The Role of Serial Sampling in the Diagnosis of Growth Hormone Deficiency

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ABSTRACT. We analyzed 12-hour serial sampling of growth hormone (GH) levels in two cohorts of short children: 96 children referred to a university endocrine clinic or studied on a research protocol and 825 children in the National Cooperative Growth Study of children treated with exogenous GH.

The mean 12-hour GH levels correlated with growth velocity in 60 children with normal height and growth velocity in the university study, and this correlation was stronger in the boys. The testosterone levels also correlated with growth velocity and mean 12-hour GH levels in the boys. The mean 12-hour GH levels were lower in a group of 36 children with idiopathic short stature than in the control subjects, as were the peak GH levels within 1 hour after the onset of sleep and the insulin-like growth factor I levels.

In the National Cooperative Growth Study cohort, pooled 12-hour GH levels were lower in the group with idiopathic GH deficiency ($n = 300$) than in the group with idiopathic short stature ($n = 525$), but the difference was not significant. The duration of GH treatment was the most significant predictor of change in the height SD score in both groups. Indices of spontaneous secretion of GH were not predictive of the response to GH treatment, nor were the results of provocative GH testing, the responses to GH treatment being similar in both groups over time.

We conclude that the results of GH testing must be interpreted for each patient and that several testing modalities may be helpful in finding GH insufficiency that originates at various levels of the somatotropic axis. *Pediatrics* **1998;102:521–524;** ***growth hormone, growth hormone neurosecretory dysfunction, insulin-like growth factor I, NCGS (National Cooperative Growth Study).***

ABBREVIATIONS. GH, growth hormone; IGF–I, insulin-like growth factor I; NCGS, National Cooperative Growth Study; SDS, standard deviation score(s); AUC, area under the curve.

Growth is the final biologic outcome of a complex chain of molecular events that begin in the cortex of the brain and end in the replicative machinery of the growing cell. A vital link in this chain is the pulsatile secretion of growth hormone (GH) from the pituitary gland. In this article, we describe the evidence for assessing the spontaneous secretion of GH in making the diagnosis in slowly growing children.

Studies of the spontaneous release of GH have been important in understanding the pathophysiology in a subgroup of children with poor growth but normal responses on provocative GH testing, called GH neurosecretory dysfunction.[1,2] These children have delayed bone ages and low insulin-like growth factor I (IGF–I) levels, and respond well to GH therapy. Because of these findings, studies of the spontaneous release of GH have been used diagnostically in many centers.

Over the years we have performed studies of the spontaneous release of GH in 12- and 24-hour periods as part of our clinical assessment of children with growth disorders and as part of clinical research studies. We have analyzed data from two groups of these children, otherwise healthy normally growing and slowly growing children, to evaluate the relationship of the secretion of GH to growth.

The National Cooperative Growth Study (NCGS) is a North American multicenter database of >24 000 children who have been treated with GH products manufactured by Genentech Inc (South San Francisco, CA). Data from children in whom both provocative GH testing and serial sampling had been performed were analyzed for determining which form of testing was more predictive of the response to GH treatment.[3]

METHODS

Data from two cohorts of children were analyzed: children who underwent diagnostic assessment for growth disorders or were enrolled in a clinical research protocol at the University of South Florida Pediatric Endocrine Clinic, Tampa, FL, and at All Children's Hospital, St Petersburg, FL, and children enrolled in the NCGS who underwent both provocative and serial GH testing. All of the children in the NCGS cohort were prepubertal at the time of testing; the children at the university clinic were at various stages of puberty. In both cohorts, blood was obtained every 20 minutes from 2000 to 0800 hours. An equal volume of serum from each of the samples was combined to create timed pooled specimens. All GH samples from an individual patient were measured in one assay. Serum GH concentrations were determined with double-antibody polyclonal radioimmunoassays in the university studies[4] and with Tandem immunoradiometric assay (Hybritech, San Diego, CA) in the NCGS studies. The frequency and amplitude of the pulses of GH release were analyzed by using cluster analysis.[5] Correlations were determined by using Pearson's correlation. The groups were compared by using the two-tailed Student's t test. The levels of testosterone and IGF–I were measured by radioimmunoassay (IGF–I without acid extraction). The results are presented as mean ± SD.

