The Glucagon Test in the Diagnosis of Growth Hormone Deficiency in Children With Short Stature Younger than 6 Years

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Context: Few studies have addressed the diagnostic role of the glucagon test in children with suspected GH deficiency (GHD).

Objective: The objective of the study was to investigate the diagnostic value of the glucagon test as an alternative test to insulin tolerance test (ITT) and arginine in GHD children younger than 6 yr.

Design and Setting: This study was conducted in two pediatric endocrinology centers.

Patients and Methods: Forty-eight children (median age 4.2 yr, median height −3.0 SD score) with GHD confirmed by a peak GH to ITT and arginine less than 10 μg/liter (median 4.7 and 3.4 μg/liter, respectively) underwent a glucagon stimulation test. Magnetic resonance imaging showed normal hypothalamic-pituitary anatomy in 24 children, isolated anterior pituitary hypoplasia in seven, and structural hypothalamic-pituitary abnormalities in 17.

Results: Median GH peak response to glucagon (13.5 μg/liter) was significantly higher than that observed after ITT and arginine ($P < 0.0001$). GH peak after glucagon was less than 10 μg/liter in 20 subjects (group 1) and greater than 10 μg/liter in 28 subjects (group 2) without significant clinical or biochemical differences between the two groups. Median GH peak after glucagon was similar between patients with multiple pituitary hormone deficiency and those with isolated GHD and between subjects with and without structural hypothalamic-pituitary abnormalities. The magnitude of the GH peak after glucagon was negatively correlated to age at diagnosis ($\rho = -0.636$, $P < 0.0001$).

Conclusions: This study shows that glucagon has an effective GH-releasing activity and can be used to evaluate somatotroph function in young children with short stature. Normative data for this test in young children need to be established before its use in clinical practice. **(*J Clin Endocrinol Metab* 94: 4251–4257, 2009)**

In children with short stature, the diagnosis of GH deficiency (GHD) is classically established when GH concentrations do not reach an arbitrary cutoff value (usually 7 or 10 μg/liter) after two provocative tests. However, a high proportion of patients with childhood-onset GHD show normalization of GH responses to stimulation when retested either after a few months (1) or at the attainment of adult height (2–7). In addition, we found that only patients with idiopathic GHD with structural abnormalities of the hypothalamic pituitary area had permanent GHD, whereas all other patients had normal GH secretion at retesting (7). Thus, it is a proper selection of patients as opposed to the type of provocative test that is fundamental for the discrimination of patients with permanent GHD.

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Abbreviations: APH, Anterior pituitary hypoplasia; BMI, body mass index; FT4, free T_4; GHD, GH deficiency; HP, hypothalamic-pituitary; IGHD, isolated GHD; IQR, interquartile range; ITT, insulin tolerance test; MPHD, multiple pituitary hormone deficiency; MRI, magnetic resonance imaging; SDS, SD score.

Moreover, it is well known that provocative tests are poorly reproducible and yield a great number of falsely abnormal responses, even in normal subjects (8–12). One of the major problems with stimulation tests is the lack of age- and sex-related normative data for each test. Indeed, previous studies have shown that, depending on the stimulus, maximum peak, as well as inter-individual variability, may show great inconsistency (8, 11). In this regard, few studies have addressed the accuracy of GH stimulation tests in children with GHD younger than 6 yr.

Insulin tolerance test (ITT), arginine, and clonidine are the most widely used pharmacological tests in childhood (13, 14). All of these tests have their own specific limitations in young children. ITT is contraindicated in children younger than 2 yr and is associated with frequent symptomatic hypoglycemia (13, 15). Clonidine may cause severe hypotension, and GHRH plus arginine in children younger than 6 yr with structural hypothalamic-pituitary abnormalities did not successfully distinguish them from normal children (16).

The glucagon test, although it offers some advantages in the first years of life over other pharmacological tests, has been poorly studied to date (17–20). Yet, compared with other procedures, the glucagon test is especially valuable in young patients. First, water may be given freely during the test. Second, glucagon testing causes hyperglycemia followed by a decrease in blood glucose that is usually moderate, so that an iv line is not required. Third, the glucagon test has very few side effects. Finally, the glucagon test allows the simultaneous evaluation of pituitary-adrenal axis function, has few contraindications and is well tolerated, even in patients with overt hypopituitarism.

