

Contribution of Growth Hormone and IGF-I to Early Diabetic Nephropathy in Type 1 Diabetes

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In children and adolescents with type 1 diabetes, we have reported an association between duration of puberty and the prevalence of nephromegaly and microalbuminuria (MA), which are early markers of diabetic nephropathy. Growth hormone (GH), IGF-I, testosterone, and prorenin are potential mediators of this effect. This study examined the relationship of these hormonal factors to kidney volume (KV) and MA in 155 subjects (78 males, age 13.2 ± 3.5 years [mean \pm SD]) with similar diabetes duration (6.83 ± 1.6 years) but varying pubertal experience (0–10 years). KV (by ultrasound), plasma IGF-I, testosterone, prorenin, and NaLi countertransport, and urinary albumin, urinary GH, and urinary IGF-I from three 24-h collections were measured. Multiple regression analysis showed that BSA ($P < 0.0001$) and urinary IGF-I ($P = 0.001$) were significantly associated with KV. MA subjects (albumin excretion rate 15–200 $\mu\text{g}/\text{min}$) had higher urinary IGF-I ($P = 0.005$) and urinary GH ($P = 0.05$) compared with normoalbuminuric subjects. Only 9% of the variance in urinary IGF-I could be attributed to plasma IGF-I ($r = 0.30$, $P < 0.0001$). Testosterone and prorenin were not associated with MA, but they were associated with KV in univariate analyses. The strong association of urinary IGF-I with KV, a marker for glomerular hypertrophy, and of both urinary IGF-I and urinary GH with MA suggests a role for these growth factors in the development of human diabetic nephropathy. Together, these data support animal studies that have shown that renal GH and IGF-I may contribute significantly to the pathogenesis of early diabetic nephropathy. *Diabetes* 47:1341–1346, 1998

The prevalence of early indicators of diabetic nephropathy increases during puberty (1,2). In addition, when controlled for diabetes duration, a relationship has been shown between pubertal duration and the prevalence of both microalbuminuria (MA) (1,3) and nephromegaly (1). These observations suggest that the hormonal milieu of puberty may be involved in the pathogenesis of early diabetic nephropathy.

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AER, albumin excretion rate; ANOVA, analysis of variance; BSA, bovine serum albumin; CT, countertransport; CV, coefficient of variation; GFR, glomerular filtration rate; GH, growth hormone; KV, kidney volume; MA, microalbuminuria; RIA, radioimmunoassay.

Several hormonal systems have been implicated as factors in early diabetic nephropathy. Involvement of growth hormone (GH) and IGF-I is strongly suggested from studies of the impact of GH excess (4,5) and deficiency (6,7) on kidney growth and function in various animal models, both diabetic (5–7) and nondiabetic (4). In these studies, a direct relationship has been noted between activity of GH and IGF-I and renal hypertrophy (4–7) and between GH and glomerulosclerosis (4,5). In humans, GH is known to correlate with both MA (8,9) and glomerular filtration rate (GFR) (10). Both the renotropic effect of testosterone (11) and the finding that increased vascular permeability is prevented when male diabetic rats are castrated (12) suggest that testosterone may also contribute to the increased expression of diabetic nephropathy during puberty. There is also considerable evidence implicating the intrarenal renin-angiotensin system in the early pathogenesis of diabetic nephropathy, through an effect on both renal hemodynamics (13) and renal growth (14). The significance of prorenin, a component of the renin-angiotensin system, is less certain; however, serum prorenin levels increase during puberty (15), and elevated prorenin levels have been associated with, and may precede the onset of, MA (15,16).

