Effect of Puberty on Initial Kidney Growth and Rise in Kidney IGF-I in Diabetic Rats

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Prepubertal subjects have a low incidence of diabetic nephropathy compared with duration-matched postpubertal subjects. At puberty, there is an increase in insulinlike growth factor I (IGF-I) levels, and because IGF-I has been implicated in the early kidney enlargement of experimental diabetes, we studied the development of kidney enlargement and kidney IGF-I levels in prepubertal (aged 5 wk) and postpubertal (aged 13 wk) Sprague-Dawley rats during the 7 days after induction of diabetes with streptozocin. Kidney weight in postpubertal diabetic animals was significantly greater than in postpubertal controls by day 2 (1.46 ± 0.06 vs. 1.16 ± 0.09 g, $P < 0.05$), and by day 7, kidney weight had increased by 36% (1.61 ± 0.07 vs. 1.18 ± 0.08 g, $P < 0.001$). Despite comparable blood glucose levels in the prepubertal and postpubertal diabetic rats, kidney weight in prepubertal diabetic animals was significantly greater than in prepubertal controls by 14% on day 7 only (0.84 ± 0.01 vs. 0.73 ± 0.03 g, $P < 0.05$). Kidney IGF-I content was significantly elevated in diabetic postpubertal rats, peaking on day 1 (diabetic vs. control, 1082 ± 156 vs. 543 ± 21 ng/g, $P < 0.001$) and day 2 but not in prepubertal diabetic rats. Thus, prepubertal diabetic rats have reduced and retarded kidney growth and attenuated kidney IGF-I levels, suggesting that local IGF-I accumulation may play an important role in diabetes-associated kidney enlargement. ***Diabetes*** **39:557–62, 1990**

Several studies have suggested that prepubertal diabetic subjects are less prone to microvascular complications than postpubertal subjects with equal duration of diabetes (1–8). These findings have raised the possibility that hormonal changes during puberty may modify the development of diabetic microangiopathy through changes in sex steroid and insulinlike growth factor I (IGF-I) levels.

Growth hormone and IGF-I, which mediates many of the actions of growth hormone, have been implicated in the development of diabetic complications (9–13). One of the features of diabetic nephropathy in humans (14–17) and rats (18–22) is increased kidney size. In rats, kidney size increases within 48 h of induction of diabetes with streptozocin (STZ). IGF-I is a potent mitogen in many tissues (23), and kidney IGF-I levels are also elevated in the first 48 h after induction of diabetes in the rat (12), suggesting a possible role for IGF-I in this early kidney growth.

We investigated the pattern of early kidney growth in prepubertal and postpubertal diabetic rats to evaluate whether puberty may modify this process. Kidney IGF-I levels were measured to assess the possible contribution of IGF-I to diabetes-associated kidney growth in general and to determine its role in any modifications of kidney growth associated with puberty.

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats were used for all experiments. Two groups of rats were used; the first group was aged 5 wk, weighed 105.4 ± 14.0 g (mean ± SD), and was designated prepubertal; the second group was aged 13 wk, weighed 350.7 ± 32.8 g, and was designated postpubertal. On day 0, groups of animals were carefully weight matched, and half were made diabetic by STZ injection via the tail vein after an overnight fast. Because young animals are relatively resistant to STZ (24), prepubertal animals were given 65 mg/kg STZ, whereas postpubertal animals were given 60 mg/kg. The remaining animals were injected with citrate buffer (pH 4.5) and acted as controls. Diabetic animals with plasma glucose levels between 15 and 40 mM were used in the experiments. All experiments were approved by the Austin Hospital Animal Welfare Committee.

On days 1–3 and 7 after injection, groups of four to six

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diabetic and control animals were anesthetized with pentobarbital sodium (60 mg/kg body wt), both kidneys were excised, and blood was obtained by cardiac puncture. The right kidney was weighed after decapsulation and trimming, and both kidneys were snap frozen in isopentane–dry ice. Blood was centrifuged, and plasma and tissues were stored at −70°C for later analysis.

Tissue IGF-I extraction was performed by pulverization under liquid N_2 and incubation in 1 M acetic acid on ice as described by D'Ercole and Underwood (25). Neutralization was performed with 1 M Tris, and samples were recentrifuged before assay.