The aim of this study was to investigate the diagnostic value of glucagon test in the diagnosis of GHD in young children with short stature. To this end, we evaluated the GH-releasing effect of glucagon in patients with GHD younger than 6 yr.

Patients and Methods

GH evaluation

On two different days, patients were admitted to our centers, and a heparin-locked cannula was placed in a forearm vein. Blood samples for GH were collected at time 0 and after 30, 60, 90, and 120 min after iv administration of insulin (bolus injection of 0.1 IU/kg body weight) and arginine (30 min infusion of 0.5 g/kg body weight, maximum 30 g). A nadir glucose value during ITT less than 2.2 mmol/liter (40 mg/dl) was recorded in all subjects at time 30 min. A GH peak of less than 10 μg/liter was considered diagnostic of GHD.

On a third occasion, a glucagon test was performed in all subjects after the diagnosis of GHD had been established, meaning that randomization of the three tests was not applied. Samples were obtained at time 0 and after 30, 60, 90, 120, 150, and 180 min after the im administration of 30 μg/kg glucagon (maximum 1 mg). Serum glucose was determined at each time point. All procedures were carried out between 0800 and 0830 h after overnight fasting. A single IGF-I determination was performed at the time of the first GH stimulation test.

The study was approved by the local ethical committee, and written informed consent was obtained from children's parents or their legal guardians.

Study population

Forty-eight prepubertal subjects (30 males, 18 females) with GHD confirmed by ITT and arginine stimulation test underwent a glucagon stimulation test at a median age of 4.2 yr [interquartile range (IQR) 3.5–5.0]. Their median height SD score (SDS) at diagnosis was −3.0 (IQR − 3.2 to −2.6), which increased to −1.5 SDS (IQR −1.8 to −1.4; $P < 0.0001$) after 1 yr of GH replacement. The patients' main clinical characteristics are summarized in Table 1. GH secretion studies showed similar values for median GH peak after ITT (4.7 μg/liter, IQR 2.5–6.4) and arginine (3.4 μg/liter, IQR 1.5–5.0; P = NS). Median IGF-I was −2.4 SDS (IQR − 3.2 to −1.7).

Thirty-two subjects had isolated GHD (IGHD), whereas 16 had multiple pituitary hormone deficiency (MPHD). Subjects with MPHD showed a significantly lower median height SDS at diagnosis than those with IGHD, lower median bone age, lower GH peak after ITT and arginine, and lower IGF-I SDS (Table 1). Sagittal and coronal T1-weighted magnetic resonance imaging (MRI) images with 2- to 3-mm sections were obtained in all patients, showing a normal hypothalamic-pituitary (HP) region in 24 subjects with IGHD, isolated anterior pituitary hypoplasia (APH; defined as pituitary height of less than 2 SDS) (21) in seven subjects (of whom six IGHD and one MPHD), and structural HP abnormalities (including ectopic posterior pituitary, pituitary stalk agenesis, and anterior pituitary hypoplasia) in 17 subjects (two IGHD, 15 MPHD of whom 12 with one and three with two additional hormone deficiencies).

Comparison between subjects with structural HP abnormalities and those with normal MRI or isolated APH revealed that the former group had a lower median height SDS at diagnosis, a lower median peak GH response to ITT, a lower median peak GH response to arginine and a lower IGF-I SDS (Table 1).

Additional hormone deficiencies

Subjects with central hypothyroidism [confirmed by low free T_4 (FT4) values with low or inappropriately normal TSH] were conventionally treated with L-thyroxine, appropriately adjusted to maintain FT4 in the reference range, whereas those with central adrenal insufficiency (demonstrated by basal cortisol <100 nmol/liter and/or peak cortisol to ITT <550 nmol/liter) received oral hydrocortisone at 10 mg/m^2 · d. Hypothyroid subjects were treated with L-thyroxine, and testing for GH was performed when serum FT4 levels reached normal values. None of the subjects had undergone previous GH treatment.

