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Quantitation of Urinary Growth Hormone in Children with Normal and Abnormal Growth

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ABSTRACT. Urinary growth hormone (GH) excretion was quantitated in 12-h overnight urine collections obtained from 31 control children, ages 3 to 17 yr (group 1); 21 children, ages 5 to 19 yr with GH deficiency (group 2), and 30 subjects, ages 10 to 18 yr with idiopathic growth failure and normal GH stimulation tests (group 3). The output of urinary GH was measured in one acromegalic woman. The authenticity of urinary GH, 22 kDa, was confirmed by high-performance liquid chromatography. The elution pattern of urinary GH was identical to that of biosynthetic and pituitary-derived GH. The immunoreactive profiles characterized by monoclonal immunoradiometric GH assay and standard GH radioimmunoassay were identical. The quantity of GH (mean ± SEM per kg body weight) in group 1 (0.27 ± 0.02 ng/kg) was significantly greater than group 2 (0.08 ± 0.02 ng/kg) or group 3 (0.17 ± 0.02 ng/kg, $p < 0.01$). Approximately 50% of the subjects in group 3 had urinary GH measurements indistinguishable from those observed in the GH-deficient population. Twelve hypopituitary patients (group 2) excreted significantly greater amounts of urinary GH in the first 12 h after GH administration compared to the baseline period (0.41 ± 0.07 *versus* 0.12 ± 0.02 ng/kg, $p < 0.01$). Markedly elevated output of urinary GH (2.0 ng/kg) was documented in one acromegalic patient. The data suggest that measurements of urinary GH may be a useful, simple, and noninvasive screening test for identifying patients with GH deficiency or excess. (*Pediatr Res* 23: 89–92, 1988)

Abbreviations

GH, growth hormone
hGH, human GH
RIA, radioimmunoassay
HPLC, high-performance liquid chromatography
NHPP, National Hormone and Pituitary Program
IRMA, immunoradiometric assay

Many studies have suggested that children with idiopathic growth failure may be misjudged to be GH sufficient because they have normal GH responses to standard provocative agents (1–4). In this population, the diagnosis of GH deficiency has been established by protocols that assess spontaneous GH secretion. One method requires serial blood sampling over 24 h; those with low mean plasma GH concentrations are presumed to have GH neurosecretory dysfunction (2, 4). The other method, based on continuous blood withdrawal from an indwelling venous catheter, has identified a population with low integrated plasma concentrations of GH (3, 5, 6). Neither method is used in small infants and children because the amount of blood required exceeds the limits of safety. From a practical viewpoint, these tests are uncomfortable, costly, and require special support services. Futhermore, many children with idiopathic growth failure have values that overlap with both the control and hypopitiuitary subjects (2–4, 6).

Given the shortcomings of current diagnostic protocols, this study was initiated to determine if measurements of urinary GH excretion might aid in the diagnosis of GH deficiency. Prior to 1970, attempts to quantitate GH in urine failed because the assays lacked sensitivity and because interfering substances led to overestimation and widely discrepant results (7–13). We used a modification of the Hanssen procedure in which urine first is dialyzed, then concentrated by lyophilization after which GH is measured in a double antibody RIA (13). Compared to former techniques this method gives greater specificity and sensitivity. Herein the authenticity of urine GH was confirmed by using HPLC.

The three study groups included normal statured children, children with classical GH deficiency, and children with idiopathic growth failure and normal GH responses to provocative tests. Urine collections from 12 of the hypopituitary subjects were evaluated before and after an injection of biosynthetic GH. In addition urinary GH was measured in one acromegalic patient.

MATERIALS AND METHODS

Patients. Eighty-two children participated in this study after their parents gave written, informed consent. Age, sex, and pubertal status of the participants are shown in Table 1. The subjects were divided into the following three groups. *Group 1.* Thirty-one healthy children ages 3 to 17 yr who were growing between the 5th–95th percentile for height and weight served as controls. Nineteen were prepubertal and 12 were pubertal, Tanner stage 2–5. *Group 2.* Twenty-one children, ages 5 to 15 yr, with GH deficiency based on a peak GH of less than 8 ng/ml after two or more stimulation tests (insulin-induced hypoglycemia, arginine infusion, clonidine or L-dopa) were in group 2. Fourteen children were severely deficient (peak GH <4 ng/ml) and seven were partially deficient, peak GH >4 and <7.9 ng/ml). Eleven were prepubertal and 10 were pubertal (Tanner stage 2–4). The data from the severely and partially deficient were pooled because they were not significantly different. Urinary GH output after an intramuscular injection of biosynthetic *Escherichia coli*-derived methionyl GH (mean dose 0.05 ± 0.01 mg/

