Diagnostic Significance of Urinary Growth Hormone Measurements in Children with Growth Failure: Correlation between Serum and Urine Growth Hormone[1]

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ABSTRACT. Twelve-h overnight urine and serum samples obtained simultaneously at 20-min intervals were assayed for growth hormone (GH). Ninety-one children, 5 to 16 y (Tanner stage 1 to 3) participated; group 1 were healthy children, group 2 were children with organic GH deficiency, and group 3 had idiopathic growth failure and normal GH stimulation tests. Serum pool GH concentrations in group 1 were similar to those in group 3 (3.3 ± 0.3 *versus* 3.4 ± 0.2 μg/L); group 2 had significantly lower GH concentrations (1.6 ± 0.2 μg/L). Plasma IGF-I levels were significantly greater in groups 1 (14.2 ± 2.6 nmol/L, $p < 0.001$) than in groups 2 and 3 (2.6 ± 0.5 and 5.5 ± 0.7 nmol/L, respectively). Urinary GH (mean ± SEM) standardized for body weight (μg/kg) in group 1 (0.31 ± 0.02) was significantly greater than in group 2 (0.14 ± 0.01) and group 3 (0.20 ± 0.01). However, when expressed as μg/mol creatinine, the output of GH was similar in group 1 (4.0 ± 0.3) and group 3 (3.4 ± 0.3); both groups had significantly greater output compared to group 2 (1.3 ± 0.2). Urinary IGF-I (nmol/kg) in group 1 (0.22 ± 0.02) was significantly greater than in group 2 (0.12 ± 0.01) or group 3 (0.07 ± 0.01). Urinary GH correlated with serum pool GH concentration ($r = 0.64$, $p < 0.001$). Although urinary GH output reflects endogenous GH secretion, the overlap between groups 1 and 3 precludes using urinary GH measurements as a diagnostic test for GH deficiency in children with idiopathic growth failure. (*Pediatr Res* 29: 619–622, 1991)

Abbreviations

GH, growth hormone
Cr, creatinine

Recent clinical studies have suggested that pathologically short children may have subtle deficiencies of GH that escape diagnosis because they exhibit normal GH responses to pharmacologic

stimuli. Diminished spontaneous GH secretion has been proposed as an explanation for the growth failure observed in these children (1–3). Attempts to detect low spontaneous GH secretion have utilized serial blood sampling or constant blood withdrawal over 12- to 24-h periods. Both of these methods are labor intensive and inappropriate for use in infants and young children. Recently, Rose *et al.* (4), questioned the diagnostic usefulness of serial plasma measurements because the mean 24-h plasma GH concentrations in children with idiopathic short stature were not significantly different from normal controls when the study populations were matched for stage of puberty. Furthermore, more than 50% of children with classical GH deficiency had normal spontaneous GH secretion.

We have recently reported that measurements of urinary GH and IGF-I provide quantitative information about the amount of hormone excreted in infants and children with normal and abnormal growth (5–7). We have also shown that urinary GH and IGF-I correlated positively, that urinary IGF-I is GH dependent, and that both urinary peptides are authentic based on HPLC characterization.

We hypothesized that measurement of urinary GH and IGF-I might be a useful and noninvasive method of detecting GH deficiency in pathologically short children who had normal GH responses to standard provocative tests. To test this hypothesis, we simultaneously measured urinary GH output and pooled plasma GH concentrations derived from overnight serial blood sampling at 20-min intervals in children with either normal stature, organic hypopituitarism, or idiopathic growth failure. The data derived from plasma and urine GH measurements were analyzed statistically to learn whether urinary GH output reflected mean plasma GH concentration and whether subtle deficiencies of GH can be identified in children with idiopathic growth failure. In this study, IGF-I was also quantitated in urine and plasma.

MATERIALS AND METHODS

Ninety-one children participated in this study after their parents gave written informed consent. Age, sex, and pubertal status of the study participants are shown in Table 1. The subjects were divided into the following three groups. Group 1 consisted of 32 healthy children (ages 7–15 y) growing between the 5th and 95th percentile for height and weight who served as controls. Group 2 consisted of 13 children with organic GH deficiency (ages 5–13 y) due to prior cranial irradiation for brain tumors or leukemia. All children were GH deficient based on a peak GH of less

than 10 µg/L after two or more standard stimulation tests (insulin-induced hypoglycemia, L-arginine infusion or L-dopa). Group 3 consisted of 46 children (ages 5–17 y) with idiopathic growth failure whose heights were greater than −2 SD and who had growth rates less than 5 cm/y. Their peak GH responses were >10 µg/L to one of two or more GH-provocative tests.

All patients were admitted to the Special Studies unit the afternoon of the test procedure. Spontaneous overnight GH secretion was assessed by obtaining blood samples every 20 min for a 12-h period (2000–0800 h). Simultaneously, overnight urine collections (2000–0800 h) were obtained in a plastic container and kept at 4°C throughout the collection period.

