Comparison between Insulin-Induced Hypoglycemia and Growth Hormone (GH)-Releasing Hormone + Arginine as Provocative Tests for the Diagnosis of GH Deficiency in Adults*

G. AIMARETTI, G. CORNELI, P. RAZZORE, S. BELLONE, C. BAFFONI, E. ARVAT, F. CAMANNI, AND E. GHIGO
Division of Endocrinology, Department of Internal Medicine, University of Turin, 10126 Turin Italy

ABSTRACT
There is now wide consensus that, within an appropriate clinical context, GH deficiency (GHD) in adults must be shown biochemically by provocative testing of GH secretion and that appropriate cut-off limits have to be defined for each provocative test. Insulin-induced hypoglycemia (ITT) is indicated as the test of choice, and severe GHD, to be treated with recombinant human GH replacement, is defined by a GH peak response to ITT of less than 3 μg/L. GHRH + arginine (GHRH + ARG) is one of the most promising tests in alternative to ITT. In fact, it has been reported as a potent, reproducible, and age-independent test and that it is able to distinguish between GHD and normal adults. The aim of the present study was to compare the GH response to ITT and GHRH + ARG in a large group of hypopituitary adults (n = 40; 29 male and 11 female; age: 36.4 ± 2.1 yr). The third centile limit of the peak GH response to ITT has been reported as 5 μg/L, whereas in our lab, that to GHRH + ARG is 16.5 μg/L. In hypopituitary adults, the mean peak GH response to ITT (1.5 ± 0.2 μg/L; range: 0.1–8.5 μg/L) was lower (P < 0.001) than that to GHRH + ARG (3.0 ± 0.4 μg/L; range 0.1–12.0 μg/L), though there was positive correlation (r = 0.61, P < 0.001) between the GH responses to the 2 tests. The peak GH response to GHRH + ARG, but not that to ITT, was positively (though weakly) associated with insulin-like growth factor-I levels (r = 0.35, P < 0.03). Childhood and adult onset GHD patients, as well as patients with single and multiple pituitary insufficiencies, had similar peak GH responses to ITT or GHRH + ARG. Analyzing individual GH responses, 4/40 (10%) of the hypopituitary patients had GH peaks higher than 5 μg/L after ITT; moreover, 3 other patients (7%) had GH peaks, after ITT, higher than 3 μg/L. On the other hand, after GHRH + ARG, all patients had GH peaks lower than 16.5 μg/L, whereas 21/40 (52.5%) had GH peaks higher than 3 μg/L. Because 3 μg/L is the arbitrary cut-off for ITT, the third centile limit of which is 5 μg/L, we arbitrarily considered 9 μg/L as the cut-off point for GHRH + ARG. It is noteworthy that 37/40 (92.5%) patients had a GH peak, after GHRH + ARG, below this limit. In conclusion, our present results confirm that the ITT test is a reliable provocative test for the diagnosis of adult GHD, whereas they show that the GHRH + ARG test is, at least, as sensitive as the ITT test (provided that appropriate cut-off limits are considered). Note that even the arbitrary cut-off point below which severe GHD is demonstrated has to be appropriate to the potency of the test. (J Clin Endocrinol Metab 83: 1615–1618, 1998)

IT HAS BEEN clearly demonstrated that adults with GH deficiency (GHD) have impaired health, which improves with GH replacement. In fact, GHD in adulthood leads to impairment in body composition and structure functions (such as increased abdominal fat mass, reduced lean body mass, lowered exercise capacity, impaired cardiac function, reduced bone mineral content), as well as to deranged lipoprotein and carbohydrate metabolism (1–6). Moreover, shortened life expectancy, caused by increased cardiovascular morbidity, has been recorded in hypopituitaric patients with GHD (7, 8). Many authors (1–7) have showed the beneficial effects of replacement therapy on metabolism and body composition; and now, many countries have already approved the use of recombinant human growth hormone (rhGH) in adults with severe GHD.

There is now wide consensus that, within an appropriate clinical context, GHD in adults must be shown biochemically, by single provocative testing, provided that a reproducible test with clear normative limits is available (9–12). This statement is based on evidence that the assays of insulin-like growth factor-I (IGF-I) and IGF binding protein-3 per se do not establish the diagnosis of adult GHD. In fact, normal IGF-I and/or IGF binding protein-3 levels do not exclude GHD. This is caused by significant overlap between normal and GHD subjects for these parameters (9, 10, 13). Also, the evaluation of spontaneous GH secretion over 24 h has no diagnostic value in adulthood (9). In fact, it is clearly reduced in GHD, but there is a clear overlap between normal and GHD subjects (even when ultrasensitive GH assays are used) (14).

