Evaluation of adult growth hormone deficiency: current and future perspectives

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Introduction

Physiological growth hormone (GH) secretion is pulsatile, and pulsatile secretion accounts for > 85% of total daily GH secretion. Due to its pulsatile secretion, serum GH levels vary between peaks and troughs. In addition, GH secretion is influenced by factors such as nutrition, sleep patterns, and physical activity. Peripheral GH actions are primarily mediated through IGF-I synthesized in the liver. Insulin-like growth factor-I (IGF-I) has a longer half-life in the circulation than GH and is considered to provide an integrated measure of GH secretion. However, serum IGF-I levels frequently overlap in adults with GH deficiency (GHD) and normal subjects. Furthermore, serum IGF-I levels decline with normal aging, and tend to be low in obesity and in patients with non-alcoholic fatty liver disease. For these reasons, a diagnosis of adult GHD cannot be established by a single random measurement of serum GH or IGF-I level, but is dependent on the demonstration of a subnormal rise in peak serum GH level in response to one or more dynamic stimulation tests.

Diagnosis of adult GH deficiency: current perspective

The diagnosis of adult GH deficiency is challenging because of the lack of a single biological end-point such as growth failure. Hence, the confirmation of adult GHD needs to rely on biochemical stimulatory 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 (1) , the validity of the chosen test and its appropriate cut-off limits, and the availability of local resources and expertise. In addition, GH immunoassay results may vary between different assay methods . Reasons for the variability in GH assay results include the heterogeneity of the analyte, the availability of different

preparations for calibration, and the interference from matrix components such as GH-binding protein (2) . Furthermore, the reporting of results in mass units or international units together with the application of variable conversion factors may lead to confusion. The current international reference standard advocated by the Growth Research Society (GRS) for GH assays is International Reference Preparation 88/624 (1 mg = 3.0 U) and for IGF-I assays is International Reference Preparation 87/518, but the society acknowledges that further comparative studies are needed to achieve standardization of GH and IGF-I assays (3) . T he GRS also calls for assay manufacturers to publish the validation of their assays, which should include specification of the GH isoforms detected (20 kDa GH, 22 kDa GH, and other isoforms) and the presence or absence of any effects due to GH binding protein, and mandates that GH and IGF-I results should be expressed in mass, not international units (3) .

Current published 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 (3-5) . The exception is with patients with ≥ 3 pituitary hormone deficiencies and low serum IGF-I levels < -2 standard deviation scores (SDS) in the appropriate clinical context, where the diagnosis of adult GHD is made without requiring stimulatory testing. Otherwise serum IGF-I levels should not be used alone to diagnose adult GHD and the 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 accepted as the gold standard test for evaluation of GH deficiency and is endorsed by several consensus guidelines (3-5) . However, the caveat with this test is that it is labor intensive, unpleasant for some patients, has potential risks, and is contraindicated in the elderly and in patients with seizure disorders and coronary artery disease. Thus, there remains a real unmet medical need for an alternative test to the ITT that is safe and reliable.

F ollowing the publication of several validation studies (6-9) and recommendations from current consensus guidelines (3-5) , the GH-releasing hormone (GHRH) combined with arginine (GHRH-arginine) test was regarded as the most reliable alternative GH stimulation test to the ITT in diagnosing adult GHD. However, when EMD Serono Inc. discontinued production of GHRH analog (Geref ®) in the United States in 2008 (10) , the Endocrine Society (5) and the American Association of Clinical Endocrinologists (4) endorsed the role of the glucagon stimulation test (GST) as the alternative test when the ITT is contraindicated.

Stimulatory tests used in diagnosing adult GH deficiency

A) INSULIN TOLERANCE TEST

Since the initial report in 1966 (11) and later seminal work in 1969 by Plumpton and Besser (12) , the ITT has become a valuable tool in the investigation of pituitary function and is widely considered as the gold standard test for the assessment of GHD in adults. One advantage of this test is that it can also simultaneously assess the hypothalamic-pituitary-adrenal (HPA) axis for adrenal insufficiency.