Plasma GH, IGF-I, and sex steroids (testosterone and estradiol) increase during puberty (17). Whereas children, and especially adolescents, with type 1 diabetes have significantly higher serum GH levels than nondiabetic individuals (18,19), plasma IGF-I levels are reported to be similar to or lower than nondiabetic subjects (18,19). This discrepancy between GH and IGF-I levels indicates a degree of GH resistance within the liver (19), an effect which may be mediated by lack of portal insulin (20). The importance of IGF-I in the pathogenesis of microvascular disease has been questioned, given that plasma IGF-I levels are not increased in diabetic subjects (21) and that IGF-I transgenic mice with high plasma IGF-I do not develop severe glomerulosclerosis (4). However, IGF-I levels in various tissues, such as kidney and bone, often differ from plasma levels (22,23). This implies that local production and metabolism of IGF-I within the kidney may be the most important determinant of its renal effects (24). Whereas urinary IGF-I concentrations correlate with plasma IGF-I (25), renal tissue and plasma levels differ (22,23), suggesting that urinary IGF-I probably reflects both plasma and renal IGF-I. Urinary GH levels are known to reflect GH secretion over the collection period (26). Thus, measurement of these hormones in urine permits an assessment of the renal IGF-I environment and provides an integrated assessment of GH secretion.

Two studies have addressed the relationship of the GH/IGF-I axis and diabetic nephropathy in the peripubertal period in humans (27,28). Salardi et al. (27) found that arginine-stimu-

lated GH response was associated with albumin excretion rate (AER), but not renal length, by ultrasound. Another study found that plasma IGF-I levels were marginally significantly lower in microalbuminuric compared with normoalbuminuric diabetic adolescents (28). No studies have comprehensively examined the relationship of the GH/IGF-I axis to kidney size and AER in diabetic nephropathy. The aim of this study was to examine, in children and adolescents with type 1 diabetes of 5–10 years duration, the relationship between early markers of diabetic nephropathy (kidney volume [KV] and MA) and GH and IGF-I, as reflected by urinary excretion rates and plasma levels of IGF-I, testosterone, and prorenin.

RESEARCH DESIGN AND METHODS

Study population. A total of 177 children and adolescents with type 1 diabetes of 5–10 years duration were recruited from the diabetes clinic at the Hospital for Sick Children in Toronto, Canada. We have previously reported on the impact of pubertal duration on KV, MA, and NaLi countertransport (CT) in this cohort (1). Most subjects had urinary GH ($n = 155$) and urinary IGF-I ($n = 150$) measured in at least two (two or three) 24-h urine collections, and they comprise the study population reported here. There were 78 males and 77 females with a mean age of 13.2 ± 3.5 (SD) years (range 6.9–22.1) and a mean diabetes duration of 6.8 ± 1.6 years (range 5–10 years). Subjects were divided into three groups based on pubertal duration: group 1: prepubertal at diagnosis and study; group 2: prepubertal at diagnosis, pubertal at study; and group 3: pubertal at diagnosis and study. Control GH and IGF-I excretion rates were determined from three overnight urine collections provided by 45 healthy normal-statured nondiabetic children (mean age 12.4 ± 4.1 years; range 4.9–19.4; 24 males). Control subjects were divided into three pubertal groups estimated by age: group 1 ($n = 16$): prepubertal girls <10 and boys <11 years of age; group 2 ($n = 18$): pubertal girls 10–15 and boys 11–16.5 years of age; and group 3 ($n = 11$): postpubertal girls >15 and boys >16.5 years of age. Informed consent was obtained from subjects 16 years of age, while in younger subjects, consent and assent were obtained from the parents and subjects, respectively. The study was approved by the Human Research Ethics Review Board at the Hospital for Sick Children.

Study protocol. The protocol has been described previously (1). Briefly, at entry to the study, pubertal staging was established by a pediatric endocrinologist, according to the ratings of Tanner (29). KV was measured by ultrasound. Blood was obtained for measurement of HbA_{1c}, prorenin, testosterone, NaLi CT, and IGF-I. Within 1 month of study entry, subjects completed three 24-h urine collections for determination of albumin, creatinine, GH, and IGF-I excretion rates. Demographic data were obtained by chart review.