At present, the diagnosis of adult GHD is established only by provocative testing of GH secretion, and insulin-induced hypoglycemia (ITT) is indicated as the test of choice (1, 9, 11–13, 15). The lowest limit of GH response to ITT in normal subjects has been reported as 5 μg/L by some (but not by other) authors (1, 9, 15–17). Severe GHD to be treated with rhGH replacement is defined by a GH peak to ITT lower than the arbitrary cut-off of 3 μg/L (11, 12). Contraindication to
ITT are ischemic heart disease, seizure disorders, and aging (12).

Alternative provocative tests of GH secretion have been proposed and have to be used with appropriate cut-off limits (10, 12, 18–20). The diagnostic value of clonidine provocative testing is limited, whereas arginine (ARG) and glucagon alone could be useful tests but are less discriminatory than ITT (18, 21). Testing with GHRH alone has no diagnostic value (22); but when GHRH is given in combination with ARG, it becomes the most useful, reproducible, and age-independent provocative test to evaluate the maximal secretory capacity of somatotrope cells (10, 22, 23). In our lab, the third centile limit of the GH response to GHRH+ARG test across human lifespan is 16.5 μg/L, and our previous data showed that it reproducibly distinguishes between normal and GHD adult and elderly subjects (10, 23). Based on this evidence, the GHRH+ARG test has been proposed as the most promising alternative to ITT for the diagnosis of GHD (11).

The aim of the present study was to compare the diagnostic reliability of ITT and the GHRH+ARG test in a large population of hypopituitary adults.

**Subjects and Methods**

Forty adult hypopituitary patients (n = 40; 29 male and 11 female, age: 36.4 ± 2.1 yr) were studied. Among them, 21 had acquired adult onset GHD: 13 of them had panhypopituitarism, whereas the others had 1–2 deficiencies other than GH.

Nineteen patients had childhood onset (CO) GHD: eight of them had isolated GHD, whereas the others had panhypopituitarism already diagnosed and treated in childhood. In CO-GHD, the diagnosis had been already demonstrated in childhood by failure to respond to two classical provocative tests.

The etiologies of multiple hypopituitarism other than GH (n = 32) were the following: 24 with pituitary tumors and craniopharyngioma before and/or after pituitary surgery or radiotherapy; 4 with idiopathic hypopituitarism; 2 with posttraumatic hypopituitarism; 1 with Sheehan syndrome; and 1 with histiocytosis.

No patient received rhGH for at least 3 months before testing, whereas all patients with pituitary insufficiencies other than GH had been in optimized replacement therapy for at least 3 months with thyroid hormone, cortisone acetate, gonadal steroids, and (deamino-cys, D-ARG)-vasopressin (DDAVP, desmopressin) when appropriate.

All patients underwent the following tests, in the morning after an overnight fasting, at least 3 days apart: 1) ITT (regular insulin, Actrapid Novo-Nordisk Denmark: 0.1 U/kg iv at 0 min); and 2) GHRH (GHRH29, GEREF, Serono, Italy; 1 μg/kg iv at 0 min) + ARG (ARG hydrochloride, 0.5 g/kg iv over 30 min from 0 to +30 min).

Blood samples were taken every 15 min from –15 to +90 min.

Serum GH levels were assayed at each time point by immunoradiometric assay (HGH-CTK, Sorin, Italy). All samples from an individual subject were analyzed together. The sensitivity of the method was 0.15 μg/L. The inter- and intraassay coefficients of variation were 5.1–7.5% and 2.6–5.4%, respectively, at GH levels of 2.9–42.4 and 2.8–41.2 μg/L, respectively. In our laboratory, the third centile limit of normal peak GH response to GHRH+ARG from young adulthood to aging is 16.5 μg/L (evaluated in a population of 74 normal subjects, age 20–80 yr) (10). For ITT, we considered 5 μg/L as the third centile limit of normal peak GH response, based on data in the literature. During ITT, glucose measurement was performed, and a minimum plasma glucose level of 2.2 mmol/L or less was detected (9).

