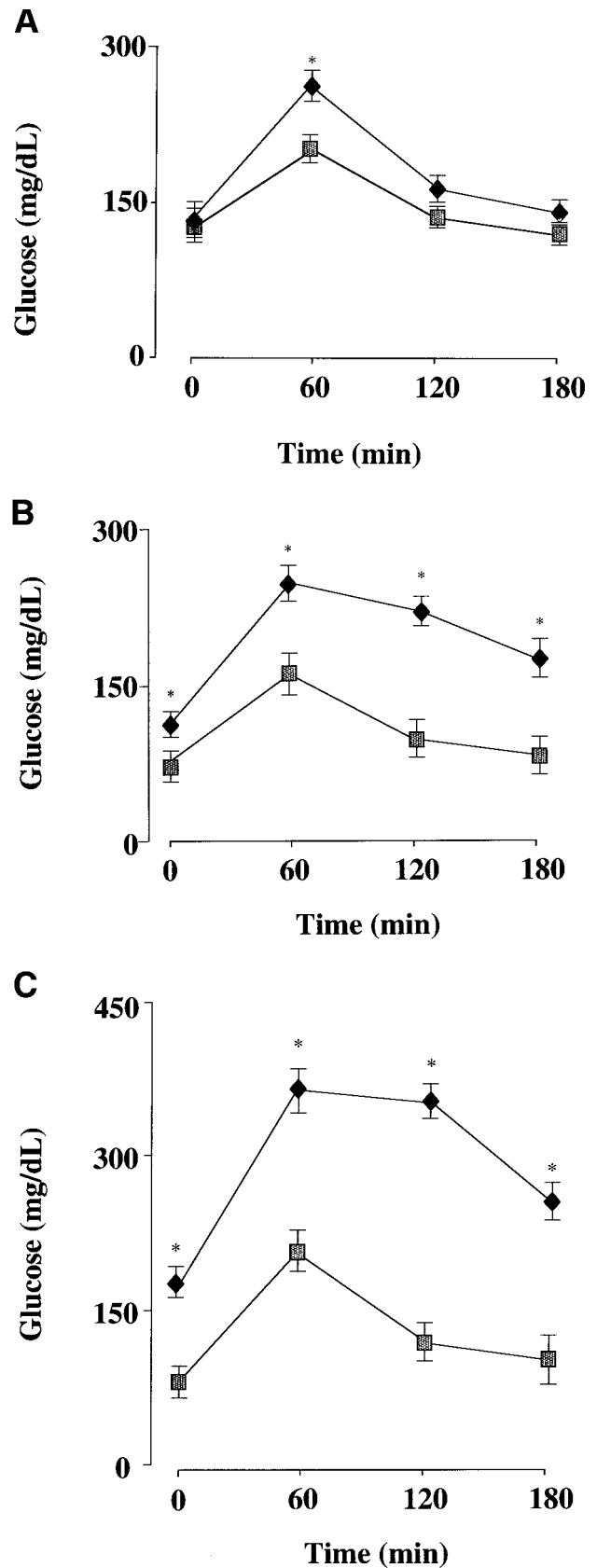


FIG. 2. Blood glucose levels during an intraperitoneal glucose tolerance test at 1 week (A), 7 weeks (B), and 15 weeks (C) of age in IUGR (\blacklozenge) and sham-operated control (\square) rats. Values are the means \pm SE from 35 animals from each group at each age. * $P < 0.05$ vs. sham-operated control rats.