Assays methods

Serum GH and IGF-I were measured by chemiluminescent immunometric assay (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA). The sensitivity of the method was 0.01 μg/liter for GH and 2.6 nmol/liter for IGF-I. The intra- and interassay coefficients of variation for the GH test were respectively 4.2–6.6 and 2.9–4.6% at GH levels of 2.6–17 μg/liter. The intra- and interassay coefficients of variation for IGF-I were

TABLE 1. Clinical, biochemical and MRI features at GHD diagnosis in the study population overall and according to peak GH responses to glucagon

	Overall	IGHD	MPHD	*P*	Normal MRI-APH	Structural HP abnormalities	*P*	Group 1 Peak GH < 10 μg/liter	Group 2 Peak GH > 10 μg/liter	*P*
Subjects (male/female)	48 (30/18)	32	16		31	17		20 (14/6)	28 (16/12)	
Age (yr)	4.2 (3.5–5.0)	4.3 (3.6–5.3)	4.0 (3.2–4.6)	NS	4.2 (3.5–5.2)	4.0 (3.3–4.6)	NS	4.6 (3.7–5.5)	4.1 (3.3–4.6)	NS
Bone age (yr)	2.0 (1.5–3.0)	2.5 (2.0–3.3)	1.8 (1.2–2.0)	0.0048	2.5 (2.0–3.4)	2.0 (1.2–2.1)	0.0088	2.4 (2.0–3.3)	2.0 (1.4–3.0)	NS
Height (cm)	88.1 (84.2–94.9)	88.6 (84.4–95.4)	86.8 (80.5–91.3)	NS	88.5 (84.4–95.2)	87.0 (81–92.4)	NS	89.1 (84.8–97.0)	87 (81.8–93.4)	NS
Height at diagnosis (SDS)	−3.0 (−3.2 to −2.6)	−2.8 (−3.0 to −2.6)	−3.2 (−3.4 to −3.1)	0.0007	−2.8 (−3.0 to −2.6)	−3.2(−3.5 to −3.0)	0.0011	−2.8 (−3.1 to −2.6)	−3.0 (−3.4 to −2.7)	NS
Height after 1 yr GH replacement (SDS)	−1.5 (−1.8 to −1.4)	−1.5 (−1.7 to −1.4)	−1.5 (−2.0 to −1.5)	NS	−1.5 (−1.7 to −1.4)	−1.5 (−2.0 to −1.5)	NS	−1.5 (−1.7 to −1.5)	−1.5 (−1.9 to −1.4)	NS
BMI (kg/m^2)	13.1 (12.8–13.6)	13.2 (13.0–13.7)	12.9 (12.6–13.6)	NS	13.2 (13.0–13.7)	12.8 (12.5–13.5)	NS	13.1 (12.9–13.6)	13.2 (12.7–13.7)	NS
Target height (SDS)	−0.2 (−0.6 to 0.4)	−0.2 (−0.4 to 0.5)	−0.3 (−0.7 to 0.3)	NS	−0.2 (−0.5 to 0.4)	−0.1 (0.6 to 0.4)	NS	0.1 (−0.3 to 0.4)	−0.3 (−0.7 to 0.5)	NS
Peak GH to ITT (μg/liter)	4.7 (2.5–6.4)	5.8 (3.5–6.7)	2.3 (1.2–3.9)	0.0002	5.8 (3.6–6.7)	2.5 (1.3–3.6)	<0.0001	4.7 (3.0–6.1)	3.5 (2.3 to 6.5)	NS
Peak GH to arginine (μg/liter)	3.4 (1.5–5.0)	4.5 (2.6–5.7)	1.8 (1.1–2.9)	0.0004	4.6 (2.3–5.7)	2.1 (1.1–3.3)	0.0014	3.8 (2.4–4.9)	2.7 (1.5–5.1)	NS
Peak GH to glucagon (μg/liter)	13.5 (8.3–16.3)	12.6 (8.3–15.7)	14.2 (8.3–17.5)	NS	13.3 (8.4–16.0)	14.0 (8.1–17.2)	NS	8.2 (7.6–8.5)	15.3 (14.1–17.9)	<0.0001
IGF-I (SDS)	−2.4 (−3.2 to −1.7)	−1.9 (−2.4 to −1.5)	−3.3 (−3.7 to −3.1)	<0.0001	−1.9 (−2.3 to −1.5)	−3.3 (−3.9 to −3.1)	0.0003	−2.0 (−2.9 to −1.4)	−2.5 (−3.3 to −2.0)	NS
Hormone defects (IGHD/MPHD)	32/16	32/0	0/16		30/1	2/15		14/6	18/10	
MRI (normal/APH/EPP+APH+PSA)	24/7/17	24/6/2	0/1/15		24/7/0	0/0/17		11/2/7	13/5/10	

