CLINICAL STUDY

Diagnosis of GH deficiency in the transition period: accuracy of insulin tolerance test and insulin-like growth factor-I measurement

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Abstract

Objective: A consensus exists that severe growth hormone deficiency (GHD) in adults is defined by a peak GH response to insulin-induced hypoglycemia (insulin tolerance test, ITT) of less than 3 µg/l based on a cohort of subjects with a mean age of 45 years.

Design and methods: By considering one of the following two criteria for the diagnosis of probable permanent GHD, i.e. the severity of GHD (suggested by the presence of multiple pituitary hormone deficiencies (MPHD)) or the magnetic resonance (MR) imaging identification of structural hypothalamic–pituitary abnormalities, 26 patients (17 males, 9 females, mean age 20.8±2.3 years, range 17–25 years) were selected for re-evaluation of the GH response to ITT and their IGF-I concentration. Eight subjects had isolated GHD (IGHD) and 18 had MPHD. Normative data for peak GH were obtained after ITT in 39 healthy subjects (mean age 21.2±4.4 years, range 15.1–30.0 years) and the reference range for IGF-I was calculated using normative data from 117 healthy individuals.

Results: Mean peak GH response to ITT was significantly lower in the 26 patients (1.8±2.0 µg/l, range 0.1–6.1 µg/l) compared with the 39 controls (18.5±15.5 µg/l, range 6.1–84.0 µg/l; $P < 0.0001$). One subject with septo-optic dysplasia had a peak GH response of 6.1 µg/l that overlapped the lowest peak GH response obtained in normal subjects. There was an overlap for IGF-I SDS between subjects with IGHD and MPHD, as well as with normal controls. The diagnostic accuracy of a peak GH response of 6.1 µg/l showed a 96% sensitivity with 100% specificity. The maximum diagnostic accuracy with IGF-I SDS was obtained with a cut-off of –1.7 SDS (sensitivity 77%, specificity 100%) while an IGF-I ≤ –2.0 SDS showed a sensitivity of 62%.

Conclusion: Our data show that the cut-off value of the peak GH response to ITT of less than 3 µg/l or 5 µg/l and of IGF-I of less than –2.0 SDS are too restrictive for the diagnosis of permanent GH deficiency in the transition period. We suggest that permanent GHD could be investigated more accurately by means of an integrated analysis of clinical history, the presence of MPHD, IGF-I concentration and the MR imaging findings of structural hypothalamic–pituitary abnormalities.

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Introduction

The syndrome of growth hormone deficiency (GHD) in adults has only recently been characterized as a specific clinical entity (1). It is now widely recognized that GHD in adults is associated with metabolic consequences such as increased adiposity, adverse serum lipid profiles, reduced exercise capacity, reduced bone mineral density, reduced insulin sensitivity, decreased psychological well-being and premature mortality for cardiovascular diseases (2, 3). Growth hormone (GH) replacement

therapy induces favorable changes in metabolic indices and improves body composition, bone density and remodeling, physical and cardiac performance and overall quality of life, although there is an absence of evidence of an improvement in mortality outcome (4, 5).

Patients with childhood-onset GHD may need to continue GH replacement after the attainment of adult height. Nevertheless, studies have shown that many subjects are no longer GH-deficient when retested at adolescence (6–10). This raises questions about the

most appropriate method and cut-off value for diagnosing GHD after completion of growth and puberty.

The diagnosis of GHD in adults with hypothalamic–pituitary disease is based on provocative GH tests and, in 1998, the Growth Hormone Research Society published consensus guidelines for the diagnosis and treatment of adult GHD (11). The consensus guidelines recommended that severe GH deficiency in adults be defined as a peak GH response of less than $3 \mu\text{g/l}$ after an insulin-induced hypoglycemia test (insulin tolerance test, ITT). This is supported by the work of Hoffman *et al.* (12) performed in 23 subjects with GHD at a mean age of 45 years and in 35 normal subjects at a mean age of 47 years who had respectively a peak GH response to ITT of less than $3 \mu\text{g/l}$ and more than $5 \mu\text{g/l}$. Indeed, by using the peak GH cut-off of $5.1 \mu\text{g/l}$ after ITT, a sensitivity of 96% and a specificity of 92% were achieved in adult GHD at a mean age of 48 years (13).