Measurements. KV (right + left) of each subject and of 92 control subjects was measured as previously described (1). KV was calculated based on the formula for the volume of an ellipsoid (30). Nephromegaly was defined as KV >300 ml/1.73 m² (31). Urinary albumin was measured by immunoturbidimetry (Roche, Nutley, NJ), and AERs were calculated from the concentration, volume, and time of the collection. Median values of two or three collections were used in the analyses. Microalbuminuria was defined as an AER of 15–200 µg/min in at least two 24-h collections.

HbA_{1c} was determined by high-pressure liquid chromatography after removal of the labile fraction (32). The nondiabetic range is 4–6%. NaLi CT was measured in erythrocytes by the method of Canessa et al. (33) and was expressed as millimoles per liter of erythrocytes per hour (normal <0.40 mmol/l · h⁻¹) (34). Active renin was determined first by radioimmunoassay (RIA) (Sanofi Diagnostics Pasteur, Marnes la Coquette, France). Total renin was then determined after activation of prorenin with trypsin, and prorenin was calculated by subtraction of active renin from total renin (16). Testosterone was measured by coated tube RIA (DPC, Los Angeles, CA).

Urinary GH concentration was measured using the Nichols Institute Chemiluminescence urinary GH double antibody immunoassay (San Juan Capistrano, CA) as previously reported (35). Urine samples were preserved with bovine serum albumin (BSA) and sodium azide, frozen at -70°C , and assayed within 12 months. Samples were eluted through a desalination column, then incubated overnight with mouse monoclonal antibody coupled to polystyrene beads. After a wash step, acridium ester-labeled goat polyclonal antibodies were added. Hydrogen peroxide and sodium hydroxide triggers were added, and light emission was measured. Urinary GH concentrations were expressed in picograms per milliliter, and excretion rates were calculated in nanograms per day as the mean of the two or three 24-h samples. The intra-assay coefficients of variation (CVs) at urinary GH concentrations of 14.6 and 20.8 pg/ml were 6.8 ($n = 21$) and 5.4% ($n = 21$), respectively. The interassay CVs for urinary GH concentrations of 14.7 and 21.7 pg/ml were 7.8 ($n = 25$) and 6.5% ($n = 25$), respectively.

IGF-I was measured by the method of Rosenfeld (36). For urinary IGF-I, BSA and sodium azide were added to aliquots of urine to achieve a final concentration of 1 g/l. The aliquots were centrifuged, and the two or three samples from each patient were pooled. The final sample was dialyzed against distilled water for 48 h (water changed three times) using Spectra/Por 3 membranes (MWCO:3,500) (Spectrum, Los Angeles, CA), then it was lyophilized in a Virtis Lyophilizer (Gardener, NJ). Lyophilized samples were reconstituted with phosphate buffer containing 0.5% human serum albumin (Albumar-25; Armour Pharmaceutical, Kankakee, IL) and 0.9% NaCl and then stored at -20°C . Aliquots of these samples were brought to 1 ml in 1% formic acid and subjected to size exclusion chromatography through a G-50 Sephadex (Pharmacia, Piscataway, NJ) column with 1% formic acid. The IGF-I fractions were collected in 1 ml of 1% BSA and lyophilized. After reconstitution in 0.01 mol/l HCl, IGF-I concentration was measured using the Nichols Institute Diagnostics RIA. Urinary IGF-I concentration was measured in nanograms per milliliter, and excretion rates were calculated in nanograms per day. The intra-assay CV was 5.1% at a urinary IGF-I concentration of 2.4 ng/ml ($n = 6$). The interassay CVs for urinary IGF-I concentrations of 1.2 and 2.6 ng/ml were 5.7 ($n = 6$) and 4.1% ($n = 9$), respectively. Western blot analysis was performed in our laboratory and confirmed in an independent laboratory (36) to assess the adequacy of separation of IGF-I from its binding proteins (36,37). IGF-I binding proteins were present in the early fractions eluted from the column, but no binding proteins were detectable in the 42- to 70-ml fractions used for measurement of urinary IGF-I. The mean recovery rate of IGF-I for the procedure, tested at different concentrations, was 95.3% (range 87.4–108.5%; CV = 8.8%; $n = 7$). Plasma IGF-I was collected in heparinized tubes and diluted 1:20 with 1% formic acid, and samples were applied to the Sephadex column. The samples were further diluted 1:10 with assay buffer. The remainder of the procedure was as for urinary IGF-I. The intra-assay CV for a plasma IGF-I concentration of 220 ng/ml was 2.9% ($n = 20$). The interassay CV for a plasma IGF-I concentration of 240 ng/ml was 5.1% ($n = 9$).

