Urinary Excretion of IGF-I and Growth Hormone in Children With IDDM

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OBJECTIVE — To compare the urinary output of insulinlike growth factor I (IGF-I) and growth hormone (GH) in prepubertal and pubertal children with insulin-dependent diabetes mellitus (IDDM) versus nondiabetic subjects and to analyze the relationship between the urinary excretion of these peptides and degree of metabolic control.

RESEARCH DESIGN AND METHODS — Group 1 included 30 IDDM patients who had had diabetes for 4.9 ± 0.7 yr and had normal renal function (mean age 11.6 ± 0.9 yr); group 2 consisted of 31 control subjects (mean age 9.2 ± 0.6 yr). Sensitive radioimmunoassays were used to measure IGF-I and GH in urine aliquots from 12-h timed overnight collections that had been dialyzed, concentrated 50-fold, and lyophilized.

RESULTS — Significantly lower IGF-I and GH outputs per kilogram body weight per 12 h were observed in IDDM subjects compared with control subjects. When data were expressed per kilogram of body weight, no difference was observed between the urinary output of IGF-I and GH between prepubertal and pubertal subjects within group 1 or group 2. The prepubertal children had significantly lower HbA_1 than the pubertal population; however, no correlation was found between urinary output of IGF-I or GH and HbA_1. A positive correlation was observed between urinary IGF-I and GH ($r = 0.85$, $P < .001$).

CONCLUSIONS — Patients with long-standing IDDM excrete significantly lower urinary levels of IGF-I and GH compared with normal subjects. Serial measurements of these peptides from onset of IDDM are needed to define whether the changes observed are present at diagnosis or are secondary to duration of disease.

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Low plasma insulinlike growth factor I (IGF-I) and variable plasma growth hormone (GH) concentrations have been observed in children with poorly controlled insulin-dependent diabetes mellitus (IDDM; 1,2). In a previous study of older IDDM patients, Hansen reported that both plasma concentrations and urinary excretion of GH were elevated during ketoacidosis, fell with insulin therapy, but remained higher than in nondiabetic control subjects (3). Hansen (3) concluded that degree of diabetic control, age, and duration of disease influenced both plasma and urinary GH values.

To our knowledge, urinary excretion of GH and IGF-I have not been simultaneously evaluated in children with IDDM. We previously reported that urinary GH and IGF-I excretion are significantly reduced in hypopituitary children compared with normal control subjects. Urinary levels of IGF-I were GH-dependent and correlated positively with those of GH (4,5).

The aim of this study was to compare the urinary output of IGF-I and GH in prepubertal and pubertal children with IDDM with normal control subjects and to analyze the relationship between the urinary measurements of these peptides and the degree of metabolic control and somatic growth in IDDM.

RESEARCH DESIGN AND METHODS

— Mean ± SD age, sex, pubertal status, height, and weight of the participants are shown in Table 1. Group 1 included 30 children with IDDM, who were further subdivided into prepubertal and pubertal. The pubertal patients had been diagnosed as having IDDM for 5.7 ± 1.1 yr before study and were receiving a mean total insulin dose of 0.90 ± 0.07 U/kg in two injections/day. The duration of IDDM in the prepubertal subjects was 3.8 ± 0.85 yr; they were receiving a mean insulin dose of 0.74 ± 0.10 U/kg in two daily injections. Group 2 consisted of 32 healthy, normally statured children. Most of the con-

Table 1—*Clinical and demographic data of the study population*

	(M/F)	AGE (YR)	HEIGHT (SD U)	WEIGHT (SD U)
GROUP 1				
ALL DIABETICS	16/14	11.6 ± 0.9	0.69 ± .22	1.28 ± .19
1A PREPUBERTAL	7/6	7.3 ± 0.9	1.43 ± .28	1.72 ± .27
1B PUBERTAL	9/8	14.8 ± 0.6	0.11 ± .27	0.94 ± .24
GROUP 2				
ALL CONTROLS	18/14	9.2 ± 0.6	0.92 ± .21	0.54 ± .17
2A PREPUBERTAL	10/9	6.8 ± 0.5	0.69 ± .28	0.38 ± .24
2B PUBERTAL	8/5	12.8 ± 0.6	1.24 ± .30	0.78 ± .22

Values are means ± SD.

trol group in this study was composed of children included in our earlier study. The control and diabetic urines were obtained, processed, and assayed in the same time frame. The mean height, weight, and body surface area in the diabetic population were not significantly different from control subjects.