Plasma IGF-I was assayed after acid-ethanol extraction (26). Recombinant IGF-I (Amgen, Thousand Oaks, CA) was iodinated by the iodogen method (27) and purified on a 1 × 25-cm Sephadex G-50 column (Pharmacia, Sydney), followed by further purification by hydrophobic interaction chromatography on a 1 × 5-cm column of octyl-Sepharose CL-4B (Pharmacia) (28). Antibody UBK487 (raised by L. Underwood and J.J. Van Wyk, Division of Pediatric Endocrinology, Univ. of North Carolina, Chapel Hill) was donated by the National Hormone and Pituitary Program (Univ. of Maryland School of Medicine, Baltimore). All constituents were made up in buffer containing 200 mg/L protamine sulfate, 4.15 g/L sodium phosphate (monobasic), 3.72 g/L EDTA, 0.02% sodium azide, and Tween 20 (0.50 g/L, pH 7.5; Ajax, Auburn, Australia). Separation was achieved with sheep anti-rabbit antibody, with normal rabbit plasma as carrier and 10% polyethylene glycol (final). Antibody-bound activity was counted. The minimum detectable concentration was 0.2 ng/ml, and the intra-assay coefficient of variation was 9.3% at a level of 1330 ng/ml for plasma and 8.5% at a level of 196 ng/g for tissue. All samples to be compared were analyzed in the same assay.

Plasma glucose was measured by the glucose oxidase method. Rats were allowed to feed freely until the time of operation, so that glucose levels represent a random nonfasting measurement. Plasma ketones were measured with Keto-Diastix (Miles, Mulgrave, Australia). Testosterone was assayed by radioimmunoassay with antibody from Farmos Diagnostica (Turku, Finland), standards from Steraloids (Wilton, NH), and tracer from Amersham (North Ryde, Australia).

All values are expressed as means ± SE unless stated otherwise. Before statistical analysis of kidney IGF-I levels, data were logarithmically transformed to stabilize variances. Kidney weight, kidney IGF-I concentration, and plasma glucose and total body weight changes were initially analyzed by two-way analysis of variance. If the overall analysis was significant at the 0.05 level, group means were compared by the protected least significant difference method (29). Testosterone levels in prepubertal and postpubertal rats were compared by unpaired *t* test. Testosterone levels in prepubertal rats were compared by one-way analysis of variance. The independent contributions of duration of diabetes and glucose levels to kidney size were analyzed by multiple regression. Data analyses were carried out with the Statview 512+ package (Brainpower, Calabasas, CA) on an Apple Macintosh SE computer (Cupertino, CA).

RESULTS

Analysis of rat weights on day 0 demonstrated that groups were adequately matched (postpubertal: $F = 0.5$, df = 3 and 35, $P = 0.69$, for day and $F = 0.3$, df = 1 and 35, $P = 0.60$, for diabetes; prepubertal: $F = 1.3$, df = 3 and 31, $P = 0.30$, for day and $F = 0.001$, df = 1 and 31, $P = 0.99$, for diabetes). Testosterone levels were significantly lower in prepubertal than postpubertal control rats (2.6 ± 0.3 vs. 16.3 ± 1.6 nM, $t = 9.7$, $P = 0.0001$), confirming their pubertal status (Tables 1 and 2). Plasma IGF-I levels were also lower in the younger rats, although these levels rose to adult levels by day 7, suggesting that the IGF-I surge associated with puberty had begun. Plasma testosterone levels were higher in prepubertal control rats on day 7 than days 0–3, confirming the onset of puberty (4.1 ± 0.3 vs. 2.1 ± 0.5 nM, $F = 10.6$, df = 1 and 20, $P = 0.004$).

Despite comparable blood glucose levels in the prepubertal and postpubertal diabetic rats ($F = 1.5$, df = 1 and 34, $P = 0.24$; Tables 1 and 2), the pattern of kidney enlargement was different in these groups. Overall, kidney weight was greater in the postpubertal diabetic rats than postpu-

TABLE 1
Effects of diabetes on plasma glucose, total body weight, plasma insulinlike growth factor I (IGF-I), and testosterone in postpubertal rats

