Glucagon Stimulation Testing in Adult Growth Hormone Deficiency—A 2014 Update

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Abstract

Growth hormone deficiency (GHD) is a well-recognized clinical syndrome in adults, and its diagnosis is established through one or more GH stimulation tests. The decision to perform GH stimulation testing should be based on medical history and clinical findings, and using appropriate GH stimulation test/s for biochemical confirmation. The insulin tolerance test (ITT) remains the diagnostic test of choice; however, this test is labor intensive, contraindicated in the elderly and in adults with seizure disorders and ischemic heart disease, can be unpleasant for the patient, and is potentially hazardous. With the discontinuation of the growth hormone releasing hormone (GHRH) analog (Geref®) in the US in 2008, the glucagon stimulation test (GST) has gained increasing popularity as the alternative test to the ITT because of its availability, reproducibility, safety, lack of influence by gender and hypothalamic cause of GH deficiency (GHD), and relatively few contraindications. In this article, recommendations for performing this test, the potential drawbacks in conducting and caveats in interpreting this test, and its future perspectives are discussed.

Keywords

Growth hormone, glucagon, insulin tolerance test, growth hormone releasing hormone plus arginine, diagnosis, adult growth hormone deficiency

Disclosure: Kevin CJ Yuen, MD, FRCP (UK), has received research support from Pfizer, Novo Nordisk, and Versartis.
Received: May 5, 2014 **Accepted**: May 21, 2014 **Citation**: *US Endocrinology*, 2014;10(1):75–8 *DOI: 10.17925/USE.2014.10.01.75*
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Growth hormone deficiency (GHD) in adults is characterized by alterations in body composition, carbohydrate and lipid metabolism, bone mineral density, cardiovascular risk profile, and quality of life.[1] Treatment with GH replacement has been shown to improve many, but not all, of these abnormalities.[2] By contrast, untreated GHD is associated with increased mortality and morbidity that was previously observed in adults with hypopituitarism.[3,4] These findings were substantiated in two large surveys based on national Danish registries,[5,6] where the morbidity of adults with GHD was found to be approximately threefold higher than that of a healthy population. This result was independent of gender and applied to patients with childhood-onset and adult-onset GHD, with mortality of childhood-onset GHD far exceeding that of adult-onset GH deficient patients.[6]

Current published consensus guidelines recommend the evaluation of adult GHD to be based on clinical findings, medical history and using the appropriate GH stimulation test for biochemical confirmation,[7–9] with the exception of patients with three or more pituitary hormone deficiencies and low serum insulin-like growth factor 1 (IGF-1) levels.[10] Serum IGF-1 levels should not be used alone to diagnose adult GHD and the maximum or peak GH secretion following GH stimulation testing is used as a surrogate of the capacity of the pituitary to release GH. The insulin tolerance test (ITT) is widely considered as the gold standard test for evaluation of GH deficiency and has been endorsed by several consensus guidelines.[7–9,11] However, this test is labor intensive, may be unpleasant for some patients, has potential risks, and is contraindicated in elderly patients and in patients with seizure disorders and ischemic heart disease. Thus, there remains a real unmet medical need for an alternative test to the ITT that is safe yet reliable. For this reason, several other dynamic tests have been proposed such as arginine (ARG), combined GH releasing hormone plus ARG (GHRH-ARG), levodopa (L-DOPA) in spite of data indicating poor performance of some of these tests for evaluation of adult GHD.[10,12] A potential alternative to the ITT is the glucagon stimulation test (GST) that has been used extensively in the UK,[13] and is now increasingly utilized in the US.[14]

Historical Perspective of the Use of Glucagon Stimulation Test in Diagnosing Adult Growth Hormone Deficiency

Following the publication of several validation studies[12,15–17] and recommendations from current consensus guidelines,[7–9,11] the GHRH-ARG test was accepted as the most reliable alternative GH stimulation test to the ITT in diagnosing adult GHD. However, when EMD Serono, Inc decided to discontinue the manufacture of recombinant GHRH (Geref®) in the US in July 2008,[18] this inevitably left a significant gap for an alternative reliable test for evaluation of patients suspected to have GHD, in place of the GHRH-ARG test. This is particularly important for endocrinologists in the US who are not comfortable or do not have the necessary logistic or staff support to conduct ITTs in their office or patients who have contraindications to hypoglycemia in whom the ITT would be inappropriate. With the lack of reliable GH stimulation tests available in the US, we have recommended

