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Research paper

The insulin-like growth factor-I (IGF-I) generation test as an indicator of growth hormone status

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ABSTRACT

OBJECTIVE: The aim of the study was to evaluate the IGF-I generation test (IGF-I gen) as a possible indirect test of Growth Hormone (GH) secretory status. METHODS: Sixty-five GH deficient (GHD 1 and 2) and 86 control children were studied. Children in the GHD-1 subgroup (n=33) had low GH values (<10μg/L) after clonidine and levo-dopa while those in the GHD-2 subgroup (n=32) had normal GH values after pharmacologic provocation but low 24-hour GH secretory rates compared to 187 Normal Statured (NS) children. Of the 86 controls, who underwent IGF-I gen, 50 were NS and 36 Short-Statured (SS). Serum IGF-I was measured prior to and daily during hGH administration (hGH 0.033 mg/kg/day x 4 days). RESULTS: The prepubertal and pubertal GHD-1 and GHD-2 children had low baseline IGF-I values but their peak IGF-I values during the IGF-I gen reached those of the controls. The percent increase of IGF-I during the test was greater in the GHD groups than in the controls; in the prepubertal groups: 516±58% in the GHD-1, 433±50 % in the GHD-2, 106±12 % in the NS, and 102±18 % in the SS (p=0.001); in the pubertal groups: 191±28 % in the GHD-1, 141±20 % in the GHD-2, 48±8 % in the NS, and 61±17 % in the SS (p=0.003). CONCLUSIONS: The IGF-I response during the IGF-I gen seems to reflect the GH status in children.

Key Words: GH deficiency, Growth delay, IGF-I, IGF-I generation test, Short stature

INTRODUCTION

Growth hormone plays a major role in skeletal growth and exerts important effects on the metabolism of proteins, lipids and carbohydrates mostly through its production of insulin-like growth factor I (IGF-I).[1] The diagnosis of Growth Hormone Deficiency (GHD) during childhood, the first sign of which is usually growth delay, is of crucial importance for the future well-being of the affected individual because besides short stature, untreated GHD can also cause problems such as hypercholesterolemia, obesity, muscle weak-

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ness, or impaired quality of life during adulthood. In routine clinical practice the assessment of GH secretion is accomplished primarily by pharmacologic provocation of GH release. Pharmacologic provocation alone, however, may sometimes be misleading and the diagnosis of GH deficiency can be missed.[2,3]

Assessment of spontaneous 24-hour (24hr) GH secretion can frequently aid greatly in diagnosing GH deficiency if the growth failure persists in the presence of low serum IGF-I and normal GH concentrations after pharmacologic provocation.[2-7] One needs to keep in mind that the 24hr GH secretion rates have been shown to vary inversely with the Body Mass Index (BMI) and that daily GH production rates in obese adult men is reduced to one fourth that in normal weight individuals.[8,9] Nevertheless, despite the markedly lower GH secretion levels, obese children usually have physiologic growth with normal levels of total IGF-I and IGF Binding Protein-3 (IGFBP-3) and increased levels of free IGF-I and GH Binding Protein (GHBP).[10,11] Therefore, an obese child with growth failure and low IGF-I concentrations in spite of a physiologic response of GH to provocation warrants further investigation. However, 24hr GH testing is somewhat difficult and expensive requiring an organized inpatient pediatric endocrine unit. It also requires the adherence to certain tedious guidelines so that the results can validly depict the entire 24hr profile of GH spontaneous secretion. The prerequisites of high quality 24hr GH testing require that: 1) the child is admitted to the hospital the evening before the testing for sleep acclimation to the hospital environment so that on the night of the 24hr testing the child can have normal uninterrupted sleep, descend into normal duration Stage III-IV sleep and thereby generate the maximal physiologic nocturnal GH peaks;[2,12] 2) the child is kept active during the day of testing and not restricted to bed, except for mealtime and sleep periods, so that the GH peaks related to activity are not missed;[2] and 3) the 24hr GH concentration results are compared to the normative 24hr GH data of the same type of GH assay from a large normal-statured control population. Unfortunately, only a minority of the many studies of 24hr spontaneous GH secretion testing thus far reported have adhered to all these prerequisites of high quality testing. This has resulted in contradictory results in both short- statured[2-7,9-11] and normal- statured[2,5-7,13-17] children. Thus, even though the properly designed 24hr profile of spontaneous GH secretion is most probably the gold-standard physiologic test of GH endogenous secretion and invaluable in GH research, the conflicting results generated by improper testing have caused this technique to be discounted for clinical use. Therefore, in clinical practice it would be very helpful to have an easier method of GH testing which could also reliably reflect abnormal 24hr spontaneous GH secretion.