	Day				
	0	1	2	3	7
Plasma glucose (mM)					
Diabetic		30.9 ± 1.3*	26.0 ± 0.6*	29.1 ± 3.1*	34.5 ± 1.4*
Control	10.4 ± 0.5	11.2 ± 0.1	10.6 ± 0.3	11.0 ± 0.4	10.1 ± 0.4
Total body weight (g)					
Diabetic		328 ± 10†	296 ± 8*	334 ± 11†	327 ± 13‡
Control	341 ± 18	380 ± 9	370 ± 13	387 ± 22	388 ± 22
Right kidney weight (g)					
Diabetic		1.17 ± 0.06	1.32 ± 0.06§	1.46 ± 0.06‡	1.61 ± 0.07*
Control	1.16 ± 0.07	1.22 ± 0.05	1.12 ± 0.05	1.16 ± 0.09	1.18 ± 0.08
Plasma IGF-I (ng/ml)					
Diabetic		709 ± 169	579 ± 115†	765 ± 194§	408 ± 123*
Control	782 ± 57	1001 ± 70	1316 ± 241	1460 ± 183	1167 ± 112
Testosterone (nM)					
Diabetic		4.3 ± 0.3	3.6 ± 1.7*	2.1 ± 0.3*	3.5 ± 0.4§
Control	17.2 ± 5.2	17.7 ± 7.9	17.2 ± 2.1	17.4 ± 1.8	10.5 ± 1.4

*$P < 0.001$, †$P < 0.02$, ‡$P < 0.01$, §$P < 0.05$, vs. control rats.

TABLE 2
Effects of diabetes on plasma glucose, total body weight, plasma insulinlike growth factor I (IGF-I), and testosterone in prepubertal rats

	Day				
	0	1	2	3	7
Plasma glucose (mM)					
Diabetic		30.4 ± 3.0*	28.6 ± 2.7*	28.4 ± 2.2*	25.4 ± 3.2*
Control	9.5 ± 0.3	9.7 ± 0.6	8.8 ± 0.4	7.9 ± 1.5	8.7 ± 0.6
Total body weight (g)					
Diabetic		119 ± 5	114 ± 8†	121 ± 10	157 ± 7†
Control	115 ± 7	124 ± 6	133 ± 2	134 ± 4	176 ± 6
Right kidney weight (g)					
Diabetic		0.63 ± 0.03	0.59 ± 0.04	0.63 ± 0.04	0.84 ± 0.01‡
Control	0.54 ± 0.02	0.58 ± 0.04	0.57 ± 0.02	0.62 ± 0.02	0.73 ± 0.03
Plasma IGF-I (ng/ml)					
Diabetic		749 ± 137	360 ± 45§	490 ± 152‡	998 ± 212
Control	601 ± 123	544 ± 52	920 ± 100	843 ± 174	1243 ± 67
Testosterone (nM)					
Diabetic		1.7 ± 0.5	1.7 ± 0.5	1.1 ± 0.2	5.7 ± 0.6
Control	2.4 ± 0.9	2.0 ± 0.3	2.0 ± 0.3	2.1 ± 0.2	4.1 ± 0.3

*$P < 0.001$, †$P < 0.01$, ‡$P < 0.05$, §$P < 0.002$, vs. control rats.

bertal controls ($F = 20.8$, df = 1 and 35, $P = 0.0001$), and in these rats, diabetes had a significant effect on the pattern of kidney growth with time (time × diabetes interaction, $F = 4.4$, df = 3 and 35, $P = 0.01$). In the postpubertal rats, comparison of kidney weights from diabetic and control rats on each day revealed a significant difference by day 2 (diabetic vs. control, 1.46 ± 0.06 vs. 1.16 ± 0.09 g, respectively, $P < 0.05$), and by day 7, there was a 36% increase in kidney weight in diabetic rats (1.61 ± 0.07 vs. 1.18 ± 0.08 g, $P < 0.01$; Fig. 1*A*). By contrast, the kidney weight of diabetic prepubertal rats was marginally greater than that of prepubertal controls overall ($F = 4.2$, df = 1 and 30, $P = 0.05$), and daily comparisons revealed a significant increase in diabetic rats on day 7 alone and then by only 14% (0.84 ± 0.01 vs. 0.73 ± 0.03 g, $P < 0.05$; Fig. 1*B*). The increase in kidney weight in diabetic rats over their respective control rats on each day was significantly greater overall in postpubertal than prepubertal rats ($F = 8.3$, df = 1 and 34, $P = 0.007$; Fig. 2).