However, for the transition period, the diagnostic usefulness of ITT in defining GHD of childhood onset has not yet been clearly assessed. Moreover, the negative correlation between age at the time of re-evaluation of GH status and peak GH response after a stimulation test in subjects with childhood-onset GHD (14, 15), suggests that the diagnostic cut-off value of the GH peak that should be adopted in the age range

between 16 and 25 years has yet to be definitively established.

As a consequence of the foregoing, we decided to evaluate the diagnostic accuracy of ITT in a cohort of 60 subjects with childhood-onset GHD. The reliability of serum insulin-like growth factor-I (IGF-I) and the potential role of pituitary magnetic resonance (MR) imaging in the definition of permanent GHD in the transition period were taken into account.

Subjects and methods

Patients

By considering one of the following two criteria for the diagnosis of probable permanent GHD, i.e. the severity of hypopituitarism (suggested by the presence of multiple pituitary hormone deficiencies) or the MR identification of structural hypothalamic–pituitary abnormalities, 26 patients (17 males, 9 females, mean age 20.8 ± 2.3 years, range 17–25 years) out of 60 with childhood-onset GHD (mean age of 19.7 ± 2.5 years, range 16–25 years) were enrolled in a multi-center study (six Pediatric Endocrinology Centers and one Adult Endocrinology Center). The study aimed to re-evaluate GH status after completion of growth and puberty (see Tables 1 and 2). The diagnosis

Table 1 Characteristics of 60 subjects with childhood-onset GH deficiency at time of re-evaluation of GH secretion. Results are expressed as means $\pm$ s.d.

		Anterior pituitary deficits		Etiology		
		IGHD	MPHD	Congenital GHD	Acquired GHD	Idiopathic GHD
Number	60	42	18	17	9	34
Age (years)	19.7 ± 2.5	19.0 ± 2.3	21.3 ± 2.2	20.8 ± 2.2	21.0 ± 2.5	18.8 ± 2.3
Sex (M/F)	37/23	25/17	12/6	12/5	5/4	20/14
Height (cm)	162.1 ± 10.3	160.9 ± 10.0	165.0 ± 10.8	162.6 ± 10.6	166.6 ± 12.3	160.7 ± 9.6
Weight (kg)	59.0 ± 15.4	56.4 ± 13.1	64.9 ± 18.7	61.4 ± 17.6	68.1 ± 22.1	55.3 ± 10.6
BMI (kg/m^2)	22.3 ± 4.8	21.8 ± 4.9	23.5 ± 4.5	23.2 ± 6.3	24.0 ± 4.4	21.4 ± 3.8

GHD, GH deficiency; IGHG, isolated GHD; MPHD, multiple pituitary hormone deficiency.

Table 2 Characteristics and results of 26 subjects with hypothalamic-pituitary abnormalities at MR imaging and/or multiple pituitary hormone deficiency. Results are expressed as means $\pm$ s.d.

		Anterior pituitary deficits		Etiology	
		IGHD	MPHD	Congenital GHD	Acquired GHD
Number	26	8	18	17	9
Age (years)	20.8 ± 2.3	19.8 ± 2.1	21.3 ± 2.2	20.8 ± 2.2	21.0 ± 2.5
Sex (M/F)	17/9	5/3	12/6	12/5	5/4
Height (cm)	164.0 ± 11.1	161.7 ± 12.2	165.0 ± 10.8	162.6 ± 10.6	166.6 ± 12.3
Weight (kg)	63.7 ± 19.1	61.0 ± 20.9	64.9 ± 18.7	61.4 ± 17.6	68.1 ± 22.1
BMI (kg/m^2)	23.5 ± 5.7	23.5 ± 8.1	23.5 ± 4.5	23.2 ± 6.3	24.0 ± 4.4
Mean peak GH ($\mu\text{g/l}$)	1.8 ± 2.0	2.9 ± 2.1	1.4 ± 1.8	2.3 ± 2.2	1.0 ± 1.2
Mean IGF-I ($\mu\text{g/l}$)	96.1 ± 71.3	150.8 ± 91.9	71.8 ± 44.5	117.5 ± 76.6	55.7 ± 36.6
Mean IGF-I SDS	-2.0 ± 0.8	-1.4 ± 1	-2.3 ± 0.5	-1.8 ± 0.8	-2.4 ± 0.5

GHD, GH deficiency; IGHG, isolated GHD; MPHD, multiple pituitary hormone deficiency.

of GHD during childhood was based on clinical criteria and biochemical findings of a GH response of less than 10 µg/l to at least two pharmacological provocative tests.