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Table 1. *Clinical data*

Groups	*n*	Prepubertal (male:female)	Pubertal (male:female)	Age range (yr)
1	31	19 (11:8)	12 (8:4)	3–17
2	21	11 (9:2)	10 (7:3)	5–19
3	30	15 (15:0)	15 (13:2)	10–18

kg) was evaluated in 12 GH-deficient children. The injections were given between 1700–1800 h and the urine was collected from 0 to 12 h after the injection. *Group 3.* Thirty children, ages 10 to 18 yr, with idiopathic growth failure comprised group 3. Their heights were greater than −2 SD and their growth rates were less than 4 cm/yr. Their peak GH responses were >8 ng/ml to two or more GH provocative tests. Fifteen children were prepubertal and fifteen were Tanner stage 2–4. On acromegalic adult female provided an overnight 12-h urine for GH.

Methods. Twelve-h overnight urines (1800–0800 h) were collected in plastic containers and kept at 4° C throughout the collection period. The urine was centrifuged to remove particulate matter and stored at −20° C. Hanssen's procedure was modified by adding 50 μl of 2% bovine serum albumin in 0.04 M phosphate buffer, pH 7.4 (Nutritional Biochemicals, Cleveland, OH) to a 50-ml aliquot of urine instead of 0.5 g of human serum albumin. The urine was dialyzed and concentrated 50-fold as described by Hanssen (13).

Urine GH was measured by a standard double antibody RIA method using polyclonal GH antibody and GH standards obtained from the NHPP (14, 15). The intraassay and interassay coefficient variation for GH were 2.1 and 4.0%, respectively. The lower threshold of sensitivity of the assay is 0.15 ng/ml.

Recovery studies were performed by adding known amounts of standard hGH (2.5–10 ng) to 50-ml aliquots of urine from GH-deficient subjects. The samples were dialyzed, lyophilized, and assayed in the same manner described above.

HPLC studies were performed on urine concentrations from four prepubertal subjects. An aliquot of the concentrated urine was applied to a reverse phase Vydac C_4 column (Hesperia, CA) equilibrated in 0.05% trifluoracetic acid containing 20% acetonitrile and developed with a gradient of 20–70% acetonitrile in 30 min at a flow rate of 1 ml/min. Fractions of 0.5 ml were collected, dried under vacuum in a Speed Vac (Savant Instruments, Hickville, NY) and stored at −20° C. The samples were reconstituted in 250-μl horse serum and 100 μl of the reconstituted samples were assayed for GH using both a double monoclonal IRMA (Hybritech, Inc. San Diego, CA) and our RIA.

Statistical analyses of the data were performed using SPSSPC+ (SPSS Inc., Chicago, IL). The Student's *t* test (paired or unpaired as indicated) was used for comparison.

RESULTS

Recovery experiments. The recovery of exogenous GH ranged from 80–100%.

Identification of urinary GH by HPLC. The HPLC profile of urinary GH from one prepubertal male patient is shown in Figure 1. Similar HPLC profiles were observed in the other patients. Radioimmunoassayable urinary GH utilizing an IRMA assay showed the same elution pattern as biosynthetic GH or pituitary-derived GH. When the HPLC fractions were assayed using a standard double antibody RIA the immunoreactive profiles were identical to those obtained with the IRMA method.

Patient studies. Urinary GH excretion was standardized for body weight and expressed as ng/kg/12 h. GH excretion was also standardized in terms of body surface area (ng/m^2/12 h) as well as creatinine excretion (ng/g of creatinine). The excretion of

