GROWTH HORMONE STIMULATION TESTS IN ASSESSING ADULT GROWTH HORMONE DEFICIENCY

Kevin C.J. Yuen, MD, FRCP (UK), FACE, Departments of Neuroendocrinology and Neurosurgery, Barrow Neurological Institute, Phoenix, AZ 85013, United States. kevin.yuen@dignityhealth.org

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ABSTRACT

Adult growth hormone deficiency (GHD) is a clinical syndrome that can manifest either as isolated or associated with additional pituitary hormone deficiencies. Its clinical features are subtle and nonspecific, requiring GH stimulation testing to arrive at a correct diagnosis. However, diagnosing adult GHD can be challenging due to the episodic and pulsatile endogenous GH secretion, concurrently modified by age, gender, and body mass index. Hence, a GH stimulation test is often required to establish the diagnosis, and should only be considered if there is a clinical suspicion of GHD and the intention to treat if the diagnosis is confirmed. Currently, there is no ideal stimulation test and the decision to perform a GH stimulation test must factor in the validity of the chosen test, the appropriate GH cut-points, and the availability of local resources and expertise. For now, the insulin tolerance test remains the gold standard test, while the glucagon stimulation test and macimorelin test are reasonable alternatives to the insulin tolerance test, whereas the arginine test is no longer recommended because arginine is a poor GH secretagogue that requires a very low peak GH cut-point of 0.4 μg/L. In this chapter, we discuss published evidence of the GH stimulation tests used in the United States and the inherent caveats and limitations of each individual test. We propose utilizing the lower GH cut-point to 1μg/L for the glucagon stimulation test to improve its diagnostic accuracy in some overweight and all obese patients based on the clinical suspicion of having adult GHD, and summarize current knowledge and change of status of availability of the oral macimorelin test in the United States.

INTRODUCTION

Physiological growth hormone (GH) secretion from the anterior pituitary gland is episodic, pulsatile, and accounts for > 85% of total daily GH secretion (1). Due to its pulsatility, serum GH levels vary between peaks and troughs, with very low levels between pulses. Hypothalamic growth hormone–releasing hormone (GHRH) and somatostatin traverse the hypothalamic–pituitary portal system to stimulate and suppress GH production, respectively, by signaling through specific somatotroph cell-surface G protein-coupled receptors (2), while gastric-derived ghrelin also stimulates GH secretion and synergizes the action of GHRH (3). Additionally, other factors such as gender, nutritional status, sleep patterns, physical activity, and metabolic and hormonal signals from other endocrine glands, including glucocorticoids, thyroid hormones, and sex steroids, also play an important role in modulating day-to-day GH secretion (1). Growth hormone regulates its own secretion by a feedback mechanism that involves other peripheral mediators, such as insulin-like growth factor-I (IGF-I), free fatty acids, glucose, and insulin (4). Peripheral GH actions are primarily mediated through IGF-I synthesized mainly by the liver.

Because IGF-I has a longer half-life in the circulation than GH, it is considered to provide an integrated measure of GH secretion. Like GH, serum IGF-I levels decline with aging (5), and tend to be low in obesity (6) and in patients with non-alcoholic fatty liver disease (7) that may overlap with the levels observed in younger GH–deficient patients. Hence, for these reasons, the diagnosis of adult GH deficiency (GHD) cannot be established in most patients by a random single measurement of serum GH or IGF-I level.

DIAGNOSIS OF ADULT GH DEFICIENCY: CURRENT PERSPECTIVE

Adult GHD is a rare heterogeneous disorder that commonly results from a variety of organic causes, including hypothalamic-pituitary tumors and/or their treatment, head trauma, and infiltrative diseases (8). This condition is characterized by decreased lean body mass and increased fat mass, dyslipidemia, cardiac dysfunction, decreased fibrinolysis and premature atherosclerosis, decreased muscle strength and exercise capacity, decreased bone mineral density, increased insulin resistance, and impaired quality of life (9). Treatment with GH replacement improves many, but not all, of these abnormalities (10, 11). However, due to the high cost of GH replacement (GH costs approximately $18,000 to $30,000 per year depending on the dose and brand used) (12) and concerns of potential long-term safety risks, particularly the development of diabetes mellitus, cancer and tumor recurrence, it is imperative that an accurate biochemical diagnosis is made so that appropriate GH replacement is offered to adults who are GH-deficient, and not for non-approved conditions (e.g., aging and sporting enhancement) (13, 14).

