

Adverse Effects of Hyperglycemia on Kidney Development in Rats

In Vivo and in Vitro Studies

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Congenital malformations occur more frequently in the offspring of diabetic mothers. These in vivo and in vitro studies investigate the potential adverse effects of hyperglycemia on kidney development in the rat. Female rats were made hyperglycemic throughout gestation with a single injection of streptozotocin (STZ) on day 0 of gestation, or for a short period encompassing the early stage of renal organogenesis by infusing glucose from gestational days 12–16. Kidney development in the pups was assessed by determining the total number of nephrons formed in the kidney. The number of nephrons was significantly reduced (10–35%) in the pups from STZ-treated dams, as a function of hyperglycemia. There were also fewer nephrons in pups from dams given glucose infusion whose hyperglycemia was transiently higher on day 13 of gestation. The in vitro experiments were done on metanephroi removed from 14-day-old fetuses and grown for 6 days in medium containing 0, 6.9, 13.8, or 27.5 mmol/l glucose. The development of explants grown in 0, 13.8, and 27.5 mmol/l glucose was impaired compared with that of explants grown in the 6.9 mmol/l control medium, showing that the glucose concentration must be closely controlled to ensure optimum in vitro metanephros development. Thus, exposure to hyperglycemia in utero can cause a nephron deficit, which in turn may have renal consequences later in life. *Diabetes* 48:2240–2245, 1999

Congenital malformations are more frequent in the children of mothers with diabetes (1–3). Similarly, increased incidence of developmental defects is more frequent in the offspring of animals with experimentally induced diabetes (4–9), as well as in whole embryos cultured under diabetes-like conditions (5,10–14). These malformations result from developmental defects occurring in early organogenesis (2,15). They include failure of neural tube closure, caudal regression syndrome, and urogenital abnormalities, which can be as severe as renal agenesis (2,16–18), and are the main causes of perinatal mor-

ality in the offspring of diabetic mothers (16,19). Although in vitro studies have shown that high concentrations of glucose can induce dysmorphogenesis of the embryonic kidney (20), the possible adverse effects of exposure to hyperglycemia in utero on kidney development, especially with regard to nephrogenesis, has not been evaluated. The number of nephrons per kidney is characteristic of a given species and, in humans, is definitively established during fetal life. Nephrogenesis results from inductive interactions between the ureteric bud, an epithelial outgrowth of the wolffian duct, and the metanephrogenic mesenchyme. Dichotomous divisions of the ureteric bud form the collecting tubules, and the induced mesenchyme condenses and is converted into epithelium to form the nephrons (21). Various factors can reduce the number of nephrons formed in utero (22–25), and defects in nephrogenesis leading to a permanent nephron deficit influence the rate of progression of acquired renal disease (26,27) and may favor the development of hypertension (28). It is, therefore, important to determine whether exposure to the diabetic environment in utero influences nephrogenesis and the final number of nephrons. We have examined this question through in vivo and in vitro studies in rats.

We first investigated the consequences of exposure to hyperglycemia throughout gestation using experimental diabetes induced by streptozotocin (STZ). We then determined whether a short period of hyperglycemia covering the onset of nephrogenesis was sufficient to cause irreversible defects, since early stages of nephrogenesis may be critical for the genesis of a nephron deficit (29). Pregnant rats were given continuous glucose infusion for 4 days only, starting on gestational day 12, which is when renal organogenesis starts in this species. The final number of nephrons was determined in the kidney of 14-day-old pups in both studies because nephron formation continues until a few days after birth (30). Lastly, we investigated the ability of the metanephros to grow and differentiate in vitro in media containing various concentrations of glucose.

RESEARCH DESIGN AND METHODS

Animals. Female Sprague-Dawley rats, weighing 200–300 g were given free access to water and standard laboratory pellets (UAR Laboratory, Villemoisson sur Orge, France). They were caged overnight with a male, and vaginal smears were taken the following morning. The day a positive smear was obtained was designated as day 0 of gestation.

STZ-induced diabetic pregnant rats. Pregnant females were made diabetic on day 0 of gestation by a single intraperitoneal injection of STZ (Sigma, Saint Quentin, Fallavier, France) in 0.4 mol/l citrate buffer, pH 4.5. Control animals were

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HPA, helix pomatia agglutinin; PNA, peanut agglutinin; STZ, streptozotocin.

given an equivalent amount of citrate buffer. The diabetic state was checked by measuring the plasma glucose concentration 3 days after STZ injection. STZ pregnant females were allocated to one of two groups: the STZ35 group, in which females were given 35 mg/kg body wt, and the STZ45 group, in which females were given 45 mg/kg body wt. Only pregnant females whose plasma glucose was 15–19 mmol/l in the STZ35 group and >20 mmol/l in the STZ45 group (31,32) were included. Plasma glucose concentrations were then determined every 2 days.