Sagittal and coronal T1-weighted MR images obtained using 2–3 mm sections, revealed hypothalamic–pituitary abnormalities compatible with congenital GHD (CGHD) in 14 subjects (anterior pituitary hypoplasia, ectopic posterior pituitary gland and pituitary stalk agenesis, septo-optic dysplasia). Acquired GH deficiency (AGHD) was diagnosed in nine subjects in whom MR imaging had detected brain tumors (craniopharyngioma, germinoma, adrenocorticotropin (ACTH)-secreting adenoma, $n = 8$) or pituitary stalk infiltration (Langerhans cell histiocytosis, $n = 1$).

Eight subjects had isolated GHD (IGHD) and 18 had multiple pituitary hormone deficiency (MPHD); four subjects had one (thyrotropin (TSH), $n = 2$ or luteinizing hormone/follicle-stimulating hormone (LH/FSH), $n = 2$), and 14 subjects had three additional anterior pituitary hormone deficits (TSH + ACTH + LH/FSH); 8 had vasopressin (AVP) deficiency. The main clinical characteristics are summarized in Tables 2 and 3. Subjects with MPHD were receiving conventional replacement therapy for pituitary deficits: L-thyroxine 75–200 µg per day, hydrocortisone 20–25 mg per day in two to three separate doses, testosterone enanthate 150–250 mg intramuscularly every 2 or 3 weeks for

males and ethinyl estradiol (first 21 days, 5 µg/day orally, $n = 2$) or transdermal 17β-estradiol patches ($n = 8$) with medroxyprogesterone acetate (5 to 10 mg, 12th to 21st days) for females; desmopressin acetate (DDAVP), two or three times daily, was administered either intranasally or orally in cases with central diabetes insipidus.

Study design

The clinical protocol was approved by the appropriate review boards and written informed consent for all procedures was obtained from subjects or their parents or guardians before enrolment. In all subjects, recombinant human GH treatment was discontinued when growth velocity during the previous year had dropped to <1 cm. GH secretion was re-evaluated by means of ITT at least 3 months after the time of GH discontinuation. All subjects were measured for height and weight and their body mass index (BMI) was calculated.

All testing took place between 0800 and 0900 h following overnight fasting. A heparin-lock cannula was placed in one forearm vein for blood sampling. Soluble insulin (0.1 U/kg) was given intravenously at time 0, and venous blood samples for GH and blood glucose determinations were obtained at 0, 30, 60, 90, and 120 min. Serum samples were taken for determination of IGF-I. After centrifugation at 4 °C, the plasma was

Table 3 Characteristics of 26 subjects with hypothalamic-pituitary abnormalities at MR imaging and/or multiple pituitary hormone deficiency.