Data are shown as median and IQR (25th to 75th percentiles). EPP, Ectopic posterior pituitary; PSA, pituitary stalk agenesis; NS, not significant.

3.4 and 7.1%, respectively. IGF-I SDS was calculated using the normative data for the method as previously described (22). All samples from each individual subject were analyzed together at the same time, after centrifugation at 4 C, plasma separation, and storage at −20 C. Glycemia determination was automatically performed with hexokinase catalyzed-glucose oxidase method.

Statistical analysis

The study population was divided into two groups, according to their GH peak response to glucagon, less than 10 μg/liter (group 1, Table 2) or greater than 10 μg/liter (group 2, Table 3). Comparisons between groups were performed using the Mann-Whitney *U* test (when comparing two groups) or the Kruskal-Wallis test (when comparing more than two groups), with Bonferroni adjustment where appropriate. Moreover, the median and IQRs (the distance between the 25th and 75th percentile, encompassing the middle 50% of observations) were used as descriptive statistics. The correlation between variables was evaluated by the Spearman rank correlation coefficient (ρ). $P < 0.05$ was considered statistically significant. All tests were two sided. Statistical analyses were performed using Stata for Windows statistical software (Stata release 9.2; Stata Corp., College Station, TX).

Results

GH response to glucagon

The median GH peak response to glucagon was significantly higher than that observed after both ITT and arginine (Fig. 1). A GH peak after glucagon of less than 10 μg/liter was recorded in 20 subjects (14 males, six females; group 1), whereas that of greater than 10 μg/liter was observed in 28 patients (16 males, 12 females; group 2). The individual characteristics of these patients are reported in Tables 2 and 3.

Clinical and biochemical parameters at diagnosis were similar between the two groups, with the single exception of GH peak after glucagon (Table 1). No statistically significant correlation was found either between peak GH to glucagon and the nadir value of glycemia obtained during the test (in the study population overall and in both groups) or between GH and glycemia values at all time points (data not shown). No gender differences were found either in the cohort as a whole or in the two groups considered separately. In nine of the 48 subjects (18.7%), GH peaked at time 90 min; in 33 of the 48 subjects (68.8%), the peak occurred at time 120 min; and in six of the 48 (12.5%), it was observed at time 150 min.

Comparisons according to hormonal and MRI features

The median GH peak responses to glucagon were not statistically different, either between MPHD and IGHD or between subjects with structural HP abnormalities at MRI and subjects with normal MRI or isolated APH, as shown in Table 1.

TABLE 2. Clinical and peak GH response to ITT, arginine and glucagon, hormonal, and MRI findings in group 1 children with peak GH response to glucagon lower than 10 μg/liter at GHD diagnosis