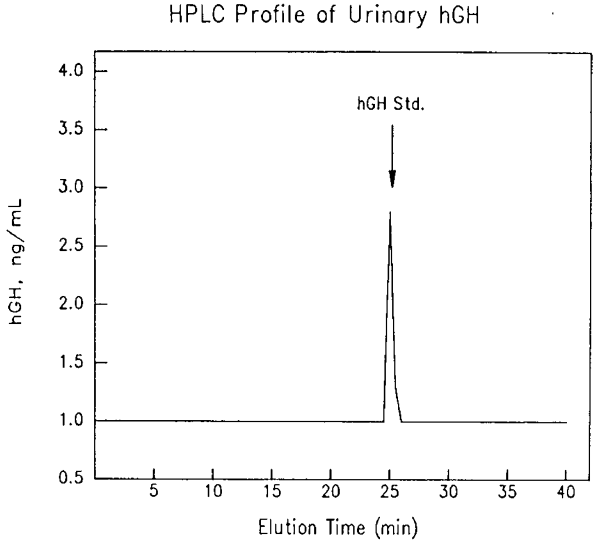

Fig. 1. HPLC elution pattern of urinary GH is identical to biosynthetic GH standard.

urinary GH (mean ± SEM) was significantly greater in group 1 (0.27 ± 0.02 ng/kg/12 h) than group 2 (0.08 ± 0.02 ng/kg/12 h) or group 3 (0.17 ± 0.02 ng/kg/12 h, $p < 0.01$) (Table 2). Children with classical GH deficiency (group 2) excrete significantly lower amounts of GH ($p < 0.01$) than children with idiopathic growth failure (group 3). Individual urinary GH values of participants in the three groups are shown in Figure 2.

When the data were expressed in terms of body surface area (ng/m^2) significantly greater output ($p < 0.01$) was also observed in children of group 1 (7.62 ± 0.06 ng/m^2) compared to group 2 (2.65 ± 0.34) or group 3 (5.13 ± 0.61). Children in group 2 excreted significantly lower amounts of GH than children in group 3 ($p < 0.01$).

Standardization of data according to creatinine excretion again showed a significantly higher output of urinary GH ($p < 0.01$) in children of group 1 (43.2 ± 6.9 ng/g of creatinine) compared to group 2 (23.7 ± 3.8 ng/g of creatinine) or group 3 (21.9 ± 2.8 ng/g of creatinine). No significant difference was observed between the GH-deficient subjects (group 2) and children with idiopathic growth failure (group 3).

Prepubertal and pubertal children in each of the three groups excreted similar amounts of urinary GH when the data were standardized for body weight, body surface area, and per g of creatinine excretion.

Twelve hypopituitary patients (group 2) excreted significantly greater amounts of urinary GH (0.41 ± 0.07 ng/kg) in the first 12 h after GH administration compared to the baseline period (0.12 ± 0.02 ng/kg, $p < 0.01$).

Increased excretion of urine GH was observed in one partially treated acromegalic woman (2.0 ng/kg/12 h); her plasma GH concentration was 46 ng/ml.

DISCUSSION

After 1970, interest in quantitating urinary GH waned because available assays lacked the sensitivity needed to measure the low concentrations of GH in urine. Also, the presence of low molecular weight molecules resulted in overestimation of urinary GH excretion (11). In 1972, Hanssen (13) demonstrated that prior dialysis of urine eliminated nonspecific interference from salts and urea. Hanssen (13) also lyophilized the specimen to yield a 50-fold concentrate. These procedures improved the specificity and sensitivity of the GH assay. Quantitation of urinary GH was carried out on 18 adults; nine controls, three hypopituitary, and six acromegalic subjects. Two of the hypopituitary patients had values that fell below the sensitivity of his RIA (0.39 ng/ml) (13).

Table 2. *Urinary GH excretion per 12 h standardized for body wt, body surface area, and urinary creatinine excretion (mean ± SEM)**

Group	n	Mean GH (ng/kg)	Mean GH (ng/m²)	Mean GH (ng/g creatinine)
1 Normal controls	31	0.27 ± 0.02	7.62 ± 0.56	43.2 ± 6.9
2 GH deficient	21	0.08 ± 0.02	2.65 ± 0.34	23.7 ± 3.8
3 Idiopathic growth failure	30	0.17 ± 0.02	5.13 ± 0.61	21.9 ± 2.8

* Significance: group 1 *versus* 2 or 3, $p < 0.01$ for all cases; group 2 *versus* 3, $p < 0.02$ GH (ng/kg), GH (ng/m²).