Subject	Age (yr)	Sex	Etiology	Anterior pituitary deficit	MR imaging	Peak GH (µg/l)	IGF-I (µg/l)	IGF-I SDS
1	17	M	CGHD	GH	EPP, APH, PSA	2.6	180.0	–1.1
2	18	F	CGHD	GH, TSH	SOD	0.1	156.0	–1.3
3	18	M	CGHD	GH	EPP, APH, PSA	1.0	59.0	–1.9
4	18	F	AGHD	GH, LH/FSH, AVP	Thick stalk/LCH	0.1	45.0	–2.0
5	18	M	AGHD	GH, LH/FSH, ACTH, TSH	Adenoma	1.0	80.0	–1.8
6	19	F	CGHD	GH	EPP, APH, PSA	1.3	255.0	–0.6
7	20	M	CGHD	GH, LH/FSH, ACTH, TSH	EPP, APH, PSA	0.1	96.0	–2.0
8	20	M	CGHD	GH	EPP, APH, PSA	0.5	67.0	–2.4
9	20	F	CGHD	GH	EPP, APH, PSA	2.2	97.0	–2.0
10	20	M	CGHD	GH	EPP, APH, PSA	4.8	218.0	–0.6
11	20	F	CGHD	GH	SOD	6.1	275.0	0.1
12	20	M	AGHD	GH, LH/FSH, ACTH, TSH, AVP	Craniopharyngioma	0.8	97.0	–2.0
13	20	M	AGHD	GH, LH/FSH, ACTH, TSH, AVP	Craniopharyngioma	1.0	20.0	–2.9
14	20	F	AGHD	GH, LH/FSH, ACTH, TSH, AVP	Germinoma	2.2	64.0	–2.4
15	21	F	CGHD	GH, LH/FSH, ACTH, TSH	NE	0.1	60.0	–2.5
16	21	M	CGHD	GH, LH/FSH, ACTH, TSH	NE	5.4	37.0	–2.7
17	21	F	AGHD	GH, LH/FSH, ACTH, TSH, AVP	Craniopharyngioma	0.1	54.0	–2.5
18	22	M	CGHD	GH, LH/FSH, ACTH, TSH	EPP, APH, PSA	0.1	85.0	–2.2
19	22	M	CGHD	GH, LH/FSH	EPP, APH, PSA	1.9	150.0	–1.4
20	23	M	CGHD	GH, TSH	EPP, APH, PSA	1.2	87.0	–2.1
21	23	M	CGHD	GH, LH/FSH, ACTH, TSH, AVP	SOD	5.6	109.0	–1.9
22	23	F	AGHD	GH, LH/FSH, ACTH, TSH, AVP	Craniopharyngioma	3.5	10.0	–3.1
23	24	M	CGHD	GH	EPP, APH, PSA	4.7	55.0	–2.5
24	24	M	AGHD	GH, LH/FSH, ACTH, TSH, AVP	Germinoma	0.2	114.0	–1.8
25	25	M	CGHD	GH, LH/FSH, ACTH, TSH	NE	0.7	12.0	–3.0
26	25	M	AGHD	GH, LH/FSH, ACTH, TSH	Craniopharyngioma	0.2	17.0	–3.0

CGHD, congenital GH deficiency; AGHD, acquired GH deficiency; GH, growth hormone; TSH, thyrotropin; LH/FSH, luteinizing hormone/follicle-stimulating hormone; ACTH, adrenocorticotropin; AVP, vasopressin; APH, anterior pituitary hypoplasia; EPP, ectopic posterior pituitary; PSA, pituitary stalk agenesis; SOD, septo-optic dysplasia; LCH, Langerhans cell histiocytosis; Adenoma, post-operative ACTH-secreting adenoma; NE, not evaluated.

separated and kept frozen at -20°C until used. Serum GH and IGF-I concentrations were centrally measured at the laboratory of the Division of Endocrinology and Metabolism, University of Turin.

Normative data for peak GH were obtained after ITT in 39 healthy subjects (25 males, 14 females, mean age 21.2 ± 4.4 years, range 15.1–30.0 years) with BMI $21.6 \pm 2.7 \text{ kg/m}^2$ ($P = \text{not significant}$) who were not affected by endocrinopathy.

The reference range for IGF-I was calculated using normative data from 117 healthy Italian individuals who had completed pubertal development (mean age 19.3 ± 4.2 years, range 14–25 years); 60 with a mean age of 15.5 ± 1.1 years (range 14–19.9 years) and 57 with a mean age of 23.3 ± 1.7 years (range 20–25 years). The cohort was stratified into two gender-based age ranges (14–19.9 and 20–25 years); IGF-I age-adjusted standard deviation scores (SDS) were calculated. The data were normally distributed (Kolmogorov-Smirnov test, $P = 0.467$).

Procedures

Serum GH was assayed by an IRMA method (HGH-CTK, Diasorin, Saluggia, Italy). All samples from each individual subject were analyzed together. The sensitivity of the method was $0.15 \mu\text{g/l}$. The inter- and intra-assay coefficients of variation were 3.5–4.4% and 5.1–7.5% respectively at GH levels of 1.98–41.92 and $2.99\text{--}42.45 \mu\text{g/l}$ respectively. The international standard was 80/505, $1 \text{ ng/ml} = 2 \mu\text{UI}$. Serum IGF-I was assayed by an RIA method (Pantec, Turin, Italy) after acid-ethanol extraction to avoid interference by binding proteins. The sensitivity of the method was $0.1 \mu\text{g/l}$. The inter- and intra-assay coefficients of variation were 8.8–10.8% and 5.0–9.5% respectively at IGF-I levels of 79.6–766.4 and $79.4\text{--}712.5 \mu\text{g/l}$ respectively.