Sex	Age (yr)	Bone age (yr)	Height (SDS)	BMI (kg/m^2)	Peak GH (μg/liter)			Nadir glycemia to ITT (mg/dl)	IGF-I (SDS)	Hormone defects	MRI	Target height (SDS)
					ITT	Arginine	Glucagon					
M	3.2	2.0	−3.1	13.8	5.2	6.1	8.9	32	−2.4	GH	Normal	−1.2
M	3.3	2.0	−3.0	12.9	5.8	3.2	9.4	29	−2.6	GH	Normal	−0.4
M	3.4	2.0	−3.0	13.3	7.9	4.0	9.2	40	−2.2	GH	APH	0.2
F	3.6	1.5	−2.7	13.3	3.1	4.7	8.5	29	−1.7	GH	Normal	0.1
F	3.7	2.0	−2.6	13.6	4.6	6.1	8.3	31	−1.4	GH	Normal	−0.3
M	3.7	2.5	−3.0	13.2	7.7	4.2	8.5	34	−1.5	GH	APH	−0.2
M	3.8	1.0	−3.2	14.1	1.4	1.8	8.4	26	−3.3	GH, TSH	EPP, APH, PSA	0.9
F	3.9	1.5	−3.4	12.9	6.3	4.1	8.2	22	−3.5	GH, ACTH	EPP, APH, PSA	−0.6
M	4.1	2.5	−2.8	12.8	8.9	5.7	8.3	31	−1.7	GH	Normal	0.4
F	4.5	1.5	−3.0	13.5	1.5	1.0	7.6	19	−3.1	GH, ACTH	EPP, APH, PSA	0.2
M	4.6	2.0	−3.1	12.7	2.9	3.2	7.1	28	−3.0	GH, ACTH	EPP, APH, PSA	0.1
F	4.9	3.0	−2.5	13.0	6.4	3.3	7.9	28	−1.4	GH	Normal	−0.4
F	5.0	2.5	−2.6	13.1	1.3	4.4	8.1	34	−1.1	GH	Normal	−0.2
M	5.3	3.5	−2.5	14.2	4.7	6.8	7.6	37	−1.3	GH	Normal	0.5
M	5.4	3.5	−2.5	13.1	5.8	2.2	7.5	29	−1.2	GH	Normal	0.0
M	5.5	2.3	−3.1	12.7	0.5	0.5	8.6	28	−3.2	GH, TSH, ACTH	EPP, APH, PSA	−0.3
M	5.6	3.5	−2.7	12.3	5.5	3.5	4.2	24	−2.9	GH, ACTH	EPP, APH, PSA	0.7
M	5.7	4.0	−2.4	13.9	4.7	5.1	7.9	28	−1.1	GH	Normal	0.6
M	5.8	2.5	−2.8	11.8	4.1	2.5	3.6	29	−2.9	GH	EPP, APH, PSA	−0.1
M	5.9	4.0	−2.8	13.0	3.6	1.3	6.8	29	−1.6	GH	Normal	0.4

EPP, Ectopic posterior pituitary; F, female; M, male; PSA, pituitary stalk agenesis.

Correlations

A significant negative correlation was observed between age at diagnosis and the GH peak response to glucagon in the study population overall ($\rho = -0.636$, $P < 0.0001$) as well as in the two separate groups (group 1, $\rho = -0.796$, $P = 0.0005$; group 2, $\rho = -0.879$, $P < 0.0001$) (Fig. 2). Age at diagnosis was also positively correlated with IGF-I SDS in all 48 subjects ($\rho = 0.411$, $P = 0.0049$) and in group 2 patients ($\rho = 0.524$, $P = 0.0065$). Height SDS at diagnosis showed a positive correlation with the GH peak after arginine ($\rho = 0.323$, $P = 0.027$) and IGF-I SDS ($\rho = 0.767$, $P < 0.0001$; $\rho = 0.856$, $P = 0.0002$; $\rho =$

TABLE 3. Clinical and peak GH response to ITT, arginine, and glucagon, hormonal, and MRI findings in group 2 children with peak GH response to glucagon higher than 10 μg/liter at GHD diagnosis