Glucose-infused pregnant rats. The flexible technique for infusion of unrestrained pregnant rats developed by Nicolaidis et al. (33) was used (34,35). The rats were fitted with an intracardiac catheter on day 8 of gestation ($n = 11$). Infusion was started on day 12 and continued until day 16 of gestation, i.e., during the early stages of fetal nephrogenesis. Sterile 30% hypertonic glucose was infused at 40 μ l/min to maintain the maternal plasma glucose at \sim 11 mmol/l. The control rats were infused with glucose-free solution ($n = 6$).

All the rats were allowed to deliver their litters spontaneously. At 4 h after delivery, the pups were weighed, and the litters were reduced to eight animals. Pups born to the glucose-infused rats were kept with their mothers. Pups born to rats with STZ-induced diabetes were placed with control foster mothers to avoid postnatal malnutrition due to the poor breast feeding of the mother. The pups were weighed and anesthetized by an intraperitoneal injection of sodium pentobarbital (5 mg/100g body wt) when they were 14 days old. The left kidney was removed, weighed, and prepared for nephron counting.

Blood samples. Maternal blood samples were taken from the cut tip of the tail during pregnancy. Plasma glucose and insulin concentrations were measured every 2 days in the morning in the three pregnant rats of the STZ35, STZ45, and control groups. In the infused glucose experiments, plasma glucose and insulin were measured three times a day during the infusion period in the 11 glucose-infused pregnant rats and in the 6 control animals.

Plasma glucose concentrations were determined immediately after sampling by the glucose-oxidase method using a glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin concentrations were measured using a radioimmunoassay kit (Sorin, Rome, Italy). The lower limit of the assay was 19.5 pmol/l, with a coefficient of variation within and between assays of 6%.

Nephron count. The nephrons in the left kidneys of the 14-day-old rats were counted as previously described (36). Briefly, whole kidneys were incubated in 50% hydrochloric acid (6 mol/l HCl) for 45 min at 37°C, rinsed with tap water, and stored overnight at 4°C in a volumetric flask. The kidneys were mechanically dissociated, and the tubules and glomeruli were suspended in water. Three 0.5-ml aliquots were taken, and the glomeruli were counted by three different investigators who were unaware of the origin of the specimens.

Metanephric organ culture. Metanephroi were cultured as previously described (37). Briefly, the metanephroi explanted from 14-day-old fetuses were placed on a 0.8- μ m polycarbonate filter (Millipore, Saint-Quentin-en-Yvelines, France) floating on a defined serum-free medium and incubated for 6 days in 35-mm Petri dishes at 37 \pm 0.5°C in a humidified incubator with 5% CO₂. The defined glucose-free medium consisted of L15 Leibovitz medium plus HEPES (15 mmol/l), sodium bicarbonate (45 mmol/l, pH 7.45), transferrin (6.2 \times 10⁻⁸ mol/l), selenium (6.8 \times 10⁻⁹ mol/l), triiodothyronine (2 \times 10⁻⁹ mol/l), and prostaglandin E1 (7 \times 10⁻⁸ mol/l). All reagents were purchased from Sigma.

A stock solution of D-glucose (2.77 mol/l) was prepared in culture medium and stored at -20°C. One metanephros from each fetus was grown in the control medium containing 6.9 mmol/l glucose, a concentration we routinely use for organ culture. The other metanephros was grown in glucose-free medium or in glucose-supplemented medium (concentration: 13.8 or 27.5 mmol/l). The media were changed daily.

Nephron counting and growth assessment in metanephric organ culture. Nephrons formed in vitro were counted after labeling the glomerular structures in the explanted metanephroi using specific lectin-binding sites located on podocyte membranes (38). Briefly, the explanted metanephroi were fixed in 2%

paraformaldehyde in phosphate-buffered saline, detached from the filter, permeabilized with saponin, and labeled with fluorescein-coupled helix pomatia agglutinin (HPA), which stains tubules and glomeruli, and rhodamine-coupled peanut agglutinin (PNA), which stains only glomeruli. Nephrons were counted independently by two investigators using an Optiphot microscope (Nikon, Champigny-sur-Marne, France). The growth of the explanted metanephroi was then determined by measuring their protein content. The labeled metanephroi were placed in individual tubes containing 0.5 ml distilled water, rinsed, and sonicated for 15 s. The protein content was measured in duplicate by the procedure of Lowry et al. (39) as modified by Larson et al. (40), using bovine serum albumin as standard.

Statistics. All values are expressed as means \pm SE. Control and hyperglycemic groups were compared by Student's unpaired *t* test. In the STZ experiments, the intergroup significance was determined by analysis of variance. Wilcoxon's test was used to compare paired in vitro data. $P < 0.05$ was considered significant.

RESULTS

In vivo experiments

STZ-induced diabetic pregnant rats. The mean maternal plasma glucose remained almost constant in the two groups throughout gestation (Table 1). It was about three times higher in the STZ35 group and four times higher in the STZ45 group than in control animals. Differences between the three groups were highly significant. Both groups of STZ rats had lower plasma insulin than did control animals. The maternal insulinemia in the STZ45 group was significantly lower than that in the STZ35 group ($P < 0.05$).