The IGF-I generation test (IGF-I gen) has classically been used to diagnose GH insensitivity (GHI), Laron Syndrome, a disorder with low IGF-I serum concentrations which do not significantly increase after biosynthetic human GH (hGH) administration.[18-24] It is an invaluable test for diagnosing GHI with a high reproducibility in adults and children.[25]

The IGF-I gen has also been used to investigate the relationship between the IGF-I gen response and the growth responses during hGH therapy in children with GH deficiency diagnosed by pharmacologic provocation testing. Rudman et al[26] first studied the IGF I response after a therapeutic trial of ten days with hGH in GHD patients and found a robust increase of IGF-I after a few days of hGH treatment. Several other authors have also reported a similar brisk response of IGF-I in children with classic GHD during hGH administration for several days.[27-30] Our group reported that an enhanced response of IGF-I and IGFBP-3 during a 5-day IGF-I and IGFBP-3 generation test is diagnostic for GHD in children with β-thalassemia major.[31] A recent report also showed an increased IGFBP-3 response in the IGF-I gen in children with GHD.[32] Other studies have also shown a positive relationship between the response of IGF-I and the growth response to hGH therapy in GHD children.[19,33] Nevertheless this is not a uniform finding.[27-29,34]

Since an enhanced response of IGF-I during the IGF-I gen is found in children with classic GH deficiency, diagnosed by an abnormal GH response to pharmacologic provocation testing, we designed a study to assess the ability of the IGF-I gen to predict GH deficiency in children with GH neurosecretory dysfunction, namely with normal GH on pharmaco-

logic provocation but abnormal 24hr GH secretory rates.

SUBJECTS AND METHODS

Subjects

Three groups of control children were recruited into the study. The first and second control groups, which served as controls for the IGF-I gen, consisted of 86 control children [50 normal-statured (NS) and 36 short-statured (SS) normal children], who were healthy and age- and puberty-matched (Table 1). All control children, both NS and SS, had had normal growth velocities for at least two years before the IGF-I gen. Both groups were from the Division of Pediatric Endocrinology and Diabetes of the University Hospital of Patras, Greece. The third group of control children consisted of 187 normal-statured children who served as the control group for the 24hr endogenous GH secretion studies; 90 children [50 Tanner I (25M/25F), 20 Tanner II (10M/10F), and 20 Tanner III (10M/10F)] from the Division of Pediatric Endocrinology and Diabetes, University Hospital of Patras, Greece and 97 children [54 Tanner I (27M/27F), 27 Tanner II (13M/14F) and 16 Tanner III (7M/9F)] from the Pediatric Endocrine Unit of the Kaplan Medical Center, Rehovot, Israel. Sixty-five children with short-stature, (heights > –2SD), abnormal growth velocities and isolated GH deficiency were recruited into the study from the Division of Pediatric Endocrinology and Diabetes of the University Hospital of Patras, Greece. They were divided into two subgroups according to the results of the tests applied to diagnose GH deficiency: 1) GHD-1 subgroup with classic growth hormone deficiency (n=33), namely maximum peak GH responses after provocation with two pharmacologic agents (clonidine and levo-dopa) less than 10 μg/L and 2) GHD-2 subgroup with growth hormone neurosecretory dysfunction (n=32), namely maximum peak GH concentrations after provocation with the aforementioned pharmacologic agents higher than 10 μg/L, and low spontaneous 24hr GH secretion rates. The standard deviation scores (SDS) of the height, weight and BMI of the GHD children are described in Table 2. None of the patients had malignancies, panhypopituitarism or chronic diseases and all the children were euthyroid when tested. None of the patients had Magnetic Resonance Imaging abnormalities.

Parental informed consent and children's assent were obtained from the patients and the control children studied in both Greece and Israel. The Ethics Committees of the University Hospital of Patras, Greece and the Kaplan Medical Center, in Rehovot, Israel, approved the study.

GH provocation tests

All tests were started between 08:30-09:00h after an overnight fast. Blood samples were obtained before (0min) and at 30, 60, 90 and 120min after oral administration of Clonidine (0.15 mg/m^2). This was followed by oral administration of Levo-Dopa (125-500 mg) and further blood sampling at 30, 60, 90 and 120min after the ingestion of Levo-Dopa was carried out.

24-hour spontaneous GH secretion

All the GHD-2 and the children which served as controls for the 24hr endogenous GH secretion testing were admitted into the respective Pediatric

Table 1. Characteristics of the 86 control children (50 normal and 36 short-statured) studied with the IGF-I generation test. The height of the short-statured normal children differed significantly from the height of the normal-statured control children (p=0.003).

	Pubertal	Normal-statured children				Short-statured children			
Gender	Stage	n	Age (years)	Height (SDS)	Weight (SDS)	n	Age (years)	Height (SDS)	Weight (SDS)
Male	I	12	8.3 ± 0.4	0.48 ± 0.20	0.29 ± 0.10	12	10.1 ± 0.5	-2.60 ± 0.10	-1.44 ± 0.23
Male	II	10	11.3 ± 0.2	0.25 ± 0.30	0.50 ± 0.13	6	13.2 ± 0.3	-2.45 ± 0.30	-1.26 ± 0.10
Male	III	5	12.3 ± 0.3	0.30 ± 0.20	0.10 ± 0.19	8	14.3 ± 0.7	-2.71 ± 0.20	-1.34 ± 0.18
Female	I	10	8.4 ± 0.3	0.37 ± 0.16	0.36 ± 0.12	4	8.5 ± 0.2	-2.50 ± 0.10	-1.55 ± 0.10
Female	II	9	11.1 ±0.5	0.10 ± 0.30	0.11 ± 0.10	4	12.5 ± 0.6	-2.55 ± 0.30	-1.38 ± 0.15
Female	III	4	12.5 ± 0.5	0.20 ± 0.40	0.51 ± 0.30	2	13.8 ± 0.5	-2.69 ± 0.50	-1.50 ± 0.08