Sex	Age (yr)	Bone age (yr)	Height (SDS)	BMI (kg/m^2)	Peak GH (μg/liter)			Nadir glycemia (mg/dl)	IGF-I (SDS)	Hormone defects	MRI	Target height (SDS)
					ITT	Arginine	Glucagon					
M	2.4	1.5	−3.4	11.5	2.5	2.4	21.5	27	−3.9	GH, TSH, ACTH	EPP, APH, PSA	−0.5
F	2.5	1.0	−3.2	12.8	1.0	1.8	18.2	34	−3.4	GH, TSH	EPP, APH, PSA	−0.1
F	2.5	1.0	−3.4	13.2	5.3	1.2	24.6	35	−2.9	GH, TSH	APH	−0.7
M	2.6	1.0	−3.0	11.8	6.8	5.3	19.3	29	−2.4	GH	Normal	−0.2
F	2.7	1.0	−3.7	12.6	3.4	3.7	22.3	39	−4.2	GH	EPP, APH, PSA	0.5
M	2.8	0.5	−3.9	12.5	3.0	2.1	18.3	40	−4.0	GH, TSH	EPP, APH, PSA	1.0
M	3.0	1.0	−3.0	13.7	6.4	5.7	16.2	36	−2.8	GH	Normal	−0.5
F	3.5	1.5	−3.0	13.5	3.6	4.8	16.3	35	−2.2	GH	APH	0.7
M	3.5	2.0	−3.3	12.9	1.7	1.1	16.8	24	−3.3	GH, ACTH	EPP, APH, PSA	−0.7
M	3.5	2.0	−3.0	13.8	7.8	6.3	18.0	33	−2.1	GH	Normal	−0.3
F	3.7	2.5	−2.7	14.0	8.3	5.2	15.2	30	−1.4	GH	APH	0.7
M	3.8	1.3	−3.1	14.0	0.9	0.5	15.4	29	−4.2	GH, TSH	EPP, APH, PSA	−1.1
F	3.9	2.0	−3.1	13.1	3.1	4.6	17.8	34	−2.1	GH	Normal	−0.7
M	4.0	2.0	−3.0	13.3	2.5	1.0	15.1	23	−4.6	GH, TSH, ACTH	EPP, APH, PSA	−1.2
M	4.1	2.3	−3.9	13.0	6.5	2.7	17.3	41	−2.5	GH	Normal	1.2
M	4.2	2.3	−3.2	13.7	1.5	0.5	14.5	32	−2.1	GH	Normal	−0.7
M	4.3	3.0	−2.2	13.3	6.5	8.6	13.3	32	−1.6	GH	APH	−0.4
F	4.3	2.0	−3.7	13.8	4.8	4.7	14.4	36	−3.3	GH, TSH	EPP, APH, PSA	−0.2
M	4.4	2.0	−4.6	12.9	5.8	1.5	13.6	33	−3.0	GH	Normal	−1.5
M	4.4	3.0	−2.2	13.1	3.2	1.5	16.7	31	−2.0	GH	Normal	0.8
F	4.5	3.0	−3.6	13.6	0.9	2.6	14.0	34	−3.4	GH, TSH	EPP, APH, PSA	−0.6
M	4.6	3.5	−2.8	12.4	6.9	4.2	14.8	29	−2.3	GH	Normal	−0.3
F	4.7	2.0	−2.6	12.5	2.1	1.4	13.3	37	−2.9	GH, TSH	EPP, APH, PSA	0.4
F	4.8	2.0	−2.6	12.4	3.0	1.5	14.1	29	−1.6	GH	Normal	−0.8
F	5.2	3.0	−2.9	13.3	6.2	7.2	11.0	32	−1.5	GH	Normal	−1.0
M	5.6	4.0	−2.6	14.1	2.1	1.0	13.9	39	−1.9	GH	Normal	0.2
F	5.7	4.0	−2.6	13.4	7.8	6.2	14.2	36	−2.0	GH	APH	0.5
M	5.9	4.5	−2.1	13.1	5.7	4.9	11.9	31	−1.9	GH	Normal	−0.2

EPP, Ectopic posterior pituitary; F, female; M, male; PSA, pituitary stalk agenesis.

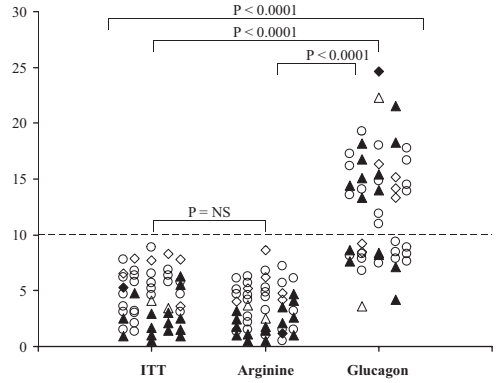

FIG. 1. Distribution of peak GH response to ITT, arginine and glucagon in 48 subjects affected by: ○, normal MRI and IGHD; △, anterior pituitary hypoplasia and IGHD; ▲, anterior pituitary hypoplasia and MPHD; ◇, structural hypothalamic-pituitary abnormalities and IGHD; ◆, structural hypothalamic-pituitary abnormalities and MPHD.