The duration of gestation, the number of newborns per litter, and the birth weight of the STZ35 rats did not differ from those of control animals, but the final number of nephrons per kidney was 15% lower than in controls (Table 2). The STZ45 females delivered their litters 12 h earlier than did the controls, and the birth weight of the pups was 13% lower. The nephron count was 22% lower in the pups of the STZ45 group, significantly lower than that of the STZ35 pups.

Glucose-infused pregnant rats. The duration of gestation, the number of newborns per litter, and the birth weight of the hyperglycemic and control groups did not differ. The mean plasma glucose for the 4-day infusion was about double in the 11 glucose-infused rats than in the 6 control animals, and their mean plasma insulin was five times higher than that of controls (Table 3). The plasma glucose profile for the 4-day infusion period revealed that hyperglycemia remained practically unchanged (11.3 \pm 0.49 mmol/l) in 5 of 11 hyperglycemic females: these five females were referred to as hyperglycemic group 1 (HG1). In contrast, plasma glucose increased more (16.8 \pm 1.49 mmol/l) on day 13 of gestation, at the beginning of the infusion period, and decreased on day 14 to \sim 11 mmol/l in the six others: these females were referred to as hyperglycemic group 2 (HG2) (Fig. 1). Postnatal growth was the same for all pups in each group. The final

TABLE 1
Blood glucose and insulin in control and STZ-induced diabetic pregnant rats and the birth weight of the offspring in each group

	Blood glucose (mmol/l)	Blood insulin (pmol/l)	Birth weight (g)
Control	5.38 \pm 0.28 (3)	171 \pm 11 (3)	6.06 \pm 0.04 (12)
STZ35	18.2 \pm 0.4 (3)*	99 \pm 4 (3)*	6.10 \pm 0.07 (12)
STZ45	24.6 \pm 0.5 (3)*†	80 \pm 3 (3)*‡	5.28 \pm 0.12 (12)*‡

Data are means \pm SE (n). * $P < 0.001$ compared with control; † $P < 0.05$, ‡ $P < 0.001$ compared with STZ35.

TABLE 2
Body weight, kidney weight, and number of nephrons in 14-day-old pups of control and STZ mothers

	<i>n</i>	Body weight (g)	Kidney weight (mg)	Number of nephrons
Control	12	35.2 \pm 0.8	210 \pm 4	36,100 \pm 860
STZ35	12	33.4 \pm 0.5	201 \pm 5	30,839 \pm 667*
STZ45	12	26.1 \pm 1.4†‡	155 \pm 5*§	28,453 \pm 840†

Data are means \pm SE. * $P < 0.01$ and † $P < 0.001$ compared with control; ‡ $P < 0.001$, § $P < 0.05$, and || $P < 0.01$ compared with STZ35.

TABLE 3

Blood glucose and insulin in control and hyperglycemic pregnant rats (HG) and the birth weight of the offspring in each group

	Blood glucose (mmol/l)	Blood insulin (pmol/l)	Birth weight (g)
Control	5.0 ± 0.17 (6)	126 ± 6 (6)	5.86 ± 0.09 (24)
HG	11.6 ± 0.8* (11)	564 ± 36* (11)	5.85 ± 0.09 (44)

Data are means ± SE (*n*). **P* < 0.001 compared with control.

number of nephrons, determined 2 weeks after birth in four pups from each litter, was the same in control and HG1 pups, but HG2 pups had 20% fewer (Table 4, Fig. 2).

In vitro experiments. The morphology of the metanephroi grown in organ culture at various concentrations of glucose is shown in Fig. 3. In vitro development was optimal in the standard medium glucose concentration (6.9 mmol/l) (Fig. 3B). Rat metanephroi did develop in glucose-free medium, but less well than in control cultures (Fig. 3A). Metanephroi grown in 13.8 mmol/l glucose were significantly smaller and contained fewer nephrons (Fig. 3C). Ureteric bud branching morphogenesis was inhibited by 27.5 mmol/l glucose, and no nephrons at all were formed (Fig. 3D).

Figure 4 shows the quantification of growth and differentiation of explants. Those grown in glucose-free medium and in medium containing 13.8 mmol/l glucose underwent 75% less in vitro nephrogenesis and 55% less metanephric growth (*P* < 0.01 for both) than explants grown in 6.9 mmol/l glucose control medium. In vitro metanephros development and differentiation failed completely in medium containing 27.5 mmol/l glucose.