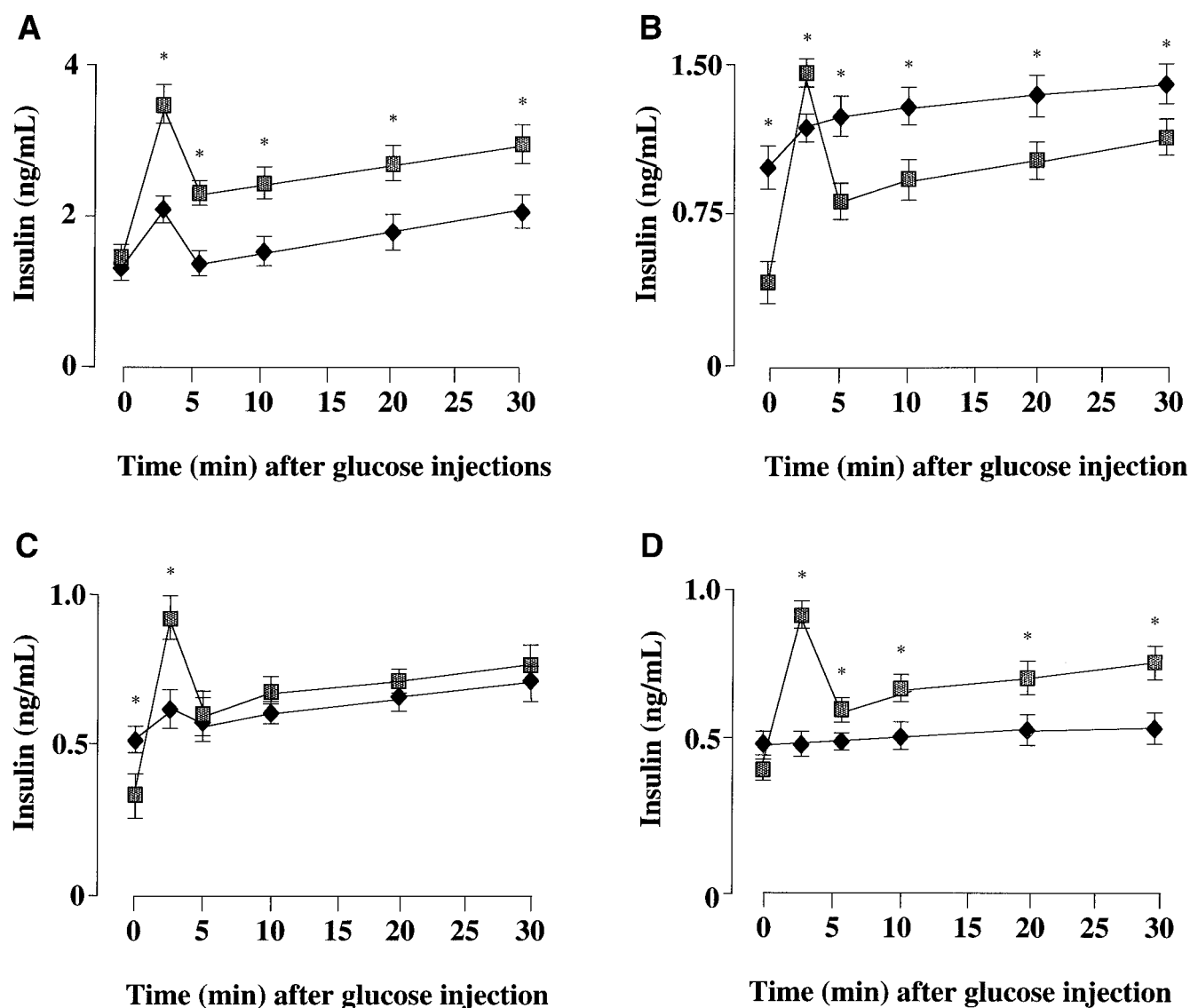


FIG. 3. Serum insulin levels after intraperitoneal injection of glucose at 1 week (A), 7 weeks (B), 15 weeks (C), and 26 weeks (D) in IUGR (◆) and sham-operated control (▣) rats. Values are the means \pm SE from 35 animals from each group at each age. * $P < 0.05$ vs. sham-operated control rats.

with a 50% decrease in blood glucose in controls after insulin was administered (Fig. 6B).

