Is Lack of Recombinant Growth Hormone (GH)-Releasing Hormone in the United States a Setback or Time to Consider Glucagon Testing for Adult GH Deficiency?

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Context: The use of the combined GHRH and arginine (GHRH-ARG) test has gained increasing acceptance in the United States as a reliable alternative test to the insulin tolerance test (ITT) for diagnosing adult GH deficiency (GHD). In July 2008, the only manufacturer of recombinant GHRH in the United States, EMD Serono, Inc., announced the discontinuation of Geref, thus raising the question of which reliable alternative GH stimulation test should practicing endocrinologists be considering in place of the GHRH-ARG test. In this article, we review the existing published data and consensus guidelines and provide recommendations for alternative stimulation tests to the GHRH-ARG test.

Evidence Acquisition: The major source of data acquisition included PubMed search strategies and personal experience of the authors from clinical experience.

Evidence Synthesis: Previous consensus guidelines and previous data assessing the reliability and discriminatory value of the GHRH-ARG, glucagon, ARG, and GH secretagogues on assessing GH reserve are discussed. Our recommendations for performing the glucagon stimulation test, potential drawbacks in conducting this test, and caveats in interpreting this test are also discussed.

Conclusions: The ITT should remain the test of choice in diagnosing adult GHD. However, when the ITT is not desirable and recombinant GHRH remains unavailable in the United States, we recommend the alternative to the GHRH-ARG test to be the glucagon stimulation test, based on its reliability and availability. Nevertheless, further studies into alternative GH stimulation tests that are available in the United States, comparable, and simpler to perform than the ITT in diagnosing adult GHD are still needed. (J Clin Endocrinol Metab 94: 2702–2707, 2009)
most reliable alternative GH stimulation test to the insulin tolerance test (ITT) in diagnosing adult GHD. The rationale for using the GHRH-ARG test to diagnose adult GHD is based on the assumption that in healthy normals, GHRH triggers the release of GH from the pituitary somatotroph cells, whereas ARG is believed to potentiate GHRH-stimulated somatotroph secretion via inhibition of hypothalamic somatostatin release (10, 11). This combination of both direct stimulation and reduction of inhibitory somatostatin results in a robust release of GH from the pituitary gland (4–6). In pituitary GHD, the acute responsiveness of the pituitary somatotroph cells is diminished and fails to mount an adequate GH response to GHRH or ARG. With the use of the GHRH-ARG test in clinical practice reported to be on the rise in the last decade in the United States (12), the decision made by EMD Serono, Inc., to discontinue the manufacture of Geref has inevitably raised the question of which reliable alternative GH stimulation test should be used in place of the GHRH-ARG test when recombinant GHRH is unavailable. This is especially relevant to practicing endocrinologists who are not equipped with the facilities, resources, and personnel to conduct the ITT in an office setting. It is also an important question for patients who have contraindications to hypoglycemia such as a history of seizure disorder or coronary artery disease, or who prefer to avoid the unpleasant hypoglycemic side effects of the ITT. Thus, there remains a clinical demand for an alternative test to the ITT that is safe and reliable. In this article, we offer our interpretation of the existing data and provide recommendations for alternative stimulation tests to the GHRH-ARG test, at least until there is access to recombinant GHRH from another source or a better GH secretagogue that becomes available in the United States in the future.

Update on Current GH Stimulation Tests Commonly Used in the United States for Diagnosing Adult GHD

The syndrome of adult GHD is now considered a well-recognized clinical entity, associated with a number of metabolic abnormalities (13). Current published consensus guidelines recommend that the evaluation of this syndrome with provocative biochemical testing should be considered in patients with appropriate clinical context that includes evidence of hypothalamic-pituitary disease, previous history of cranial irradiation, previous history of traumatic brain injury or subarachnoid hemorrhage, or patients with childhood onset GHD (8, 9). In patients with at least three pituitary hormone deficiencies and a low serum IGF-I level, no further testing is required because the probability of adult GHD being documented on stimulation testing is well over 90% (14). However, if the patient has two or fewer pituitary hormone deficiencies with normal or low serum IGF-I levels, then further GH stimulation testing is required (8, 9).

In the GH Research Society (GRS) guidelines first published in 1998 (15), the ITT was considered the test of choice, followed by the glucagon and ARG tests as acceptable alternatives, whereas the GHRH-ARG test was then proposed as a promising option. In 2006, The Endocrine Society guidelines indicated that the ITT and the GHRH-ARG test have adequate sensitivity and specificity for the diagnosis of adult GHD, with the exception of recent hypothalamic injury, when the latter may be misleading (9).