SDS=Standard Deviation Score

Table 2. Characteristics of the 65 growth hormone deficient children (33 children with abnormal GH provocation test results: GHD-1 and 32 children with abnormal 24h spontaneous GH secretion test results: GHD-2). The heights of the growth hormone deficient children differed significantly from those of the normal- statured control children (p=0.001).

		GHD-1 children					GHD-2 children				
Sex	Pubertal Stage	n	Age (years)	Height (SDS)	Weight (SDS)	BMI (SDS)	n	Age (years)	Height (SDS)	Weight (SDS)	BMI (SDS)
M	I	9	10.1±0.7	-3.65±0.40	-2.50±0.12	1.46±0.20	14	10.60±0.50	-3.42±0.20	-2.02±0.27	1.42±0.30
M	II	7	12.7±0.5	-2.85±0.20	-2.09±0.15	1.31±0.15	5	12.90±0.80	-2.61±0.20	-1.04±0.77	1.08±0.50
M	III	6	13.9±0.2	-3.15±0.20	-2.68±0.01	1.33±0.05	3	14.30±0.10	-2.98±0.20	-1.55±0.01	0.88±0.04
F	I	5	8.1±0.9	-3.25±0. 40	-2.07±0.23	1.39±0.50	6	9.60±0.60	-3.00±0.70	-1.05±0.70	1.00±0.40
F	II	4	11.9±0.4	-3.05±0.10	-1.95±0.13	1.21±0.3	3	12.08±0.01	-2.69±0.01	- 1.89±0.57	1.49±0.01
F	III	2	12.8±0.2	-2.75±0.30	-1.68±0.11	1.22±0.05	1	12.80	-2.55	- 1.85	1.52

SDS=Standard Deviation Score

Endocrine Units in Greece and in Israel for the 24hr testing at 20:00h the evening before the testing in order to become acclimated to the hospital environment. A heparinized needle was inserted at 08:30h the next morning, on the day of the testing. The 24hr studies of spontaneous GH secretion were initiated at 09:00h the day of the testing using a continuous withdrawal pump system, with a nonthrombogenic catheter (ConFlo, Carmeda, Sweden). Blood samples were collected in heparinized tubes attached to the withdrawal pump every 30min, for 24 hours, without interrupting the children's sleep. The children were encouraged to walk and play during the day of testing and to stay in bed only during the sleep and the mealtime periods. All children received a regular diet consisting of three meals.

IGF-I generation test

The 5-day IGF-I gen was performed in the GHD-1 children one month after the GH provocative studies, in the GHD-2 one month after the 24hr GH studies and randomly in the control children. Blood samples were obtained daily for five days at 09:00h and human GH (hGH, Genotropin, Pfizer, Sweden) was administered daily for four days subcutaneously at a dose of 0.03 mg/kg/day immediately after the first blood sampling. All samples were placed on ice, immediately centrifuged in a refrigerated centrifuge and stored at –35° C until tested.

GH treatment

All the GHD-1 and the GHD-2 children were treated with hGH (0.2 mg/kg/week), by daily subcutaneous injections for three years, immediately following the IGF-I gen and they have been followed regularly on a six-month basis in the Pediatric Endocrine Outpatient Division of the University Hospital of Patras.

Hormone Assays

Serum GH was measured at the University Hospital of the Patras Laboratory of Clinical Nuclear Medicine using a polyclonal radioimmunoassay (RIA) (Sorin Biomedica, Italy), with a sensitivity of 0.1 ng/ml and an intra- and inter-assay coefficient of variation of 8.5% and 9.5%, respectively. Serum GH was also measured at the Kaplan Medical Center using a similar polyclonal RIA (Incstar, Stillwater, Min., USA), with a sensitivity of 0.1 ng/ml and an intra- and inter-assay coefficient of variation of 9% and 10%, respectively. The inter-laboratory coefficient of variation between the laboratories in Greece and Israel during quality control testing of the same samples was 10.5%. Serum IGF-I was measured by using a commercial RIA (Nichol's Institute, San Juan, Capistrano, CA, USA) after acid/ethanol extraction. The sensitivity of the assay was 0.8 ng/ml and the intra- and inter-assay coefficients of variation were 4.6% and 7.5%, respectively.