For the clinician, establishing the diagnosis of adult GHD is challenging because of the lack of a single biological end-point (e.g., growth failure in children with GHD). Other biochemical measurements like IGF-I, IGF-binding protein-3, or GH secretion over a 24-hour period have shown poor diagnostic value as there is an overlap between healthy and adults with GHD, particularly in adults > 40 years of age (5, 15). Hence, a GH stimulation test is often required to establish the diagnosis, and should only be considered if there is a clinical suspicion of GHD and the intention to treat if the diagnosis is confirmed. Currently, there is no ideal stimulation test as each test has its pros and cons, and the decision to consider performing a GH stimulation test to diagnose adult GHD must factor in the validity of the chosen test and its GH cut-points, and the availability of local resources and expertise.

Clinical practice guidelines recommend the evaluation of adult GHD to be based on medical history, clinical findings, and utilizing the appropriate GH stimulation test for biochemical confirmation (8, 16-18). The exception of when GH stimulation testing can be exempted include those with organic hypothalamic-pituitary disease with ≥ 3 pituitary hormone deficiencies and low serum IGF-I levels [< -2.0 standard deviation scores (SDS)] (19), patients with genetic defects affecting the hypothalamic-pituitary axes, and those with hypothalamic-pituitary structural brain defects (8, 16, 18). Evaluation for adult GHD should not be performed in patients with no evidence of a suggestive history, e.g., sellar/parasellar mass lesion or a history of a hypothalamic–pituitary insult, such as surgery, radiation therapy, head trauma, or brain tumor. Conversely, GH stimulation testing should not be performed in patients with commonly encountered, generalized, nonspecific symptoms of weakness, frailty, fatigue, or weight gain, without a history of organic hypothalamic/pituitary disease, as such patients are unlikely to benefit from GH therapy (8, 16, 18). These considerations are important for the clinician when deciding which patients to consider testing for possible adult GHD.

All GH stimulation tests are based on the concept that a GH secretagogue agent acutely stimulates pituitary GH secretion, and peak serum GH levels are detected by sequential blood sampling of serum GH levels after administration of the agent. The desired criteria of an ideal GH stimulation test should include the following: the ability to accurately and reliably differentiate adults with GHD from GH-sufficient individuals, high reproducibility, safety with minimal side-effects, affordability, and short test duration. It should also not be unpleasant to the patient and it should be simple to perform.

The insulin tolerance test (ITT) has historically been accepted as the gold-standard test for the assessment of adult GHD provided adequate hypoglycemia (blood glucose <40 mg/dL) is achieved (8, 16, 17). However, multiple drawbacks associated with the ITT hamper its wider use (20), and they include the requirement of close medical supervision by a physician throughout the test, the possibility of inducing severe life-threatening hypoglycemia, and the potential of causing seizures and altered consciousness resulting from neuroglycopenia in certain susceptible sub-populations. This test is also contraindicated in the elderly (> 65 years of age) and in patients who are at risk of and/or with a history of cardio-/cerebrovascular disease and seizures.

Finding a reliable alternative to the ITT for the diagnosis of adult GHD has been challenging. When the GHRH-arginine test was available in the United States before EMD Serono discontinued manufacturing the GHRH analog (Geref[@]) in November 2008 (8, 16, 17), GHRH-arginine test became the most acceptable alternative to the ITT. Since then, the glucagon stimulation test (GST) has grown in popularity replacing the GHRH-arginine test as the test of choice if the ITT cannot be performed or is contraindicated (21). Previous studies have examined the diagnostic utility of the GST for adult GHD, but these studies have either not taken body mass index (BMI) into consideration (22, 23) or included only controls with normal BMIs (24, 25). Several recent retrospective studies have questioned the diagnostic accuracy of the GST when the GH cut-point of 3μg/L is applied to overweight/obese adults (26-29) and in those with glucose intolerance (28, 29), while Hamrahian et al. (30) demonstrated in a prospective study of 28 patients by comparing the GST to the ITT that a lower GH cut-point of 1 μg/L improved its diagnostic accuracy with a 92% sensitivity and 100% specificity.