A 50-mL aliquot of urine was centrifuged to remove particulate matter and subsequently dialyzed, lyophilized, and reconstituted in 1 mL 0.04 M phosphate buffer, ph 7.4, containing 0.5% purified serum albumin (albuminar 25; Armour Pharmaceutical Co., Kankakee, IL), and 0.9% sodium chloride as previously described (5–7).

GH in serum and urine concentrates were measured in duplicate by a standard double antibody RIA method using polyclonal GH antibody and GH standards obtained from the National Hormone and Pituitary Program (8, 9). The intraassay and interassay coefficients of variation for GH were 2.1 and 4%, respectively.

IGF-I in plasma and urine concentrates were quantitated in duplicate using RIA kits purchased from Nichols Institute Diagnostics (San Juan Capistrano, CA). The intra- and interassay coefficients of variation were 5.7 and 8.6%, respectively.

The data were analyzed using analysis of variance with post hoc comparisons based on the Student-Newman-Keuls test.

RESULTS

There was no significant difference observed in the serum pool GH values obtained from healthy children (3.3 ± 0.2 µg/L) and those from children with idiopathic growth failure (3.4 ± 0.2 µg/L) (Table 2). Patients with organic GH deficiency had significantly lower serum pool GH values (1.6 ± 0.2) compared to children in groups 1 and 3 ($p < 0.05$). A scattergram of the individual serum pool GH values for the participants in the three groups is shown in Figure 1.

Urinary GH output was standardized for body weight (µg/kg/12 h) and grams of Cr (µg/mol Cr). The mean (± SEM) for each of the study groups is shown in Table 2. Based on body weight, urinary GH output was significantly greater in the healthy controls (0.31 ± 0.02 µg/kg) than in children with organic GH deficiency (0.14 ± 0.01 µg/kg) or children with idiopathic growth
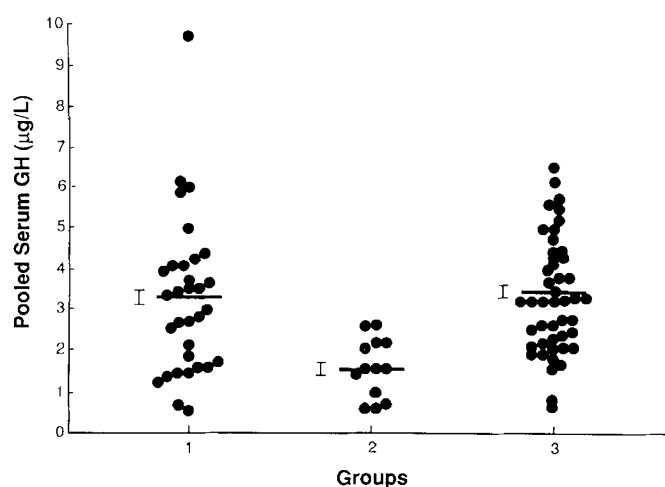

Fig. 1. Pooled serum GH concentrations in healthy controls (*group 1*), GH-deficient subjects (*group 2*), and children with idiopathic growth failure (*group 3*). The *horizontal* and *vertical lines* indicate the mean ± SEM.
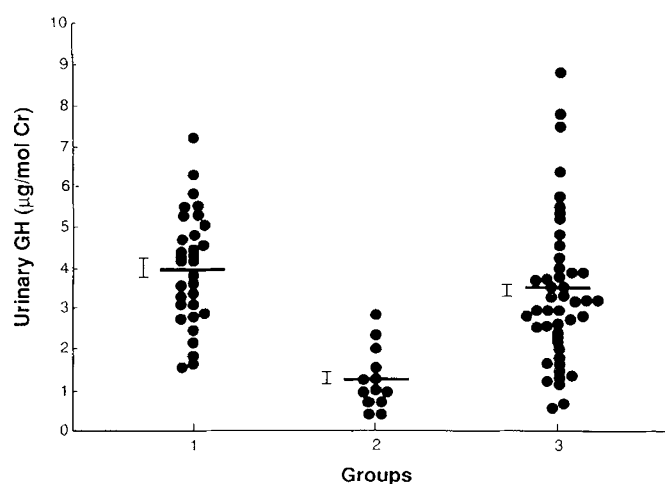

Fig. 2. Mean (± SEM) urinary GH values in the three study groups.

failure (0.20 ± 0.01 µg/kg, $p < 0.05$). There was no significant difference in children of group 2 *versus* group 3.

When the data were standardized according to Cr excretion, children in group 1 (4.0 ± 0.3 µg/mol Cr) had significantly higher urinary GH output compared to subjects in group 2 (1.3 ± 0.2 µg/mol Cr). However, the output of urinary GH in group 3 (3.4 ± 0.3 µg/mol Cr) was no longer significantly different from group 1. Figure 2 illustrates the degree of overlap observed among the groups.