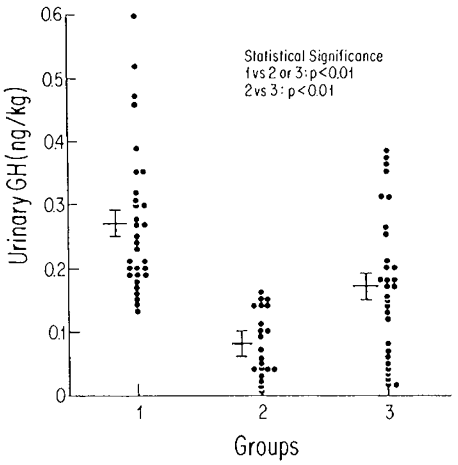

Fig. 2. Output of urinary GH in 12-h overnight urine collections obtained from healthy children (group 1), subjects with GH deficiency (group 2), children with idiopathic growth failure (group 3). *Bars* indicate the mean ± SEM. Approximately one-quarter of the hypopituitary patients had values that overlapped with the control group.

For reasons cited previously, we set out to reevaluate the merits of measuring urinary GH excretion in children with normal and abnormal growth using a slight modification of the Hanssen procedure and an assay with improved sensitivity.

The authenticity of urinary GH, molecular weight 22 kDa was confirmed by HPLC in the present study. Similar conclusions about the molecular weight of urinary GH were reported by investigators who used polyacrylamide gel electrophoresis or Sephadex gel filtration (13, 16). We determined that the HPLC elution profile of urinary GH was identical to both biosynthetic and pituitary-derived GH standards. Furthermore, immunoreactivity of the HPLC fractions were assayed using a double monoclonal IRMA technique that recognizes only intact GH and the standard GH polyclonal assay (NHPP). The immunoreactive GH profiles defined by the two assays were identical.

The output of urinary GH in hypopituitary children given an injection of biosynthetic GH was significantly greater than the value observed before treatment. Less than 0.001% of administered biosynthetic GH was measured in urine collected from 12 of our hypopituitary children. Similar estimates have been reported previously (13).

In the large cohort of children who participated in our study, we observed that mean urinary GH excretion was significantly greater in control subjects compared to the children with either GH deficiency or idiopathic growth failure. This difference was observed irrespective of the parameters used to standardize the data. Some authors have standardized urinary output of GH on the basis of creatinine excretion to compensate for inaccuracy of urine collection (17, 18, 19). However, previous reports have documented that protein synthesis and creatinine excretions are lower in hypopituitary children than in normal children (20, 21). Thus expressing renal output of GH per g of urinary creatinine is likely to result in falsely high values. This may explain the lack of statistical difference between groups 2 and 3 when GH concentrations are expressed per g of creatinine.

Approximately 50% of the children with idiopathic growth failure had urinary GH values that were similar to those of children with classical GH deficiency. It would appear that the group consisting of children with idiopathic growth failure is a heterogeneous population and that almost one-half of the children may have GH deficiency. The rest of the children in group 3 who had urinary GH values in the normal range may have a bioinactive GH molecule, unrecognized nutritional defects, or receptor problems. The overlap of individual urinary GH measurments among the groups resembles the spread of individual plasma GH concentrations determined by constant blood withdrawal (integrated GH concentration) or by serial blood sampling over 24 h in similar populations of children (2–4, 6). Less overlap of urinary GH values was observed between the normal and hypopituitary patients.

Based on the intergroup differences observed in this study and the reduced cost and discomfort of the test, it would appear that measurement of urinary GH may prove to be useful in screening patients with suspected GH deficiency or GH excess. Although this approach has inherent problems relative to accuracy of urine collection, it permits assessment of spontaneous output of GH over time in very small infants and children. During the course of our studies an even more sensitive assay for urinary GH was described by Hashida *et al.* (17) that uses a sandwich horseradish peroxidase enzyme fluorophotmetry technique; the sensitivity of the assay was increased to 0.03 ng/ml. The range of values observed in our patients was similar to the range of urinary GH per g of creatinine reported by Hashida and coworkers in 37 healthy children (18, 19). Significantly lower output of urinary GH was found in five hypopituitary children (18).

We recently reported that quantitation of urinary Somatomedin C/IGF-I provides diagnostic information about patients with GH deficiency and GH excess (22, 23). Additional investigations are required in children with normal and abnormal growth to determine whether combined measurements of GH and Somatomedin C/IGF-I in urine will aid in recognizing specific defects in the pathway of GH secretion and action.

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