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Urinary growth hormone excretion as measured by a sensitive immunochemiluminometric assay

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SUMMARY. A sensitive immunochemiluminometric assay with a detection limit of 1·1 μU/L was developed for the measurement of urinary growth hormone (UGH). The assay was shown to be specific and precise. There was a good correlation between serum growth hormone (GH) and UGH concentrations in 20 patients with acromegaly and six volunteers following an intravenous injection of recombinant GH. We concluded therefore that UGH measurements appear to provide a satisfactory index of GH secretion. The use of the assay in the investigation of growth disorders was assessed. We studied 11 pre-pubertal children, six of normal stature, and five of short stature, over a 6-month period. Sequential fortnightly measurements of UGH were carried out and height velocity was determined. The children of short stature grew at a slower rate and excreted less GH than the children of normal stature. However, we observed considerable within-individual variability in GH excretion in both groups (CV 22–98%). We therefore recommend that sequential UGH analyses should be carried out and the results interpreted in conjunction with growth measurements. However, further investigations into the renal handling of GH are needed to establish optimum sampling regimes.

Additional key phrases: acromegaly; short stature; growth velocity

The ready availability of recombinant growth hormone (GH) has led to a renewed interest in the pathophysiology of growth. There are various tests used for the assessment of GH secretion. As GH secretion is episodic, a single plasma sample yields little information. Serial 24 h or overnight blood sampling may overcome difficulties in interpreting data by achieving a direct measure of hormone secretion. However, these tests can be time consuming, costly and may exceed the limits of safety especially in small children.[1] GH provocative tests elicit GH secretion by use of agents such as GH releasing hormone, insulin, arginine or clonidine. Though these tests reflect the pituitary's potential for releasing GH they do not necessarily provide an index of endogenous GH secretion under physiological conditions.[2] This has called into question the diagnostic relevance of provocative testing. Recently, with the advent of more sensitive immunometric assays, urinary GH measurements have been put forward as an alternative non-invasive test, since they may provide integrated values of secreted GH and thus offer a physiological measure of a patient's GH secretion.[3] The application of urine GH measurements in the investigation of growth disorders has been carried out by various workers. Some studies have shown that urine GH levels are significantly greater in normal children than in short children with GH deficiency diagnosed by GH provocative tests.[4,5] Others, however, have reported an overlap in levels between normal and GH deficient children and a group of children of short stature but with 'normal' response to provocative tests.[1,6] However, considerable intra-individual variation in GH excretion has been reported[7] and this may therefore limit the clinical usefulness of urinary GH measurements. The aim of the study was to confirm that urine GH measured by a sensitive immunochemiluminometric assay provided a satisfactory reflection of secreted GH and to assess the value of the test in the investigation of growth abnormalities in children.

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MATERIALS AND METHODS

UGH analysis

Urine GH was measured by a highly sensitive immunochemiluminometric assay (ICMA) comprising a monoclonal GH antibody labelled with an acridinium ester and polyclonal anti-GH antibody immobilized on to coated tubes. The monoclonal antibody was a generous gift from Dr G Beastall, Department of Biochemistry, Glasgow Royal Infirmary, Glasgow and the polyclonal antibody was obtained from the Scottish Antibody Production Unit, Carluke, Lanarkshire, UK. The assay had good sensitivity with a minimum detection limit of 0·55 ng or 1·1 μU/L and has been shown by reverse-phase HPLC to detect the 22 kDa pituitary GH. The basic assay protocol is shown in Fig. 1. The precision profile[8] derived from 143 separate data points is shown in Fig. 2. The inter-assay imprecision was good with a CV of 14%, 11% and 8% at GH concentrations of 5·0 μU/L, 10 μU/L and 25 μU/L, respectively. Increasing urea and NaCl concentrations were shown to give rise to an apparent decrease in GH added to the urine samples, so sample pre-treatment prior to analysis was necessary by overnight dialysis against 20 volumes of assay buffer (phosphate buffer saline with 2·5 g of bovine serum albumin (BSA) and 0·5 g of sodium azide pH 7·4) at 4°C. BSA (2·5 g/L) was added to the urine samples before dialysis to avoid GH adhering to the dialysis membrane.

