Endocrine, *vol. 15, no. 1, 29–38, June 2001* 0969–711X/01/15:29–38/$12.50

Growth Hormone–Releasing Hormone Combined with Arginine or Growth Hormone Secretagogues for the Diagnosis of Growth Hormone Deficiency in Adults

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Insulin-induced hypoglycemia (ITT) is currently the "gold-standard" test for the diagnosis of adult growth hormone deficiency (GHD). ITT is often contraindicated, however, particularly in conditions that are also common in patients with suspected GHD. Used alone, GH-releasing hormone (GHRH) has no diagnostic value owing to within-subject variability and the inability to distinguish GHD from normal subjects. When combined with arginine, however, GHRH becomes a potent and reproducible test, which is unaffected by gender and aging, showing excellent specificity. The GHRH+ arginine (ARG) test distinguishes GHD patients from normal subjects and is at least as sensitive as ITT, provided that appropriate cutoff limits are considered. Its reliability for retesting GHD has also been demonstrated. The GHRH+ARG test can also be performed in a shorter procedure, resulting in potential for cost reduction. Synthetic GH secretagogues (GHSs) possess a strong and reproducible GH-releasing effect and synergize with GHRH. The combination of GHRH and a peptidyl GHS, such as hexarelin or GH-releasing peptide-6, has recently been shown as another reliable test for the diagnosis of adult GHD, again provided that the cutoff limit is appropriate to the potency of the test. Thus, GHRH combined with either arginine or GHS is a potential tool for the diagnosis of adult GHD.

Key Words: Growth hormone deficiency; provocative tests; growth hormone–releasing hormone; arginine; growth hormone; secretagogues; insulin-induced hypoglycemia.

Introduction

Adults with growth hormone deficiency (GHD) have impaired health, which improves with GH replacement. GHD in adulthood leads to impairment in body composition and function, as well as to deranged lipoprotein and carbohydrate metabolism leading to increased cardiovascular morbidity *(1,2)*. Based on evidence that GHD in adulthood is a new syndrome that benefits from GH replacement, health authorities in many countries have approved the therapeutic use of GH in hypopituitaric patients with severe GHD *(3,4)*.

GHD is a common finding in adults with acquired hypothalamus-pituitary diseases or, alternatively, is the persistence of a congenital or acquired somatotroph defect that had been diagnosed in childhood because of short stature and/or impaired growth velocity *(3–6)*.

Within an appropriate clinical context, GHD in adults must be shown biochemically by single provocative testing, and insulin-induced hypoglycemia (ITT) has been indicated as the test of choice *(3,7)*. Because ITT has been questioned owing to poor reproducibility and common contraindications, the need for reliable alternative tests with appropriate cutoff limits has been emphasized *(3,4,8)*.

Although GH-releasing hormone (GHRH) alone is not a reliable provocative test *(9–12)*, the diagnostic value of this neurohormone in combination with substances that truly potentiate its GH-releasing activity (e.g., arginine, pyridostigmine, and synthetic GH secretagogues [GHSs]) has been clearly shown *(11–17)*. The GHRH+arginine (ARG) test has already been indicated as the best alternative to ITT *(3, 8)*; moreover, the usefulness of testing with GHRH+GHS has also been reported more recently *(18–20)*.

The aim of this article is to review data showing that testing with GHRH in combination with arginine or GHS represents a reliable diagnostic tool that is preferable to ITT for the diagnosis of adult GHD.

Current Indications for Diagnosis of Adult GHD

Following the consensus guidelines of the GH Research Society and those of the American Association of Clinical Endocrinologists (AACE), within an appropriate clinical context, GHD in adults must be shown biochemically by single provocative testing *(3,8)*.

The measurement of insulin-like growth factor-1 (IGF-1) and IGF-binding protein-3 (IGFBP-3), as well as of spontaneous mean GH concentration, does not establish the diagnosis of adult GHD *(3,7,8,21)*. In fact, mean IGF-1, IGFBP-3, and 24-h mean GH concentrations (even when

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measured by ultrasensitive assay method) are lower in GHD than in normal adults; however, there is significant overlap between the two groups for these parameters *(7,13,22–24)*. The statement that IGF-1 is useless for the diagnosis of adult GHD has recently been toned down; in fact, it has been reported that low IGF-I levels in hypopituitaric patients with multiple anterior pituitary deficiencies may have definitive diagnostic value *(25)*. Our own unpublished results agree with this assumption.

Among provocative tests, ITT is indicated as that of choice *(3,8,26,27)*, and severe GHD to be treated with rhGH replacement is defined by a GH peak lower than the arbitrary cutoff of 3 μg/L *(3,8,25,26)*. As defined by various studies in normal subjects *(7,12,27,28)*, the 3 μg/L cutoff represents the 1st centile limit of the normal GH response, while 5 μg/L is the 3rd centile limit. By assuming 3 μg/L to be the cutoff below which severe GHD is present, it has been demonstrated that ITT distinguishes normal from GH-deficient adults *(1,4,7,14–17,27)*, and it is this evidence that has suggested ITT to be the diagnostic test of choice. However, the reliability of ITT has been questioned by some investigators because of poor within-subject reproducibility *(28–32)*. Moreover, it is reasonable to suspect that the assumption that ITT provides diagnosis of GHD owing to both pituitaric and hypothalamic defects is not definitively reliable. Indeed, ITT induces GH release via activation of hypothalamic mechanisms including hyperactivation of GHRH-secreting neurons and inhibition of somatostatin release *(10,33)*; however, these central mechanisms of action do not protect this test from within-subject variability and false positive responses for GHD in normal subjects *(12,29,30)*.