RESULTS

Normally Growing Children

A control population from the university clinic was defined by a growth velocity standard deviation

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score (SDS) of −1.0 or greater; a height within 3.0 SD units of the mean; and the lack of a recognized syndrome, cranial irradiation, precocious puberty, or obesity (body mass index >95th percentile for age). Sixty children (33 boys, 36 prepubertal) met these criteria, ranging in age from 3.8 to 17.8 years.

The height SDS in the control group did not correlate with any parameters of GH secretion, but growth velocity correlated with the mean level of GH in 12 hours ($r = .37$; $P < .01$) (Table 1), as well as with area under the curve (AUC) ($r = .35$), number of peaks of GH ≥5.0 μg/L ($r = .39$), and peak GH concentration within 1 hour from the onset of sleep ($r = .32$). The correlation of growth velocity and the mean level of GH in 12 hours was particularly strong in the boys ($r = .59$), and the pooled testosterone level correlated strongly with both growth velocity ($r = .66$) and the mean level of GH in 12 hours ($r = .51$).

Slowly Growing Short Children Without GH Deficiency

The results of the studies in 36 children in the university cohort who had unexplained slow growth (height SDS, <−3.0; growth velocity SDS, <−1.0; maximum stimulated GH level, ≥10.0 μg/L; no systemic illness) were compared with results in the control subjects.

The mean level of GH in 12 hours was significantly lower in the slowly growing children than in the control subjects (4.1 ± 1.8 vs 5.6 ± 3.4 μg/L; $P = .02$), as was the number of GH peaks in 24 hours (6.8 ± 2.0 vs 8.1 ± 2.4 peaks; $P = .04$). The number of GH peaks ≥5.0 μg/L and the mean amplitude of the peaks were not different between the groups. The peak GH concentration after the onset of sleep was significantly lower in the slowly growing group (16.5 ± 10.5 vs 21.2 ± 11.3 μg/L; $P = .04$), as was the mean IGF–I concentration (0.7 ± 0.6 vs 1.4 ± 1.0 U/mL; $P = .0003$).

Short Children in the NCGS Cohort

Children with short stature in the NCGS cohort were classified as having idiopathic GH deficiency ($n = 300$) if their maximum stimulated GH level was <10 μg/L and as having idiopathic short stature ($n = 525$) if it was ≥10 μg/L.[3] The two groups were similar in age, bone age, height SDS, dose of GH, and duration of GH treatment. The pooled GH concentrations were lower in the group with idiopathic GH deficiency than in those with idiopathic short stature (1.6 ± 1.0 vs 2.1 ± 1.2 μg/L), but the difference was not significant. A correlation analysis indicated that the duration of GH treatment was the most significant predictor of change in height SDS in all the children; indices of the spontaneous secretion of GH were not predictive of the response to GH treatment. As shown in Fig 1, the results of provocative GH testing also were not predictive, because the responses to GH treatment were similar in the two groups over time.

DISCUSSION

We report on two distinct cohorts of children who were assessed for the spontaneous secretion of GH. Because of the use of different clinical parameters and GH measurement assays, it is not possible to compare directly the results from the two groups.

Our results indicate that abnormalities of GH secretion can be seen in children who have normal results on provocative GH tests but are growing slowly. The spontaneous nocturnal secretion of GH was low in the slowly growing short children with normal results on provocative tests, and this appeared to be mediated largely by a decrease in the sleep-associated release of GH. The amplitude of the

TABLE 1. Correlations in the Control Population

	All Subjects	Boys	Girls	Prepubertal
Growth velocity with mean 24-hour GH level				
r	.24	.52	.24	.15
P	NS	<.005	NS	NS
n	47	30	17	26
Growth velocity with mean 12-hour GH level				
r	.37	.59	.08	.21
P	<.01	<.001	NS	NS
n	50	30	20	29
Growth velocity with PM testosterone level				
r	.57	.66	.43	.29
P	<.0001	<.0001	NS	NS
n	50	30	20	29
Mean 12-hour GH level with PM testosterone level				
r	.28	.51	.01	.16
P	<.05	<.005	NS	NS
n	60	33	27	36
Mean 24-hour GH level with IGF–I level				
r	.53	.41	.58	.26
P	<.0001	<.05	<.05	NS
n	49	31	18	26
Mean 12-hour GH level with IGF–I level				
r	.46	.37	.47	.39
P	<.001	<.05	<.05	<.05
n	53	31	22	29

The IGF–I level is the average of two determinations done at 8 am and 8 pm.