Table 1: Recommended Protocol for Performing the Glucagon Stimulation Test in Assessing Growth Hormone Reserve in Adults

Contraindications
Malnourished patients or patients who have not eaten for >48 hours
Precautions
Patients may feel nauseous during and after the test (administration of intravenous anti-emetics can be considered)
Late hypoglycemia may occur (patients should be advised to eat small and frequent meals after completion of the test)
Procedure
Ensure patient is fasted from midnight
Weigh patient
Patient in recumbent position and intravenous cannula inserted for intravenous access between 8 am to 9 am
Glucagon administered intramuscularly 1 mg (1.5 mg if patient weighs more than 90 kg)
Sampling and Measurements
Serum GH and capillary blood glucose levels at 0, 30, 60, 90, 120, 150, 180, 210, and 240 minutes
Normal Response
Blood glucose: usually rises to peak around 90 minutes and then gradually declines (not used to interpret the test)
Serum GH: peak GH levels tend to occur between 120 to 180 minutes with GH levels peaking to above 3 ng/ml
Interpretation
In adults with GHD, peak GH levels fails to rise above 3 ng/ml

GH = growth hormone; GHD = growth hormone deficiency.

the GST as the alternative test to the ITT for diagnosing adult GHD based on its availability, reproducibility, safety, lack of influence by gender and hypothalamic cause of GHD, and relatively few contraindications.[14]

Analyzing the data of 13,167 GH-deficient patients enrolled in the KIMS pharmaco-epidemiologic database (Pfizer International Metabolic Database) from its inception to the end of 2008, Brabant et al. addressed the question of whether there were regional differences in the use of different biochemical tests to diagnose adult GHD in six large European countries and the US.[13] This analysis revealed striking regional variations in the approach to GH stimulation testing. The ITT was the most popular test used in 44.3 % of all countries but was less popular (13.3 %) in the US ranking second after the ARG test, whereas the GST was ranked third in the US, being the most popular in the UK (29.9 %) and the least popular in Germany and the Netherlands (0.1 %). However, the unavailability of the GHRH-ARG test in the US since 2008 has resulted in a change in recent years with the GST being more frequently used as the alternative test to the ITT.[14]

The utilization of the GST for the assessment of GH reserve was first described in 1969 by Mitchell et al.[19] Since then, the GST has been shown by various investigators to have a GH secretory potency that is similar to or only slightly less than the ITT, suggesting that it is more reliable than other classic agents such as ARG or clonidine for separating GHD patients from normal subjects.[20–24] The true mechanism by which glucagon induces GH release is still a mystery. Some of the hypothesized mechanisms include the glycemic fluctuations during the test where blood glucose levels increase initially before decreasing later in the test,[25] the generation of a peptidyl fragment associated with the GH- and adrenocorticotropic hormone (ACTH)-releasing activity,[26] and the induction of norepinephrine secretion in stimulating GH release via α-receptors.[27] It has also been previously demonstrated that glucagon stimulates GH release more effectively when administered intramuscularly or subcutaneously compared with the intravenous route.[23]