Table 1 Recommended protocol for performing the ITT.

CONTRAINDICATIONS :

History of epileptic seizures, coronary artery disease, pregnancy or age > 55 years.

PRECAUTIONS:

Patients commonly develop neuroglycopenic symptoms during the test and should be encouraged to report these symptoms (administration of IV anti-emetics can be considered).

Late hypoglycaemia may occur (patients should be advised to eat small and frequent meals after completion of the test).

PROCEDURE:

Ensure patient is fasted from midnight for 8-10 hours from solids and liquids (except water).

Withhold all morning medications (if the HPA axis is simultaneously assessed, then glucocorticoids should be withheld ≥ 12 hours before testing).

Weigh patient.

*Place IV cannula in both forearms.

**Administer IV human Regular insulin by IV push (standard dose: 0.05-0.1 units/kg for non-diabetic subjects with a BMI < 30 kg/m ^{2} and high dose: 0.15-0.3 units/kg for subjects with a BMI ≥ 30 kg/m ^{2} and subjects with insulin resistance).

SAMPLING AND MEASUREMENTS:

Baseline

Blood is drawn for glucose measurement with a glucometer.

Blood draw for baseline glucose, GH and IGF-I (cortisol and ACTH, if HPA axis is assessed simultaneously) levels will be sent to the laboratory for further analysis.

During the test

Blood samples are drawn from the IV line every 5-10 mins for measurement of glucose levels using a glucometer.

Signs and symptoms of neuroglycopenia are recorded.

When blood glucose levels from the glucometer approaches 45 mg/dL (2.5 mmol/L), blood samples are sent to the laboratory for measurements of blood glucose levels.

When symptomatic hypoglycemia is achieved (laboratory blood glucose < 40 mg/dL or 2.2 mmol/L), a blood sample is drawn and 4 additional blood samples are collected to measure glucose and GH (+/- cortisol if the HPA axis is assessed simultaneously) levels at 20, 40, 60, and 80 min.

The patient can begin drinking orange juice and eat to raise his/her blood glucose levels (IV 100 ml of 5% Dextrose can be administered if the patient cannot tolerate oral intake due to nausea or vomiting).

At the end of the test

Blood glucose levels measured from the glucometer should increase to levels > 70 mg/dL (3.9 mmol/L) before the patient is discharged from the testing unit.

INTERPRETATION *:*

If adequate (symptomatic) hypoglycemia is not achieved (< 40 mg/dL or 2.2 mmol/L), then GHD cannot be diagnosed.

Peak serum GH levels **< 5 µg/L** at any time point during the hypoglycemic phase of the test is diagnostic of adult GHD.

ACTH: adrenocorticotropic hormone, IV: intravenous.

**Two IV lines are placed is because one IV line is used for the administration of insulin bolus and possibly for administration of IV 5% Dextrose administration if the patient requires resuscitation from hypoglycemia, while the other IV line is used for repeated blood draws.*

***In certain patients with BMIs > 30 kg/m ^{2} who appear muscular with enhanced insulin sensitivity, clinical discretion is required in deciding the insulin dose for these patients. A dose of 0.05-0.1 units/kg may be more appropriate preventing severe hypoglycaemia.*

B) GLUCAGON STIMULATION TEST

The use of the GST for the assessment of GH reserve was first described in 1969 by Mitchell *et al* . (13) . 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 arginine or clonidine for differentiating GH-deficient patients from normal subjects (14-18) . The GST has been validated with the ITT in assessing GH reserve (15 , 19 , 20) and hypothalamic-pituitary-adrenal (HPA) (21 , 22) axes in children, but the true mechanism/s of how glucagon induces GH release remains unclear. With the unavailability of the GHRH-arginine test as the alternative test to the ITT in the United States

since 2008, the GST has since been increasingly used because of its availability, reproducibility, safety, and lack of influence by gender and hypothalamic cause of GHD (23) .