To examine the possible influence of the severity of diabetes as measured by blood glucose on the development of kidney enlargement, we analyzed the relationship between kidney size, days since STZ injection, and glucose by multiple regression analysis. This revealed that, whereas duration of diabetes was a strong independent predictor of kidney size in both prepubertal and postpubertal diabetic rats, blood glucose was not (postpubertal: duration, $t = 3.95$, $P = 0.0008$; glucose, $t = 0.24$, $P = 0.81$; prepubertal: duration, $t = 4.56$, $P = 0.0003$; glucose, $t = 0.86$, $P = 0.40$; Fig. 3). In postpubertal rats, the effect of duration of diabetes

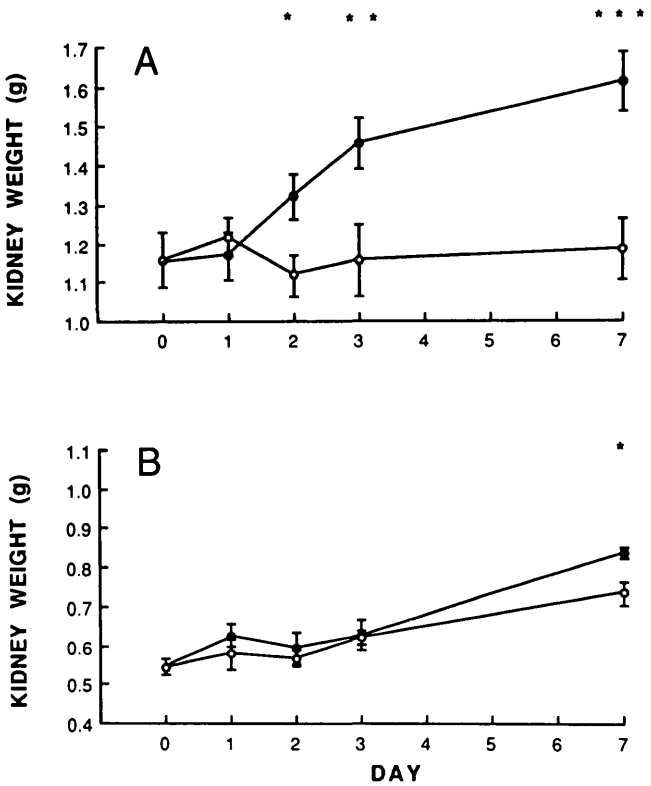

FIG. 1. Right kidney weight in diabetic (●) and control (○) postpubertal (*A*) and prepubertal (*B*) rats. Values are means ± SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, diabetic vs. control.

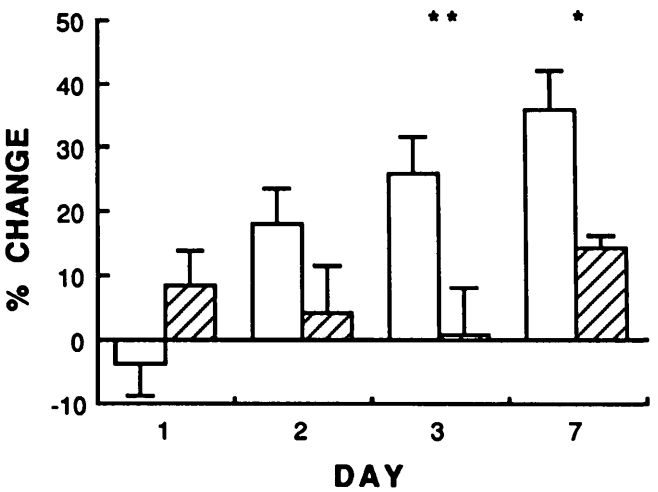

FIG. 2. Percent change in kidney weight in diabetic versus control postpubertal (*open bars*) and prepubertal (*hatched bars*) rats. *$P < 0.02$, **$P < 0.01$, postpubertal vs. prepubertal.