Serum IGF-I levels was assayed basally by RIA (Nichols Institute of Diagnostics, San Juan Capistrano, CA) after acid-ethanol extraction, to avoid interference by binding proteins. The sensitivity of the method was 0.1 μg/L. The inter- and intraassay coefficients of variation were 8.8–10.8% and 5.0–9.5%, respectively, at IGF-I levels of 79.6–776.4 and 79.4–712.5 μg/L, respectively. Age-adjusted 3rd centile limits of normality for IGF-I levels in our laboratory (data derived from 336 normal subjects from 20–80 yr old) are: 108.5 μg/L between 20–30 yr, 129.8 μg/L between 31–40 yr, 72.3 μg/L between 41–50 yr, 62.4 μg/L between 51–60 yr, 41.5 μg/L between 61–70 yr, and 24.7 μg/L between 71–80 yr.

Student’s paired t test and linear regression analysis were used for statistical analysis of the data.

Results (mean ± sem) are expressed as absolute values for GH, as well as for IGF-I (μg/L).

**Results**

Mean IGF-I levels were 74.6 ± 8.3, with a range between 7.0–200.0 μg/L. Sixty-two percent of the patients had IGF-I levels below the age-adjusted normal range, but the percentage was 91% for patients between 20–40 yr old.

The 3rd centile limit of the peak GH response to ITT was assumed to be 5 μg/L, based on data from literature (1, 9), whereas in our laboratory, the response to GHRH+ARG provocation was 16.5 μg/L.

The mean peak GH response to ITT (1.5 ± 0.2 μg/L, range 0.1–8.5 μg/L) was lower (P < 0.001) than that to GHRH+ARG (3.0 ± 0.4 μg/L, range 0.1–12.0 μg/L) (Fig. 1). The peak GH responses to the two tests were positively associated (r = 0.61, P < 0.0001), whereas both were independent of age. The peak GH response to GHRH+ARG (r = 0.35, P < 0.03) [but not the response to ITT (r = 0.2, P not significant)] was positively, though weakly, associated with IGF-I levels. The mean peak GH responses to ITT and GHRH+ARG in CO-GHD (1.3 ± 0.3 and 3.0 ± 0.6 μg/L, respectively) were similar to those in adult onset-GHD (1.8 ± 0.4 and 3.1 ± 0.6 μg/L, respectively). Similarly, the mean peak GH responses in patients with panhypopituitarism (1.9 ± 0.7 and 2.9 ± 0.8 μg/L, respectively) were similar to those in patients with one to two pituitary hormonal insufficiencies other than GH (1.8 ± 0.5 and 3.7 ± 1.0 μg/L, respectively).

Analyzing individual GH peaks, 4/40 (10%) of the patients had GH peaks higher than 5 μg/L after ITT; moreover, 3 other patients (7%) had GH peaks, after ITT, higher than 3 μg/L (Figs. 2 and 3). On the other hand, after GHRH+ARG,

![FIG. 1. Mean and individual GH peaks, after ITT or GHRH+ARG, in 40 hypopituitary patients.](image-url)
all patients had GH peaks lower than 16.5 μg/L, whereas 21/40 (52.5%) had GH peaks higher than 3 μg/L. It is noteworthy that 73% of the patients with GH peak higher than 3 μg/L after GHRH+ARG had GH peaks below this limit after ITT (Fig. 2 and 3).

Because 3 μg/L is the arbitrary cut-off for ITT, the third centile limit of which is 5 μg/L, we arbitrarily considered 9 μg/L as the cut-off point for GHRH+ARG. It is noteworthy that 37/40 (92.5%) of the patients had GH peaks, after GHRH+ARG, below this limit (including 6/7 patients with GH peaks more than 3 μg/L after ITT (Figs. 2 and 3). Note that those patients with GH peaks higher than 3 μg/L after ITT or 9 μg/L after GHRH+ARG had multiple pituitary insufficiencies.

No significant side effects were observed with both tests. Only mild tachycardia and sweating (after ITT) and transient facial flushing (after GHRH) occurred in the majority of patients. However, no test had to be stopped.

Discussion

Our present results confirm that ITT is a reliable provocative test for the diagnosis of adult GHD, and they show that the GHRH+ARG test is, at least, as sensitive as ITT, provided that appropriate cut-off limits are considered. The GH response to both tests is positively correlated, but GHRH+ARG is a more potent stimulus of GH secretion than ITT, even in GHD patients. Moreover, the GH response to GHRH+ARG, but not that to ITT, is positively associated with IGF-I levels.