STATISTICAL METHODS

A multi-parameter deconvolution analysis was used to derive relevant estimates of 24hr GH secretion

from the 24hr serum GH measurements. Clearance function was modeled by a mono-exponential function (a one compartment model), with a unique rate constant for each subject.[9,35-39]

Deconvolution analysis was used for the estimation of the following parameters of GH secretion and elimination: 1) number and temporal location of the secretory episodes; 2) amplitude (maximal secretory rate in each episode); 3) secretory burst half duration (duration of the secretory rate episode at half maximal amplitude); 4) constant basal secretion; and 5) GH half-life (GH HL), corresponding to the disappearance rate by simultaneous fitting to the experimental data.

To account for the fact that data consisted of GH concentration measurements collected with a constant withdrawal pump, the simultaneous estimation of secretion and elimination parameters by nonlinear least squares had to be modified accordingly. The way the blood samples were collected implies that every sample provided an estimation of the mean hormone concentration during the 30-minute interval of the sample collection rather than an instant sample at the specific moment. In contrast, the reconvolution curve, predicted by the specific model, yields the instant values of the hormone concentration at the corresponding time points. If the experimental data points (mean concentrations) have been collected at distinct t_i points equally spaced by T, the corresponding model predicted mean concentration for each time interval can be estimated by the following formula:

$$\overline{y(t_i)} = \frac{1}{T} \int_{t_i}^{t_i} C(t)$$

where C(t) represents the reconvolution predicted concentration.

The model predicted mean concentration for each time interval was used with a nonlinear least squares method for the estimation of the profile specific secretion parameters. This is an unavoidable modification to the calculations if one wants to be in concordance with the physiological meaning of the experimental data collected.

The quantity of GH secreted per burst was calculated as the analytical integral of the deconvolution-resolved secretory burst. The total mass of GH secreted in 24 hours was calculated assuming a distribution volume of 7.9% of body weight. To account for the delayed growth of the GHD-2 children and the parallel increase in body size and the total distribution volume that occur with age, GH secretion values were also normalized for body surface area (BSA).

Data are presented as mean ± SE, unless otherwise stated. Measurements derived from the deconvolution analysis were compared by one-way ANOVA after logarithmic transformation. For multiple group comparisons, the Bonferroni adjustment to the critical values was used in order to compensate for multiple comparisons. Mean GH concentrations were compared without prior transformation.

Statistical significance was set at $p<0.05$. For comparisons of the IGF-I concentrations of the IGF-I generation test of the different groups Student's t test was used.

RESULTS

GH provocation tests

The GHD-1 children had a GH response to pharmacologic provocation as follows: 1) a maximum GH peak of 5.5 ± 1.3 μg/L following L-Dopa administration; and 2) a maximum GH peak of 6.3 ± 0.9 μg/L following clonidine administration.

The GHD-2 children had a normal GH response to pharmacologic provocation as follows: 1) a maximum GH peak of 15.7 ± 1.9 μg/L following L-Dopa administration; and 2) a maximum GH peak of 22.3 ± 4.3 μg/L, following clonidine administration.

Spontaneous GH secretion tests

Deconvolution-derived estimates of the 24hr secretory profiles in all male and female normal-statured control, GHD-1 and GHD-2 children are summarized in Table 3. The GHD-1 and GHD-2 children had significantly lower mean 24hr GH concentrations and GH secretion rates than the normal-statured control children of the same Tanner stage. The GHD-1 children had 24hr GH concentrations and GH secretion rates that were comparable to those of the GHD-2 children with no significant differences in these two parameters between the two GHD groups.

IGF-I generation test

The results of the basal and peak IGF-I serum

Table 3. Twenty-four hour GH testing results derived by deconvolution analysis of 187 normal-statured control children and 32 GHD-2 and 33 GHD-1 children with GH deficiency (values are presented as mean±standard error, SEM) (normal vs. GHD: * = p<0.004, # = p<0.01, † = <0.03)

Parameter	Tanner I			Tanner II			Tanner III		
	Normal	GHD2	GHD1	Normal	GHD2	GHD1	Normal	GHD2	GHD1
Males (n)	**52**	**14**	**9**	**23**	**5**	**7**	**17**	**3**	**6**
Mean Serum 24h GH Concentration (µg/L)	4.03±0.2	2.13±0.2*	1.99±0.4*	4.17±0.1	2.26±0.5*	2.14±0.3*	5.11±0.3	2.84±0.1*	2.79±0.5*
Number of Secretory Bursts/24h	8.9±0.4	9.1±0.8	8.9±0.9	10.8±0.4	7.20±0.6*	6.95±0.9*	12.9±0.1	12±0.8	11.8±0.9
Daily GH Secretion Rate (µg/L/24h)	218±13	165±15#	158±25#	275±17	117±29#	114±35#	312±34	208±22#	201±31#
Total GH Secretion (µg/24h)	524±53	339±36*	325±42*	681±46	361±14*	355±23*	1119±108	506±78*	501±82*
Females (n)	**52**	**6**	**5**	**24**	**3**	**4**	**19**	**1**	**2**
Mean Serum 24h GH Concentration (µg/L)	4.13±0.20	2.13±0.3*	1.96±0.4*	5.16±0.1	3.02±0.1*	2.91±0.3*	5.83±0.1	3.83	3.55±0.3*
Number of Secretory Bursts/24h	10.1±0.50	11.4±0.6	10.8±0.9	11.2±0.50	7.5±0.30*	7.0±0.5*	11.1±1	11	10.8±0.5
Daily GH Secretion Rate (µg/L/24h)	245±14	173±30†	170±21†	361±21	193±18†	188±25†	327±27	206†	200±4†
Total GH Secretion (µg/24h)	611±62	373±68*	369±55*	1023±129	431±77*	426±68*	1059±143	535	519±18*