Statistical analysis. Baseline characteristics of the diabetic subjects were compared by analysis of variance (ANOVA). Pearson's correlation coefficient was used to compare urinary IGF-I and plasma IGF-I or urinary GH. Hormonal values among pubertal groups of diabetic subjects and control subjects were compared by two-way ANOVA, followed by contrast tests to compare pair-wise means. For urinary IGF-I, the plot of residuals violated the assumption of normality, so the data were transformed using the natural logarithm. Multiple regression analysis was used to examine the association between KV and hormonal and other factors (e.g., HbA_{1c}, NaLi CT). Differences between subjects with and without MA were examined using logistic regression analysis controlling for sex, BSA, and pubertal duration. For analyses involving testosterone, males and females were analyzed separately. Values of $P < 0.05$ were considered statistically significant. Results are expressed as mean \pm SE, unless specified otherwise.

RESULTS

Demographic and hormonal characteristics of type 1 diabetic subjects. Table 1 shows the characteristics of the subjects as a whole and grouped by pubertal status. Mean (\pm SD) HbA_{1c} for the entire group was $8.8 \pm 1.4\%$ ($n = 155$). The overall prevalence of nephromegaly and MA was 26 and 9% ($n = 155$), respectively. As previously reported in these subjects, nephromegaly and MA increased with increasing pubertal duration (1). In the entire cohort with diabetes, the mean GH excretion rate was 14.0 ± 0.9 ng/day ($n = 155$), and the mean IGF-I excretion rate was 328 ± 20 ng/day ($n = 150$). These excretion rates were higher than those in the nondiabetic control subjects: urinary GH, 9.0 ± 0.6 ng/day ($P = 0.004$) and urinary IGF-I, 157.5 ± 15.0 ng/day ($P < 0.0001$); these were determined from overnight collections and corrected to 24 h. Differences in urinary IGF-I between diabetic subjects and control subjects were significant in all three pubertal groups, whereas the differences in urinary GH were significant only in the pubertal group (Fig. 1). Correlational analysis with Pearson coefficients showed that urinary GH and urinary IGF-I were significantly correlated ($r = 0.22$; $P < 0.007$). The correlation between plasma IGF-I and urinary IGF-I was also significant but small ($r = 0.30$; $P = 0.0001$); thus, only 9% of the variance in urinary IGF-I was attributable to plasma IGF-I.

Table 2 shows the hormonal measurements by pubertal status in subjects with diabetes. All hormonal values (urinary GH,

TABLE 1
Characteristics of type 1 diabetic subjects

	All subjects	Group 1	Group 2	Group 3
<i>n</i>	155	55	63	37
Age (years)	13.2 ± 3.5	9.9 ± 1.5	13.6 ± 2.1	17.5 ± 2.1
Pubertal duration (years)	2.7 ± 3.0	0	2.4 ± 1.8	6.9 ± 1.9
Sex (M/F)	78/77	33/22	35/28	10/27
IDDM duration (years)	6.8 ± 1.6	6.5 ± 1.5	7.1 ± 1.5	6.9 ± 1.9
Current HbA _{1c} (%)	8.8 ± 1.4	8.6 ± 1.2	9.0 ± 1.2	8.8 ± 1.9
Prevalence of nephromegaly	41 (26)	7 (13)	19 (30)	15 (41)
Prevalence of MA	14 (9.1)	0	6 (9.5)	8 (21.6)