Statistical analysis

The normality of data distribution was assessed by the Shapiro-Wilk test and the homogeneity of variances was assessed by Levene's test; data are presented as means and standard deviation (s.d.). Receiver operating curve (Roc) analysis was used for the evaluation of the cut-off point. Comparison of mean values among different categories of the considered variables was performed using Student's *t*-test (in cases of comparison of 2 means) or analysis of variance and Least Significant Differences test (in cases of more than 2 means). Correlations between IGF-I, peak GH, IGF-I and age were analyzed with Pearson's coefficient. A *P* value of less than 0.05 was considered statistically significant. All tests were two-sided. Analyses were performed with Statistics for Windows software (2003 version) (StatSoft, Inc., Tulsa, OK, USA).

Results

There were no statistically significant differences in adult height, weight or BMI between subjects with IGHD and MPHD and between those with congenital and acquired GHD (Table 2).

Insulin tolerance test

All 26 subjects and 39 controls had blood glucose with a nadir of less than 2.2 mmol/l during ITT (data not shown); symptomatic hypoglycemia including tachycardia, sweating and/or sense of hunger, was documented in 16 subjects. The clinical and laboratory findings for the 26 subjects are reported in Tables 2 and 3.

Mean peak GH response to ITT was significantly lower in the 26 patients ($1.8 \pm 2.0 \mu\text{g/l}$) compared with the 39 controls ($18.5 \pm 15.5 \mu\text{g/l}$, $P < 0.0001$). In particular, peak GH response to ITT ranged between $0.1 \mu\text{g/l}$ and $6.1 \mu\text{g/l}$ in the potentially affected subjects and between $6.1 \mu\text{g/l}$ and $84.0 \mu\text{g/l}$ in the controls. No significant statistical differences of mean peak GH values were found between subjects with IGHD, MPHD, CGHD and AGHD. Figure 1 shows the peak GH response after ITT in 26 patients compared with the 39 controls. One subject with septo-optic dysplasia (case 11) had a peak GH response of $6.1 \mu\text{g/l}$ that overlapped the lowest peak GH response obtained in normal controls (Table 3). The correlation analysis between peak GH response and age in the 26 subjects showed no significant age-related decline ($r = 0.10$).

IGF-I

There were no gender differences in mean IGF-I concentration and IGF-I SDS within the two age-stratified groups of controls (14–19.9 and 20–25 years). Mean serum IGF-I concentrations and IGF-I SDS were statistically lower in the 26 subjects ($96.1 \pm 71.3 \mu\text{g/l}$, -2.0 ± 0.8 respectively) compared with the 117 healthy controls ($309.9 \pm 130.0 \mu\text{g/l}$, 0.0 ± 1.0 respectively, $P < 0.0001$). The value of -1.7 SDS was the lowest normal value obtained from controls.

Mean IGF-I concentrations and mean IGF-I SDS were significantly higher in the eight subjects with IGHD compared with the 18 subjects with MPHD ($P = 0.006$), while mean IGF-I concentrations were higher in CGHD compared with AGHD patients ($P = 0.03$) (Table 2).

Figure 2 shows IGF-I SDS in the 26 subjects compared with the 117 normal controls. There was an overlap for IGF-I SDS between subjects with IGHD and MPHD, as well as with normal controls.

The correlation analysis between IGF-I SDS and age in the 26 subjects showed a significant age-related decline ($r = -0.49$, $P = 0.01$).

Figure 1 Results of ITT in 26 subjects with isolated GH deficiency (○) or multiple pituitary hormone deficiency (●) and in 39 age- and sex-matched controls (△); *n* = number of subjects. Note logarithmic scale for peak GH in A and linear scale for GH cut-off between 3 and 8 µg/l in B.

Diagnostic accuracy

Based on the normative data of the 39 and 117 controls, the diagnostic usefulness, respectively, of ITT and a single IGF-I measurement was taken into consideration.

The diagnostic accuracy of a peak GH response of 6.1 µg/l after ITT showed a 96% sensitivity with 100% specificity. By adopting the current published cut-off GH response of <3 µg/l and <5 µg/l, 11% and 23% of the subjects respectively would have been wrongly excluded (Table 4) from a correct diagnosis.