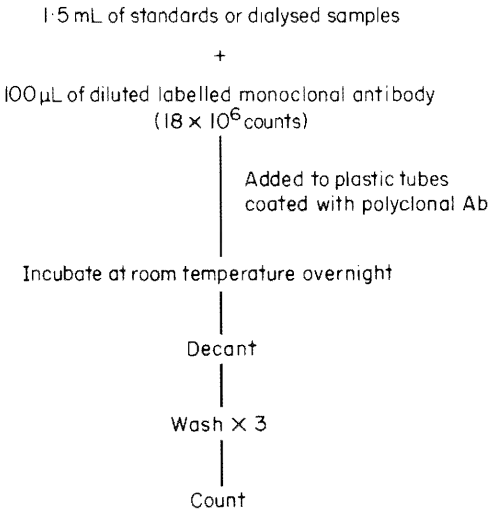

FIGURE 1. *Basic protocol for the immunochemiluminometric assay for urinary growth hormone measurements.*

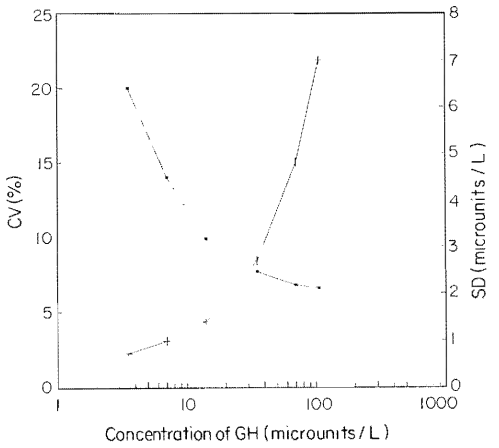

FIGURE 2. *Precision profile of the immunochemiluminometric for urinary growth hormone measurements.* □ = *CV (%);* + = *SD (microunits/L).*

Recovery of added GH ranged from 85 to 90%. The urine samples were stable at −70°C for at least 6 months. There was significant cross-reactivity (3·5%) with human placental lactogen (HPL). However, for the purpose of our study this was not clinically relevant. Serum GH concentrations were measured by a sensitive ICMA as previously described by Weeks and Woodhead.[9]

Patients

To validate the urinary GH assay 20 patients with either acromegaly or a suspected diagnosis of acromegaly were studied. Eleven of the patients with acromegaly had been treated with either radiotherapy or were receiving somatostatin (Group I). Five patients had been taken off somatostatin and their GH secretion was being re-assessed (Group II). The remaining four patients were being investigated for acromegaly on clinical grounds (Group III). Blood was taken at hourly intervals, for 6 h, for GH measurements with a simultaneous urine collection. Five patients with suspected hypopituitarism who underwent an insulin stress test (IST) were also studied (Group IV). Urine was collected from each of the five patients immediately after the test. Six healthy adults were given an intravenous injection of 2·0 IU of synthetic GH. Blood and urine for GH measurements was collected at 15 min and 30 min intervals, respectively, for 3 h.

To assess the degree of intra-individual variation in urinary GH secretion and its effect in the investigation of growth disorders we studied six

pre-pubertal children aged 5·4–12·5 years whose stature fell on or above the fiftieth centile of the standard British Charts (Group A). Five short slowly growing pre-pubertal children aged 6·5–11·4 years whose stature fell below the third centile were also recruited (Group B). Only one of the children in this group (Subject 2) had been tested by the insulin provocation test and a normal response had been found. The children were urged to keep all urine samples passed during the night in addition to the first voiding morning urine specimen. Thus, the urine obtained represented an overnight collection. This was carried out at fortnightly intervals for a period of 6 months. The volumes were noted and aliquots stored for urinary GH measurements. All urine samples on each individual were analysed in one batch. Height velocity was derived over the 6-month period on all children.

Statistical analysis

The relation between serum and urine GH levels was determined by Spearman's rank correlation. The difference between mean height velocity and urine GH levels in the two groups of children was analysed by the Mann–Whitney *U*-test.