0.707, $P = 0.0002$ in the entire cohort and in groups 1 and 2, respectively), whereas it was negatively related to the GH peak after glucagon in the study population overall ($\rho = -0.446$, $P = 0.0022$) and in group 2 ($\rho = -0.483$, $P = 0.0121$). No significant correlation was found between body mass index (BMI) and the peak GH response to any of the provocative tests or with IGF-I.

IGF-I SDS was significantly correlated with peak GH to ITT ($\rho = 0.415$, $P = 0.0045$ in the entire cohort, $\rho = 0.495$, $P = 0.0101$ in group 2), to arginine ($\rho = 0.483$, $P = 0.0009$; $\rho = 0.514$, $P = 0.0251$; $\rho = 0.423$, $P = 0.0279$ in the overall population and in groups 1 and 2, respectively) and to glucagon ($\rho = -0.395$, $P = 0.0067$ in the entire cohort; $\rho = -0.452$, $P = 0.0188$ in group 2). There was a significant positive correlation between GH peak after ITT and arginine ($\rho = 0.653$, $P < 0.0001$), whereas no correlation was observed between the peak GH response to glucagon and the response after ITT or arginine.

Discussion

Stimulation tests have been [1]sed for decades to assess GH secretion, with cutoff for normal responses arbitrarily set at 5, 7, or 10 μg/liter. However, provocative tests are flawed by the absence of age- and sex-related normative data, poor reproducibility, and the amount of falsely abnormal responses frequently observed in both affected and normal subjects (8–12). This variability has been attributed to the periodic secretion of somatostatin, which may influence somatotroph response to the stimulus (23). Furthermore, GH responses to stimulation may also be influenced by the pattern of GH secretion preceding the stimulus, *i.e.* whether the latter is administered during a spontaneous trough or peak of GH secretion (23). Sex steroid priming in prepubertal children has been proposed to reduce the number of false-positive results (24, 25). However, priming with sex steroids in prepubertal children remains a controversial issue because the procedure is nonphysiological and nonstandardized, and, once again, definitive cutoff limits are not available.

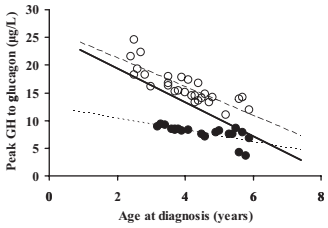

FIG. 2. Spearman rank correlation between age at diagnosis and peak GH response to glucagon at GHD diagnosis: ●, subjects with peak GH to glucagon less than 10 μg/liter (group 1); ○, subjects with peak GH to glucagon greater than 10 μg/liter (group 2); *solid line*, correlation in the study population overall, $\rho = -0.636$, $P < 0.001$; *dashed line*, correlation in group 1 subjects, $\rho = -0.796$, $P < 0.0005$; *broken line*, correlation in group 2 subjects, $\rho = -0.879$, $P < 0.0001$.

Among the various GH stimulation tests, the glucagon test has been poorly studied in the pediatric population. Previous studies have shown that the im administration of glucagon is a reliable tool for studying GH secretion (17–20). As with most pharmacological tests, however, normal age- and gender-related values have not yet been established. Furthermore, a thorough characterization of the patients was never performed in the few published studies, and the GH assay used in these studies is now obsolete. In the present study, we investigated the diagnostic value of the glucagon test in young children with GHD. All patients were younger than 6 yr of age, the diagnosis of GHD was established on the basis of two stimulation tests (ITT and arginine), and all underwent MRI studies of the hypothalamic-pituitary area, with abnormal results found in 17 patients. In this cohort, the median GH peak after glucagon was significantly higher than that observed after either ITT or arginine. Surprisingly, the GH peak after glucagon was higher than 10 μg/liter in 28 of 48 patients (58.3%), 15 of whom had hypothalamic and/or pituitary stalk involvement and/or MPHD.