The rate of nausea, vomiting and headaches during GST ranges from less than 10% (15) to 34% (24) . In our experience of 425 fixed-dose GSTs performed at 5 academic centers in the United States, the main side-effects reported were nausea (37.2%), vomiting (2.4%), hunger, headaches, sleepiness, body chills, lightheadedness, and abdominal cramping that occurred mainly between 60-210 mins, and that most of these events were mild or moderate in severity that resolved by 240 mins of the test (25) .

The diagnosis of secondary adrenal insufficiency may be challenging particularly in patients with recent pituitary surgery or cranial irradiation when the adrenal cortex may still be responsive to stress but the hypothalamic-pituitary function is compromised. A number of studies have investigated the utility of GST in evaluating the HPA axis (24 , 26 , 27) but to date, there is no accurate validation on the peak cortisol cut-points that can reliably diagnose secondary adrenal insufficiency. In patients where there is a possibility of secondary adrenal insufficiency, we propose measuring cortisol levels during the GST. If peak cortisol levels during the GSTare **> 20 µg/dL** , we do not advocate further testing of the HPA axis, but if peak cortisol levels are **≤ 20 µg/dL** , we recommend further testing of the HPA axis with either an ITT or an ACTH stimulation test.

Table 2 Recommended protocol for performing the GST.

CONTRAINDICATIONS :

Malnourished patients or patients who have not eaten for > 48 h.

PRECAUTIONS:

Patients may feel nauseous during and after the test (administration of IV anti-emetics can be considered).

Late hypoglycemia may occur (patients should be advised to eat small and frequent meals after completion of the test).

No peak GH responses have been studied using the GST in normal subjects > 70 years and none of the previous studies included patients with diabetes mellitus. Therefore, caution should be exercised when interpreting normal GST results in these patients. If the suspicion of GHD remains high in these patients, it is reasonable to consider using a second GH stimulatory test

PROCEDURE:

Ensure patient is fasted from midnight for 8-10 hours from solids and liquids (except water).

Withhold all morning medications (if the HPA axis is simultaneously assessed, then

glucocorticoids should be withheld ≥ 12 hours before testing).

Weigh patient.

Place IV cannula in one forearm.

Administer glucagon 1 mg (1.5 mg if patient weighs more than 90 kg) as an IM bolus.

SAMPLING AND MEASUREMENTS:

Serum GH (+/- ACTH at baseline and cortisol at all time points if the HPA axis is assessed simultaneously) and capillary blood glucose levels* are measured at baseline, 30, 60, 90, 120, 150, 180, 210 and 240 mins.

INTERPRETATION:

Peak serum GH levels tend to occur between 120-180 mins.

Peak GH levels **< 3 µg/L** at any time point during testing is diagnostic of adult GHD.

IM: intramuscular.

**Blood glucose levels are monitored for late hypoglycemia and not used to interpret the test . While the lowest blood glucose level with the GST in the literature was reported at 37 mg/dL (13) , in our experience, we rarely observed blood glucose levels falling below 40 mg/dL with this test (25) .*

C) GHRH-ARGININE TEST

The GHRH-arginine test been validated in several studies as a reliable alternative test when the ITT is contraindicated or impractical (7 , 8) , and is endorsed by the Endocrine Society (5) , the American Association of Clinical Endocrinologists (4) , and the GRS (3) . Arginine potentiates and reduces variability in GHRH-stimulated GH secretion by inhibiting release of somatostatin from the hypothalamus. In 2008, EMD Serono, the sole distributor of GHRH analog (Geref ®) in the United States, discontinued its production leaving a need for an alternative test (10 , 23) . Stratum Medical Corporation (San Diego, CA, United States) is currently developing GHRH Diagnostic, which is chemically identical to Geref ® . However, GHRH Diagnostic is currently only available for investigational use under its Investigational New Drug registration with the United States Food and Drug Administration (PIND 106, 573) (28) .