DISCUSSION

Uteroplacental insufficiency limits the availability of substrates to the fetus and retards growth during gestation. Several lines of evidence suggest that it may also have permanent consequences after birth. Epidemiological studies have revealed strong statistical links between poor fetal growth and the subsequent development of type 2 diabetes in adulthood (1–6). These observations have been made in a large number of populations worldwide. Barker, Osmond, and Hales conducted some of the earliest studies in England that explored the association between neonatal anthropometrics and later development of glucose intolerance and diabetes in individuals aged 50–64 years (1,2). They described a significant correlation of decreased birth weight with impaired glucose tolerance, hypertension, and diabetes independent of adult body mass. A similar association was found among participants in the

San Antonio Heart study (4). The prevalence of diabetes in 50- to 60-year-old men in Uppsala, Sweden, was inversely related to weight and ponderal index at birth (11). In Pima Indians, who are known for their high prevalence of diabetes, the development of type 2 diabetes in offspring is related to both extremes of birth weight (12). The most recent study was conducted in a cohort of women in the Nurses' Health Study who were free of diabetes at baseline (6). After adjustment for adult BMI, age, and maternal history of diabetes, the relative risk for type 2 diabetes was nearly twofold higher in women whose birth weights were <5.5 pounds (6). These findings have led to the "fetal origins" hypothesis, which suggests that an adverse intrauterine environment would program or imprint the development of fetal tissues, permanently determining responses, producing later dysfunction and disease (13).

Data from animal models of intrauterine growth retardation induced by malnutrition support the concept that poor fetal growth has permanent consequences in adulthood. A low protein diet (~ 40 – 50% of normal intake) fed