The magnitude of the GH peak was inversely related to age, suggesting that the GH response to glucagon may be age dependent. This observation contrasts with the findings of Chanoine *et al.* (26) and those of Johnstone and Cheetham (27), who found a positive correlation with age in normal children aged 0.5–12 months and in short normal prepubertal children, respectively. Differences in the study populations (normal *vs.* GHD children) may explain these discrepancies. Furthermore, it is worth noting that in patients with congenital GHD, endocrinopathies can evolve with time, a phenomenon that might explain the age-related decrease of GH secretion. In our study, the mean GH response to glucagon was higher than that observed after ITT or arginine. This may indicate that the

GH-releasing effect of glucagon in young children with congenital GHD is greater than that of ITT and arginine, and thus, the same cutoff levels cannot be adopted. As a matter of fact, using the classically adopted criteria, 28 of our patients would have been regarded as normal, *i.e.* with a GH peak greater than 10 μg/liter.

The capability of glucagon to induce a higher GH response in the younger children in our study may also be related to its mechanism of action, which, at present, is still poorly understood. It is unlikely that the glucagon-induced GH release is mediated by changes in glucose concentrations because this was already excluded by previous studies in both adults (28–30) and children (27), and, in this study, the GH peaks were clearly not associated with serum glucose variations. Another possible mechanism by which glucagon stimulates GH secretion is via activation of central noradrenergic pathways. In this regard, glucagon has been shown to induce noradrenaline release in healthy subjects (31). The increase in noradrenaline release may trigger GH secretion via α-adrenergic receptors because administration of a β-blocker enhances GH secretion (20, 32). Different age- and disease-related maturation/activity of the neuroendocrine pathways through which glucagon exerts its GH-releasing action may explain these observations.

We have previously shown that the GHRH plus arginine test, a potent stimulatory test for GH secretion, which reportedly explores the maximum GH secretory reserve (11), may yield false-negative responses in young patients with GHD (16). In fact, we found a normal GH response in a number of GHD children younger than 6 yr with GHD and structural abnormalities of the hypothalamic pituitary area. This observation, coupled with the results of the present study, suggests that GH responses to pharmacological tests in young children with GHD may be different from those observed in older age groups. Whether this applies only to GHD children or whether it perhaps extends to normal children as well requires further investigation.

Strich *et al.* (33) have recently shown that not only is the magnitude of the GH peak after glucagon important but also that the timing of the peak is meaningful. They have shown that a GH peak at a time other than 90 or 120 min may be an important indicator of GH deficiency. In our study, the maximum GH peak was observed between 90 and 120 min in the vast majority of patients. Only six of 48 patients (12.5%) had a GH peak at a different time point. These findings contradict those of Strich *et al.* because all of our patients had a previously established diagnosis of GHD.

IGF-I measurement is of great aid in the diagnosis of GHD (34). Although a normal IGF-I concentration does not always exclude a diagnosis of GHD in young children, low IGF-I in well-nourished subjects is strongly indicative of GHD (13). Indeed, many of our patients had a very low IGF-I concentration associated with a GH peak of less than 10 μg/liter after both ITT and arginine. In addition, 17 of 48 (35.4%) also had structural hypothalamic-pituitary abnormalities at MRI, a finding compatible with congenital hypopituitarism. The fact that the GH response to glucagon was normal, *i.e.* higher than 10 μg/liter, does not contradict the diagnosis. Instead, this observation supports our view that GH responses to glucagon (as observed in this study) and to GHRH plus arginine (16) may yield false-negative results when the cutoffs of 10 or 20 μg/liter for normal responses, respectively, are considered. This plainly reinforces the need for normative data for GH response to pharmacological tests in this age group.

Our study does have some limitations. First, we cannot evaluate the diagnostic accuracy of the glucagon test because a gold standard for the GH response to pharmacological stimulation in this age group is not unanimously accepted. Second, because our study lacks a control group, we believe that the results and the specificity of GH cutoff would be strengthened by including data on the response to a glucagon test in a group of normal children. Even with these limitations, however, our study shows for the first time the efficacy of the glucagon test in stimulating GH secretion in a previously well-characterized cohort of young children with GHD, and it also contributes to our knowledge of the glucagon test's mechanism of action. In addition, it shows that in these patients the cutoff limit of 10 μg/liter for a normal response cannot be used.

In conclusion, we have shown that the glucagon test is a potent test for GH secretion in young children with GHD. Normative data for this test in young children need to be established before its use in clinical practice.

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