Table 3 Recommended protocol for performing the GHRH-arginine test.

CONTRAINDICATIONS :

Allergy to GHRH analog and arginine.

PRECAUTIONS:

Transient sensation of body warmth and/or flushing due to GHRH.

Other side effects of GHRH are nausea, headache, experiencing a strange taste in the mouth, and transient hypotension.

PROCEDURE:

Ensure patient is fasted from midnight for 8-10 hours from solids and liquids (except water).

Withhold all morning medications.

Weigh patient.

Place IV cannula in one forearm.

Administer GHRH analog (1 µg/kg) [GHRH 1-29 (Geref ® , Serono, Inc., Norwell, MA, United States] as an IV bolus, and arginine hydrochloride 30 g simultaneously as an IV infusion from 0-30 min.

SAMPLING AND MEASUREMENTS:

Baseline

Blood is drawn for laboratory measurements of GH and IGF-I levels.

During the test

Serum GH levels are measured at 30, 60, 90 and 120 min.

INTERPRETATION:

Two important factors must be taken into consideration when interpreting the GHRH-arginine test:

1. Because GHRH directly stimulates somatotroph cells, this test can yield misleadingly normal responses in patients with a hypothalamic GHD (e.g., patients with previous cranial radiotherapy or hypothalamic tumors) (29) .
2. Cut-points to diagnose adult GHD are dependent on BMI. Peak serum GH levels **< 11.0 µg/L, 8.0 µg/L** and **4.0 µL** at any time point during testing in patients with BMIs ≥ 30 kg/m ^{2} , 25-30 kg/m ^{2} , and < 25 kg/m ^{2} , respectively, is diagnostic of adult GHD (4) .

D) ARGININE TEST

Arginine alone is less reliable than the ITT or GHRH-arginine test (7) , and the mean peak GH response to ARG alone is lower than in the ITT or GST, even in normal lean subjects (18) . The diagnostic reliability of ARG alone has been previously questioned (7 , 14) . Thus, the Endocrine Society (5) and American Association of Clinical Endocrinologist (4) guidelines recommend utilizing this test only when the ITT and the GST is contraindicated or if glucagon is unavailable.

Table 4 Recommended protocol for performing the arginine test.

CONTRAINDICATIONS :

Allergy to arginine.

PRECAUTIONS:

Nausea, vomiting, headache, and flushing.

If the solution extravasates, local skin irritation may occur.

PROCEDURE:

Ensure patient is fasted from midnight for 8-10 hours from solids and liquids (except water)

Withhold all morning medications.

Weigh patient.

Place IV cannula in one forearm.

Administer arginine 0.5 g/kg (maximum 30 g) as an IV infusion over 30 min.

SAMPLING AND MEASUREMENTS:

Baseline

Blood is drawn for laboratory measurements of GH and IGF-I levels.

During the test

Serum GH levels are measured at 30, 60, 90 and 120 min.

INTERPRETATION:

Peak GH levels **< 0.4 µg/L** at any time point during testing is diagnostic of adult GHD (7) .