concentrations and the maximum percent increase of the IGF-I levels from the basal level during the IGF-I gen in the NS and SS prepubertal control children, and in the prepubertal GHD-1 and GHD-2 children are illustrated in Figure 1. The results of the basal and peak IGF-I serum concentrations and the maximum percent increase of the IGF-I levels from the basal level during the IGF-I gen in the NS and SS pubertal control children, and in the pubertal GHD-1 and GHD-2 children are illustrated in Figure 2. The results from the Tanner stage II and III children were averaged under the heading of "pubertal" because there was no significant difference between the results of Tanner II and III.

The children in both the prepubertal and pubertal GHD-1 and GHD-2 groups had significantly lower baseline IGF-I concentrations than the NS and SS control children of the corresponding pubertal status (prepubertal: p= 0.001 and pubertal: p= 0.003). However, all the GHD-1 and GHD-2 children had peak IGF-I concentrations that were comparable to those of the control children of the same pubertal status (p= NS). Thus, the percent increase of the IGF-I from the basal IGF-I was significantly greater in the prepubertal (p= 0.001) and pubertal (p= 0.003) GH deficient children (516±58 % in the GHD-1 and 433±50 % in the GHD-2 prepubertal children and 191±28 % in the GHD-1 and 141±20 % in the GHD-2 pubertal children) than in both groups of normal control children of the corresponding Tanner stages (106±12 % in the NS and 102±18 % in the SS prepubertal children and 48±8 % in the NS and 61±17 % in the SS pubertal children). There was no overlapping in the values of the percent increase of IGF-I of the prepubertal GHD-1 and GHD-2 groups with the prepubertal NS and SS groups. There was a small overlapping between the percent increase of IGF-I of the pubertal GHD-1 and GHD-2 groups with the pubertal NS and SS groups. The pubertal children in

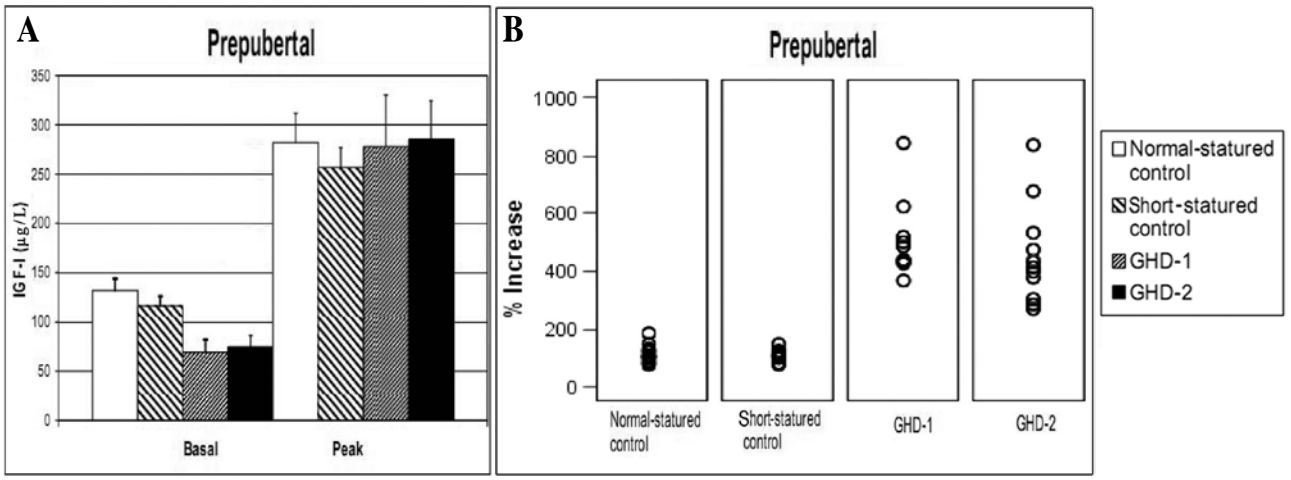

Figure 1. A. The basal IGF-I concentrations of the prepubertal GHD-1 and GHD-2 children are lower than those of the prepubertal normal-statured and short-statured control children (p=0.001), whereas the peak IGF-I concentrations during the IGF-I generation test of all four groups are comparable. **B.** The percent increase of IGF-I in the prepubertal GHD-1 and GHD-2 children during the test are higher than those in the prepubertal normal-statured and short-statured control children (p=0.001).