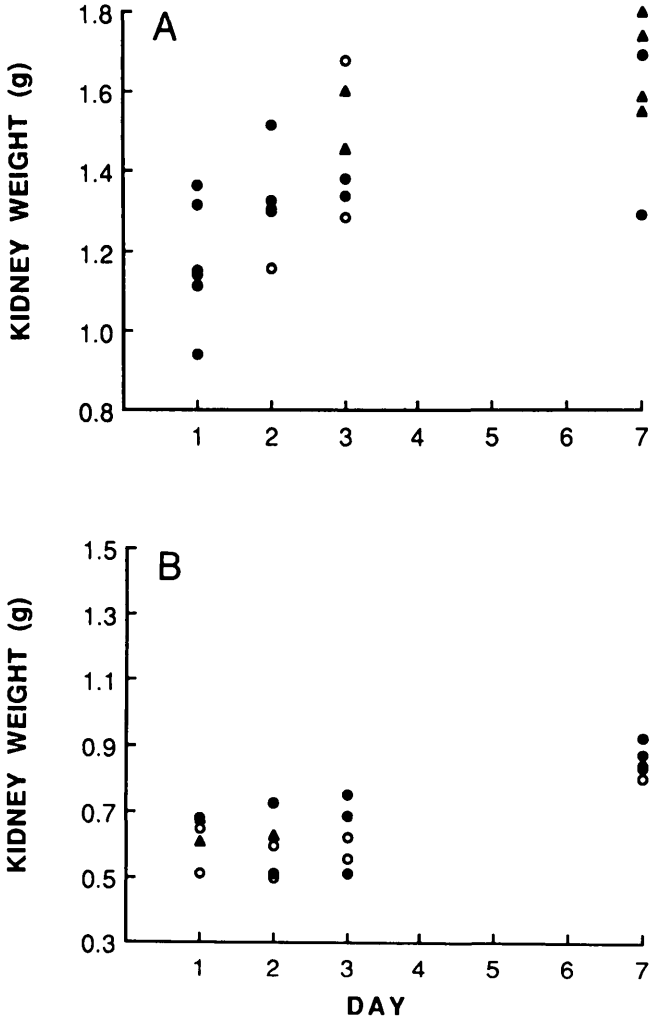

FIG. 3. Relationship between blood glucose and kidney weight in postpubertal (*A*) and prepubertal (*B*) diabetic rats on days 1–7 of experiment. ○, 15–25 mM plasma glucose; ●, 25–35 mM plasma glucose; ▲, 35–40 mM plasma glucose.

on kidney size was independent of normal growth, because kidney size in postpubertal control animals did not increase during the experiment. In contrast, this effect in prepubertal rats was predominantly a reflection of normal growth, because kidney weight in prepubertal control animals increased by 35% during the experiment.

Kidney IGF-I levels were elevated in the postpubertal diabetic rats compared with postpubertal controls (F = 5.4, df = 1 and 33, P = 0.03), and the presence of diabetes had a significant effect on the pattern of kidney IGF-I levels with time (time × diabetes interaction, F = 6.0, df = 3 and 33, P = 0.002; Fig. 4*A*). The levels of kidney IGF-I in the postpubertal diabetic animals peaked on day 1 (diabetic vs. control, 1082 ± 156 vs. 543 ± 21 ng/g, $P < 0.01$). However, in prepubertal rats, kidney IGF-I levels were no different in diabetic rats than in their respective controls (F = 0.3, df = 1 and 28, P = 0.58; Fig. 4*B*). The tissue IGF-I levels in the latter rats rose in parallel with the plasma levels throughout the 7 days.

Total weight was decreased in both postpubertal and prepubertal diabetic rats, although there was no overall difference in the degree of weight loss in the two groups (F = 0.6, df = 1 and 34, P = 0.44). By day 7, postpubertal diabetic rats weighed 24.3 ± 2.6% less than postpubertal control rats compared to a 19.0 ± 2.5% difference in the prepubertal rats (Tables 1 and 2). None of the rats in the study had significant ketonemia.

DISCUSSION

Several studies have suggested that diabetic prepubertal children may be protected from the development of complications until after they have passed through puberty. In 1961, Larsson et al. (1) found that age of onset of diabetes was related to the development of angiopathy, with onset of diabetes <5 yr of age being associated with lower complication rates. They attributed this to a putative "hormonal atherogenic factor" associated with puberty (1). Krolewski et al. (2) showed that the cumulative incidence rates of persistent proteinuria and end-stage kidney failure in subjects with insulin-dependent diabetes mellitus (IDDM), when expressed according to duration of diabetes, are lower in children diagnosed at <10 yr of age than in older children. However, when expressed in terms of attained age, the rates are similar, suggesting a disease-free period in the younger children. Dahlquist and Rudberg (3) found that children <13 yr of age with diabetes of 5 yr duration had significantly lower albumin excretion rates than older children and that microalbuminuria was only found in subjects aged ≥16 yr. Mathiesen et al. (4) also found microalbuminuria only in patients aged ≥15 yr in a cohort of children with IDDM. Several