Urinary GH output correlated positively with the serum pool GH concentrations ($r = 0.64$, $p < 0.001$) (Fig. 3). The strongest correlation between urinary GH output and serum pool levels was observed in group 1 ($r = 0.73$, $p < 0.0$).

Plasma IGF-I levels and urinary IGF-I output expressed as nmol/kg and nmol/mol of Cr for the three study groups are shown in Table 3. Plasma IGF-I levels in group 1 (14.2 ± 2.5 nmol/L) were significantly greater compared to those of children with organic GH deficiency (2.6 ± 0.5 nmol/L) or children with idiopathic growth failure (5.5 ± 0.7 nmol/L, $p < 0.05$).

Urinary IGF-I output was significantly higher in group 1 (0.22 ± 0.02 nmol/kg; 3.0 ± 0.3 nmol/mol Cr) compared to group 2 (0.12 ± 0.01 nmol/kg; 1.1 ± 0.3 nmol/mol Cr) or group 3 (0.07 ± 0.01 nmol/kg; 1.2 ± 0.10 nmol/mol Cr). The low renal output of IGF-I in groups 2 and 3 was not significantly different when expressed in terms of body weight or Cr excretion. Figure 4

Table 1. *Clinical data of study participants*

Groups	n	Tanner stage	Age (y)	SD Units Height	SD Units Weight
1	32	1–3	10.6 ± 0.3	1.0 ± 0.2	0.8 ± 0.2
2	13	1–3	8.1 ± 0.7	-0.9 ± 0.4	-0.1 ± 0.3
3	46	1–3	11.6 ± 0.5	-2.4 ± 0.1	-1.2 ± 0.1

Table 2. *Urinary and serum GH in healthy controls, GH-deficient children, and subjects with idiopathic growth failure*

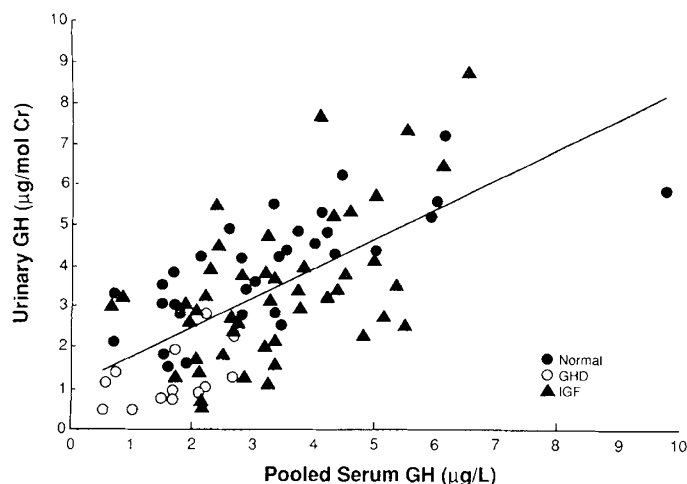| Groups	Urine GH (µg/kg)	Urine GH (µg/mol Cr)	Serum GH (µg/L)
1	0.31 ± 0.02	4.0 ± 0.3	3.3 ± 0.3
2	0.14 ± 0.01	1.3 ± 0.2	1.6 ± 0.2
3	0.20 ± 0.01	3.4 ± 0.3	3.4 ± 0.02
	$F = 17.0$	$F = 14.3$	$F = 7.2$
	$p < 0.01$	$p < 0.01$	$p < 0.01$
SNK*	1 *vs* 2, 3	2 *vs* 1, 3	2 *vs* 1, 3

* SNK, Student-Newman-Keuls test.

Fig. 3. Correlation between urinary GH excretion and pooled serum GH concentrations ($r = 0.64$; $p < 0.001$).

Table 3. *Urinary and plasma IGF-I in healthy controls, GH-deficient children, and subjects with idiopathic growth failure*