Data are means ± SD, *n*, or *n* (%). Group 1: prepubertal at diagnosis and study; group 2: prepubertal at diagnosis, pubertal at study; group 3: pubertal at diagnosis and study. Nephromegaly defined as KV >300 ml/1.73 m²; *P* < 0.0001 for group differences by ANOVA. MA is defined as AER = 15–200 µg/min; *P* < 0.001 for group differences by Kruskal-Wallis test.

urinary IGF-I, plasma IGF-I, testosterone, and prorenin) were significantly higher in the two pubertal groups compared with the prepubertal group. Urinary GH was lower in the postpubertal subjects than in pubertal subjects; however, urinary IGF-I levels did not differ in these two groups. Mean plasma IGF-I was 309 ± 13 ng/ml and was highest in the mid-pubertal group. Testosterone levels increased as expected for pubertal duration.

Hormone measurements and KV. In univariate analysis, prorenin (*P* < 0.0001), testosterone (*P* < 0.0001), urinary GH (*P* = 0.009), and urinary IGF-I (*P* < 0.0001) were significantly associated with KV, while plasma IGF-I approached significance (*P* = 0.053). After controlling for BSA, sex, and pubertal duration, only urinary IGF-I remained significantly associated with KV (*P* = 0.0005), while the association of KV and prorenin approached significance (*P* = 0.054). Multiple regression analysis with KV as the dependent variable and urinary GH, urinary IGF-I, plasma IGF-I, prorenin, testosterone, sex, pubertal duration, BSA, current HbA_{1c}, and NaLi as independent variables showed a significant association between KV and urinary IGF-I (*P* = 0.001) as well as BSA (*P* < 0.0001). **Hormone measurements and MA.** Mean urinary IGF-I and urinary GH excretion rates were higher in MA subjects than in normoalbuminuric subjects (645 ± 110 vs. 294 ± 17 ng/day [*P* < 0.0001] and 22.6 ± 2.8 vs. 12.4 ± 0.7 ng/day [*P* < 0.005], respectively) (Fig. 2). Logistic regression analysis controlling for sex, BSA, and pubertal duration showed that subjects with MA had significantly higher urinary excretion of both IGF-I (*P* = 0.005) and GH (*P* = 0.05) compared with normoalbuminuric subjects. The odds ratio for MA increased 3.5-fold (95% CI 1.5–8.1) with each 350 ng/day increment in urinary IGF-I (the difference between the means of the two groups). Testosterone, prorenin, and plasma IGF-I were not significantly associated with MA.

DISCUSSION

Glomerular hypertrophy is thought to be one of the key early changes in the development of diabetic nephropathy (5,38). The link between glomerular hypertrophy and diabetic