RESULTS

There was a highly significant correlation ($r = 0{\cdot}90$; $P < 0{\cdot}001$) in the 20 patients with either a suspected or proven diagnosis of acromegaly and the five patients undergoing an IST between the mean serum GH levels and their urinary GH excretion per hour (Fig. 3). In Group I a wide range of serum and urine GH levels were observed depending on the success of the treatment; serum GH [mean (SD)] were $6{\cdot}649 \times 10^3$ ($5{\cdot}442 \times 10^3$) μU/L and urine GH excretion [mean (SD)] were $1{\cdot}280 \times 10^3$ ($8{\cdot}80 \times 10^2$) nU/h. Patients in Group II had the highest urine and serum GH concentrations, with serum GH ranging from $4{\cdot}8 \times 10^4$ to $1{\cdot}29 \times 10^5$ μU/L and urine GH excretion from $8{\cdot}1 \times 10^3$ to $3{\cdot}5 \times 10^4$ nU/h. Only one of the patients in Group III was found to have acromegaly, with a mean serum GH concentration of $2{\cdot}4 \times 10^4$ μU/L and a urine GH excretion of $4{\cdot}0 \times 10^2$ nU/h and one of the five patients undergoing the IST (Group IV) had an abnormal response diagnostic of hypopituitarism. In response to an intravenous bolus injection of 2 IU of biosynthetic GH in six healthy men, there was a rapid rise in serum GH followed by clearance of the hormone from the circulation. A similar pattern of change was reflected in urine GH concentrations measured in 30 min urine collections indicating a relationship between serum and urine GH (Fig. 4). Approximately 0·0027% (0·0019)% [mean (SD)] of injected GH was excreted in urine over the collection period.

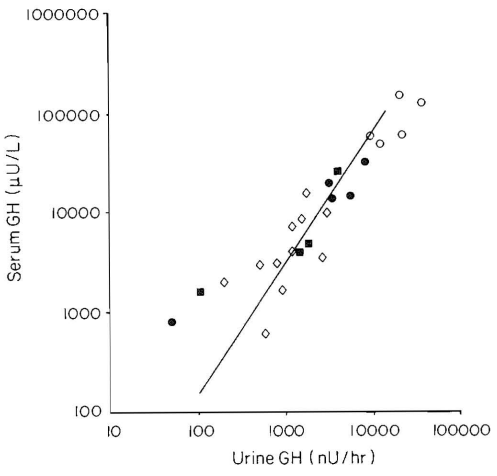

FIGURE 3. *The relation between mean serum growth hormone (GH) concentration levels and urinary GH excretion per hour (r = 0·90; P < 0·001) in the four groups of patients. Group I = ◇, Group II = ○; Group III = ■; Group IV = ●.*

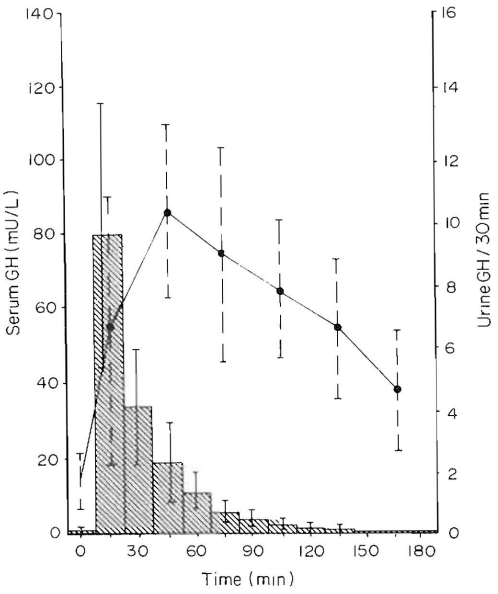

FIGURE 4. *Mean urinary growth hormone (GH) and serum GH concentrations following an intravenous bolus injection of 2·0 IU of biosynthetic GH in six healthy male volunteers. ▨ = serum GH; ● = urine GH.*