DISCUSSION

This study demonstrates that exposure to hyperglycemia in utero impairs nephron formation in the rat. The STZ used to cause maternal hyperglycemia during gestation crosses the placenta, but has a half-life of only ~15 min (41). STZ given to the maternal rat on day 0 of gestation is, therefore, unlikely to have adverse effects on the renal organogenesis that began on day 13. STZ given later in gestation may directly affect fetal organogenesis. The diabetic status induced by STZ is permanent. Therefore, STZ cannot be used to induce transient hyperglycemia during the period of early nephrogenesis. We used the glucose infusion method for that purpose. Because previous studies have shown that intrauterine growth retardation is associated with a permanent nephron deficit in humans and animals (23,42,43), fetal growth was carefully checked in our models. There was no fetal growth retardation in either the glucose infusion group or in the STZ35 group. The 13% reduction in birth body weight in the STZ45 group may be due to their early delivery. We cannot exclude, however, that in addition to the effect of hyperglycemia, other alterations resulting from sustained metabolic decompensation of diabetes (44) contributed to the impaired development of nephrons and sustained growth retardation in this particular model of severely diabetic mothers.

All of the STZ pups had fewer-than-normal nephrons, which correlated with the degree of hyperglycemia. Hyperglycemia induced by glucose infusion was less severe than that induced by STZ. In this model of glucose infusion, how-

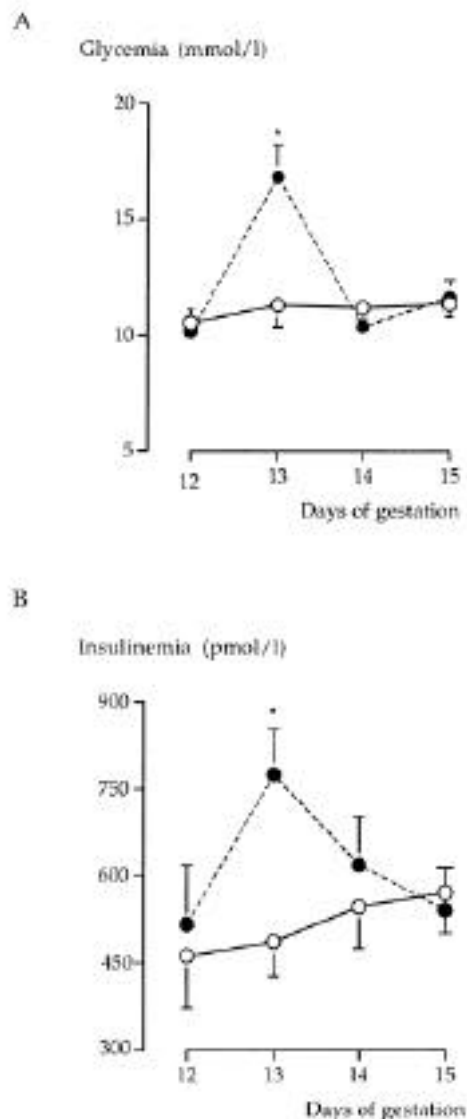


FIG. 1. Evolution of maternal glycemia (A) and insulinemia (B) during the glucose infusion period. ○, HG1 group; ●, HG2 group. Values are means ± SE. **P* < 0.05 compared with the HG1 group.

ever, pups whose mothers had a transiently elevated hyperglycemia on day 13 of gestation had a nephron deficit. This hyperglycemia may have been critical for inducing nephron deficit. Garnham et al. (45) have reported abnormal development in cultured whole embryos exposed to high concentrations of glucose for brief periods during early organogenesis. Despite efforts to control diabetes during pregnancy, such transient elevation of glycemia is known to occur in the absence of symptoms and may, therefore, have similar consequences for nephrogenesis in humans.

The in vitro experiments demonstrate that optimum metanephros growth and differentiation is obtained only within a narrow range of glucose concentration. The 14-day fetal kidney contained no glomeruli at the time of explantation, and very few nephron anlagen had been induced. The fact that metanephroi were able to grow and form some nephrons in the absence of glucose in culture medium suggests that they drew glucose from the intracellular stores of

TABLE 4
Body weight, kidney weight, and number of nephrons in 14-day-old pups of control and hyperglycemic (HG) mothers

	<i>n</i>	Body weight (g)	Kidney weight (mg)	Number of nephrons
Control	24	28.7 ± 0.8	174 ± 6	37,047 ± 410
HG1	20	29.9 ± 1.0	170 ± 5	37,887 ± 319
HG2	24	28.3 ± 0.8	167 ± 4	29,546 ± 688*†

Data are means ± SE. **P* < 0.001 compared with control; †*P* < 0.001 compared with HG1.

glycogen known to be present in the developing fetal kidney (46). However, the poorer development in control medium than in metanephroi may reflect exhaustion of glycogen stores. The poor development of the metanephros in the glucose-free medium suggests that sustained maternal hypoglycemia may adversely affect renal development. Others have reported that maternal hypoglycemia leads to developmental abnormalities in the rat embryo (47–49). Metanephroi grown in medium containing high glucose concentration showed reduced nephrogenesis. Nephron formation in the metanephric mesenchyme depends on the branching capacity of the ureteric bud (29). Since direct exposure of metanephric explants to elevated concentrations of glucose resulted in branching dysmorphogenesis of the ureteric bud, this is likely to have been the cause of the nephron deficit. Dysmorphogenesis was most marked after exposure to 27.5 mmol/l glucose, which led to complete failure of both growth and nephrogenesis. Similar impairment of nephrogenesis in vitro was recently reported for the mouse (20). However, the kidney was exposed to glucose concentrations higher than those used in the present study. Thus, glucose concentration must be closely controlled to ensure optimum renal differentiation in vitro. High environmental glucose may alter extracellular matrix components and so lead to dysmorphogenesis. Epithelial:mesenchymal interactions, which are essential for the formation of the nephrons, are modulated by numerous factors, including the extracellular matrix (50,51). Recent studies have reported that exposure of metanephroi