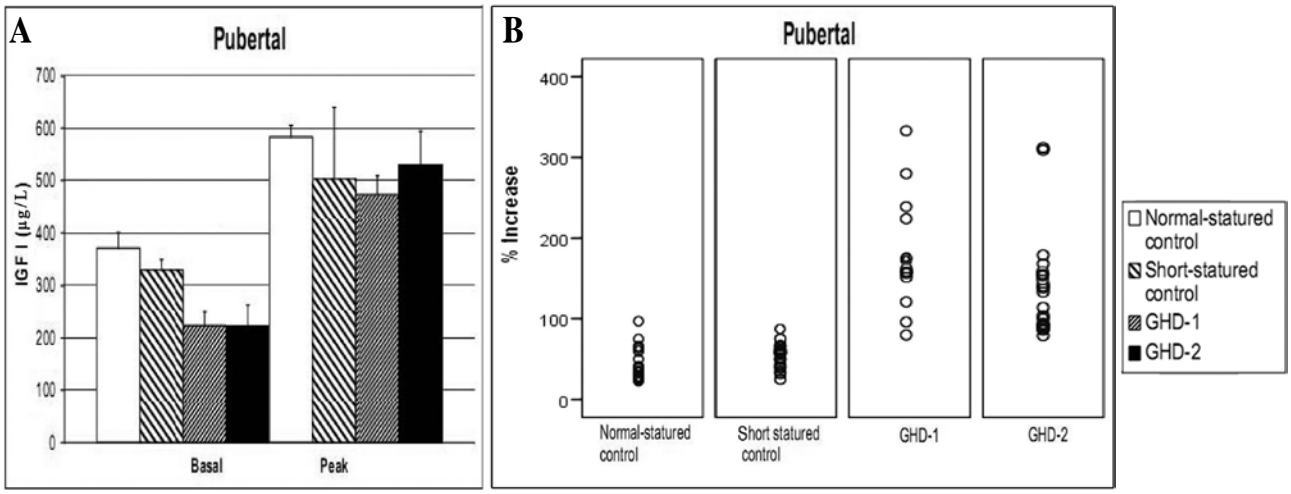

Figure 2. A. The basal IGF-I concentrations of the pubertal GHD-1 and GHD-2 children are lower than those of the pubertal normal-statured and short-statured control children (p=0.001), whereas the peak IGF-I concentrations during the IGF-I generation test of all four groups are comparable. **B.** The percent increase of IGF-I in the pubertal GHD-1 and GHD-2 children during the test are higher than those in the pubertal normal-statured and short-statured control children (p=0.003).

the control and GHD groups had higher basal IGF-I concentrations and higher peak IGF-I concentrations during the IGF-I generation test than the corresponding prepubertal children (p= 0.001). Conversely, the percent increase of the IGF-I concentrations was significantly lower in the pubertal than in the prepubertal children (p= 0.02).

The mean values of the IGF-I concentrations (mIGF-I) during each day of the IGF-I gen for the four groups of prepubertal children are illustrated in Figure 3 and for the pubertal children in Figure 4. The patterns of increase of the mIGF-I during the IGF-I gen were different in the individual groups as follows: 1) the prepubertal NS and SS controls started out at normal mIGF-I concentrations (the SS mIGF-I levels were 13% lower than those of the NS with no significant difference between the basal mIGF-I concentrations in the two groups) and subsequently

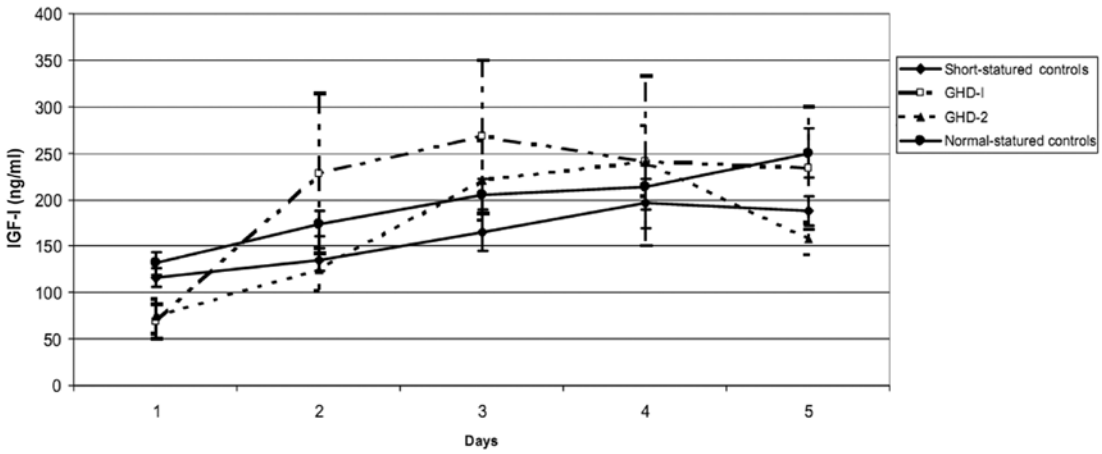

Figure 3. The IGF-I concentrations during the 5-day IGF-I generation test of the prepubertal GHD-1 and GHD-2 children showed a steeper upward slope than those of the prepubertal normal-statured and short-statured control children. The values are expressed as mean ± SEM.