The maximum diagnostic accuracy with IGF-I SDS was obtained with a cut-off of −1.7 SDS (sensitivity 77%, specificity 100%) (Table 4). If the traditionally-used IGF-I cut-off of ≤ −2.0 SDS had been adopted, 38% of the affected subjects (sensitivity 62%) would have been wrongly classified (Table 4). Diagnostic accuracy was not improved by a combination of peak GH and IGF-I measurements, because the patient with a

peak GH response of 6.1 µg/l was not even picked-up by the IGF-I SDS (case 11, +0.1) values.

Discussion

Our data confirm that a group of subjects with hypopituitarism of childhood onset have a high likelihood of being affected by severe GHD when retested at the completion of growth and puberty (6–10). Based on the assumption that children with MPHD and congenital or acquired GHD are likely to be GH deficient in adult life (9, 16), 43% of our cohort of 60 subjects had clinical and/or imaging features suggestive of permanent GHD.

The diagnosis of GHD remains challenging in adults, although a cut-off peak GH response to ITT has been established (12). In particular, consensus guidelines for the diagnosis of adult GHD recommended that severe GH deficiency in adults be defined as a peak GH response of less than 3 µg/l after ITT (11). Another study by Biller *et al.* in adult patients (13) demonstrated that a peak GH cut-off of 5.1 µg/l after ITT has a sensitivity of 96% and a specificity of 92% in defining GHD. However, both of these studies were conducted in GHD subjects with a mean age of 45 and 48 years respectively (11, 13). Moreover, the cut-off points of 3 or 5 µg/l have often been used in clinical practice regardless of the type of stimulation test adopted (14, 16–18).

Twenty of our subjects (76%) had peak GH responses of less than 3 µg/l, a cut-off point compatible with persistent severe GH deficiency (11, 12, 19). More specifically, 23 of the 27 subjects (85%) had structural hypothalamic–pituitary abnormalities confirming that ITT is a reliable provocative test for diagnosing GHD in the age range between 16 and 25 years. Of particular relevance is the finding that six subjects, three with isolated GHD and congenital hypothalamic–pituitary

Figure 2 Results of IGF-I SDS in 26 subjects with isolated GH deficiency (○) or multiple pituitary hormone deficiency (●) compared with 117 normal age- and sex-matched controls (shown in two separate columns for optimal viewing) (△); *n* = number of subjects.

Table 4 Sensitivity and specificity of ITT and IGF-I SDS.

Test	Gold standard	Diagnostic criterion	Mean (95% confidence interval)	
			Sensitivity (%)	Specificity (%)
GH ($\mu\text{g/l}$)	HP abnormalities/MPHD	$< 6.1^a$	96 (80–99)	100 (91–100)
		< 5.0	89 (70–97)	100 (91–100)
		< 3.0	77 (56–91)	100 (91–100)
IGF-I SDS ($\mu\text{g/l}$)	HP abnormalities/MPHD	$< -1.7^a$ (127 $\mu\text{g/l}$)	77 (56–91)	100 (97–100)
		≤ -2.0 (45 $\mu\text{g/l}$)	62 (41–80)	100 (97–100)

HP, hypothalamic-pituitary abnormalities at MR imaging; MPHD, multiple pituitary hormone deficiency. ^aCut-off value defining the maximum sensitivity and specificity.

abnormalities, a marker of permanent GHD (9), and three with MPHD had peak GH values of between 3.5 and 6.1 $\mu\text{g/l}$; three had a GH response greater than 5 $\mu\text{g/l}$, of whom two had MPHD. The present study is the first attempt to make a valuable contribution to the highly discussed issue of establishing normative data for peak GH responses after ITT in the transition period. In particular, the peak GH response of 6.1 $\mu\text{g/l}$ after ITT in young adults was twice that reported by Hoffman *et al.* (12) and that recommended by the Growth Hormone Research Society (11). Our results clearly suggest that a cut-off peak for GH of less than 3 $\mu\text{g/l}$ following ITT is too restrictive for diagnosing permanent GHD of childhood onset in the transition period.