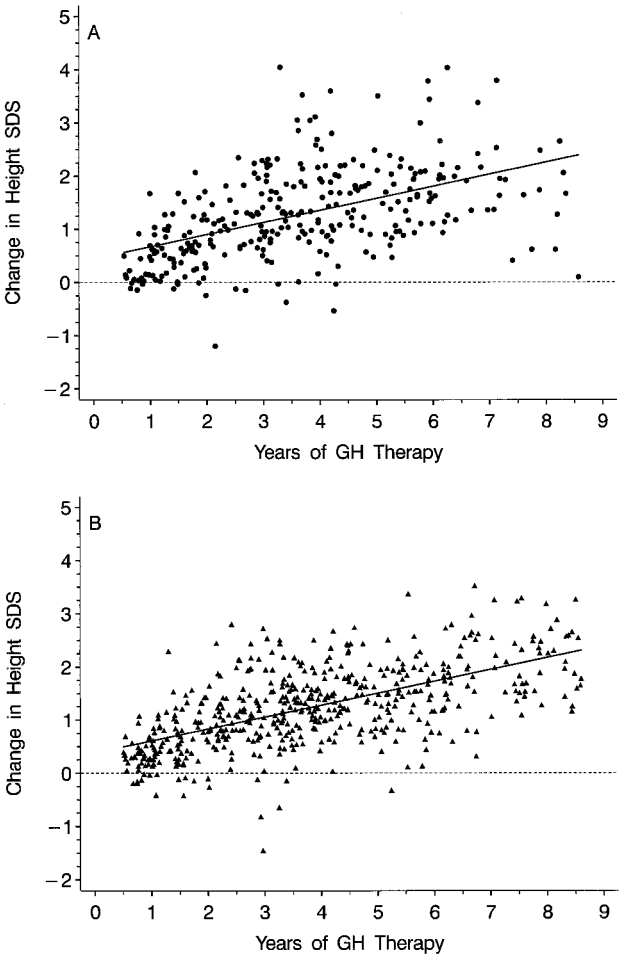

Fig 1. Long-term changes in height SDS during GH therapy in children with idiopathic growth hormone deficiency (maximum stimulated GH level, <10 μg/L) (A), and with idiopathic short stature (maximum stimulated GH level, ≥10 μg/L) (B).

GH pulses in these children was not different from that in the control subjects, but the mean frequency of the pulses was less by an average of 1.3 peaks during a 24-hour period. These factors, and potentially others, result in lower average IGF–I concentrations.

These results are in agreement with our earlier observations that slowly growing short children with normal results on provocative GH tests responded well to GH treatment.[1] Zadik and associates[6] reported on a group of short children with normal responses on provocative GH tests but with integrated GH concentrations obtained by constant exfusion similar to those found in children with GH deficiency. Kerrigan and colleagues[7] examined the dynamics of GH secretion in a group of short boys with delayed bone ages. Using deconvolutional analysis, they found lower GH mass (μg/L_v) and secretion rates than in short and normal-stature boys without delayed bone age. Other conditions that have been shown to be associated with decreased spontaneous secretion of GH are cranial irradiation,[8] gonadal dysgenesis,[9] Russell–Silver syndrome,[10] thalassemia major,[11] and hypothyroidism.[12]

The height SDS in our subjects did not correlate with the mean 12-hour secretion of GH. Albertsson-Wikland and Rosberg found a relationship between spontaneous secretion of GH and height SDS,[13] but this may be attributable either to the larger number of tall subjects in their study or to the use of different characteristics of spontaneous secretion (AUC above the baseline).