The major limitation of ITT as a diagnostic tool is that hypoglycemia is not a safe test. In fact, it is contraindicated in certain clinical situations, such as in elderly subjects, as well as in the presence of ischemic heart disease and seizure disorders. Thus, unfortunately, ITT is often contraindicated because these clinical conditions are very common in patients with suspected acquired GHD *(1–3,8,26)*.

The need for reliable alternative tests with appropriate cutoff limits has been emphasized *(3,8)*. Based on the experience in pediatric endocrinology, attention was first focused on the classic provocative tests of GH secretion.

The diagnostic value of clonidine provocative testing has been shown to be very limited. On the other hand, arginine and glucagon alone could be useful but are less discriminatory than ITT *(34–37)*. However, the poor within-subject reproducibility of these classic provocative tests is well recognized *(4,11,12,29,30)*. Poor reproducibility favors false negative and false positive responses, and this probably explains why some researchers suggested that in adults as well as in children suspected for GHD, the diagnosis should rely on lack of GH response to two provocative tests *(4,38,39)*.

Testing with GHRH alone has no diagnostic value *(4,11, 15,16)*. In fact, the mean GH response to the maximal effective dose of GHRH in normal subjects shows dramatic intraindividual variability *(9,40)*. This marked variability is likely owing to spontaneous variations in the hypothalamic somatostatinergic tone *(10,33)*.

The very poor within-subject reproducibility of the somatotroph response to GHRH explains why testing with this neurohormone alone does not distinguish GHD from normal subjects in adulthood as well as in childhood *(11,12)*. Moreover, it is a common opinion that testing with GHRH alone can induce a clear GH response even in hypopituitaric patients with GHD owing to hypothalamic defect, thus missing appropriate diagnosis *(4)*. This assumption derives from the demonstration that short children with isolated GHD shown by lack of GH response to classic provocative tests can normally respond to GHRH *(41–44)*. This possibility seems more unlikely in adults with acquired severe GHD or in subjects who had been diagnosed with severe GHD in childhood.

Testing with the combined administration of GHRH and arginine has been recommended as the most promising alternative to ITT *(3,8)*. In the following paragraphs, we focus on the justification for this recommendation in the GHRS Consensus Guidelines in 1998. In addition, we consider more recent evidence suggesting that this test could be preferable to ITT as a first-choice diagnostic test for GHD in adults.

The GHRH+ARG Test

Rational Basis for Assuming GHRH+ARG as Provocative Test of Somatotroph Function

The spontaneous fluctuations in the hypothalamic somatostatin release are the likely explanation for the marked intraindividual variability of the somatotroph responsiveness to GHRH given alone *(10,33)*. Given alone, GHRH is unable to reliably explore the secretory capacity of somatotroph cells and has no diagnositc reliability *(4,11,15,16)*. That GHRH may elicit no GH response owing to a phase of somatostatinergic hypertone has been clearly shown in animals in which somatostatin antiserum or hypothalamic deafferentation abolished somatostatinergic activity on somatotroph; in fact, the removal of somatostatin influence allows a strong and reproducible stimulatory effect of GHRH on GH secretion *(10)*. Obviously, these experimental approaches are not feasible in humans; however, similar evidence has been provided in humans by testing with GHRH in combination with cholinergic agonists *(33,45)*. There is direct evidence that acetylcholine inhibits hypothalamic somatostatin release *(33)*, and cholinergic agonists such as pyridostigmine and neostigmine truly potentiate the GH response to GHRH, abolishing the intraindividual variability of the somatotroph responsiveness to the neurohormone *(16,40)*. GHRH in combination with pyridostigmine has been shown as one of the most powerful provocative tests of GH secretion and has been proposed for the diagnosis of

GHD in adulthood as well as in childhood *(11–15)*. Unfortunately, the potentiating effect of pyridostigmine on the GH response to GHRH decreases with aging *(13,16)*, and it has been shown that a GHRH+pyridostig-mine test distinguishes GHD from normal subjects in young adulthood but not in elderly subjects *(13)*. Moreover, pyridostigmine often induces classic cholinergic side effects and is contraindicated in clinical situations such as in elderly subjects and in the presence of arrhythmic heart disease *(46)*.

These clinical limitations reduced the diagnostic reliability of the combined test with GHRH and pyridostigmine but suggested that the combination of GHRH with another substance able to reproducibly potentiate its GH-releasing effect independently of age would represent the best provocative test of somatotroph function. Of course, safety should be another key point.