E) GHRELIN MIMETICS

The reliability of testing with GH secretagogues such as GH-releasing peptide-2 alone (30) , GH-releasing peptide-6 alone and combined GH-releasing peptide-6 plus GHRH (31) in comparison with the ITT has been previously evaluated. These agents work on the same concept as the GHRH-arginine test in stimulating pituitary GH release by mimicking the activity of the natural GH secretagogue receptor ligand (i.e. ghrelin) to stimulate endogenous GHRH and to block somatostatin action. The advantage of these agents is that because many of these are nonpeptides or contain D-amino acids, they are able to resist proteolysis, and therefore can be active when administered orally. The limitation, 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 (32) . Previous studies have reported that GHRP-6 has been tested alone and in combination with GHRH as a provocative test for adult GHD (31-33) , and these studies have demonstrated its accuracy comparable to ITT, although with varying cut-points. The only side-effect reported was flushing. In these studies, GHRP-6 was administered as an IV injection, and peak GH levels occur at 15–30 min; significantly earlier than in other GH provocative tests. Peak GH levels in the GHRH-GHRP-6 test are unaffected by age, sex or increased BMI (33) . In addition, the GHRP-6 test is highly specific (34) , but less sensitive than the ITT for the diagnosis of adult GHD, and of little utility in the diagnosis of adrenal insufficiency (35) . In another study comparing GHRP-2 with ITT in adults with GHD and controls, a diagnostic cut-point of **15 µg/L** with GHRP-2 corresponded to a cut-point of 3 µg/L with the ITT (30) . The results were reproducible on repeated testing, and the GH peak after GHRP-2 typically occurred within 1 h. However, none of these agents are currently available commercially in the United States.

Future perspectives

Recent studies have indicated that further refinements to the GST are still necessary to improve the sensitivity and specificity of this test. More recently, we reported a 2-year experience of fixed and weight-based dosing of 515 GSTs conducted at 5 academic centers in the United States and explored the potential of the GST in testing the HPA axis (25) . In this study, we found that the weight-based dosing regimen induced higher peak and nadir glucose levels, with peak GH and peak cortisol levels occurring later in the test compared to the fixed dosing regimen. Vomiting was more prevalent in the weight-based regimen, and age, BMI and glucose tolerance may impact glucagon-induced GH and cortisol secretion. Overall, the GST was well-tolerated and can be performed as an out-patient; however further studies are required to determine whether GSTs may falsely diagnose GHD in patients with fasting hyperglycemia, and/or high BMIs. Thus, to improve the diagnostic reliability of the GST especially in patients with glucose intolerance and in those with high BMIs, a priming agent may be required to combine with the GST with appropriate cut-points to improve its sensitivity and specificity, similar to the GHRH in priming the arginine test. Until such data becomes available, we recommend that a second GH stimulation test should still be considered for such patients.

Currently, a multicentre study has just been completed assessing the diagnostic efficacy of a novel oral GH secretagogue AEZS-130 (Macimorelin, Aeterna Zentaris, Inc., Basking Ridge, NJ, United States) compared to the GHRH-arginine test in 50 adults with GHD and 48 healthy controls (36) . The optimal GH cut-point was **6.8 µg/L** for patients with BMIs < 30 kg/m ^{2} , and **2.7 µg/L** for patients with BMI ≥ 30 kg/m ^{2} . These cut-points yielded 82% sensitivity, 92% specificity, and 87% accuracy at the 60 min time point of the test. These results are promising as they demonstrate the safety, convenience and comparable efficacy of oral Macimorelin to the GHRH-arginine test in diagnosing adult GHD.

Conclusion

In line with recently published consensus guidelines (3-5) , the ITT should remain as the test of reference due to its greatest diagnostic accuracy, even in patients with suspected hypothalamic GHD. We recommend the GST as the alternative test to the ITT for diagnosing adult GHD because of its availability, reproducibility, safety, relatively few contraindications and the lack of influence by gender and hypothalamic cause of GHD. Despite some studies demonstrating the comparability of the GST to the ITT in assessing the HPA axis (21 , 22) , further refinements to the cortisol cut-points for the GST are still required to improve the diagnostic accuracy of the GST in assessing cortisol secretion in adults. 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 the ITT, would make this test more appealing. While previous studies have shown that the GST could be shortened from 4 to 3 hours and yet maintain its diagnostic utility (24 , 27) , until further prospective data becomes available, we recommend that the GST be conducted over 4 hours to ensure that delayed peak GH responses and late hypoglycemia are not missed. Recent GH stimulatory studies using newly formulated ghrelin mimetics appear promising as these tests are simple, effective and well-tolerated. With further validation and when they eventually become available commercially, these agents may potentially become the tests of choice for diagnosing adult GHD.

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