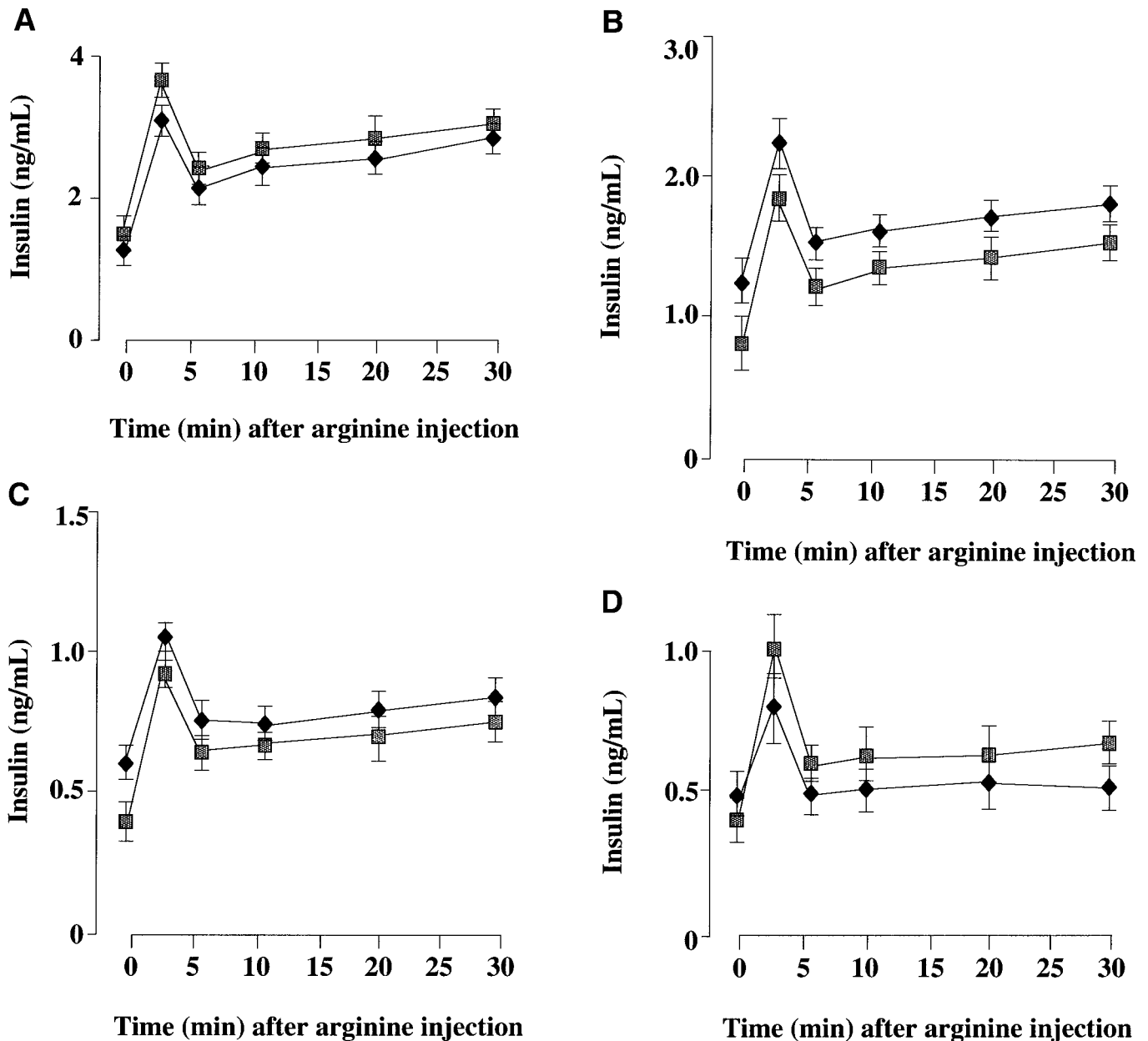


FIG. 4. Serum insulin levels after intraperitoneal injection of arginine at 1 week (A), 7 weeks (B), 15 weeks (C), and 26 weeks (D) in IUGR (◆) and sham-operated control (▣) rats. Values are the means \pm SE from 20 animals from each group at each age. * $P < 0.05$ vs. sham-operated control rats.

to the pregnant animal throughout gestation and lactation induces severe growth retardation in the offspring (14–19). β -Cell mass is reduced in the fetus and remains reduced throughout life (14,15). However, unlike the IUGR animals described in our studies, protein-restricted rats do not develop diabetes. The mechanisms underlying this phenomenon are unclear. Protein deprivation appears to preferentially affect the β -cell. Early in life, protein-deprived animals are more insulin-sensitive than controls (16). It is likely that the combination of insulin resistance and β -cell dysfunction are necessary to induce the phenotype of type 2 diabetes in the growth-retarded rat.

To extend these experimental studies of growth retardation, we have developed a model of intrauterine growth retardation in the rat that does in fact lead to diabetes in later life. This model of fetal growth retardation has many

advantages over other animal models: 1) bilateral uterine artery ligation induces uteroplacental insufficiency, one of the most common causes of human intrauterine growth retardation; 2) growth-retarded fetal rats have critical features of a metabolic profile characteristic of growth-retarded human fetuses, i.e., decreased levels of glucose, insulin, IGF-I, amino acids, and oxygen (7–9); and 3) most importantly, IUGR rats develop diabetes with a phenotype remarkably similar to that observed in humans with type 2 diabetes, i.e., progressive dysfunction in insulin secretion and insulin action. Because of this IUGR model's similarity to human growth retardation and subsequent disease states, the IUGR rat represents one of the best experimental tools for studying the impact of uteroplacental insufficiency on the evolution of diabetes.

The basis of type 2 diabetes in humans is incompletely

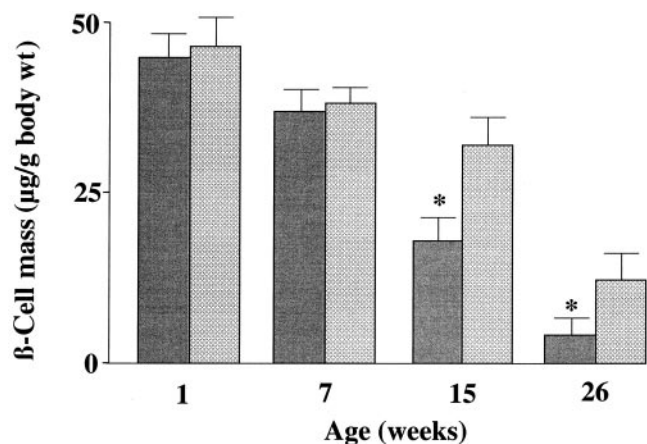


FIG. 5. β -Cell mass determined by point-counting in IUGR and sham-operated control rats. Data are expressed as β -cell mass (microgram) per gram of body weight. Values are the means \pm SE from 10 animals at each age in each group. * $P < 0.05$ vs. sham-operated control rats.