Arginine is an amino acid whose GH-releasing effect was demonstrated more than 30 yr ago *(47,48)*. Arginine alone is still one of the most considered classic provocative tests for the diagnosis of GHD *(4,11,12,36,38)*. The mechanisms of action by which arginine induces GH release have never been definitively clarified, but it is now clear that it probably acts as a neurotransmitter at the central level *(10,33,49)*. There is evidence that arginine does not act at the pituitary level and truly potentiates the GH response to GHRH, making it a powerful and reproducible stimulus of somatotroph secretion *(11–13,16,50)*. This evidence led to the hypothesis that arginine acts via inhibition of hypothalamic somatostatin release, but this hypothesis has never been tested directly; in fact, arginine possesses weak stimulatory effect, if any, in animals, rendering animal models useless in terms of reliability in this context *(10)*.

Indirect evidence, however, supports the hypothesis that arginine has somatostatin-mediated action. The potentiating effect of arginine on the GH response to GHRH overlaps with that of pyridostigmine; the combined administration of arginine and pyridostigmine does not induce any interaction *(45,51,52)*. Moreover, the GH-releasing effect of arginine as well as that of pyridostigmine is refractory to the inhibitory effect of glucose and free fatty acids and to that of rhGH *(16,53,54)*. The inhibitory effects of these later factors operate at least partially via enhanced hypothalamic somatostatin release *(10,16,33)*; thus, arginine, like pyridostigmine, is able to counteract the negative feedback action exerted by GH as well as that exerted by metabolic factors.

Independently of the mechanisms of action, this evidence demonstrated that testing with GHRH in combination with arginine or pyridostigmine represents a very powerful and reproducible stimulus of GH secretion.

To provide preference for the combination of GHRH with arginine, it has also been shown that the GH response to this stimulus is also refractory to the negative feedback action of IGF-1 *(53)* and, above all, is basically independent of aging *(51)*. The GH response to GHRH+ARG in elderly subjects has been found preserved and similar to that recorded in young adults *(13,51)* and normally growing children *(11)*.

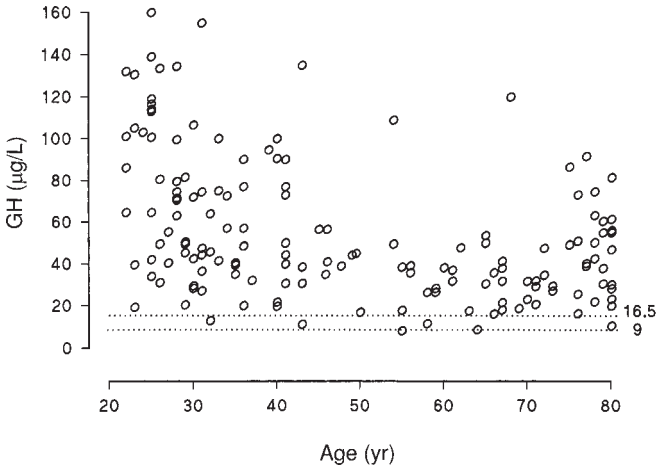

Fig. 1. Peak GH responses to GHRH+ARG test in normal and elderly subjects (n = 157) (dotted lines represent the 3rd centile limit).

The rational basis for considering GHRH+ARG as a reliable provocative test of somatotroph function is therefore its potency, reproducibility, and refractoriness to the majority of negative influences on GH secretion.

Definition of Normal GH Response and Within-Subject Reproducibility

Based on the assumption that GHRH+ARG could be reliable as a provocative test of somatotroph function, we decided to define, in our laboratory, the normative limits of the GH response in adults and elderly subjects; those in a large population of normally growing children had been already established *(11)*. In fact, provocative tests alternative to ITT must be used with appropriate cutoff limits *(3,8)*.

The determination of absolute GH levels depends on assay methods, which show considerable differences *(55)*. In our laboratory, normative values of the peak GH response to GHRH+ARG were defined by an immunoradiometric assay method (HGH-CTK, Sorin, Italy) to assay serum GH levels. This method uses monoclonal antibodies. The sensitivity of our method is 0.15 µg/L; the inter- and intraassay coefficients of variation are 5.1–7.5 and 2.6–5.4%, respectively.

As a test procedure, the following schedule was adopted: acute administration of GHRH (GHRH-29), 1 µg/kg as iv bolus at 0 min, followed by a 30-min iv infusion of 0.5 g/kg of arginine (arginine hydrochloride); blood samples were generally taken every 15 min from −15 to +90 to 120 min.

The 3rd and 1st centile limits of the peak GH response to GHRH+ARG were defined in a population of 157 normal subjects (79 males). The age range was 20–80 yr, with 72 subjects age 20–40 yr, 39 age 41–65 yr, and 46 above age 65. All subjects had body mass index (BMI) ranging from 19 to 26 kg/m^2 (Fig. 1).