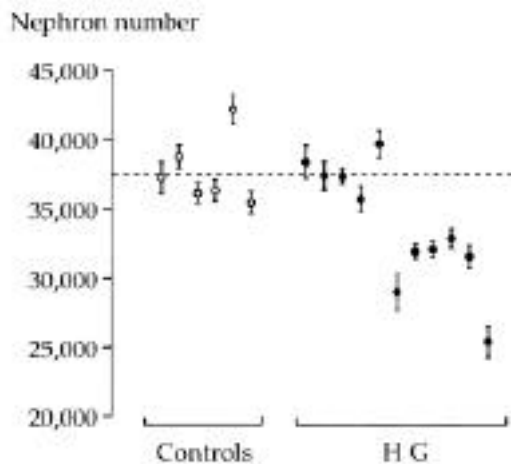


FIG. 2. Number of nephrons in the litters from control and hyperglycemic (HG) groups. The dotted line represents the mean value observed in the control group. Data are means ± SE.

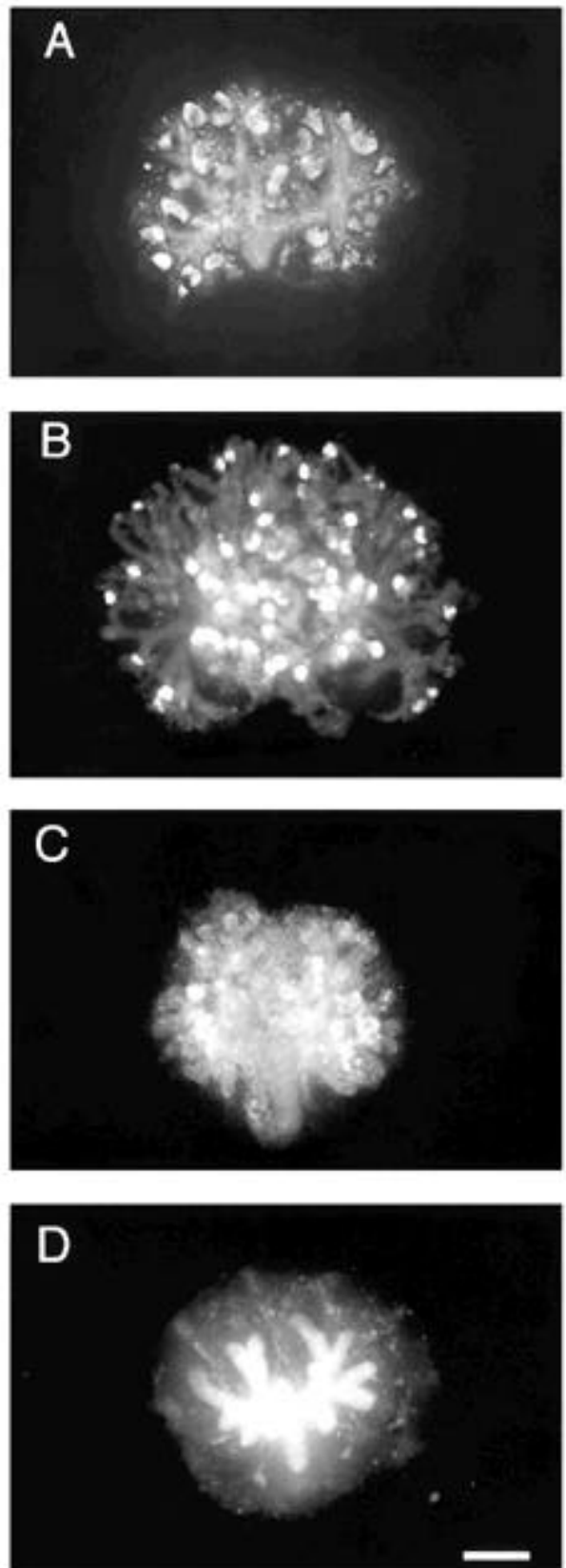


FIG. 3. In vitro metanephros development in either glucose-free (A) or glucose-supplemented medium (concentrations: 6.9 [B], 13.8 [C], or 27.5 [D] mmol/l). Metanephroi were grown for 6 days and subsequently processed for lectin labeling. Glomerular and tubular structures were visualized with HPA and PNA. The bar represents 250 μ m.