The ITT is still widely regarded as the gold standard test for diagnosing adult GHD despite concerns about its safety (16, 17), reproducibility, and specificity (18–20). Nevertheless, the test has several limitations in clinical practice. The test is contraindicated in adults with seizure disorders and patients with ischemic heart disease, and it is relatively contraindicated in the elderly because the underlying concern is that occult vascular disease increases with age, making the stress of hypoglycemia more of a potential risk in older subjects. The hypoglycemia induced during an ITT also causes an adrenergic response that may be unpleasant for some patients. Furthermore, the ITT requires close monitoring by trained medical personnel to ensure that adequate hypoglycemia (blood glucose nadir level <40 mg/dl) is achieved without inducing dangerous neuroglycopenia and that correction of the insulin-induced hypoglycemia is appropriately instituted. For all of these reasons, sensitive and reliable alternative GH stimulation tests are needed.

Previous studies by Aimaretti et al. (4), Biller et al. (5), and Corneli et al. (6) have shown that the GHRH-ARG test demonstrated excellent sensitivity and specificity both in childhood-onset and in adult-onset GHD, assuming appropriate cutoff limits are employed. The recommendation to use the GHRH-ARG test by the recent consensus guidelines from the GRS (8) and The Endocrine Society (9) is based on the review of several studies published since 1998 (4–7) demonstrating that this test is well validated in adults with 95% sensitivity and 91% specificity at a GH cutoff of 4.1 μg/liter and compares very well to the ITT. The advantage of the GHRH-ARG test is that this test is easier to perform than the ITT in an office setting, is generally well-tolerated, has a favorable safety profile, and has few contraindications (i.e. poor vascular access and sensitivity to GHRH). Furthermore, the peak GH response to GHRH-ARG stimulation has been shown to be independent of age, and there is less inter- and intraindividual variability compared with other stimulation tests (5). However, the GHRH-ARG test has its limitations. Because GHRH directly stimulates the pituitary, it can yield misleadingly normal responses in hypothalamic GHD because the defect is bypassed when exogenous GHRH directly stimulates the pituitary somatotroph cells (21, 22). The peak GH response to GHRH-ARG stimulation is also blunted in healthy obese subjects (23–25), whereas it may be exuberant in certain patient populations such as those with fibromyalgia (26). With the confounding effect of excess weight on peak GH levels, the GHRH-ARG test is not as reliable as other tests such as the insulin tolerance test (ITT) and the GHRH-ARG test have adequate sensitivity and specificity for the diagnosis of adult GHD, with the exception of recent hypothalamic injury, when the latter may be misleading (9).

With the publication of the consensus guidelines by the GRS in 1998 (15) and updated in 2007 (8), and The Endocrine Society in 2006 (9), the GHRH-ARG test has gradually gained increasing acceptance in clinical practice in the United States as the alternative test of choice to the ITT to diagnose pituitary GHD.
Recent data from the Hypopituitary Control and Complications Study surveillance database further confirm that although the ITT is still the most widely used test, the use of the GHRH plus ARG as a diagnostic test was reported to have increased from 1.4 to 21.2% between 1996 and 2005, whereas the use of other less validated tests such as ARG alone, L-dopa, and clonidine have declined from 29.2 to 13.5%, from 11.1 to 5.8%, and from 5.5 to 1.3%, respectively, over the same time period (12).

Recommended Approach to Diagnosing Adult GHD When GHRH Is Not Available

The diagnosis of adult GHD has proved to be challenging because of the lack of a single biological end-point such as growth failure, and therefore, the confirmation of adult GHD largely depends on biochemical provocative testing. Clearly, there is no ideal stimulation test, and we recommend that the decision to embark on a stimulation test to diagnose adult GHD must factor in the appropriate clinical context of each individual patient together with the number of pituitary hormone deficiencies plus serum IGF-I level (14), the validity of the chosen test and its appropriate cutoff limits, the sensitivity of the GH assay, and the availability of local resources and expertise.

Based on available data, our recommended test for diagnosing adult GHD when an ITT is not desirable and recombinant GHRH is not available is the glucagon stimulation test (GST). Glucagon was first reported in the 1970s to have a high degree of accuracy in discriminating between normal and hypopituitary subjects (29–31). A number of studies have since shown that the GST is capable of stimulating not only GH but also ACTH release (32–34); however, further data regarding the validity of this test in assessing the hypothalamic-pituitary-adrenal axis are still required.