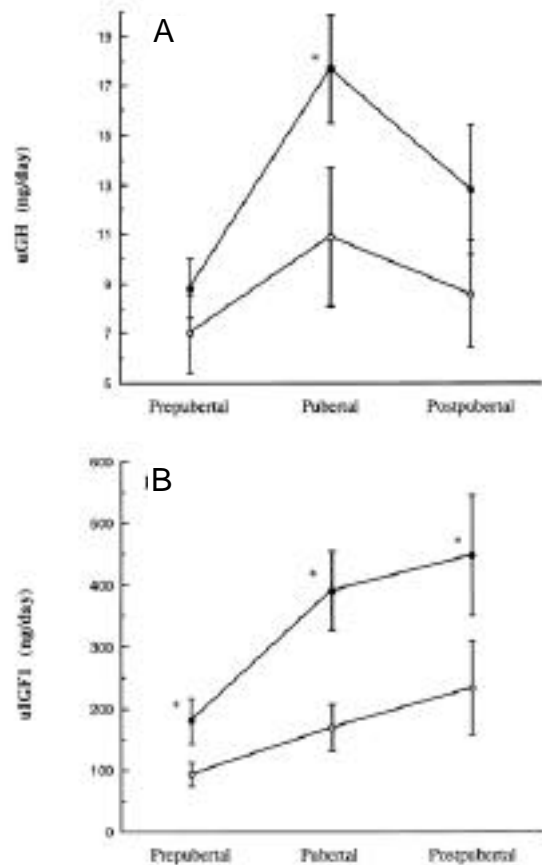


FIG. 1. Urinary GH (uGH) (A) and urinary IGF-I (uIGF1) (B) in diabetic subjects and control subjects by pubertal group. ●, diabetic subjects; ○, control subjects. Error bars represent 95% CIs. **P* < 0.005 for diabetic subjects versus control subjects within pubertal groups.

nephropathy is underlined by the finding of larger glomeruli in microalbuminuric subjects compared with normoalbuminuric subjects (39) and by the association between kidney size and MA (1). Puberty is characterized by significant changes in the hormonal milieu, particularly in GH, IGF-I, and sex steroid secretion (17). Evidence linking these hormones to renal/glomerular growth in various animal models (5,6,11,40) suggests that these hormonal factors may also contribute to the renal growth and emergence of early diabetic nephropathy during puberty in humans.

Among diabetic subjects, the pubertal group had the highest GH excretion rates. Pubertal subjects with diabetes also had significantly higher urinary GH than pubertal control subjects, while levels in pre- and postpubertal diabetic and control subjects did not differ. These findings are consistent with most previous reports of serum GH (18,19) and some (41,42), but not all, reports of urinary GH (43). Urinary IGF-I was higher in the two pubertal groups of diabetic subjects compared with prepubertal diabetic subjects, and urinary IGF-I levels were increased in subjects with diabetes compared with control subjects within each pubertal group. These findings contrast with a single previous report that found lower levels of urinary IGF-I in diabetic children than in control subjects (43). Plasma IGF-I levels in our cohort were similar to or slightly higher than those reported in type 1 diabetes (18,19). Differences from previous reports may be accounted for by our use of an IGF-I assay

TABLE 2
Hormonal measurements by pubertal status

Hormonal factors	All subjects	Group 1	Group 2	Group 3
Urinary GH (ng/day)	14.0 ± 0.9	8.7 ± 0.6	17.7 ± 1.8	12.8 ± 1.3
Urinary IGF-I (ng/day)	328 ± 20	180 ± 18	390 ± 32*	448 ± 49
Plasma IGF-I (ng/ml)	309 ± 13	201 ± 15	399 ± 21	329 ± 21
Prorenin (pg/ml)	122 ± 3	109 ± 4	127 ± 5†	145 ± 7
Testosterone				
Male (nmol/l)		0.8 ± 0.2	11.0 ± 1.1	18.7 ± 1.4
Female (nmol/l)		0.7 ± 0.1	1.2 ± 0.1	1.7 ± 0.1

Data are means ± SD. All group differences are significant at $P < 0.01$ to $P < 0.0001$, except as noted. *NS group 2 versus group 3; † $P = 0.05$ group 2 versus group 3.

method that effectively separates IGF-I from its binding proteins (36). It is interesting to note that pre- and postpubertal diabetic subjects excreted more IGF-I than control subjects despite there being no difference in GH excretion in these groups. These observations should be confirmed given that there are some limitations of the control group data, since pubertal stage was estimated based on age. We also assumed that there is no variation between day and night IGF-I excretion rates, an assumption which seems justified because IGF-I has a long plasma half-life and shows little diurnal variation (44).