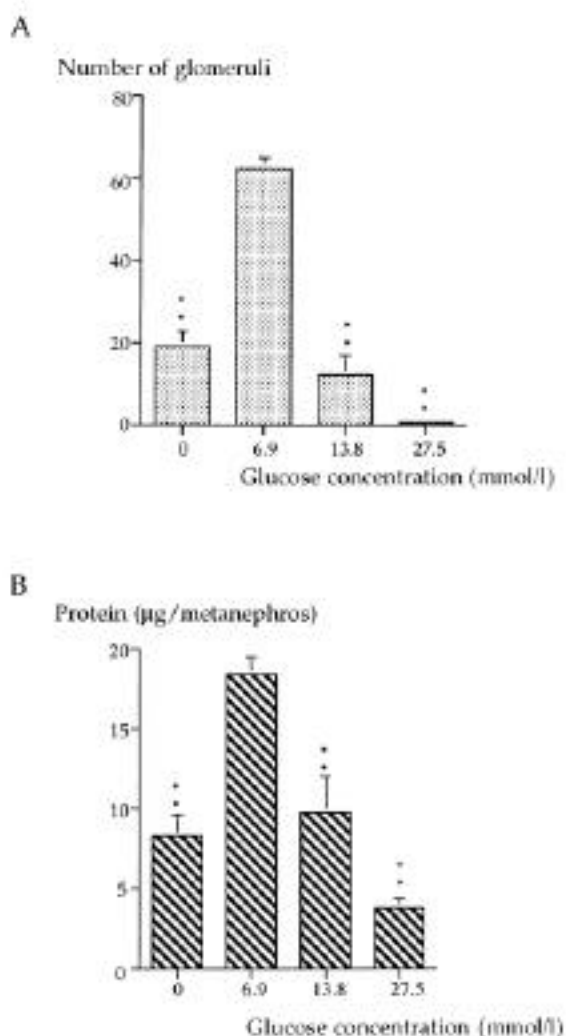


FIG. 4. Quantitative analysis of the effect of glucose on in vitro development of metanephroi. The metanephroi explanted from 14-day-old fetuses were grown for 6 days in the absence or presence of different concentrations of glucose. Differentiation was analyzed by counting the total number of glomeruli present within the metanephroi (A). Growth was assayed by protein content determination (B). Values are means \pm SE. $^*P < 0.01$ compared with 6.9 mmol/l glucose concentration.

to high glucose caused alterations in extracellular matrix components, including proteoglycan and laminin, which may explain the dysmorphogenesis of the embryonic kidney (52,53). In addition, Cagliero et al. (54) have shown that maternal diabetes also increases the synthesis of extracellular matrix components in developing embryos. Impaired nephrogenesis in the offspring of hyperglycemic mothers may thus be due to alterations in the composition of extracellular matrix.

The clinical relevance of the present findings is highlighted by recent observations that even a moderate congenital nephron deficit is a potential risk factor for progression in patients with chronic renal disease (25–28). Some have also suggested that a reduced number of nephrons favors the development of hypertension (28). Diabetic Pima Indians exposed in utero to a diabetic environment are at increased risk of elevated urinary excretion of albumin than are diabetic Pima Indians exposed to a normal intrauterine environment (55). This is consistent with the hypothesis of

long-term renal consequences in individuals born to diabetic mothers. The latter study shows an effect of the intrauterine environment itself, independent of the effects of other currently recognized susceptibility factors for renal disease that cluster in Pima Indians. Our data suggest that these patients may have acquired a nephron deficit in utero, due to a high glucose environment. This is supported by the fact that a large glomerular size, possibly reflecting an adaptation to fewer nephrons, was found in Pima Indians (56).

This study thus identifies maternal diabetes as a novel risk factor for inborn nephron deficit, in addition to other factors, such as fetal growth retardation, vitamin A deficiency, and drug exposure, already reported to alter nephrogenesis. It points out the particular risk of high blood glucose during the critical period of nephron formation, and particularly in early stages of this process. This calls for a strict control of blood glucose in diabetic pregnancy.