The three studies utilizing the GST by Gomez et al.,[28] Conceicao et al.[22] and Berg et al.[21] evaluated GHD in patients with pituitary disorders. The first two studies[22,28] were prospective studies that compared the diagnostic characteristics of GST to ITT and included a control group which was matched for age and sex in both studies and for body mass index (BMI) in one study.[28] Using receiver operated curve (ROC) analysis, both studies proposed a peak GH cut-off value of 3 ng/ml as the best cutpoint with the highest combined sensitivity and specificity to differentiate between patients with GHD and healthy controls.[28,29] Additionally, Gomez et al.[28] found no correlation among age, sex, and BMI with peak GH levels in patients with hypopituitarism, but there was a significant negative correlation between age ($r=-0.389$; $p=0.0075$) and BMI ($r=-0.329$; $p=0.025$) with peak GH levels in healthy controls. It is important to note that the GH-deficient adults in this study had higher BMIs than the healthy controls; nevertheless, these data suggest that there is a potential association between relative, but not functional, GH deficiency of obesity and aging with BMI. By contrast, the study by Conceicao et al.[22] demonstrated that peak GH levels were not affected by age in either the control or patient group, and that there were no gender differences. It is, however, noteworthy that in the study by Gomez et al.,[28] the dose of glucagon administered was 1 mg for subjects that weighed 90 kg or less and 1.5 mg for subjects that weighed more than 90 kg whereas in the study by Conceicao et al.,[22] all subjects received 1 mg of glucagon. Furthermore, there were slightly more females in the study by Conceicao et al.[22] compared with the Gomez et al.[28] study. On the other hand, the study by Berg et al. was a retrospective study that revealed an optimal peak GH cut-off value of 2.5 ng/ml with 95 % sensitivity and 79 % specificity using ROC analysis.[21] This study also reported lower peak GH levels with GST compared with ITT (5.1 versus 6.7 ng/ml; $p<0.01$), but a significant positive correlation between peak GH levels during ITT and GST ($r=0.88$; $p<0.0001$). Additionally, no correlation between BMI and age to peak GH responses were observed, peak GH responses occurred mainly between 120 and 180 minutes consistent with previous studies,[27,30] and that, overall, the GST was a well tolerated test. Nevertheless, these[21,22,28] and previous studies[20,23,24,31] did not specifically evaluate patients with glucose intolerance and frank diabetes and, for this reason, the characteristic of the GST and its reliability in testing for GHD in this population remains unclear. To address this question, we evaluated GSTs performed in 515 patients, and found that BMI, fasting, peak, and nadir glucose levels correlated negatively with peak GH levels.[32] These data therefore suggest that lower GH cutpoints may be needed if the GST is to be used to reliably evaluate the GH reserve in hyperglycemic overweight/obese patients, but requires further confirmation with larger prospective studies. This is clinically relevant because performing ITT in patients with diabetes and/or obesity can be challenging and may not be safe especially as large insulin doses are usually required to induce symptomatic hypoglycemia in these patients with underlying insulin resistance.

Other Considerations in Performing and Interpreting the Data of the Glucagon Stimulation Test

Unlike GHD in children, 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-1 level,[10] the validity of the chosen test and its appropriate cut-off limits, the sensitivity of the GH assay, and the availability of local resources and expertise.

The GST is a simple and safe test to perform (see *Table 1*). 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, while Geref® and ARG are approximately $80–$130 per single 50 µg and $10–$12 per single 30 g dose, respectively). Glucagon appears to be well-tolerated and is only relatively contraindicated in patients with malnourishment or have not eaten for more than 48 hours due to concern of prolonged hypoglycemia and those with pheochromocytoma in whom a significant exacerbation of blood pressure may be observed.[25]

The GST was initially described as a 4-hour test in older studies,[33,34] but more recent studies have suggested that the test could be shortened to a 3-hour test, and that serum GH levels can be evaluated between 3 to 5 time points only (0, 90, 120, 150, and 180 minutes) as the majority of GH peaks occurred between 120 and 180 minutes (85 %).[27,31] In a study by Orme et al. comparing standard and simplified GST (0, 150, and 180 minutes), 75 % of discordant GH results were due to a peak GH level occurring at 210 minutes.[31] Accordingly, the authors proposed that the diagnostic utility of the simplified GST could be improved further by drawing an additional blood sample at 210 minutes when assessing GH deficiency. The audit by Leong et al.[27] reported that the GST could be shortened by omitting the 240-minute blood sample. In this study of 414 patients, the majority of peak GH levels occurred between 120 and 180 minutes (85 %), and five patients had their peak GH levels recorded at 240 minutes. Hence, it is still not clear whether the ideal timing of the GST is 3 versus 4 hours, and continuing the test for 4 hours may be advisable, at least until there are more definitive data available. This also allows monitoring for late hypoglycemia, although truly low blood glucose levels are not common. While the lowest blood glucose level with the GST in the literature was reported at 37 mg/dl,[19] overall, the occurrence of hypoglycemia with blood glucose levels lower than 40 mg/dl during GSTs are somewhat rare events.[21,27]