In this document, we will discuss published evidence of the GH stimulation tests used in the United States and the inherent caveats and limitations of each individual test. The lower GH cut-point of 1 μg/L for the GST should be utilized to improve its diagnostic accuracy in some overweight and all obese patients. We will also summarize current knowledge of the oral macimorelin test as the only approved diagnostic test for adult GHD by the United States Food and Drug Administration (FDA) and the European Medicines Agency, and its change in status of availability in the United States.

GENERAL LIMITATIONS AND IMPORTANT CAVEATS WHEN INTERPRETING GH STIMULATION TESTS

The responses to all GH stimulation tests show intra-individual variability, and the GH cut-points vary depending on the test used. For the ITT and GST, the cut-points advocated by previous consensus guidelines were 3-5 μg/L and 2.5-3 μg/L, respectively (8, 16). Other GH stimulatory agents such as clonidine, L-DOPA, and arginine are weaker GH secretagogues, and would require very low GH cut-points with utilization of sensitive GH assays to

achieve adequate specificity (e.g., arginine of 0.4 µg/L) (31). Hence, these tests are not recommended in the United States (8, 16). Other limitations include the relative lack of validated normative data based on age, gender, BMI, glycemic status, and the paucity of data for specific etiologies of adult GHD that have recently been described, such as traumatic brain injury, subarachnoid hemorrhage, ischemic stroke, and central nervous system infections (32, 33).

One of the caveats in interpreting the results of GH stimulation tests is that adult GHD itself is complicated by an increased susceptibility to central obesity (34). Obesity *per se* is a state of relative GHD (35-40), and earlier physiologic studies in obese individuals have shown that spontaneous GH secretion is reduced, GH clearance is enhanced, and stimulated GH secretion is reduced (40-42). Conversely, serum IGF-I levels are unaffected, or even increased, and this discordance is related to the increased hepatic GH responsiveness (43). The decreased serum GH levels in obesity up-regulate GH receptor and sensitivity. Furthermore, non-alcoholic fatty liver disease and non-alcoholic steatohepatitis are now recognized as being highly prevalent in overweight and obese adults with GHD (44), with consequent lower serum IGF-I levels being associated with increased severity of the disease (7). Thus, these data suggest that BMI-specific cut-points should be considered when testing patients for adult GHD. Table 1 summarizes the accepted GH cut-points for the GH stimulation tests used in the United States, as recommended by different consensus guidelines.

Table 1. Accepted GH Cut-Points (µg/L) for GH Stimulation Tests Used in the United States by Different Consensus Guidelines for Diagnosis of Adult GHD

	GRS 2007 (17)	AACE 2009 (16)	ES 2011 (8)	AACE 2019 (18)
ITT	< 3.0	≤ 5.0	< 3.0 to 5.0	≤ 5.0
GHRH-arginine - BMI < 25 kg/m^2 - BMI 25-30 kg/m^2	 < 11.0 < 8.0	 ≤ 11.0 ≤ 8.0	 < 11.0 < 8.0	No recommendation as not commercially available in the United
Glucagon - BMI < 25 kg/m^2 - BMI 25-30 kg/m^2	 < 3.0 < 3.0	 ≤ 3.0 ≤ 3.0	 < 3.0 < 3.0	 ≤ 3.0 ≤ 3.0[1] or ≤ 1.0[2]
Macimorelin	Not commercially available in	Not commercially available in 2009	Not commercially available in 2011	≤ 2.8
Arginine	Not recommended to be used	≤ 0.4	Not recommended to be used	No longer recommended to be used

[1]GH cut-point of ≤ 3.0 µg/L for patients with a high pre-test probability; [2]GH cut-point of ≤ 1.0 µg/L for patients with a low pre-test probability.