We report for the first time in humans with type 1 diabetes a strong association between urinary IGF-I (a surrogate for renal IGF-I) and KV (a marker of glomerular hypertrophy) (45). In animal models, induction of diabetes results in the rapid development of nephromegaly, accompanied by an increase in renal IGF-I levels despite unchanged or decreased plasma IGF-I levels (6,7,24). Circumstances that limit this increase in renal IGF-I, such as GH deficiency or somatostatin administration, limit the development of glomerular hypertrophy (6,7,46). On the other hand, elevations in plasma IGF-I, as seen in IGF-I transgenic mice (4) or diabetic rats given IGF-I infusions (47), promote the development of glomerular/renal hypertrophy. Furthermore, prepubertal diabetic rats do not develop the accumulation of renal IGF-I or the increase in KV and diabetic nephropathy that occurs in postpubertal diabetic rats (24,48). Thus, the evidence in experimental diabetes strongly implicates renal IGF-I as an important factor in the development of nephromegaly and the pubertal milieu as a mediator of this effect. Our finding that urinary IGF-I, and not plasma IGF-I, was associated with KV in linear regression analysis is consistent with these data.

The lack of an association between urinary GH and KV in our subjects contrasts with animal studies that show a role of GH in kidney growth (4,5,7). This role for GH is further supported by the finding that treatment with a somatostatin analog reduces both kidney size and GFR in adults with type 1 diabetes (49). In our study, the correlation between urinary GH and urinary IGF-I may explain our failure to find an association between urinary GH and KV in multivariate analysis when both urinary GH and urinary IGF-I were in the model. Alternatively, renal IGF-I may be the more direct factor in determining the extent of kidney growth.

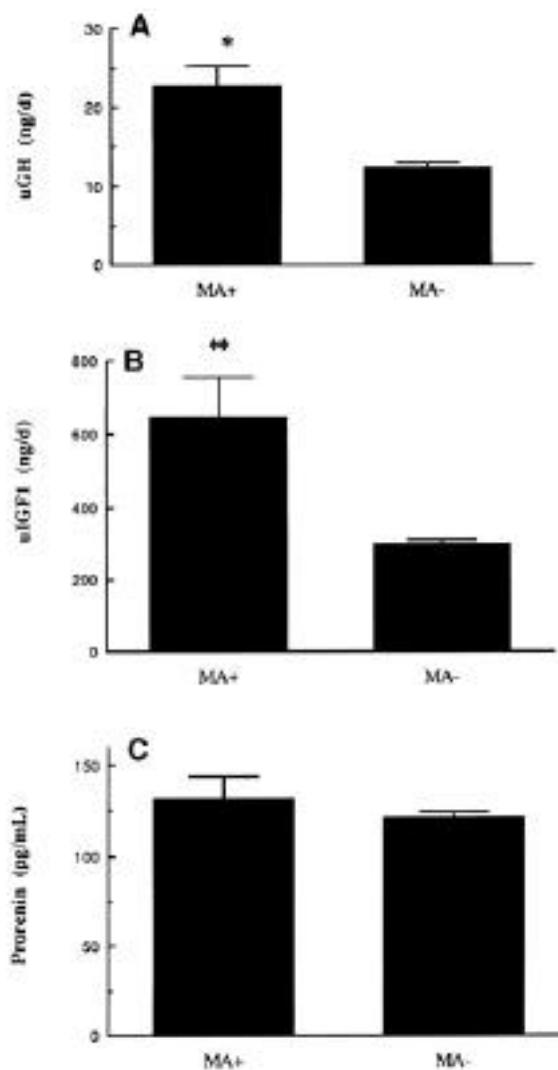


FIG. 2. Hormonal factors in subjects with (+) and without (-) MA compared by logistic regression analysis, controlling for sex, BSA, and pubertal duration. **A:** Urinary GH (uGH) versus MA; **B:** urinary IGF-I (uIGF1) versus MA; **C:** prorenin versus MA. * $P = 0.05$; ** $P = 0.005$.