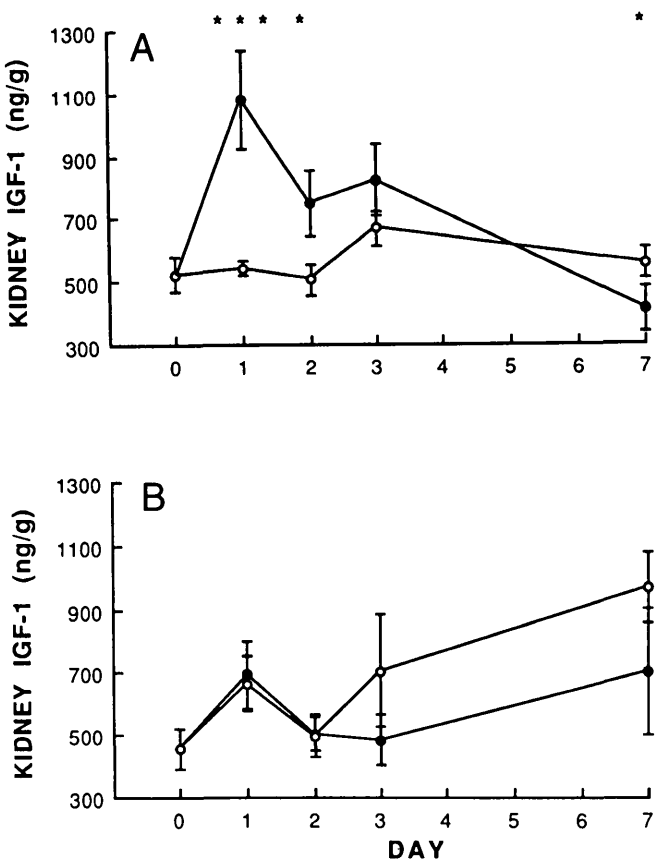

FIG. 4. Kidney insulinlike growth factor I (IGF-I) concentrations in diabetic (●) and control (○) postpubertal (*A*) and prepubertal (*B*) rats. Values are means ± SE. *$P < 0.05$,*$P < 0.001$, diabetic vs. control.**

studies had similar findings with respect to retinopathy (5–8). Capillary basement membrane thickening is a feature of diabetic microangiopathy, and this process is related to the level of glycemia in postpubertal but not pubertal diabetic subjects (30).

Several hormonal changes with possible implications for the development of diabetic nephropathy take place at puberty. First, there is a rise in sex steroid levels (31), which have been shown to exert an important effect on potential pathogenic mechanisms in experimental diabetic nephropathy such as the polyol pathway. Diabetes is associated with an increase in vascular permeability, which is greatest in tissues predisposed to diabetic vascular disease in humans, including the kidney and eye. Castration has been shown to prevent this increase in vascular permeability, which has been attributed to a reduction in polyol-pathway abnormalities (32). Castration in diabetic rats is also associated with increased collagen solubility, suggesting that castration reduces collagen cross-linking in diabetes (33).

A second association with puberty is a rise in IGF-I levels (31), which has also been linked to aspects of diabetic microangiopathy. Flyvbjerg et al. (12) found that the early kidney enlargement associated with experimental diabetes is associated with a rise in kidney IGF-I levels. Administration of a somatostatin analogue suppresses the increase in both kidney IGF-I levels and kidney growth in diabetic rats without altering blood glucose levels, suggesting a role for IGF-I in the process of kidney growth (13). Diabetic subjects with proliferative retinopathy have greater plasma IGF-I levels than subjects without this complication (11). Nondiabetic transgenic mice that express the gene for growth hormone, growth hormone–releasing hormone, or IGF-I develop histological changes similar to those seen in diabetic nephropathy, although the changes are more severe in the first two models. This may reflect differences in the magnitude of hormone overproduction, changes in IGF-binding protein concentrations, or a growth hormone–dependent, IGF-I–independent mechanism (34,35). Specific receptors for IGF-I are found on mouse mesangial cells, and IGF-I is a potent mitogen for these cells (36). It is therefore possible that IGF-I contributes to the mesangial hyperplasia that is a feature of diabetic nephropathy.