AACE, American Association of Clinical Endocrinologists; BMI, body mass index; ES, Endocrine Society; GHRH, growth hormone releasing hormone; GRS, Growth Hormone Research Society; ITT, insulin tolerance test

GROWTH HORMONE STIMULATION TESTS USED IN DIAGNOSING ADULT GH DEFICIENCY

Insulin Tolerance Test

The ITT remains accepted as the gold standard test for the assessment of adult GHD, with a GH cut-point of 3-5 μg/L when adequate hypoglycemia (blood glucose < 40 mg/dL) is achieved (8, 16, 17). This GH cut-point was originally proposed by Hoffman *et al.* (45) in 1994 based on GH responses to insulin-induced hypoglycemia, mean 24-hour GH levels derived from 20-min sampling, and serum IGF-I and IGFBP-3 levels in 23 patients considered GH-deficient due to organic pituitary disease, and in 35 sex-matched normal subjects of similar age and BMI. The ranges of stimulated peak GH responses separated GH-deficient (0.2-3.1 μg/L) from GH-sufficient (5.3-42.5 μg/L) patients. However, an overlap in mean 24-hour GH, IGF-I, and IGFBP-3 levels was observed, demonstrating the challenge in utilizing random single serum GH, IGF-I and IGFBP-3 levels to accurately differentiate GH-sufficiency from GHD.

Disadvantages of the ITT include the requirement of close medical supervision, may be unpleasant, and cautioned in some patients because of potential adverse effects (e.g., seizures or loss of consciousness resulting from neuroglycopenia), and contraindicated in elderly patients and in patients at risk of and/or with a history of cardio-/cerebrovascular disease and seizures. Furthermore, normoglycemic and/or hyperglycemic obese patients with insulin resistance may fail to achieve adequate hypoglycemia (46), necessitating the use of higher insulin doses (0.15-0.2 IU/kg), thus increasing the risk of delayed hypoglycemia. Although the ITT demonstrates good sensitivity, its reproducibility is another major limitation. Differences in peak GH responses have been demonstrated in healthy subjects undergoing ITT at varying times (47) and in women at different times of their menstrual cycle (48).

Table 2. 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 hypoglycemia may occur (patients should be advised to eat small and frequent meals after completion of the test).
PROCEDURE: Fast from midnight for 8-10 hours. All morning medications can be taken with water (if the HPA axis is simultaneously assessed, then glucocorticoids should be withheld ≥ 12 hours before testing). Weigh patient. [1]Place IV cannula for IV access in both forearms. [2]Administer IV human Regular insulin (standard dose: 0.05-0.1 units/kg for non-diabetic subjects with a BMI < 30 kg/m2 and high dose: 0.15-0.3 units/kg for subjects with a BMI ≥ 30 kg/m2).
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), additional blood samples are collected to measure glucose and GH (+/- cortisol if the HPA axis is assessed simultaneously) levels at 20, 25, 30, 35, 40, 60 and 90 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 adult 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.
CAUTION: If adequate (symptomatic) hypoglycemia is not achieved (< 40 mg/dL or 2.2 mmol/L), then adult GHD cannot be diagnosed.

ACTH: adrenocorticotropic hormone, HPA: hypothalamic-pituitary-adrenal, IV: intravenous.

[1]Two IV lines are placed, 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.

[2]In certain patients with BMIs > 30 kg/m^2 who appear muscular with increased 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 to prevent severe or delayed hypoglycemia.

Glucagon Stimulation Test

Glucagon is reportedly to be more potent than arginine or clonidine in stimulating GH secretion (24, 25). Glucagon is also a more potent GH secretagogue when administered intramuscularly or subcutaneously compared to the intravenous route (49). However, the mechanism/s of glucagon-induced GH stimulation remains unclear, and one hypothesis is that glucagon decreases ghrelin-independent effects of glucose or insulin variations (50).