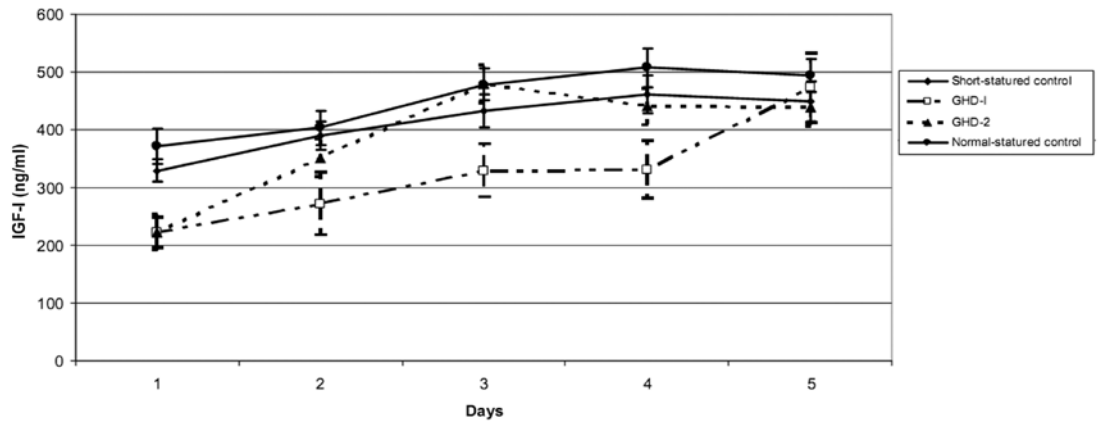

Figure 4. The IGF-I concentrations during the IGF-I generation test of the pubertal GHD-2 children showed a greater upward slope than those of the pubertal GHD-1, normal-statured and short-statured control children. The pubertal GHD-1 showed the greatest increase on day 5 of the test. The values are expressed as mean ± SEM.

increased steadily till day 5 staying essentially parallel to each other, although the SS children followed at somewhat lower levels than the NS children till day 5; the peak mIGF-I concentrations were 18% lower in the SS children as compared to those of the NS but there was no significant difference between the peak mIGF-I levels in the two groups; 2) the mIGF-I in the prepubertal GHD-1 and GHD-2 children started at significantly lower levels than in the control children ($p= 0.003$) followed by a large increase on days 2 and 3 with peak mIGF-I on day 3 in the GHD-1 and on day 4 in the GHD-2 children; in the GHD-1 children the mIGF-I subsequently decreased on day 4 and essentially leveled off on day 5, whereas in the GHD-2 the mIGF-I decreased on day 5; 3) the NS and SS pubertal controls started out at normal mIGF-I concentrations (the SS mIGF-I levels were 14 % lower than those of the NS with no significant difference between the basal mIGF-I concentrations of the 2 groups); the mIGF-I increased in parallel to each other till day 4 when they leveled off with no significant difference in the peak mIGF-I between the two groups; and (4) in the pubertal GHD-1 and GHD-2 children the mIGF-I started off at similar basal levels, significantly lower however than in the controls ($p= 0.001$), and there was a sluggish increase

of the mIGF-I in the GHD-1 children reaching peak concentrations on day 5, while in the GHD-2 children there was a brisk increase of the mIGF-I up to day 3, with a decrease on day 4.

GH treatment

The GHD-1 and GHD-2 children, both prepubertal and pubertal, had subnormal growth velocities prior to hGH, therapy. During treatment with hGH growth velocities increased significantly in both groups (Table 4). Also, the height SDS in the prepubertal and pubertal GHD-1 and GHD-2 subjects improved significantly during treatment with hGH (Table 4).

DISCUSSION

In the present study, all the prepubertal and pubertal GHD children (GHD-1 and GHD-2) had a greater increase in IGF-I values during the 5-day IGF-I generation test as compared to the normal-statured and short-statured control children. Although the baseline IGF-I concentrations in the GHD children were significantly lower, they reached similar peak IGF-I concentrations with those observed in the control children. It should be emphasized that this enhanced IGF-I response during the IGF-I generation test occurred in both GHD groups, whether the children had classic GH deficiency (GHD-1) or GH neurosecretory dysfunction (GHD-2). It might be speculated that the enhanced IGF-I response during the IGF-I generation test in the GHD groups was due to a "priming" effect of hGH on an increased amount of available "un-occupied" GH receptors present in GH deficient children.

It is also noteworthy that even though the short-statured control children in our study had similar heights to the GH deficient children, they differed from them in that they had normal growth velocities and normal basal and peak IGF-I concentrations during the IGF-I gen, similar to those of normal-statured children. It should be emphasized that our short-statured control group differed from most Idiopathic Short-Statured (ISS) children included in other IGF-I generation studies in that they had normal growth velocities, whereas the ISS children in the majority of published studies had abnormal growth velocities.