understood. However, insulin resistance and β -cell dysfunction are the two predominant and characteristic features. In humans destined to develop type 2 diabetes, defects in insulin secretion and insulin action can be detected years before the onset of clinical disease (12,20–24). In these individuals, the β -cell is capable of secreting enough insulin to both compensate for the defect in insulin action and to maintain normal glucose homeostasis. Ultimately, hyperglycemia ensues when the β -cell fails to secrete insulin in adequate amounts.

Similar to the human predestined to develop diabetes (21,23,24), first-phase insulin secretion was impaired early in life in IUGR rats, before the onset of hyperglycemia, and before a reduction in β -cell mass occurred. Although the plasma insulin levels were increased in IUGR rats for a period of time, quantitative insulin output was not appropriate for the degree of hyperglycemia, leading to the development of glucose intolerance. Impaired first-phase insulin secretion was specific to glucose, because arginine-stimulated insulin release was similar in IUGR and control rats, indicating an intact secretory apparatus. Thus, the loss of glucose-stimulated insulin response is caused by an intrinsic defect in the β -cell induced by uteroplacental insufficiency. Possible mechanisms might be related to impaired glucose sensing or defective oxidative phosphorylation of glucose, the primary signal eliciting insulin secretion by the β -cell.

Glucose intolerance was observed very early in life and was associated with defects in insulin secretion and insulin action. Eventually, impaired glucose tolerance progressed to overt diabetes in the IUGR rat because of the ultimate inability of the β -cell to compensate for secretory defects and insulin resistance. In normal human subjects, in addition to increasing insulin secretion per β -cell, elevated levels of glucose induce an expansion in β -cell mass (25–26). The latter did not occur in the IUGR rat. β -Cell mass was similar in IUGR and control rats for the first several weeks after birth, despite the IUGR rats manifesting insulin resistance and β -cell secretory defects. In adult IUGR rats, total pancreatic β -cell mass progressively declined, resulting in a further deterioration of glucose homeostasis.