Indeed, the results of our study raise questions about exactly which cut-off peak of GH response should be adopted in the diagnosis of GHD of childhood onset after the attainment of adult height. Of relevance, it has been reported that severity of GHD in adults is related to the number of additional anterior pituitary hormone deficits found (16, 20), and that organic hypopituitarism is not associated with a GH response of greater than 3 $\mu\text{g/l}$ after ITT (12). However, these findings were only partially confirmed in this study since in some of our subjects the presence of three additional anterior pituitary hormone deficits was not predictive of the 'severity' of GHD. Therefore, the discrepancy between our results and those reported by Hoffman *et al.* (12) and Biller *et al.* (13), can be explained by the subjects' age difference or by their different etiology of GHD. Unlike our cohort of subjects who were younger and with variable causes of GHD, Hoffman's and Biller's patients were older and suffered mainly from organic MPHD (12). Indeed, the BMI of Biller's patients was higher than 30 kg/m^2 compared with that of our affected subjects and controls (23.5 $\pm$ 5.7 kg/m^2 and 21.6 $\pm$ 2.7 kg/m^2 , respectively) and it is known that GH release is influenced by body composition (21).

In our study, the analysis of factors possibly affecting GH secretory status showed that a physiological age-related decline in peak GH response (22) was not observed in our subjects with permanent GHD. A significant decrease in IGF-I with age was observed, in agreement with the well-known age-associated declines

in IGF-I concentrations (23). The lack of any significant correlation between peak GH response or IGF-I values and BMI in our subjects indicated that adiposity does not affect these parameters in young adults with true, permanent GHD.

The high diagnostic accuracy of ITT in the transition period was demonstrated by the near total separation of peak GH response between control subjects and patients. A peak GH of 6.1 $\mu\text{g/l}$ provided 96% sensitivity and 100% specificity for the diagnosis of GHD. Only one subject with septo-optic dysplasia had a peak GH of 6.1 $\mu\text{g/l}$ that matched the lowest peak GH response after ITT in normal controls.

The diagnostic usefulness of age- and sex-related IGF-I values in these transition-age subjects proved disappointing because the commonly used threshold of IGF-I of ≤ -2.0 SDS (45 $\mu\text{g/l}$) in defining the 'severity' of adult GHD would have excluded more than one third of the GH-deficient subjects. On the other hand, the individual variability of IGF-I concentrations within the subjects with permanent GHD, as well as the overlap with normal controls, was high. The present study's sensitivity of IGF-I ≤ -2.0 SDS of 62% in defining severe GHD was higher than the 39% reported by Hoffman *et al.* (12) and lower than the 92% by de Boer *et al.* (1). Nonetheless, IGF-I limits of < -1.7 SDS (127 $\mu\text{g/l}$) would have identified 77% of the GH-deficient subjects with IGF-I concentrations below the lower limit of normal controls, suggesting that a low single age-stratified IGF-I measurement between the ages of 16 and 25 years in subjects with childhood-onset GHD has value in the diagnosis of permanent hypopituitarism. It is important to underline the fact that one subject with a peak GH response of 6.1 $\mu\text{g/l}$ showed an IGF-I value of +0.1 SDS which does not exclude a diagnosis of GHD (11) in agreement with several studies (11, 13, 18, 24–29). It is our opinion that since the washout period of GH treatment before re-assessment of GH secretory status was longer than 3 months (9, 30), the time effects of GH discontinuation on IGF-I values are not relevant in this case and, therefore, other regulatory factors may play a role.

In conclusion, our data clearly show that the diagnosis of GHD in the transition period represents a major clinical challenge and that a definitive GH diagnostic

threshold needs to be established. By applying the criterion of a peak growth hormone value of less than 3 µg/l or 5 µg/l, several misdiagnosed GH-deficient subjects would be wrongly excluded from a potentially beneficial renewal of GH replacement treatment. If a higher GH peak criterion is established for the diagnosis of adult GHD patients during the transition period, then clinical trials of adult replacement therapy are needed according to the alterations of body composition after discontinuation of GH treatment in adolescents with partial GHD (31). It is worthwhile to point out that in some young adults with childhood-onset hypopituitarism, a suspected diagnosis of permanent GHD could be investigated more accurately by means of an integrated analysis of clinical history, the presence of MPH, IGF-I concentration, MR imaging findings of structural hypothalamic–pituitary abnormalities and the monitoring of GH-dependent endpoints, rather than by a GH cut-off point after a stimulation test.

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