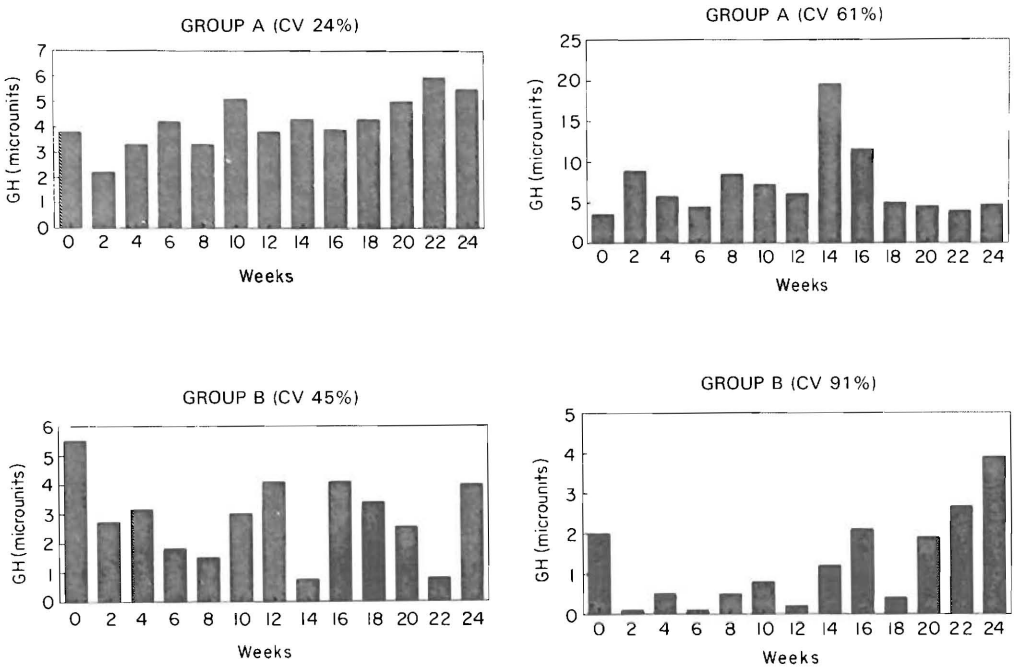

FIGURE 5. *The intra-individual variation in growth hormone excretion at fortnightly intervals in two subjects in Group A (CV 24% and 61%) and Group B (CV 45% and 91%).*

TABLE 1. *The mean urinary growth hormone levels and height velocity over a 6-month period on each child*

Subjects	1	2	3	4	5	6
Group A						
Mean (SD) (urinary GH levels μU/specimen)	2·6 (1·5)	2·8 (1·2)	7·3 (3·5)	4·2 (1·0)	7·2 (4·4)	6·0 (2·5)
Height velocity (cm/0·5 year)	4·0	3·0	2·8	3·2	4·0	3·8
Group B						
Mean (SD) (urinary GH levels μU/specimen)	2·6 (2·4)	1·3 (1·0)	4·0 (2·1)	2·9 (1·4)	1·3 (1·2)	—
Height velocity (cm/0·5 year)	2·2	2·8	1·8	2·7	3·2	—

GH = Growth hormone.

The amount of GH excreted (μU/specimen) varied considerably between the fortnightly collections obtained over the 6-month period in both groups of children. In Group A the coefficient of variation (CV) ranged from 24–61% (mean 46%). The intra-individual variation was more obvious in Group B where the CV ranged from 48 to 98% (mean 73%). This is illustrated in Fig. 5 on two subjects in Groups A and B, respectively. The mean GH excretion over the 6-month period in Group A was nearly twice that of Group B [5·27 (SD 2·39) and 2·44 (SD 1·11) μU, respectively, $P<0·05$] although there was an overlap between individuals within the two groups. The mean height velocity over the 6-month period was significantly greater in Group A than in Group B [3·46 (0·53) and 2·51 (0·48) cm/0·5 year, respectively, $P<0·05$]. The mean urinary GH levels and height velocity data over the 6-month period on each child are summarized in Table 1. There was no significant correlation between these two parameters.