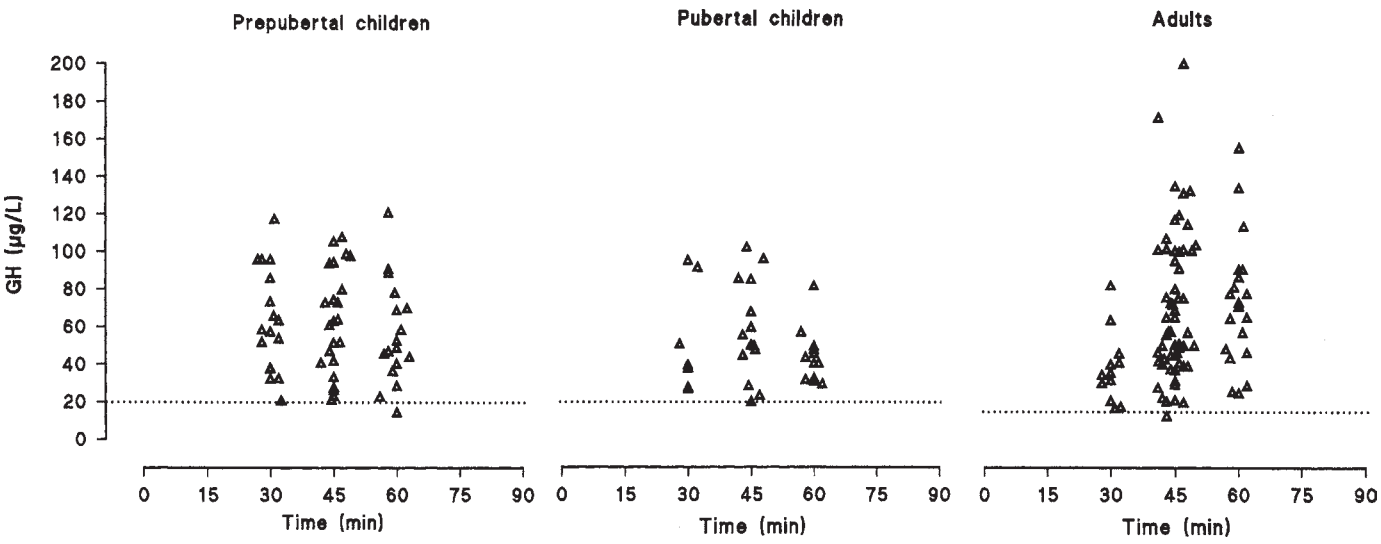

Fig. 2. Individual GH levels at +30, +45, and +60 min after GHRH+ARG test in prepubertal and pubertal children and in adults.

In this population, the 3rd centile limit of the normal peak GH response was 16.5 µg/L, while the 1st centile was 9.0 µg/L. In our hands, the specificity of the test (identifying normal subjects with certainty) was 98.7% above this latter cutoff value. Only 2 of 157 normal subjects with ideal body wt had a GH peak below 9 µg/L, and 6 of 157 had a GH peak below 16.5 µg/L (Fig. 1).

The mean peak GH response to GHRH+ARG in this population was 63.1 µg/L; no significant gender- or age-related difference was found *(12,13)*. The mean peak GH response to GHRH+ARG was clearly higher than that to the majority of other classic provocative tests *(13,56)*. Note that the normative limits of the peak GH response to ITT are one-third of those recorded for GHRH+ARG *(13,56)*. The GH response to GHRH+ARG was independent of gender.

The intraindividual variability of the GH response to GHRH+ARG in two testing sessions was evaluated in both adult and elderly normal subjects *(50)*. The intraindividual reproducibility of the peak GH response to GHRH+ARG was good in both adult and elderly subjects who, again, showed similar mean peak GH response to the test *(50)*.

Classic procedures of provocative tests include GH measurement every 15 min from baseline up to 90–180 min. We have recently shown that the procedure of the GHRH+ARG test in clinical practice can be usefully shortened, evaluating GH levels at only three timing points and in a single session. In 92 young and middle-aged normal adults, GH peaks above the 3rd centile limit (16.5 mg/L) occurred in 99% of subjects within the following timing points: +30, +45, and +60 min after GHRH administration *(57)* (Fig. 2). Thus, the GHRH+ARG test fully mantains its specificity when performed in a shortened procedure, which, of course, simplifies the clinical practice and reduces costs.

Reliability for Diagnosis of Adult GHD

The evaluation of the diagnostic reliability of the GHRH+ARG test is based on the evaluation of the sensitivity (i.e., ensuring that all patients with severe GHD are identified) and the reproducibility of the test in hypopituitaric patients suspected for GHD, compared with the gold-standard ITT test.

It has been shown that the GHRH+ARG test distinguishes adult GH-deficient patients from normal subjects and that it is at least as sensitive as ITT, provided that appropriate cutoff limits are considered *(13,16,21,56)*. In GH-deficient adults as well as in normal subjects, the mean GH response to GHRH+ARG is approximately three times higher than that to ITT *(56)*. Moreover, a strict positive association has been found between the peak GH response to GHRH+ARG and ITT in hypopituitaric patients with GHD *(24,56)*. This strictly positive correlation between GH peaks recorded after ITT and GHRH+ARG contradicts the criticism that testing with the neurohormone may miss the diagnosis of GHD owing to hypothalamic GHRH deficiency.