There have been three earlier studies that have assessed the GST in identifying adult GHD in patients with pituitary disorders (22, 23, 51). Gomez *et al.* (51) and Conceicao *et al.* (23) compared the diagnostic characteristics of GST to ITT and included a control group matched for age and sex in both studies, and for BMI in one study (51). Using receiver operating characteristic (ROC) analysis, both studies proposed that a GH cut-point of 3 μg/L provided optimal sensitivity and specificity (51, 52). Gomez *et al.* (51) also demonstrated an inverse correlation between age (R = - 0.389, *P* = 0.0075) and BMI (R = - 0.329, *P* = 0.025) with peak GH levels in healthy controls. These data suggest that there is a potential association between relative, but not organic, GHD in aging and obesity. However, this study was conducted in a European cohort, where the frequency and severity of obesity is generally to a lesser degree than in the United States (53). Conversely, Conceicao *et al.* (23) demonstrated that peak GH levels were unaffected by age in either the control or patient group, and neither were there any gender differences. Additionally, Gomez *et al*. (51) used intramuscular glucagon doses of 1 mg and 1.5 mg for body weights ≤ 90 kg and > 90 kg respectively, whereas Conceicao *et al*. (23) used intramuscular glucagon of 1 mg for all subjects. In another study, Berg *et al.* (22) demonstrated an optimal peak GH cut-point of 2.5 μg/L with 95% sensitivity and 79% specificity using ROC analysis. This study also reported lower peak GH levels with GST compared to ITT (5.1 vs 6.7 μg/L, *P* < 0.01) and a positive correlation between peak GH levels during ITT and GST (R = 0.88, *P* < 0.0001), but no correlation between BMI or age to peak GH responses (54, 55). However, these (22, 23, 51) and other earlier studies (24, 25, 49, 56) did not specifically evaluate patients with glucose intolerance; hence, the diagnostic accuracy of the GST in testing for GHD in this population remains unclear.

Advantages of the GST is its reproducibility, safety, and lack of influence by gender and hypothalamic GHD (21), whereas disadvantages include the lengthy test duration (3-4 hours), and the need for an intramuscular injection that might not appeal to some patients. Side-effects frequently reported include nausea, vomiting, and headaches ranging from < 10% (22) to 34% (54), mainly occur between 60-210 min and tend to resolve by 240 min into the test, and seem to be more pronounced in elderly subjects, where severe symptomatic hypotension, hypoglycemia, and seizures have been observed (57).

However, since the publication of the 2009 American Association of Clinical Endocrinologists (AACE) (16) and 2011 Endocrine Society (8) Clinical Practice Guidelines, there have been several studies that have suggested that the fixed-dose GST using a GH cut-point of 3 μg/L may potentially over-diagnose adult GHD in a substantial number of overweight/obese subjects and in those with glucose intolerance. In two large retrospective studies, Toogood *et al.* (58) and Yuen *et al.* (29) found an inverse correlation between BMI and peak GH during the GST, and that this relationship appeared to be strongest with BMIs between 30 and 40 kg/m^2 and seemed to plateau for those with BMIs > 40 kg/m^2 (58). Alternatively, a negative correlation between BMI and peak GH