The patients were followed regularly in a multidisciplinary diabetes program. Under parental supervision, timed overnight urines (2000–0800) were collected at home in plastic containers, kept at 4°C throughout the collection period and brought to the laboratory the next morning. The diabetic patients did not have increased urine volume compared with control subjects. Before dialysis, each urine was centrifuged to remove debris. If dialysis could not be performed immediately, the centrifuged urine was stored at −20°C. A 50-ml aliquot of urine was dialyzed against redistilled water for 36 h with 18-mm Spectropor membranes (cutoff of ~3500 M_r, Spectrum, Los Angeles, CA); lyophilized in a Virtis Lyophilizer (Virtis, Gardiner, NJ) and reconstituted in 1 ml of 0.04 M phosphate buffer (pH 7.4), containing 0.5% purified human serum albumin (Albuminar-25, Armour, Kankakee, IL), and 0.9% sodium chloride. The albumin was checked for IGF-I contamination, and none was found. The urine concentrates were not extracted. We have previously looked for IGF binding protein in the urine samples with a modification of the method described by Furlanetto (6,7). The urinary IGF-I was found to be predominantly free, similar to what was reported by Yokowa et al. (8).

IGF-I was quantitated by radioimmunoassay (RIA) kits purchased from Nichols (San Juan Capistrano, CA). The lowest limit of sensitivity was 7 mU/ml and cross-reactivity with IGF-II was 0.5–0.6%. The intra- and interassay coefficients of variation (C.V.) were 5.7 and 8.6%.

Urinary GH was measured in duplicate by a standard double-antibody (RIA) method with polyclonal GH antibody and GH standards obtained from the National Hormone and Pituitary Program (NHPP). The intra- and interassay (C.V.s) for GH were 2.1 and 4%, respectively. The sensitivity of the assay was 0.15 ng/ml (0.15 μg/L). HbA_1 was quantitated by affinity chromatography (Glyc-Affin, Isolab Akron, OH; normal range for nondiabetic control subjects equals 4–8%).

Urinary microalbumin and β_2-microglobulin excretion were measured in duplicate by RIA kits (Diagnostic Products, Los Angeles, CA; and Pharmacia, Piscataway, NJ, respectively).

Data were standardized for body weight per total output per 12 h and per body surface. We felt that standardization by gram of creatinine would not have been appropriate in our study because the data are biased by the fact that diabetic children are similar to children with GH deficiency because they excrete significantly lower amounts of creatinine per total volume compared with control subjects. This difference persists when the data were analyzed controlling for pubertal status and is not secondary to significant differences in total urine volume.

The data analysis was performed with the SPSS statistical package. When testing for differences between groups, the nonparametric Mann-Whitney *U* test was used.

RESULTS— Urinary GH (ng/kg) and IGF-I (nmol/kg) excretion in diabetic and nondiabetic children are shown in Table 2. Significantly lower excretion of GH (0.14 ± 0.02 μg/kg) was observed in the IDDM children compared with the control group (0.27 ± 0.02 μg/kg, $P < 0.001$). Within each group, there was no statistical difference in the amounts of GH (μg/kg) excreted by prepubertal or pubertal subjects (1a vs. 1b and 2a vs. 2b). However, both diabetic subgroups (groups 1a and 1b) had significantly lower output of urinary GH compared with the comparable control subgroups (groups 2a and 2b, respectively; $P < 0.01$). The data standardized by body surface area reproduce the differences described above.

Urinary IGF-I excretion was markedly suppressed in the IDDM group (0.04 ± 0.01 nmol/kg) compared with the control population (0.15 ± 0.01 nmol/kg). The urinary IGF-I output excreted by prepubertal and pubertal children within groups 1 and 2 did not differ significantly. However, both diabetic subgroups (1a and 1b) excreted significantly less urinary IGF-I compared with their control subgroups (2a and 2b, respectively; $P < 0.01$). The data standardized by body surface area are in agreement with the above data.