With respect to appropriate 3rd centile cutoff limits (5.0 and 16.5 µg/L GH peak), GHD was shown by ITT in 90% of patients and by GHRH+ARG in all patients *(56)*. Regarding appropriate 1st centile cutoff limits (3.0 and 9.0 µg/L GH peak), GHD was shown in approx 80 and 90% of patients by ITT and GHRH+ARG, respectively *(56)*.

Assuming 3 µg/L as a unique arbitrary cutoff, only 47.5% of patients could have been shown as having severe GHD by GHRH+ARG. Since the large majority of patients with GH peak above 3 µg/L after GHRH+ARG had shown a peak below 3 µg/L after ITT, it is clear that cutoff levels must be appropriate to the potency of each test. Otherwise, one should assume that ITT had often shown false positive responses indicating severe GHD. This hypothesis has to be ruled out, considering that the majority of these patients had multiple pituitary insufficiencies and low IGF-1 levels (Fig. 3).

In a number of hypopituitaric patients with GHD, the intraindividual reproducibility of the peak GH response to

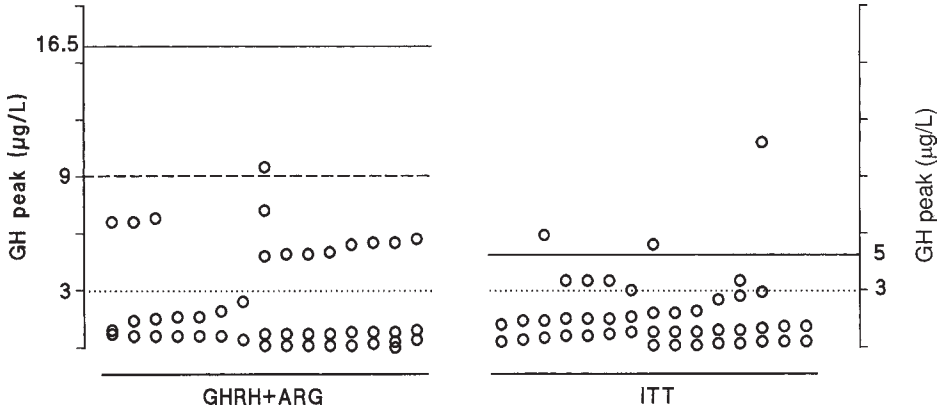

Fig. 3. Peak GH responses to GHRH+ARG or ITT tests in the same adult patients with GH deficiency (n = 44).

GHRH+ARG has also been demonstrated; all hypopituitaric patients tested twice were invariably shown as having severe GHD *(50)*.

Thus, the GHRH+ARG test is at least as sensitive as ITT, provided that appropriate cutoff limits are considered. It has been proposed that a peak GH response to GHRH+ARG below the 1st centile limit of normalcy (i.e., 9.0 µg/L) indicates the existence of severe GHD, which needs GH replacement *(56)*. The within-subject reproducibility of the peak GH response to the test in GHD as well as in normal subjects indicates that GHRH+ARG can be performed in a single test with a shortened procedure, thus reducing time and associated cost *(57)*.

Further ageement about the suggestion that the GHRH+ARG test is as reliable as ITT for the diagnosis of adult GHD *(58)* comes from more recent studies. Biller et al. *(58)* recently presented data comparing the performance of five GH stimulation tests for the diagnosis of adult GHD in patients with pituitary disease and normal control subjects matched to the pituitary patients for age, sex, BMI, and estrogen use. The five tests were ITT, GHRH+ARG, arginine alone, L-dopa, and arginine+L-dopa. Their preliminary analysis of the data suggested that the ITT and the GHRH+ARG tests had greater diagnostic accuracy than the other tests for the diagnosis of adult GHD.

We have screened a population of 200 adult hypopituitaric patients suspected of GHD with the GHRH+ARG test. In agreement with an appropriate clinical context, GHD was demonstrated in 97% of these patients and severe GHD in 93%, while 3% patients showed preserved somatotroph function (Fig. 4). A clear distinction between GHD and normal subjects has also been demonstrated in elderly subjects *(13,59)*. Interestingly, a positive association has also been shown between the peak GH response to GHRH+ARG and IGF-1 levels, bone mineral density, and cardiac performances *(60,61)*, and a negative association has been reported between the alterations in lipid metabolism and the GH response to this test *(62)*. In all, this evidence strongly points toward the reliability of this test to explore the somatotroph function and, indirectly, the activity of the GH/IGF-1 axis. The peak GH response to GHRH+ARG but not to ITT is positively associated with IGF-1 in GH-deficient adults *(24,56)*.