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REFERENCES

1. Pedersen L, Tygstrup I, Pedersen J: Congenital malformations in newborn infants of diabetic women. *Lancet* 1:1124–1126, 1964
2. Fuhrmann K, Reiher H, Semmler K, Fischer F, Fischer M, Glockner E: Prevention of congenital malformations in infants of insulin-dependent diabetic mothers. *Diabetes Care* 6:219–223, 1983
3. Martinez-Friaz M: Epidemiological analysis of outcomes of pregnancy in diabetic mothers: identification of the most frequent congenital anomalies. *Am J Med Gen* 51:108–113, 1994
4. Goldman A, Baker L, Piddington R, Marx B, Herold R, Egler J: Hyperglycemia-induced teratogenesis is mediated by a functional deficiency of arachidonic acid. *Proc Natl Acad Sci U S A* 82:8227–8231, 1985
5. Eriksson U: Importance of genetic predisposition and maternal environment for the occurrence of congenital malformation in offspring of diabetic rats. *Teratology* 37:365–374, 1988
6. Eriksson U, Thunberg L, Eriksson U: Effects of interrupted insulin treatment on fetal outcome of pregnant diabetic rats. *Diabetes* 38:764–772, 1989
7. Travers J, Pratten M, Beck F: Effects of low insulin levels on rat embryonic growth and development. *Diabetes* 38:773–778, 1989
8. Eriksson U, Borg L: Diabetes and embryonic malformations: role of substrate-induced free-oxygen radical production for dysmorphogenesis in cultured rat embryos. *Diabetes* 42:411–419, 1993
9. Buchanan T, Denno K, Sapos G, Sadler T: In vitro evidence for a multifactorial etiology with little contribution from glucose per se. *Diabetes* 43:656–660, 1994
10. Cockroft D, Coppola P: Teratogenic effect of excess glucose on head-fold rat embryos in culture. *Teratology* 16:141–146, 1977
11. Horton W, Sadler T: Effects of maternal diabetes on early embryogenesis: alterations in morphogenesis produced by the ketone body, B-hydroxybutyrate. *Diabetes* 32:610–616, 1983
12. Hod M, Star S, Passonneau J, Unterma T, Freinkel N: Effect of hyperglycemia on sorbitol and myo-inositol content of cultured rat conceptus: failure of aldose reductase inhibitors to modify myo-inositol depletion and dysmorphogenesis. *Biochem Biophys Res Commun* 140:974–980, 1986
13. Strielman P, Connors M, Metzger B: Phosphoinositide metabolism in the developing conceptus: effect of hyperglycemia and scyllo-inositol in rat embryo culture. *Diabetes* 41:989–997, 1992
14. Sadler TW: Mouse embryos in culture: models for understanding diabetes-induced embryopathies and gene function. *Int J Dev Biol* 41:291–297, 1997
15. Mills J, Baker L, Goldman A: Malformations in infants of diabetic mothers occur before the seventh gestational week: implications for treatment. *Diabetes* 28:292–293, 1979
16. Soler N, Walsh C, Malins J: Congenital malformations in infants of diabetic mothers. *Quart J Med* 45:303–313, 1976
17. Kitzmiller J, Gavin L, Peterson L: Preconception care of diabetes glycemic control prevents congenital anomalies. *JAMA* 265:731–736, 1991