Because of total volume (TV; Table 3), the overnight output of GH was significantly lower in IDDM (6.2 ± 0.02

Table 2—*Urinary growth hormone and insulinlike growth factor I (IGF-I) excretion standardized for body weight*

	N	GROWTH HORMONE (μG/KG)	IGF-I (NMOL/KG)
GROUP 1			
ALL DIABETICS	30	0.14 ± 0.2*	0.04 ± 0.01*
1A PREPUBERTAL	13	0.16 ± 0.03†	0.03 ± 0.01*
1B PUBERTAL	17	0.12 ± 0.02†	0.05 ± 0.01*
GROUP 2			
ALL CONTROLS	31	0.27 ± 0.02	0.15 ± 0.01
2A PREPUBERTAL	18	0.28 ± 0.03	0.15 ± 0.01
2B PUBERTAL	13	0.25 ± 0.03	0.16 ± 0.02

*$P < 0.01$, group 1 vs. group 2, group 1a vs. group 2a, group 1b vs. group 2b.
†$P < 0.01$, group 1a vs. group 2a, group 1b vs. group 2b.

μg/TV) than in control subjects (8.5 ± 0.9 μg/TV, $P < 0.05$). Within each group, a lower amount of GH was excreted by the prepubertal children (group 1a 4.99 ± 1.3 μg/TV, group 2a 6.18 ± 0.03 μg/TV) compared with their respective groups of pubertal children (group 1b 7.2 ± 1.3 μg/TV, group 2b 11.6 ± 1.5). The absolute GH excretion per 12 h in prepubertal diabetic subjects was lower than that observed in the control subgroup (group 1b vs. 2b, $P < 0.05$). When the IGF-I data were analyzed based on output per TV, the patterns of excretion were similar to those observed on a body weight basis, because diabetic children as a group or as subgroups, excreted significantly lower IGF-I output than comparable control groups and subgroups. Within each group, prepubertal children excreted significantly lower IGF-I than pubertal subjects.

Of the 30 IDDM patients, 10 had no detectable microalbuminuria, whereas the remaining 20 had normal values of microalbuminuria. The mean value was 1.4 ± 0.4 mg/24 h. None of the IDDM patients had measurable β_2-microglobulin.

The mean HbA_1 in group 1 was 10.9 ± 0.5%. The prepubertal children had significantly lower HbA_1 (9.4 ± 0.4%) than the pubertal population (12.0 ± 0.8%, $P < 0.001$). When the diabetic and control groups were analyzed separately, a significant correlation was found between urinary IGF-I and GH (diabetic subjects $r = 0.85$ and 0.65 for data standardized per TV and per kilogram body weight, respectively; control subjects $r = 0.70$ and 0.50 per TV and body weight, respectively). The correlation between IGF-I and GH persisted also when both study groups were combined ($r = 0.85$, $r = 0.64$, respectively; $P < 0.001$). Neither urinary GH nor IGF-I correlated with height, HbA_1, or duration of disease. A negative correlation was observed between HbA_1 and height ($r = -0.47$, $P < 0.001$).

CONCLUSIONS— In this study, children with IDDM excrete significantly lower amounts of GH and IGF-I than nondiabetic control subjects when data are expressed on the basis of body weight or per total output. This relationship persists when the IDDM population is divided into prepubertal and pubertal subgroups and compared with their respective control subgroups. Prepubertal and pubertal IDDM patients excrete significantly less GH and IGF-I per kilogram of body weight compared with their respective control groups. When both study populations were combined, a significant positive correlation was observed between urinary GH and IGF-I output; we previously reported this relationship and interpreted it as evidence supporting the somatomedin hypothesis (5–9).

Previous studies in IDDM patients have described low plasma IGF-I levels and elevated plasma GH concentrations during poor glycemic control followed by their return to normal values when metabolic control is improved. Although previous data have not been available for urinary IGF-I excretion in patients with IDDM, urinary GH levels have been reported to be abnormally elevated during ketoacidosis and to normalize during appropriate insulin therapy. In our healthy diabetic population, however, a different pattern was observed for the urinary output of GH and IGF-I; both peptides were as low as those

Table 3—*Urinary growth hormone and insulinlike growth factor I (IGF-I) excretion per 12 h*

	N	GROWTH HORMONE (μG/TOTAL VOLUME)	IGF-I (NMOL/TOTAL VOLUME)
GROUP 1			
ALL DIABETICS	30	6.24 ± 0.2*	1.9 ± 0.4†
1A PREPUBERTAL	13	4.99 ± 1.3‡	1.0 ± 0.4†‡
1B PUBERTAL	17	7.20 ± 1.3*	2.7 ± 0.5†
GROUP 2			
ALL CONTROLS	31	8.46 ± 0.9	5.0 ± 0.6
2A PREPUBERTAL	18	6.18 ± .03	3.3 ± 0.4
2B PUBERTAL	13	11.6 ± 1.5	7.5 ± 1.0