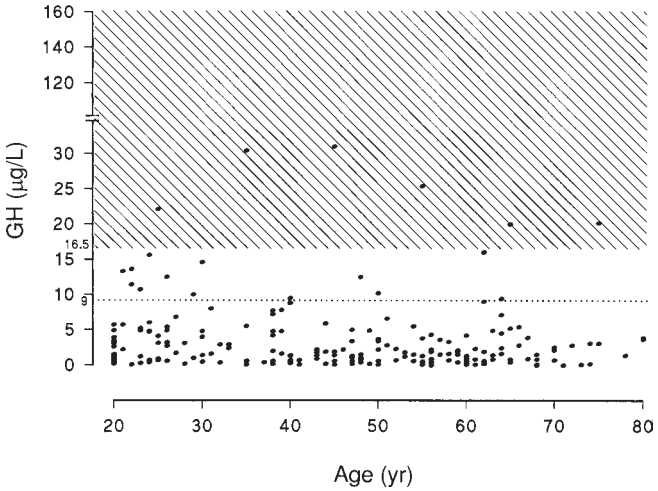

Fig. 4. Peak GH responses to GHRH+ARG test in adults and elderly with GH deficiency (n = 200). Dashed area represents the normal GH response.

Patients with childhood-onset GHD need retesting in late adolescence or young adulthood to verify whether they have to continue rhGH treatment *(3,4,6,8)*. This is achieved by provocative testing *(3,6)*. So far, retesting studies have found different percentages of persistent GHD in adulthood; however, these studies have been performed in different cohorts of GH-deficient patients with classic provocative tests that have poor reproducibility *(63)*. We have recently shown that, given appropriate cutoff limits, GHRH+ARG is as reliable as ITT for retesting patients who have undergone GH treatment in childhood. Severe GHD in adulthood is generally confirmed (>90%) by the GHRH+ARG test, as well as by ITT, in patients with organic GHD and is frequently confirmed (>50%) in patients who have been diagnosed with severe idiopathic GHD *(5)*.

Thus, studies performed to date provide evidence that GHRH+ARG is reliable for the diagnosis of GHD in adults and for retesting the transition adolescents who have suspected persistent severe GHD. It must be emphasized that GHRH+ARG has been indicated as the provocative test with the best specificity even in childhood *(11)*. It is widely accepted that the diagnosis of GHD in childhood cannot rely on provocative tests alone because slowly growing children with insufficiency of spontaneous GH secretion often show a normal response to provocative tests (the so-called GH neurosecretory dysfunction) *(64,65)*. This means that GH insufficiency cannot be definitively ruled out in children who pass provocative tests. Nevertheless, in children as well as in adults, marked impairment to a single provocative test as potent and reproducible as GHRH+ARG strongly points toward the existence of severe GHD, which should be treated by GH replacement without the need for additional endocrinologic investigation.

Another important point favoring GHRH+ARG as the first-choice test for the diagnosis of GHD is that it has an extremely good safety profile and no contraindications. The most common minor side effect is transient facial flushing induced by GHRH, which occurs in approx 30% of the subjects tested. Administering 0.5 g/kg of arginine hydrochloride over 30 min is basically devoid of side effects; increasing the speed of the infusion could induce nausea and vomiting. Chronic renal failure is basically the unique contraindication to arginine load in humans *(39)*.

Potential Limits

Because GHRH acts directly on the pituitary, its administration (both alone and in combination with arginine) could induce clear GH response even in hypopituitaric patients with GHD owing to hypothalamic GHRH defect *(4)*. Were this the case, the test could miss appropriate diagnosis. This possibility seems unlikely, however. In fact, as alluded to before (see previous section), there is a strong positive association between the peak GH after GHRH+ARG and ITT *(24,56)*. Moreover, concordant diagnosis of GHD has been shown by GHRH+ARG and ITT in a large population of hypopituitaric patients with suspected GHD, provided that appropriate cutoff levels are assumed *(56)*.

More recently, a study in children and adults with congenital hypopituitarism was conducted to clarify the relationship between the hypothalamus-pituitary morphology and the somatotroph responsiveness to GHRH+ARG as a maximal provocative test exploring the GH-releasable pool in the pituitary *(66)*. The results demonstrated that the integrity of the hypothalamus-pituitary connection (evaluated by magnetic resonance imaging) is essential for GHRH+ARG as well as for ITT to express its GH-releasing activity. The GHRH+ARG test was found to be a reliable tool for the diagnosis of GHD and induced GH responses comparable with those of the classic provocative tests. Thus, the hypothesis that GHRH+ARG explores the somatotroph function in a way different from that of classic provocative tests does not seem to be supported by clinical evidence; this implies that for GHRH+ARG, as well as for any other provocative test, the diagnostic reliability is expressed by good reproducibility, specificity, and sensitivity.

GHD is only one among several hyposecretory GH states that can occur in physiologic conditions such as in aging or in pathologic states such as obesity, Cushing syndrome, and hypothyroidism, which present with clear GH insufficiency *(53,67–72)*. In fact, these pathophysiologic conditions show clear reduction of both spontaneous and stimulated GH secretion. Thus, a key point regarding the diagnosis of GHD is represented by the ability of provocative tests to distinguish the GHD from other GH hyposecretory states *(3)*.