Kidney enlargement is a well-described feature of early IDDM in humans (14–17) and STZ-injected rats (12,18–22). In humans, increased kidney size is related to increased glomerular filtration rate and may be associated with increased risk of development of diabetic nephropathy (37). In humans, no evalution of kidney size in prepubertal diabetic subjects has been undertaken, although one study has demonstrated hyperfiltration in four of five diabetic children aged ≤12 yr (38). In rats, kidney enlargement has been confirmed in both sexes and in several different strains. Although pubertal status was not evaluated in these experiments, the rat weights suggest that they were postpubertal.

It has been suggested that kidney growth is dependent on the metabolic state of diabetic rats, so that severe hyperglycemia may retard kidney enlargement (21). It is therefore of critical importance that the severity of diabetes in the prepubertal and postpubertal rats in our study was the same. Several findings suggest this is true. The degree of weight loss in both diabetic groups relative to their respective controls was the same. Plasma glucose levels were not significantly different in both diabetic groups. Finally, the decrease in plasma IGF-I levels was similar in both diabetic groups. Additionally, the early pubertal rises in plasma and kidney IGF-I and plasma testosterone were seen in both control and diabetic groups on day 7. It is unlikely that this would have occurred if the prepubertal diabetic animals were severely catabolic.

Although it has been stated that kidney enlargement is inhibited in diabetic rats with glucose levels >25–30 mM (21), analysis of our data did not confirm this finding (Fig. 3). This may relate to differences in measurement techniques (whole-blood glucose levels are 15% lower than plasma glucose levels) and strain or sex differences. Furthermore, plasma glucose levels are increased by up to 30% in the first 20 min of pentobarbital sodium anesthesia (39), so that the glucose measurements in our study may be higher than those seen in other studies.

In male rats, plasma testosterone begins to rise at ~5 wk of age and reaches a peak at 8–9 wk before plateauing at adult levels by wk 10 (40,41). Full spermatogenesis occurs at ~7–8 wk of age (40). The pubertal plasma IGF-I surge in rats commences at ~6 wk of age and reaches the adult plateau at ~8–11 wk, with adult plasma levels approximately twice those of prepubertal rats (42). Accordingly, the younger rats in our study were prepubertal on day 0. This has been confirmed by measurement of plasma testosterone and IGF-I levels. The rise in plasma testosterone and IGF-I levels in the control groups by day 7 suggests that these animals had entered puberty. There was marked suppression of testosterone levels in the postpubertal diabetic rats, confirming the findings of other studies (43). We did not observe this finding in the prepubertal rats, which may reflect lower levels in the control animals. Interestingly, kidney IGF-I levels rose in the prepubertal control group in parallel with plasma IGF-I levels, a puberty-related phenomenon not previously described. The 13-wk-old rats were postpubertal as assessed by the above criteria. The relatively low plasma IGF-I levels in postpubertal rats on day 0 may reflect a degree of starvation due to overnight fasting and an inadequate time for a response to refeeding (these rats were operated on within 1–2 h of refeeding). The relatively large gain in body weight observed between days 0 and 1 supports this possibility.

Plasma IGF-I levels are low in STZ-induced diabetic rats (44), a finding we confirmed. However, the paracrine and autocrine effects of IGF-I appear to be more important than plasma IGF-I levels in states of tissue hypertrophy and repair (45). Additionally, growth in growth hormone–replaced hypophysectomized rats is more closely correlated with tissue than with plasma IGF-I levels (46). The increased tissue levels of IGF-I in the kidney, an organ that enlarges in diabetes, contrast strikingly with the low plasma levels and decreased hepatic production of IGF-I (47) associated with the general weight loss seen in this condition.

Our finding of suppressed kidney growth coincident with a lack of increase in kidney IGF-I levels in prepubertal diabetic animals supports the hypothesis that IGF-I may play a significant role in the development of kidney enlargement in diabetic rats. The rise in kidney IGF-I in postpubertal animals precedes kidney growth, which is consistent with a potential

causative role. The return to normal kidney IGF-I values by the 3rd day of diabetes suggests that the local role of IGF-I may be to initiate the kidney growth that may ultimately lead to kidney damage.

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