It is of interest to note that the peak IGF-I concentrations occurred more often on days 4-5 of the test in the normal control children, whereas it occurred more often on days 3 and 4 in the GH deficient children. Therefore, it seems important to measure the IGF-I concentrations on each of the five days of the IGF-I gen in order to obtain the peak response of IGF-I in all children. We have previously reported similar findings by applying the IGF-I gen in children with β-thalassemia major.[31] Our findings are in disagreement, however, with a recent study which reported that the peak IGF-I response occurs by 36 hours following a standard dose of hGH.[40]

In ISS, variable results have been reported with regard to the IGF-I response during the IGF-I gen, possibly reflecting the heterogeneity in the etiology of ISS. There are older reports indicating that there is an enhanced response of IGF-I during the generation test in children with ISS.[41,42] In contrast, another study showed that in 16 ISS patients with normal baseline IGF-I levels there was a lower than normal IGF-I response on days 5 and 8 of a 7-day IGF-I gen, pos-

Table 4. Growth velocities (CV) in cm/year and height SDS before and during hGH therapy in the GH deficient children; GHD-1: children with low GH values on provocation tests; GHD-2: children with low 24hour GH secretion (values represent mean±standard error, SEM); PrP = prepubertal; Pb = pubertal; *p<0.001, **p<0.005)

Group/ Pubertal status	Before cm/year	1st year cm/year	2nd year cm/year	3rd year cm/year
GHD-1/PrP (n=14)				
CV	2.6±0.2	8.0±1.0*	7.8±1.2*	5.8±1.1**
Height SDS	-2.8±0.2	-2.0±0.5*	-1.7±0.5*	-1.5± 0.6**
GHD-2/PrP (n=20)				
CV	3.6±0.1	10.5±1.0*	9.5±1.5*	8.0±2.0*
Height SDS	-3.3±0.2	-2.8±0.3*	-2.3±0.4*	-1.6±0.4*
GHD-1/Pb (n=19)				
CV	2.6±0.1	7.2±0.6**	7.1±0.8**	6.4±3.0**
Height SDS	-3.0±0.2	-2.5±0.2**	-2.1±0.1**	-1.7±0.2**
GHD-2/Pb (n=12)				
CV	3.6±0.5	9.3±0.4*	9.5±1.7*	7.4±0.5**
Height SDS	-2.9±0.3	-2.3±0.4*	-1.9±0.5*	-1.4±0.3**

sibly indicating a partial GH insensitivity.[25] Another study showed that the IGF-I response 24 hours after a single injection of hGH (2 mg/m^2) given to 22 prepubertal ISS children was comparable to that of the control children whose mean height though was -1.2 SD.[43] The criteria for classifying the children as ISS in these studies was only that they were short (height < -2SD) and had a normal GH response to pharmacologic provocation. In one study,[41] an enhanced IGF-I response in the generation test in children with ISS was followed by an increased growth velocity during hGH treatment for six months. Moreover, in a large study of reportedly ISS children with a subnormal endogenous GH secretion, a significantly increased IGF-I response was noted after hGH treatment.[42] Based on our findings, we may speculate that the enhanced response of IGF-I during the IGF-I generation test, previously reported in a number of children with ISS, could be attributed to undiagnosed GH neurosecretory dysfunction. This could also explain the observed increase in the growth velocity after hGH treatment in these children with "ISS". The enhanced response during the 5-day IGF-gen, in both the GHD-1 and GHD-2 children in our study groups, was associated in all cases with a very good response (catch-up growth) to long-term treatment with exogenous hGH.

In addition to being used to evaluate children with short stature, the IGF-I gen has also been used to compare GH responses in obese and tall prepubertal children. Interestingly, a recent paper reported that baseline and stimulated IGF-I values in obese and tall prepubertal children were significantly higher than those seen in normal-statured lean children 24 hours after a single injection of hGH (2 mg/m^2), although the percent IGF-I increment was not significantly different between the three groups.[43]

In conclusion, the IGF-I generation test appears to be useful in identifying children not only with GH insensitivity but also with GH deficiency both with classic GH deficiency and GH neurosecretory dysfunction. In the case of GH insensitivity, the IGF-I response during the IGF-I gen is diminished, whereas in the case of GH deficiency it is enhanced. It is an easy test to perform on an outpatient basis, which can identify GHD children who will benefit from hGH treatment.

Therefore, we suggest that, since the 5-day IGF-I generation test seems to be a good indirect test of GH deficiency in children, and especially in prepubertal and most pubertal children, it might have the potential of replacing the GH pharmacologic provocation and the 24hr spontaneous GH secretion tests. As noted, the IGF-I generation test is an easy outpatient diagnostic test that could be performed in the endocrinologist's office. One of its major advantages, especially in prepubertal and most pubertal children is its ability to indirectly diagnose patients with abnormal 24hr spontaneous GH secretion, in which the provocation tests yield normal results, without the tediousness of 24hr GH testing. In addition, the method is simple and does not cause any side effects or unpleasant symptoms in the patients. However, investigation of a larger group of GHD patients and control children will demonstrate whether or not the IGF-I generation test decidedly provides reliable results as a single indirect test of GH deficiency.

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