18. Lynch S, Wright C: Sirenomelia, limb reduction defects, cardiovascular malformation, renal agenesis in an infant born to a diabetic mother. *Clin Dysmorphol* 6:75-80, 1997
19. Lowy C, Beard R, Goldschmidt J: Congenital malformations in babies of diabetic mothers. *Diabet Med* 3:458-462, 1986
20. Kanwar Y, Liu Z, Kumar A, Usman M, Wada J, Wallner E: D-glucose-induced dysmorphogenesis of embryonic kidney. *J Clin Invest* 98:2478-2488, 1996
21. Saxen L: *Organogenesis of the Kidney*. Cambridge, U.K., Cambridge University Press, 1987
22. Gilbert T, Lelièvre-Pégorier M, Maliérou R, Meulemans A, Merlet-Bénichou C: Effects of prenatal and postnatal exposure to gentamicin on renal differentiation in the rat. *Toxicol* 43:301-313, 1987
23. Merlet-Bénichou C, Gilbert T, Muffat-Joly M, Lelièvre-Pégorier M, Leroy B: Intrauterine growth retardation leads to a permanent nephron deficit in the rat. *Pediatr Nephrol* 8:175-180, 1994
24. Lelièvre-Pégorier M, Vilar J, Ferrier M, Moreau E, Freund N, Gilbert T, Merlet-Bénichou C: Mild vitamin A deficiency leads to inborn nephron deficit in the rat. *Kidney Int* 54:1455-1462, 1998
25. Merlet-Bénichou C, Gilbert T, Vilar J, Moreau E, Freund N, Lelièvre-Pégorier M: Nephron number: variability is the rule: causes and consequences. *Lab Invest* 79:515-527, 1999
26. Gilbert T, Lelièvre-Pégorier M, Merlet-Bénichou C: Long-term effects of mild oligonephronia induced in utero by gentamicin in the rat. *Pediatr Res* 30:450-456, 1991
27. He C, Zalups RK, Henderson DA, Striker GE, Striker LJ: Molecular analysis of spontaneous glomerulosclerosis in Os/+ mice, a model with reduced nephron mass. *Am J Physiol* 269:F266-F273, 1995
28. Brenner BM, Garcia DL, Anderson S: Glomeruli and blood pressure: less of one, more of the other? *Am J Hypertens* 1:335-347, 1988
29. Gilbert T, Cibert C, Moreau E, Géraud G, Merlet-Bénichou C: Early defect in branching morphogenesis of the ureteric bud in induced nephron deficit. *Kidney Int* 50:783-795, 1996
30. Larsson L, Aperia A, Wilton P: Effect of normal development on compensatory renal growth. *Kidney Int* 18:29-35, 1980
31. Shafir E: Diabetes in animals. In *Diabetes Mellitus: Theory and Practice*. Rifkin H, Porte D, Eds. New York, Elsevier, 1989, p. 299-340
32. Siman CM, Eriksson U: Vitamin E decreases the occurrence of malformations in the offspring of diabetic rats. *Diabetes* 46:1054-1061, 1997
33. Nicolaidis S, Rowland N, Meile M, Marfaing-Jallat P, Pesze A: A flexible technique for long term infusions in unrestrained rats. *Pharmacol Biochem Behav* 2:131-136, 1974
34. Ktorza A, Girard J, Kinebanyan M, Picon L: Hyperglycemia induced by glucose infusion in the unrestrained pregnant rat during the last three days of gestation. *Diabetologia* 21:569-574, 1981
35. Freund N, Prieur B, Bismuth J, Delaval E: Effect of hyperglycemia on the polyol pathway in rat kidney during the perinatal period. *Eur J Biochem* 242:86-89, 1996
36. Merlet-Bénichou C, Lelièvre-Pégorier M, Muffat-Joly M, Augeron C: Functional and morphologic pattern of renal maturation in the developing guinea-pig. *Am J Physiol* 241:F618-F624, 1981
37. Avner E, Ellis D, Temple T, Jaffe R: Metanephric development in serum free organ culture. *In Vitro* 18:675-682, 1982
38. Gilbert T, Gaonach S, Moreau E, Merlet-Bénichou C: Defect of nephrogenesis by gentamicin in rat metanephric organ culture. *Lab Invest* 70:656-666, 1994
39. Lowry O, Rosebrough N, Farr A, Randall R: Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265-275, 1951
40. Larson E, Howlett B, Jagendorf A: Artificial reductant enhancement of the Lowry method for protein determination. *Anal Biochem* 155:243-248, 1986
41. Schein P, Loftus S: Streptozotocin: depression of mouse liver pyridine nucleotides. *Cancer Res* 28:1501-1506, 1968
42. Hinchliffe S, Lynch M, Sargent P, Howard C, Van Velzen D: The effect of intrauterine growth retardation on the development of renal nephrons. *Br J Obstet Gynaecol* 99:296-301, 1992
43. Merlet-Bénichou C, Leroy B, Gilbert T, Lelièvre-Pégorier M: Retard de croissance intra-utérin et déficit en néphrons (In French). *Médecine-Sciences* 9:777-780, 1993
44. Styrd J, Thunberg L, Nybacka O, Eriksson U: Correlations between maternal metabolism and deranged development in the offspring of normal and diabetic rats. *Pediatr Res* 37:343-353, 1995
45. Garnham E, Beck F, Clarke C, Stanisstreet M: Effects of glucose on rat embryos in culture. *Diabetologia* 25:291-295, 1983
46. Gutierrez-Correa J, Hod M, Passoneau J, Freinkel N: Glycogen and enzymes of glycogen metabolism in rat embryos and fetal organs. *Biol Neonate* 59:294-302, 1991
47. Akazawa S, Akazawa M, Hashimoto M, Yamaguchi Y, Kuriya N, Toyama K, Ueda Y, Nakanishi T, Mori T, Miyake S: Effects of hypoglycaemia on early embryogenesis in rat embryo organ culture. *Diabetologia* 30:791-796, 1987
48. Buchanan T, Schemmer J, Freinkel N: Embryotoxic effects of brief maternal insulin-hypoglycemia during organogenesis in the rat. *J Clin Invest* 78:643-649, 1986
49. Tanigawa K, Kawaguchi M, Tanaka O, Kato Y: Skeletal malformations in rat offspring: long-term effect of maternal insulin-induced hypoglycemia during organogenesis. *Diabetes* 40:1115-1121, 1991
50. Sorokin L, Ekblom P: Development of tubular and glomerular cells in the kidney. *Kidney Int* 41:657-664, 1992
51. Kanwar Y, Caron F, Kumar A, Wada J, Ota K, Wallner E: Role of extracellular matrix, growth factors and proto-oncogenes in metanephric development. *Kidney Int* 52:589-606, 1997
52. Abrass C, Spicer D, Berfield A, St. John P, Abrahamson D: Diabetes induces changes in glomerular development and laminin-B2 (s-laminin) expression. *Am J Pathol* 151:1131-1140, 1997
53. Kanwar Y, Liu Z, Wallner E: Influence of glucose on murine metanephric development and proteoglycans: morphologic and biochemical studies. *Lab Invest* 76:671-681, 1997
54. Cagliero E, Forsberg H, Sala R, Lorenzi M, Eriksson U: Maternal diabetes induces increased expression of extracellular matrix components in rat embryos. *Diabetes* 42:975-980, 1993
55. Nelson RG, Morgenstern H, Bennett PH: Intrauterine diabetes exposure and the risk of renal disease in diabetic Pima Indians. *Diabetes* 47:1489-1493, 1998
56. Schmidt K, Pesce C, Liu Q, Nelson R, Bennett P, Karnitschnig H, Striker L, Striker G: Large glomerular size in Pima Indians: lack of change with diabetic nephropathy. *J Am Soc Nephrol* 3:229-235, 1992