DISCUSSION

The aims of this study were to establish whether the GH ICMA would be sensitive enough to measure the low concentrations of GH occurring

in urine, to determine whether these concentrations adequately reflect GH secretory activity and finally to assess the usefulness of the test in the investigation of disorders of growth in children.

Our results showed that the assay is specific in measuring the 22 kDa pituitary GH molecular species as evidenced by the detection of a single peak of immunoreactivity on reverse-phase HPLC. The sensitivity of the assay was 1·1 μU/L, which compares favourably with other immunometric assays described for urine GH.[10] Intra- and inter-assay precision were also within acceptable limits across the clinically relevant range. Interference in the assay by high salt or urea concentrations, which has been observed by other groups,[3,4,11] was avoided by dialysis of samples prior to measurement. This procedure is time-consuming and cumbersome and we have, therefore, investigated alternative methods for extraction of GH using ethyl or cyano-bonded cartridges. Preliminary experiments have been disappointing, with recoveries from around 50% as compared with 85–90% using dialysis.

Our data obtained from acromegalics showed a good correlation between GH measured in urine samples and the corresponding serum concentrations ($r=0{\cdot}90$, $P<0{\cdot}001$). The urine GH levels also reflected the clinical status of the patients in the various groups; the highest levels were those observed in the patients off treatment (Group II). In the subjects who received intravenous GH, the increase in serum concentration was followed by a corresponding increase in excretion, though only a small fraction of the administered dose ($0{\cdot}0027\% \pm 0{\cdot}0019\%$) was actually excreted as has been found by others.[12] However, the implication is that in different situations GH excretion may provide a satisfactory reflection of hormone secretion.

The second part of the study involved fortnightly urinary GH measurements over a period of 6 months on a group of prepubertal children of normal and short stature. The children of short stature, although not clinically overtly GH deficient, were selected because in practice such children present difficulties in relation to both diagnosis and therapy.[13] Moreover the GH provocative tests in such clinical situations have recently been discredited.[2,16] Urine measurements which reflect secretory activity under physiological conditions offer great potential in this difficult group since they are non-invasive and can thus be used to monitor progress. We found in this study that, as a group, the children of short stature grew more slowly and excreted less GH than the control group, though there was some degree of overlap between concentrations measured in individuals within the two groups. In some studies on children with growth disorders, it has been found that short stature children with GH deficiency excreted significantly less hormone than normal children.[4,5] However, others observed an overlap between normal and GH deficient children and a group of short stature children with 'normal' responses to GH provocative testing.

Some of this earlier work may be criticized on the grounds that only single point estimates of GH excretion were made, longitudinal studies of urine GH levels were not undertaken and no attempts were made to correlate the results obtained with other indices of growth such as height velocity. We observed considerable within-individual variability when urine GH concentrations were assessed at fortnightly intervals in both groups of children studied. This variability may be attributed to several factors. First, it may result from changes in the renal tubular function[14] which in turn may be influenced by the degree of hydration of the subject. Secondly, GH secretion itself may fluctuate on a daily basis[5] and may be affected in any case by extraneous factors such as diet, exercise and stress. Finally, the imprecision of the assay itself may contribute to the variability, particularly at low GH concentrations.

Variability deriving from analytical imprecision has been minimized in this study by carrying out sequential measurements on an individual patient within a single assay. However, within-individual fluctuations present the biggest single problem in trying to relate urine GH levels to growth status. The variability in GH excretion observed in this study did not necessarily reflect long-term changes in GH secretion, since a similar degree of variability was found in daily samples from normal children (unpublished observations). This factor may limit the use of the assay as a screening test in the investigation of children with growth disorders and may partially explain the lack of correlation observed between height velocity and UGH measurements. This problem can be overcome in part by carrying out several measurements on each individual. Furthermore, growth is not solely dependent on GH secretion since it is influenced by other factors such as social economic status,[17] genetic influences[18] and nutrition. However, further investigations on the effect of changes in renal function on the renal handling of GH are needed in order to establish

optimum sampling regimes. In this way it may prove possible to establish a meaningful relationship between GH excretion and growth rate data and thus improve the diagnostic efficiency of the test.

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