The GST is simple to perform (Table 1), and glucagon is readily accessible because it is widely available for treating hypoglycemic episodes in patients with diabetes. In addition, glucagon is relatively inexpensive (the current average wholesale price of recombinant DNA glucagon is approximately $50–$70 per single 1-mg dose, whereas Geref and ARG are approximately $80–$130 per single 50-μg dose and $10–$12 per single 30-g dose, respectively). Glucagon appears to be reasonably well-tolerated, with the only contraindication being in patients who are malnourished or have not eaten for more than 48 h (33, 35). The GST was originally described as a 4-h test in older studies (29, 31), but more recent studies have suggested that it could be shortened to a 3-h test and that serum GH levels can be evaluated between three to five time points only (0, 90, 120, 150, and 180 min) because the majority of GH peaks occurred between 120 and 180 min (85%) (33, 34). Furthermore, the shortened GST appears to retain its diagnostic utility and could simplify the test in clinical practice, which reduces costs and resources. However, it is still not clear whether the ideal timing of the GST is 3 vs. 4 h, and continuing the test for 4 h may be advisable, at least until there are more data available. This also allows the monitoring for late hypoglycemia, although truly low blood glucose levels are not common. Although the lowest blood glucose level with the GST in the literature was reported at 37 mg/dl (36), in our experience, we have rarely observed blood glucose levels falling below 40 mg/dl with this test. One drawback, nevertheless, is that glucagon has to be administered im because this route of administration stimulates GH release better than the iv route (37).

Table 2 shows five publications of the GST in assessing the GH reserve in normal adults and in patients with hypothalamic-pituitary disease. Studies by Rahim et al. (38) and Aimaretti et al. (39) are the two most recent studies that have extensively compared the GH response of glucagon to different GH secretagogues in normal adults, whereas studies by Gomez et al. (40) and Conceicao et al. (41) are the only two studies that compared the GH response of the GST to the ITT in adult patients with hypothalamic-pituitary disease to determine its cutoff value and its sensitivity and specificity. In normal adults, the GST has been shown to be at least equal to the ITT in assessing the GH reserve in hypopituitary adults (38, 39), and it provided a clear separation between GH-deficient and normal adults (40, 41). To reliably identify controls and patients, Gomez et al. (40) and Conceicao et al. (41) demonstrated that the GST with a cutoff GH level of 3 μg/liter provided the best pair of sensitivity (100 and 97%, respectively) and specificity (100 and 88%, respectively) when evaluated by the receiver-operating characteristic curve analysis (Figs. 1 and 2). These two studies included age-matched normal controls and adult patients with common etiologies of GHD (40, 41). Finally, the audit by Leong et al. (33) demonstrated that the GST is safe and well tolerated in a large number of patients with hypothalamic-pituitary disease and that the duration of the GST could be shortened by omitting the 240-min blood sample.

The mechanism by which glucagon induces GH release remains unclear. Some of the hypothesized mechanisms include the glycemic fluctuations during the test where blood glucose levels increase initially before decreasing later in the test (33), the generation of a

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**TABLE 1. Recommended protocol for performing the GST in assessing GH reserve in adults**

<table>
<thead>
<tr>
<th>Procedure</th>
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<tr>
<td>Ensure patient is fasted from 2400 h</td>
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<td>Weigh patient</td>
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<tr>
<td>Patient in recumbent position and iv cannula inserted for iv access between 0800 and 0900 h</td>
</tr>
<tr>
<td>Glucagon administered im 1 mg (1.5 mg if patient weighs more than 90 kg)</td>
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**Biochemical measurements**

- **Serum GH and capillary blood glucose levels at 0, 30, 60, 90, 120, 150, 180, 210, and 240 min**

**Normal response**

- GH: rises to above 3 μg/liter
- Blood glucose: usually rises to peak around 90 min and then gradually declines (not used in the interpretation of the test)

**Interpretation**

In adults with GHD, peak GH level fails to rise above 3 μg/liter
peptidyl fragment associated with the GH- and ACTH-releasing activity (42), and the induction of norepinephrine secretion in stimulating GH release via \( \beta \)-receptors (33). Unlike the GHRH-ARG test, no correlation was observed between BMI and peak GH response to the ITT and GST in GH-deficient adults; however, an inverse relationship between BMI and age with peak GH response was found in the control subjects with the GST (40). It is important to note that the GH-deficient adults in this study had higher BMI values than the controls; nevertheless, these data suggest that there is a potential association between relative, but not functional, GHD of obesity and aging with BMI (40).