As discussed, unlike the majority of classic provocative tests *(67)*, the GH response to GHRH+ARG is preserved in normal elderly subjects *(13,59)*, and the test has been shown to be able to distinguish between GHD and normal elderly subjects *(13,59)*. Even in elderly subjects, the peak GH response to GHRH+ARG has been found to be positively associated with IGF-1 levels and negatively associated with lipids, bone markers and density, as well as cardiac performances *(60–62)*. It must be emphasized that there is now considerable evidence indicating that, in the presence of severe GHD, GH replacement can be considered even in aging patients *(3,8)*. However, ITT is contraindicated in elderly subjects because hypoglycemia and the adrenergic response to it could trigger ischemic attack *(3,8)*. Thus, GHRH+ARG is the test of choice to distinguish GHD from normal elderly subjects.

Thyroid hormones have a key role in GH synthesis and secretion *(39,72)*, and, thus, it is not surprising that the somatotroph responsiveness to provocative stimuli is reduced in hypothyroid patients *(72)*. However, it has been demonstrated that the combined administration of GHRH and arginine induces unexpected massive GH discharge in patients with primary hypothyroidism in whom the GH response to GHRH alone is impaired *(73)*. This evidence suggests that the pituitary GH-releasable pool in hypothyroid patients is not as impaired as was previously thought, and that the GH response to GHRH+ARG is not so dependent on the impaired thyroid status. Nevertheless, it is recommended that the evaluation of the secretory capacity of somatotroph cells, by whatever provocative test, be carried out following appropriate replacement therapy *(3,8)*.

Glucocorticoids have dual influence on somatotroph secretion depending on the dose and the length of exposure to high glucocorticoid levels *(74–76)*. It has been reported that, unlike other classic stimuli, GHRH+ARG induces a marked GH response even during short-term treatment with glucocorticoids *(77)*. However, there is also evidence that patients with Cushing syndrome show severe impairment of the GH response to GHRH+ARG, which is often as marked as in hypopituitaric patients with severe GHD *(70,78)*. Thus, even

GHRH+ARG, as well as other provocative tests, does not distinguish GHD from hypercortisolemic patients, but this should not be troublesome if GHD is suspected in the appropriate clinical context.

On the other hand, the confounding effect of obesity on the evaluation of somatotroph function needs particular attention owing to its common occurrence. It is well kown that somatotroph function is negatively associated with body mass *(79)*, and, in fact, both spontaneous and stimulated GH secretion are clearly reduced in obese patients *(53,68,69)*. We have also previously demonstrated that the mean GH response to GHRH+ARG is negatively associated with BMI, being markedly reduced in obese patients *(80)*. Actually, considering individual GH responses, an overlap between obese and GH-deficient patients has been found; in a considerable percentage of obese patients, the GH response to GHRH+ARG is as impaired as that in hypopituitaric patients with GHD *(80,81)*. Taking into account the fact that hypopituitaric patients are often overweight, it is stongly recommended that adult GHD be suspected in the appropriate clinical context; otherwise the distinction between adult GHD and simple obesity may be troublesome even with a provocative test as potent and reproducible as GHRH+ARG.

The marked impairment of the pituitary GH-releasable pool in obesity remains a matter for debate *(53)*. It has been suggested, however, that unexpected GHD should be suspected in adult obese patients with low IGF-1 levels, particularly in the presence of an empty sella *(81)*.

The GHRH+GHS Test

Synthetic GHS is a nonnatural molecule that mimics the activity of the natural GHS receptor ligand (i.e., ghrelin) *(19,82–86)*. Like ghrelin, synthetic GHS possesses a strong and reproducible GH-releasing effect in humans, and this evidence suggested their diagnostic usefulness as a provocative test *(19,82,84)*.

GHS mainly acts via hypothalamic mechanisms *(82)*; the GH response to GHS but not GHRH is almost abolished in patients with hypothalamus-pituitary disconnection *(20, 87,88)*. Moreover, the GH-releasing activity of GHS undergoes marked age-related variations, increasing at puberty and decreasing in aging *(82)*. This evidence implies that age-related normative values are needed to evaluate the GH response to GHS in patients with suspected GHD. In fact, the reliability of testing with GHS alone has been questioned, particularly in prepubertal children and elderly subjects *(16,82,89)*.

On the other hand, evidence that GH-releasing peptides (GHRPs) and GHRH have a true synergistic effect suggested that this combined stimulus could be more useful in provocative testing. Basically, testing with the combined administration of GHRH and GHS represents the same diagnostic approach of GHRH combined with arginine. This assumption is suggested by evidence that GHS acts, at least partially, as a functional somatostatin antagonist *(19, 82)*. The GH response to GHRH is strongly potentiated even by a very low GHS dose *(17,19,82,90,91)*. The GH response to GHRH+GHS represents one of the most potent stimuli of GH secretion *(12,17,19)* and also shows good intraindividual reproducibility *(17,19)*. Like GHRH+ARG, the GH response to GHRH+GHS is partially refractory to the inhibitory effect of glucose and free fatty acid load as well as of rhGH *(90–93)*. Unlike GHRH+ARG, however, the GH response to GHRH+GHS has been found to be dependent on aging *(16,17,82,83,89)*, but this has not been consistently recorded *(94)*. Similarly, the GH response to the combined administration of GHRH and GHS has been reported to be reduced in some studies of obese patients *(53)* but not in other studies of this patient group *(95)*. However, it remains that in aged and obese subjects, testing with GHRH+GHS shows an impressive GH discharge, and this result has to be considered a potent provocative stimulus.