We found a correlation between growth velocity and spontaneous secretion of GH in normally growing children. Other authors have found a relationship between growth rate and secretion of GH. Hindmarsh et al[14] described a positive asymptotic relationship between parameters of secretion of GH and height velocity in short prepubertal children. Spadoni et al[15] found a strong correlation (r = .74) between mean 12-hour nocturnal GH concentration and growth velocity (expressed as SD for bone age) in prepubertal children with a variety of causes of their short stature. Rose and associates[16] also found that the growth rate correlated with the mean 24-hour secretion of GH (r = .33). Rogol and colleagues[17] analyzed data from 2123 children in the NCGS who underwent serial GH sampling and found weak correlations of the secretion of GH with the growth rate in the first year of GH therapy, but the strongest correlations were with the maximum 12-hour and pooled 12-hour GH levels. The maximum stimulated GH levels did not correlate with the response to therapy at all. We found previously that the response to GH treatment in a cohort of short children in less than the first percentile in height for chronologic age correlated best with the pretreatment height velocity (r = −.67) and less well with the spontaneous or maximum stimulated GH levels (24-hour AUC, r = −.33; maximum stimulated GH level, r = −.04).[18]

In summary, we conclude from these studies that 1) growth velocity correlates with the mean 12-hour GH level in normal children, 2) that there can be subtle differences in GH secretion between slowly growing short children with normal results on provocative testing and control subjects, and 3) that neither provocative GH testing nor serial sampling is predictive of the response to GH therapy in very short children. These findings suggest that GH testing must be interpreted in the clinical context for each patient and that several testing modalities may be necessary to find GH insufficiency that originates at various levels of the somatotropic axis.

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The Role of Auxologic and Growth Factor Measurements in the Diagnosis of Growth Hormone Deficiency

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ABSTRACT. The use of auxologic measurements in the diagnosis of short stature in children has a long history in pediatric endocrinology, and they have even been used as the primary criteria in selecting children for growth hormone (GH) therapy. Certainly, an abnormality in the control of growth is more likely in short children than in children of normal stature. However, most studies have shown little or no value of auxologic criteria in differentiating short children who have classic growth hormone deficiency (GHD) from short children who do not. In National Cooperative Growth Study Substudy VI, in more than 6000 children being assessed for short stature, the overall mean height SD score was −2.5 ± 1.1 and the body mass index standard deviation score was −0.5 ± 1.4. However, there were no significant differences in these measures between the patients who were found subsequently to have GHD and those who were not. There also was no consistent difference in the growth rates between the patients with classic GHD and those short children without a diagnosis of GHD. This probably reflects the fact that we are dealing with a selected population of children who were referred for short stature and are further selecting those who are the shortest for additional investigation.

Growth factor measurements have been somewhat more useful in selecting patients with GHD and have been proposed as primary diagnostic criteria. However, in National Cooperative Growth Study Substudy VI, only small differences in the levels of insulin-like growth factor I and insulin-like growth factor binding protein 3 were seen between the patients who were selected for GH treatment and those who were not. Many studies indicate that the primary value of growth factor measurements is to exclude patients who are unlikely to have GHD or to identify those patients in whom an expedited work-up should be performed. The diagnosis of GHD remains difficult and must be based on all of the data possible and the best judgment of an experienced clinician. Even under ideal circumstances, errors of both overdiagnosis and underdiagnosis of GHD still are likely. *Pediatrics* 1998;102:524–526; *auxology, growth hormone, growth hormone deficiency, insulin-like growth factor.*

ABBREVIATIONS. GHD, growth hormone deficiency; GH, growth hormone; NCGS, National Cooperative Growth Study; SDS, standard deviation score(s); IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein.

In many cases, the diagnosis of growth hormone deficiency (GHD) is not straightforward, and the validity of the criteria currently used in the diagnosis of GHD has been criticized.[1] One illustration of the uncertainties in the diagnosis of GHD in childhood is the secular trend in the peak level of growth hormone (GH) in response to provocative stimuli that is considered diagnostic of GHD (Table 1). In the past 3 decades, the diagnostic cutoff concentration of GH has at least doubled, perhaps because of the increased availability of biosynthetic GH. Because of the difficulties in diagnosing GHD, some endocrinol-

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