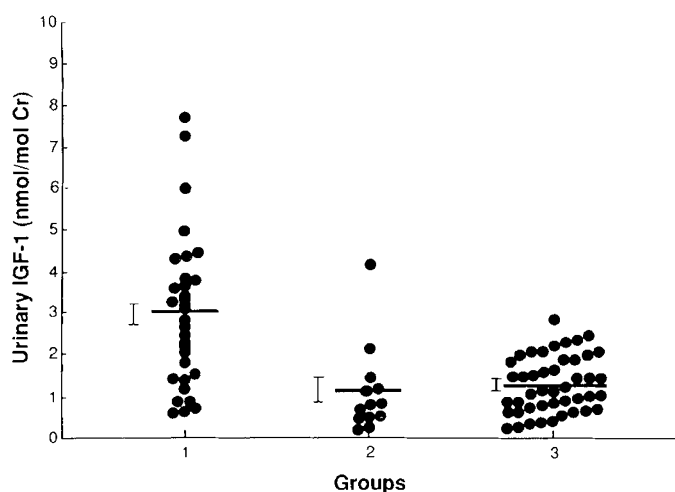| | Urine IGF-I | | Plasma IGF-I |
Groups	(nmol/kg)	(nmol/mol Cr)	(nmol/L)
1	0.22 ± 0.02	3.0 ± 0.3	14.2 ± 2.5
2	0.12 ± 0.01	1.1 ± 0.3	2.6 ± 0.5
3	0.07 ± 0.01	1.2 ± 0.1	5.5 ± 0.7
	$F = 25.5$	$F = 23.0$	$F = 16.5$
	$p < 0.01$	$p < 0.01$	$p < 0.01$
SNK*	1 *vs* 2, 3	1 *vs* 2, 3	1 *vs* 2, 3

* SNK, Student-Newman-Keuls test.

Fig. 4. Output of urinary IGF-I in the three groups. *Bars* indicate the mean ± SEM.

illustrates the individual urinary IGF-I measurements in the three study groups.

A moderately positive correlation was observed between plasma and urinary IGF-I ($r = 0.41$, $p < 0.01$).

DISCUSSION

Our study confirms that the output of GH in timed urine collections accurately reflects serum pool GH concentrations derived from serial blood samples obtained simultaneously at 20-min intervals. For the 91 participants, the highly positive correlation between serum and urinary GH values ($r = 0.64$ in

all subjects and 0.71 in control groups) compares favorably with a similar correlation reported by Sukegawa *et al.* (10) ($r = 0.81$) in 24-h studies of 25 short children and four hypopituitary patients. A positive correlation between serum and urinary GH values in our patients was observed when the data were standardized for body weight (μg/kg) or urinary Cr (μg/mol Cr). This observation strengthens the conclusions reached by our previous study, which took advantage of the noninvasive and semiquantitative features of urinary GH measurements to compare GH production throughout infancy and childhood (5, 7). Normal renal function was a requisite for measuring urinary GH output, inasmuch as the presence of glomerular or tubular disease results in spurious estimates of urinary GH excretion (11, 12).

Among our three study populations, the overlap observed in the serum pool GH concentrations and the urinary GH measurements was of such magnitude that a difference in the GH status of pathologically short children was not clearly delineated from that of control subjects. A similar problem with the overnight serial blood sampling protocol has been reported by other investigators who questioned the diagnostic validity of this test in children with idiopathic short stature (4). Although our pathologically short children in this and an earlier study had significantly lower urinary GH output than controls when the data were expressed as μg/kg of body weight, the difference was not statistically significant when the data were expressed as μg per mol of Cr (5). Because Cr excretion reflects muscle mass, lower output of urinary Cr in short or hypopituitary children tends to falsely elevate urinary GH excretion (13–15). The hypopituitary children in our present study were less severely affected compared to those described previously, hence their relatively higher outputs of urinary GH and greater degree of overlap with control children (2). Lastly, our data confirm the findings reported by others concerning the wide range and unexpectedly low values of mean serum GH concentrations as well as urinary GH outputs in normal children (16–18). This characteristic of healthy children is likely to make it extremely difficult to develop a sensitive, accurate test of subtle GH deficiency.

The labor-intensive RIA used to measure urinary GH output in our study yields data that is comparable to the results obtained with more sensitive ELISA (Hashida males 6.1–37 ng/g Cr, females 4.7–25 ng/g Cr) (19–21). Both methods indicate that urinary GH output is highest in infants and that growing children excrete more GH than adults.

Urinary IGF-I measurements, in contrast to the urinary GH results, have much less overlap among our three study groups. Although opinions differ as to the proportion of IGF-I bound to IGF-I binding proteins, their presence did not appreciably change the results of assays performed on unextracted *versus* extracted urine concentrations (unpublished observations). The significantly lower IGF-I output in our pathologically short children cannot be readily explained. This group did not appear to be nutritionally deficient by physical examination or Z scores for weight.

The positive correlation between the outputs of urinary GH and IGF-I in this study confirms the findings in an earlier investigation, which concluded that the observed relationship between these peptides in urine provides strong evidence in support of the somatomedin hypothesis.

Although urinary GH measurements alone may not sufficiently discriminate which child with idiopathic short stature will benefit from GH therapy, it is possible that additional follow-up of these children will provide information about the diagnostic value of measuring both urinary GH and IGF-I in this population. Children with classical GH deficiency can be identified using these screening tests. Also, measurements of these peptides in urine have yielded information about the relative merits of different GH doses and treatment schedules and may aid in monitoring compliance in children undergoing GH treatment (22).

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