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. Treatment with GH replacement has been shown to improve many, but not all, of these abnormalities. By contrast, untreated GHD is associated with increased mortality and morbidity that was previously observed in adults with hypopituitarism. These findings were substantiated in two large surveys based on national Danish registries, 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.

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, with the exception of patients with three or more pituitary hormone deficiencies and low serum insulin-like growth factor 1 (IGF-1) levels. 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. 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. A potential alternative to the ITT is the glucagon stimulation test (GST) that has been used extensively in the UK, and is now increasingly utilized in the US.

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

Following the publication of several validation studies and recommendations from current consensus guidelines, 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 in the US in July 2008, 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

<table>
<thead>
<tr>
<th>Contraindications</th>
<th>Precautions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malnourished patients or patients who have not eaten for &gt;48 hours</td>
<td>Patients may feel nauseous during and after the test (administration of intravenous anti-emetics can be considered)</td>
</tr>
<tr>
<td>Late hypoglycemia may occur (patients should be advised to eat small and frequent meals after completion of the test)</td>
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</tbody>
</table>

**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 fail 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. This analysis 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.27,28 and that, overall, the GST was a well tolerated test. Nevertheless, these27,28,29 and previous studies25,31,32 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. 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

**Interpretation**

Serum GH: peak GH levels tend to occur between 120 to 180 minutes with GH levels (not used to interpret the test)
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,21 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.23,24

The GST was initially described as a 4-hour test in older studies,22,23 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 more recent studies have suggested that the test could be shortened to 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 is readily available in many countries, including the US.

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 is readily available in many countries, including the US.

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 %.25 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.26 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.27 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,26 it is unclear whether hypoglycemia 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.

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.28 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.29 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.
**Pituitary Disorders**

In conclusion, in line with previously published consensus guidelines, the ITT should remain as the test of reference due to its greatest diagnostic accuracy, even in patients with suspected hypothalamic GH-RHR deficit. We recommend the GH test 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, 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 insufficiency 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 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.

14. Yuen KC, Biller BM, Morfit ME, Cook DM, Clinical review: is lack of response to GHRH-stimulation test in assessing this axis. If the GST can be shown to reliably distinguish adrenal insufficiency 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 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.