Both urinary GH and urinary IGF-I were associated with the presence of MA in this study. In two studies, both involving heterogeneous groups of mostly adults with both type 1 and 2 diabetes, a positive association was noted between AER and GH excretion rate (9,41). Serum GH has previously been related to AER in both diabetic and nondiabetic subjects (8,27,41). In acromegalic subjects, AER is moderately elevated and decreases after reduction of serum GH and IGF-I (8,50). In type 1 diabetes, stimulated GH levels correlate positively with AER (27,41). Plasma IGF-I levels tended to be lower in diabetic adolescents with MA compared with subjects without MA (28); however, we did not find a relationship between plasma IGF-I and MA. Our data, which reflect mean GH and IGF-I production over a 2- to 3-day period, strengthen the evidence of an association between GH and MA and also implicate urinary IGF-I in MA.

Although the association between urinary GH and MA that we report is consistent with animal studies that implicate GH in glomerulosclerosis and diabetic nephropathy, there is less support for a role for IGF-I in this process. GH-deficient rats

with diabetes are relatively protected from the typical renal effects of diabetes seen in GH-sufficient rats (6,7), while transgenic mice expressing excess GH develop glomerular hypertrophy, albuminuria, and glomerulosclerosis, a sequence of events similar to the evolution of diabetic nephropathy (4,5). Similarly, transgenic mice expressing excess IGF-I binding protein have elevated GH levels and develop mesangial hypertrophy and glomerulosclerosis, despite a decrease in plasma IGF-I levels (51). IGF-I transgenic mice, on the other hand, develop renal hypertrophy but only minimal glomerulosclerosis and albuminuria (4). In these transgenic mouse models, renal IGF-I levels have not been reported (4,5,51), making it difficult to evaluate the relative contributions of GH, altered GH secretion, and local renal IGF-I to the pathogenesis of diabetic nephropathy. Our findings that both urinary GH and urinary IGF-I are associated with MA suggest that in addition to GH, renal IGF-I is important in the development of MA, an effect that may be mediated by GH.

The increase in urinary IGF-I we found cannot be explained by loss through damaged glomeruli because urinary IGF-I was elevated in the entire diabetic cohort compared with control subjects. When the comparison was repeated, excluding MA subjects ($n = 14$), urinary IGF-I remained almost double that of control subjects ($P < 0.0002$, data not shown). In addition, only 9% of the variance in urinary IGF-I could be explained by variation in plasma IGF-I. IGF-I is highly (>99%) protein bound, with the largest portion circulating in a large 150-kDa ternary complex (44), and there is no evidence that this complex or other IGF-I binding proteins are extensively lost through the glomerulus in renal diseases without gross proteinuria (36). Therefore, the increase in urinary IGF-I likely reflects increased renal secretion and/or altered renal handling of IGF-I via receptors or binding proteins. GH is ~50% protein bound, and urinary levels are dependent primarily on tubular resorption/degradation (52). Tubular function appears to be mainly intact in normo- and microalbuminuric subjects (53,54), suggesting that the increase in urinary GH in our subjects reflects increased GH secretion.

Both testosterone and prorenin were associated with KV in univariate analysis. However, after controlling for sex, pubertal duration, and BSA, these associations were no longer significant. Testosterone is involved in normal pubertal growth of the kidney (11), but our results suggest that it does not contribute to the excessive growth that occurs in subjects with type 1 diabetes. Some (15,16), but not all (55), previous investigators have found a positive relationship between prorenin and MA. Testosterone increases vascular permeability in the diabetic rat model (12). We did not find an association between MA and testosterone or prorenin. Further study of these questions appears warranted, however, since the power of our study was limited by the small number of MA subjects.

In summary, in this cross-sectional investigation of the association of pubertal hormonal factors with markers of early diabetic nephropathy in children and adolescents with diabetes for 5–10 years, we report that KV is associated with urinary IGF-I and microalbuminuria is associated with both urinary IGF-I and urinary GH. These findings, taken in the context of the substantial evidence in experimental animals, further implicate the GH/IGF-I axis in the pathogenesis of human diabetic nephropathy and may help to explain the emergence of early diabetic nephropathy during puberty.

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