It is not known whether the IUGR rat's inability to

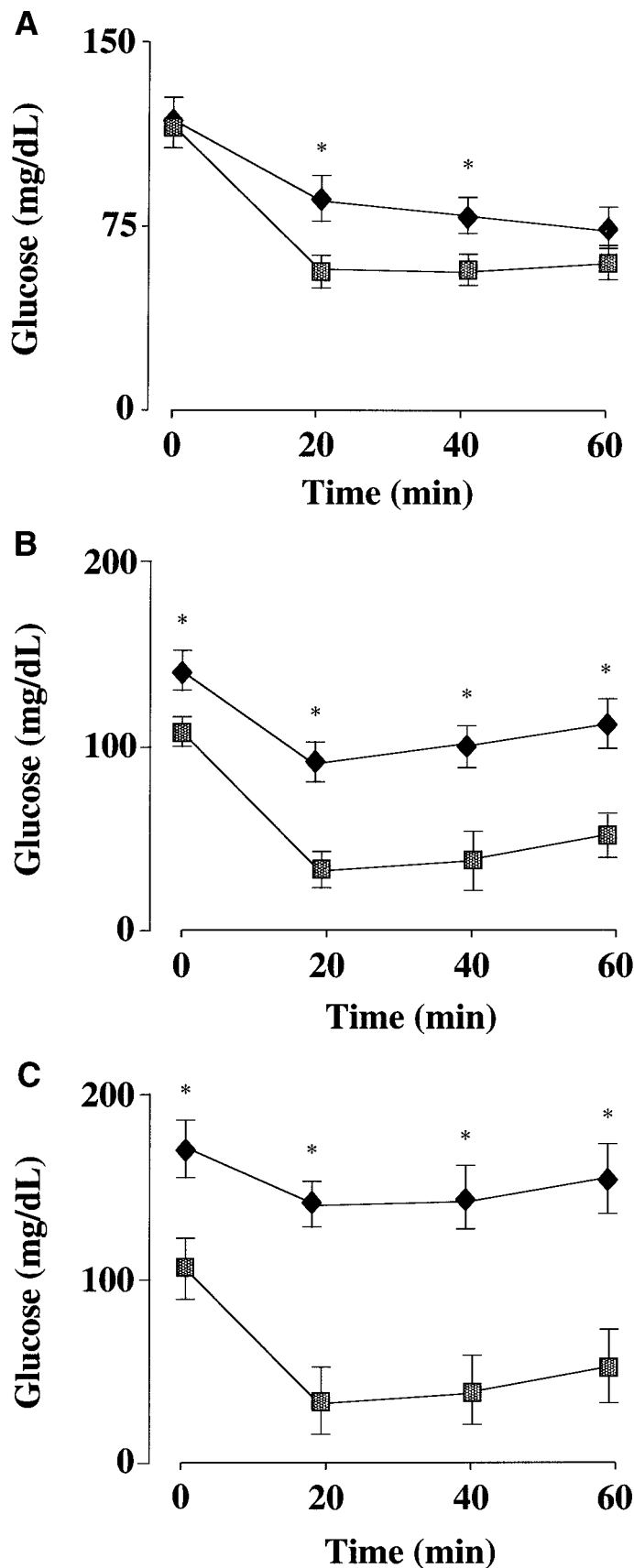


FIG. 6. Blood glucose levels during an intraperitoneal injection of insulin at 1 week (A), 7 weeks (B), and 15 weeks (C) of age in IUGR (\blacklozenge) and sham-operated control (\square) rats. Values are the means \pm SE from 35 animals from each group at each age. * $P < 0.05$ vs. sham-operated control rats.

adequately increase β -cell mass is caused by decreased proliferation or increased rates of cell death. In a model of intrauterine growth retardation induced by protein restriction during pregnancy and lactation, β -cell mass was reduced in rats exposed to the low-protein diet. This reduction was associated with decreased rates of β -cell proliferation and increased rates of apoptosis (27). In a separate study, protein malnutrition was started later in gestation and continued throughout lactation, which also resulted in reduced β -cell mass in the offspring. However, in these animals, the reduction in β -cell mass was not associated with reduced rates of proliferation. The authors hypothesized that impaired neogenesis and increased rates of apoptosis of β -cells were responsible for the observed decrease in β -cell mass (28). It is clear from these studies that intrauterine events can permanently alter the normal programming of the β -cell. The underlying cellular and molecular mechanisms remain to be elucidated.

Insulin action, as measured by insulin tolerance tests, was significantly blunted in IUGR rats. Multiple alterations in the insulin action cascade have been shown to contribute to insulin resistance in humans and animal models of type 2 diabetes. IUGR animals display impaired glucose tolerance even at 1 week of age; therefore, it is possible that the underlying defect responsible for impaired insulin action may be secondary to glucose toxicity. The relative contribution of insulin resistance to the progression of the disease remains to be elucidated.

In conclusion, our results indicate that uteroplacental insufficiency leads to the progressive development of a type 2 diabetic phenotype in IUGR rats. These animals exhibit mild peripheral insulin resistance and β -cell secretory defects very early in life (1 week of age), but have adequate compensatory insulin secretion for several weeks. However, eventually, β -cell compensation fails, and overt diabetes occurs. 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 its long-term capacity for normal growth and development. The fetus redirects its severely limited resources in ways that produce a persistent defect in the ability to use metabolic fuel (glucose). In effect, the cells are reprogrammed to enable the fetus as a whole to survive under conditions of nutrient deprivation. However, this reprogramming persists throughout life, leading to the development of type 2 diabetes in adulthood.

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