There is consensus that the diagnosis of adult GHD is established by provocative testing of GH secretion (9–10). ITT is considered the test of choice (9, 11, 12). In fact, this test has been found to be capable of distinguishing GHD from the reduced GH secretion that accompanies aging and obesity. Following consensus of opinion leaders (11, 12), severe GHD, to be treated with rhGH replacement, is defined by an arbitrary cut-off that is a GH peak response to ITT less than 3 μg/L. Note that the lowest limit of GH response to ITT in normal individuals has been reported as 5 μg/L (9). However, recently, ITT has been found to be poorly reproducible in normal subjects, thus questioning its specificity (16, 17). Moreover, ITT is contraindicated in patients with ischemic heart disease, seizure disorders, and aging (12).

Alternative provocative tests of GH secretion have been proposed, but they must be used with appropriate cut-off limits (11, 12). Among alternatives, other classical provocative tests, as well as GHRH alone, are of less (if any) value, when compared with ITT (18, 21, 22). However, when GHRH is given in combination with ARG or pyridostigmine, which probably act via inhibition of hypothalamic somatostatin release (24), it becomes the most potent and reproducible provocative test to evaluate the pituitary GH releasable pool (19, 20, 22, 23, and present data) distinguishing between normal and GHD adults (10, 20).

In contrast to GHRH+PD, the GH response to GHRH+ARG is not reduced in aging (25–27). Thus, it has been suggested that the latter stimulus could be proposed as the most promising alternative to ITT.
With respect to its third centile cut-off limit (5.0 μg/L GH peak) (9), ITT demonstrated GHD in 90% of the patients, whereas all of them had insufficient GH response to GHRH+ARG. This picture overlaps with that comparing ITT with GHRH + pyridostigmine in a large population of young and middle-aged GHD subjects (20).

With respect to the arbitrary cut-off of 3 μg/L as the limit below which severe GHD is demonstrated after ITT (11, 12), 83% of the patients were below this limit. Thus, our data confirm the diagnostic reliability of ITT, which, in our hands, gave no serious side effects.

On the other hand, with respect to the arbitrary cut-off of 3 μg/L, 47.5% of the patient population has peak GH results below this limit when tested with GHRH+ARG. Note that 73% of them had had a GH peak lower than 3 μg/L after ITT. Theoretically, one could hypothesize that ITT had given a large number (approximately 50%) of false positive responses, indicating severe GHD. However, this hypothesis is unlikely, considering that the majority of these patients had multiple pituitary insufficiencies and low IGF-I levels.

We would like to emphasize that each test must be used with appropriate cut-off limits. This means also that the arbitrary cut-off point below which severe GHD is demonstrated has to be appropriate to the potency of each provocative test. Because 3 μg/L is the arbitrary cut-off for ITT, the third centile limit of which is 5 μg/L, we arbitrarily considered 9 μg/L as the cut-off point for GHRH+ARG; note that this limit represents the first centile limit of the normal GH response to the GHRH+ARG test, in a large population of normal subjects (10).

Note that 92.5% of the GHD patients had GH peaks, after GHRH+ARG, below the limit of 9 μg/L. This population also included 6/7 of the patients with GH peaks higher than 3 μg/L after ITT. We concluded that all these patients have severe GHD; they are all now treated with rhGH and show benefit from treatment.

On the other hand, we assumed that patients with GH peaks higher than 9 μg/L (7.5%), but lower than 16.5 μg/L, after GHRH+ARG had partial GHD. At present, there is no evidence supporting the hypothesis that partial GHD in adults needs GH replacement.

Finally, we would like to emphasize that, unlike ITT, the GH response to GHRH+ARG in GHD is positively associated with IGF-I levels, which is the best marker of GH secretory status (28). This evidence reinforces the reliability of this provocative test for the diagnosis of severe adult GHD.

In conclusion, our present results show that the GHRH+ARG test is, at least, as reliable as ITT for the diagnosis of adult GHD, provided that appropriate cut-off limits are considered.

Acknowledgments

The authors wish to Dr. J. Bellone, E. Ciccarelli, and G. Sortino for their cooperation in the study; and Mrs. M. Taliano for her skillful technical assistance.

References