The reliability of testing with GHRH+GHS has been demonstrated in some studies *(12,17,18,20,96,97)*. Leal-Cerro et al. *(96)* first suggested the potential usefulness of testing with GHRH+GHRP-6 for the diagnosis of adult GHD.

The normative values and the reproducibility of testing with GHRH plus low-dose hexarelin, a hexapeptidyl GHS, have been defined in normal young adults. In a large group of hypopituitaric patients with severe GHD, the diagnostic reliability of this test was then compared with that of ITT and GHRH+ARG *(17)*. The results demonstrated that testing with GHRH in combination with a GHS such as hexarelin at low dose is as reliable as ITT and GHRH+ARG, provided that the cutoff limits are appropriate to the potency of the test.

In another study, it was reported that testing with GHRH +GHRP-2, another hexapeptidyl GHS, is reliable for the diagnosis of adult GHD *(97)*. This test showed 100% specificity and 78.6% diagnostic sensitivity even when the GH response was measured as a single assay 30 min after the administration of drug.

Very recently, the reliability of the combined test with GHRH and GHRP-6 has been clearly shown in an extensive study *(18)*. Normative values were first defined in 125 healthy individuals, and then the same number of adult patients with organic pituitary disease was studied. The GHRH+GHRP-6 test was more potent and safe than ITT in both normal subjects and hypopituitaric patients. When individually analyzed, GH peaks after GHRH+GHRP-6 were a continuum. Good distinction between GHD and normal adults was ensured by assuming a 15 μg/L GH peak as the cutoff level. However, for clinical utility, the researchers proposed that values above a 20 μg/L GH peak be considered normal and those below 10 μg/L be considered as true GHD. The diagnosis of patients with suspected hypopituitarism with a GHRH+GHRP-6-induced GH peak between 10 and 20 μg/L should be considered uncertain and the final

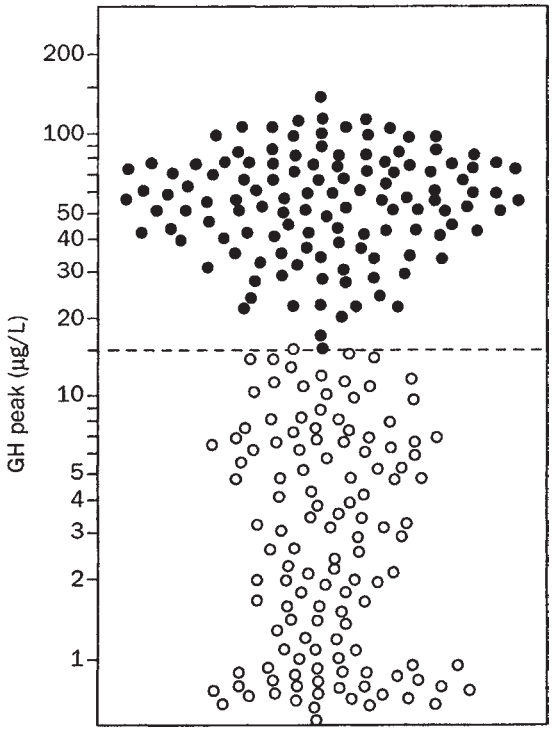

Fig. 5. Individual GHRH plus GHRP-6-mediated GH peaks in controls (●) and case ( ). (Reproduced from ref. *18* with permission.)

diagnosis based on either considering the appropriate clinical context or doing a second provocative test (Fig. 5).

As well as synthetic GHS, the natural ligand of the GHS receptor, ghrelin *(86)*, should be considered as a possible useful provocative stimulus to evaluate somatotroph function. It has already been shown in humans that ghrelin exerts a strong dose-dependent stimulatory effect on GH secretion, releasing more GH than both GHRH and synthetic GHS *(98–100)*. Moreover, while ghrelin has no interaction with synthetic GHS, it produces a true synergistic effect with GHRH *(100)*, inducing an impressive amount of GH secretion from the pituitary in normal subjects.

Conclusion

GHRH+ARG is as sensitive and as specific as ITT for the diagnosis of adult GHD, provided that appropriate cut-off limits are considered. Testing with the combination of GHRH and peptidyl GHS is another alternative to GHRH+ARG and ITT. Thus, GHRH in combination with substances such as arginine and GHS has a place as a diagnostic tool for adult GHD.

Based on this evidence, the following guideline for the hormonal diagnosis of adult GHD can be proposed. In the appropriate clinical context, the measurement of IGF-1 should be a first step. Low IGF-1 levels in patients with multiple pituitary deficits, as well as in patients with genetic total GHD, can be definitive evidence of severe GHD in adults who should not require further provocative testing. However, taking into account that normal IGF-1 levels do not rule out GHD, it is recommended that patients with suspected GHD but showing normal IGF-1 levels undergo provocative testing. Based on evidence discussed in this review, we propose that the GHRH+ARG/GHS test be considered as the first-choice diagnostic test that is devoid of side effects and risks.

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