Like other GH stimulation tests, there are also limitations associated with the GST. The 3- or 4-h GST is still longer than many other GH stimulation tests and requires an im injection that may be unappealing to some patients. Side effects of nausea, vomiting, and headaches have been reported in 10 to 34% of patients tested (Table 2) (33, 35). However, because there is a relationship between peak GH response to GHRH-ARG stimulation and ambient glucose levels (43), it is unknown whether hyperglycemia may play a part in influencing the peak GH response to glucagon stimulation. Furthermore, no peak GH responses have been studied using the GST in normal controls over the age of 70 yr, and none of the previous studies included patients with diabetes. Therefore, it is not known whether testing using the GST in subjects with diabetes is valid. Hence, caution

![FIG. 1. Receiver operating characteristics curve assessment determining the efficacy of peak GH response during the GST to identify patients and controls. The chosen peak GH cutoff of 3 \( \mu \)g/liter demonstrated the best sensitivity (100%)/specificity (100%). Adapted from Gomez et al. (40).](https://academic.oup.com/jcem/article-abstract/94/8/2702/2596349)

![FIG. 2. Peak GH response to the GST in patients (n = 33) vs. controls (n = 25). Adapted from Conceicao et al. (41).](https://academic.oup.com/jcem/article-abstract/94/8/2702/2596349)
is recommended when interpreting normal results of the GST in the patients with diabetes, and if the suspicion of GHD remains high in these patients, it is reasonable to consider using a second GH stimulation test.

Other provocative tests that have been proposed include ARG alone and GH secretagogues. ARG alone has been shown to be less reliable than the ITT or GHRH-ARG (5), and the mean peak GH response to ARG alone is lower than in the ITT or GST, even in normal lean subjects (38). The diagnostic reliability of ARG alone has been previously questioned (5, 39). Thus, we recommend that ARG alone should only be considered if the ITT and the GST are contraindicated or if glucagon is unavailable. If this test is used, appropriately low peak GH cutoffs should be employed (for 95% sensitivity, 1.4 μg/liter; for 95% specificity, 0.21 μg/liter; and to minimize misclassification in either direction, 0.4 μg/liter) (5). In contrast, the reliability of testing with GH secretagogues such as GH-releasing peptide-2 alone (44), GH-releasing peptide-6 alone, and combined GH-releasing peptide-6 plus GHRH (45) in comparison with the ITT has also been demonstrated. These agents use the same concept as the GHRH-ARG test in stimulating pituitary GH release by mimicking the activity of the natural GH secretagogue receptor ligand (i.e. ghrelin), and they appear to demonstrate a good safety profile with relatively few contraindications (46). The limitation of these GH secretagogues, however, is that these agents are more likely to explore the pituitary somatotroph releasable pool and might potentially induce misleadingly normal peak GH responses in hypothalamic GHD (47). Furthermore, these agents are not available as yet in the United States.

Conclusions

In line with recent published consensus guidelines (8, 9), we agree that the ITT remains as the test of reference due to its greatest diagnostic accuracy, even in patients with suspected hypothalamic GHRH deficit. We recommend that the alternative to the GHRH-ARG test become the GST for diagnosing adult GHD for the following reasons: 1) accurate and reliable discrimination between normal and true GHD; 2) availability; 3) reproducibility; 4) safety; and 5) lack of influence by BMI, gender, or hypothalamic cause of GHD. Despite some studies demonstrating the comparability of the GST to the ITT in assessing the hypothalamic-pituitary-adrenal axis (32, 38, 48), further larger, well-controlled studies are still needed to confirm the reliability of the GST in assessing this axis. If the GST can be shown to reliably distinguish adenohypophysis sufficiency from insufficiency, then the ability of assessing both the GH and cortisol reserve simultaneously, just as in the ITT, would make this test even more attractive. Although previous studies have shown that the GST could be shortened from 4 h to 3 h and yet maintain its diagnostic utility (33, 34), we would still recommend that the GST be conducted over 4 h with measurements every 30 min for serum GH and capillary blood glucose levels primarily to ensure that delayed peak GH responses and late hypoglycemia are not missed (Table 1). It is noteworthy that recombinant GHRH is available in Europe, manufactured by pharmaceutical companies other than EMD Serono, Inc. Hence, in the United States, the onus is now for another pharmaceutical company to take up the baton and provide recombinant GHRH so that the GHRH-ARG test can be used again. Although further studies into alternative GH stimulation tests that are available, comparable, and simpler to perform than the ITT are still required, in the current difficult situation with the unavailability of Geref in the United States, we recommend the use of the GST as the alternative test to the ITT in the place of the GHRH-ARG test for diagnosing adult GHD.

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