*$P < 0.05$, group 1 vs. group 2, group 1b vs. group 2b.
†$P < 0.001$, group 1 vs. group 2, group 1a vs. group 2a, group 1b vs. group 2b, group 2a vs. group 2b.
‡$P < 0.01$, group 1a vs. group 2a, group 1a vs. group 1b.

previously reported in GH-deficient children (4,5). The urinary GH data in our diabetic population differs from those previously reported. This difference may be explained by the younger age of our population, whereas previous data were obtained in adult patients who had IDDM for 16–46 yr. Furthermore, previous reports included patients with albuminuria, whereas our patients had no evidence of nephropathy (3). The latter point is very important in view of recent data that show that the mean GH excretion rate is higher in diabetic patients with elevated albumin excretion rate (10).

The importance of good metabolic control has been emphasized by many authors as a key factor in the normalization of plasma IGF-I and GH values (11). Increased GH levels and significantly blunted IGF-I generation tests have been observed in those patients who were in poor metabolic control as judged by high HbA_1 levels (1). Also, several authors have found that improved diabetic control, generally obtained by more effective delivery of insulin, results in normal plasma IGF-I levels (12,13). In our study, however, no correlation was observed between HbA_1 levels and urinary GH or IGF-I output. Based on the HbA_1 levels, our prepubertal diabetic children were in better control than the pubertal patients; nonetheless, tighter glycemic control in the prepubertal IDDM patients did not result in normal urinary IGF-I output.

The pubertal IDDM population did not excrete significantly higher amounts of urinary IGF-I standardized for body weight or surface area than the prepubertal patients. In children, serum IGF-I has been reported to increase during puberty with a peak observed in pubertal Tanner stage 4 for both sexes (14). Blethen et al. (15) reported that although plasma IGF-I levels in diabetic children <5 yr of age were significantly lower than in nondiabetic children, the concentrations increased progressively throughout puberty. In a previous study we documented that normal pubertal controls and pubertal children with idiopathic short stature secreted significantly higher urinary IGF-I output per TV in 24 h but comparable amounts of urinary IGF-I per kilogram body weight (5). Similarly in this study, when the data were standardized by body weight, no difference was observed in the urinary IGF-I and GH outputs between prepubertal and pubertal subjects within the control and diabetic groups. A significant difference was observed only when the data were standardized by TV. Significant differences in weight or total volume are not present between prepubertal and pubertal subjects and therefore do not account for these discrepancies. Our data are in agreement with those of Yokoya et al. (8), who observed only a slight but not significant increase in urinary IGF-I levels in pubertal Japanese children (8). The different patterns observed in serum versus urinary IGF-I levels may be explained by the fact that although serum has an abundance of IGF-I bonding protein-3 (BP-3), urine has negligible or no BP-3 and only a small amount of 40,000 M_F compared with serum. Also urinary IGF-I excretion may reflect renal production as well as filtration. The diabetic population in our study was mainly in pubertal Tanner stages 3 and 4, whereas the control subjects were mainly in Tanner stages 2 and 3. Because children in Tanner stage 2 already exhibit a significant rise in plasma IGF-I levels, the slightly different Tanner stage distribution of the subjects studied should not account for the lack of rise of urinary IGF-I during puberty (14). No correlation was found between Tanner stage and urinary IGF-I values.

The influence of nutritional status on GH and IGF-I levels has been well described in nondiabetic subjects and in patients with IDDM (16–18). The diabetic children in our study were well nourished, therefore the low urinary IGF-I values observed in our IDDM patients were not secondary to malnutrition. On the contrary, despite height and weight not being significantly different in the two groups, in the diabetic group there were few children with generous weight. To control for the possibility that urinary GH and consequently IGF-I may have been suppressed because of obesity, a correlation was run between weight or height-weight ratio and GH output. No relationship was found between the latter and the above-mentioned variables.

Further studies on urinary GH and IGF-I at diagnosis and at subsequent intervals are necessary to better define the etiology of the low values observed in our study population. Serial measurements of these peptides from onset of IDDM are needed to clarify whether the changes observed are present at diagnosis or are secondary to duration of disease.

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