The common side effects in patients with hypothalamic-pituitary disease that underwent testing with the GST included nausea, vomiting, and headaches, and have been reported to range from less than 10 %[21] to 34 %.[27] In a study of 97 normal subjects, mild nausea in approximately 30 % of the subjects, and transient vomiting and retching in about 10 % of the subjects, were the only side effects that were noted.[35] In our recent study of 515 GSTs, the main side effects reported were nausea (ranging from 37.2 to 44.4 %), hunger, headaches, sleepiness, body chills, lightheadedness, and abdominal cramping that occurred mainly between 60 and 210 minutes, with rapid resolution by 240 minutes.[32]

Like other GH stimulation tests, there are also limitations associated with the GST. The 3- or 4-hour GST is still longer than many other GH stimulation tests, and requires an intramuscular injection that may not appeal to some patients. However, as there is a relationship between peak GH response to GHRH-ARG stimulation and ambient glucose levels,[29] it is unclear 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 years and none of the previous studies included patients with frank diabetes. Therefore, it is not known whether testing using the GST in subjects with diabetes is valid. Hence, caution should be exercised when interpreting normal GST results in the patients with diabetes. 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[12] and the mean peak GH response to ARG alone is lower than in the ITT or GST, even in normal lean subjects.[24] The diagnostic reliability of ARG alone has been previously questioned.[12,20] Thus, we recommend that ARG alone should only be considered if the ITT and the GST is contraindicated or if glucagon is unavailable. If this test is used, appropriately low peak GH cut-offs should be employed (for 95 % sensitivity: 1.4 µg/l, for 95 % specificity: 0.21 µg/l, and to minimize misclassification in either direction: 0.4 µg/l).[12] By contrast, the reliability of testing with GH secretagogues such as GH-releasing peptide-2 alone,[36] GH-releasing peptide-6 alone and combined GH-releasing peptide-6 plus GHRH,[37] acylated ghrelin,[38] and macimorelin[39] in assessing for adult GHD has also been reported. These agents utilize 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 appear to demonstrate a good safety profile with relatively few contraindications.[40] The limitation, however, of these GH secretagogues 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.[41] Furthermore, these agents are not readily available in many countries, including the US.

Future Perspectives

Recent studies have indicated that further refinements to the GST may still be required to improve the sensitivity and specificity of this test. A study by Micmacher et al.[42] demonstrated in a group of healthy men above 50 years old that GH secretion in response to the GST, but not with the ITT, correlated to physiologic spontaneous GH secretion. These data indicate that GH response to the GST reflects the endogenous GH spontaneous secretion and poses the question as to whether the cutpoints of peak GH response to the GST should be age-dependent. More recently, we reported a 2-year experience of 515 GSTs conducted from five large academic centers in the US and explored the potential of weight-based (0.03 mg/kg) versus fixed dose (1–1.5 mg) regimens of the GST.[32] In this study, we found that peak and nadir glucose, and delta GH, were higher in the weight-based regimen. In both regimens, BMI, fasting, peak, and nadir glucose correlated negatively with peak GH levels. Our data demonstrated that as age, BMI, and glucose tolerance negatively correlated with peak GH levels, the weight-based regimen may be more effective than the fixed-dose regimen in older overweight glucose intolerant patients.

In conclusion, in line with recently published consensus guidelines,[7–9,11] the ITT should remain as the test of reference due to its greatest diagnostic accuracy, even in patients with suspected hypothalamic GHRH deficit. We recommend the GST as the alternative test to the ITT for diagnosing adult GHD because of its availability, reproducibility, safety, lack of influence by gender and hypothalamic cause of GHD, and relatively few contraindications. Despite some studies demonstrating the comparability of the GST to the ITT in assessing the hypothalamic–pituitary–adrenal axis,[43,44] further larger, well-controlled studies are still required to confirm the reliability of the GST in assessing this axis. If the GST can be shown to reliably distinguish adrenal 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. While previous studies have shown that the GST could be shortened from 4 to 3 hours and yet maintain its diagnostic utility,[27,31] until further data becomes available, we would still recommend that the GST be conducted over 4 hours with measurements every 30 minutes for serum GH and capillary blood glucose levels primarily to ensure that delayed peak GH responses and late hypoglycemia are not missed. ■

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