following glucagon stimulation has been reported by Gomez *et al*. (51) in healthy subjects but not in patients with underlying pituitary disease. Dichtel *et al.* (26) evaluated 3 groups of overweight/obese men, i.e., controls who were younger than the patients, patients with 3-4 pituitary hormone deficits, and patients with 1-2 pituitary hormone deficits. Using ROC analysis, the GH cut-point of 0.94 μg/L provided the optimal sensitivity (90%) and specificity (94%), whereas BMI and amount of visceral adipose tissue inversely correlated with peak GH levels in controls. Almost half of the healthy overweight/obese individuals (45%) failed the GST using the 3 μg/L GH cut-point. Diri *et al.* (27) evaluated 216 patients with pituitary disease and 26 healthy controls and compared the GST to the ITT. These investigators used a GH cut-point of 3.0 μg/L for the ITT and two GH cut-points of 3.0 μg/L and 1.07 μg/L for the GST, yielding the diagnosis of adult GHD in 86.1%, 74.5%, and 54.2 % patients, respectively. Additionally, patient age, BMI, and number of pituitary hormone deficits correlated with IGF-I and peak GH levels. Twelve out of 26 (46.2 %) healthy subjects failed the GST using a GH cut-point of 3.0 μg/L, but none when the cut-point was lowered to 1.07 μg/L. Wilson *et al.* (28) studied 42 patients with a high pre-test probability of adult GHD. After excluding 10 patients with severe GHD based on peak GH levels ≤ 0.1 μg/L, these investigators found that body weight negatively correlated with GH area under the curve (AUC) (R = -0.45; *P* = 0.01) and peak GH response (R = -0.42; *P* = 0.02) and positively correlated with nadir blood glucose levels (R = 0.48; *P* < 0.01). Conversely, nadir blood glucose levels during GSTs inversely correlated with GH AUC (r= -0.38; p=0.03) and peak GH (r= -0.37; p=0.04), implying that patients with higher nadir blood glucose levels tended to have a lesser glucagon-induced GH response. Recently, Hamrahian *et al.* (30) compared the fixed-dose GST (1 mg or 1.5 mg in patients > 90 kg body weight) and weight-based GST (WB-GST: 0.03 mg/kg) with the ITT using a GH cut-point of 3.0 μg/L. Patients with hypothalamic-pituitary disease and 1-2 (n = 14) or ≥ 3 (n = 14) pituitary hormone deficiencies, and control subjects (n = 14) matched for age, sex, estrogen status and BMI undertook the ITT, GST and WB-GST in random order. Using ROC analyses, the optimal GH cut-point was 1.0 (92% sensitivity, 100% specificity) for fixed-dose GST and 2.0 μg/L (96% sensitivity and 100% specificity) for WB-GST. Therefore, lowering the GH cut-point from 3 μg/L to 1 μg/L is important to reduce misclassifying adult GHD in overweight (BMI 25-30 kg/m^2) patients with a low pre-test probability and in obese (BMI > 30 kg/m^2) patients.

It remains unclear whether hyperglycemia influences peak GH responses to glucagon stimulation, independent of central adiposity. No peak GH responses have been studied using the GST in normal controls > 70 years of age, and none of the previous studies included patients with poorly controlled diabetes mellitus. Studies by Yuen *et al.* (29) and Wilson *et al.* (28) demonstrated that higher fasting (range 90-316 mg/dL), peak (range 156-336 mg/dL), and nadir (range 52-200 mg/dL) blood glucose levels during the GST were associated with lower peak GH responses. Therefore, stratification of GH responsiveness by the degree of glycemia will be helpful to clinicians in interpreting the GST results in patients with impaired glucose tolerance and diabetes mellitus. Because these data are currently unavailable, caution should be exercised when interpreting abnormal GST results in these patients. Further larger prospective studies are needed to address the effects of varying degrees of hyperglycemia on the ability of glucagon to stimulate GH secretion.

Table 3. Recommended Protocol for Performing the Glucagon Stimulation Test
CONTRAINDICATIONS: Malnourished patients or patients who have not eaten for > 48 hours. Severe fasting hyperglycemia > 180 mg/dL.
PRECAUTIONS: Patients may feel nauseous during the test (administration of IV anti-emetics may be considered). Late hypoglycemia may occur (patients should be advised to eat small and frequent meals after completion of the test).
PROCEDURE: Fast from midnight for 8-10 hours. All morning medications can be taken with water. Weigh patient. Place IV cannula for IV access in one forearm. Administer IM glucagon (1.0 mg if patient body weight ≤ 90 kg and 1.5 mg if patient body weight > 90 kg).
SAMPLING AND MEASUREMENTS: Blood is drawn for measurements of serum GH[1] and blood glucose[2] levels at 0, 30, 60, 90, 120, 150, 180, 210 and 240 mins.
INTERPRETATION: Peak GH levels ≤ 3.0 µg/L in normal-weight (BMI < 25 kg/m2) patients and in overweight (BMI 25-30 kg/m2) patients with a high pre-test probability, and ≤ 1.0 ug/L in overweight (BMI 25-30 kg/m2) patients with a low pre-test probability and in obese (BMI > 30 kg/m2) patients at any time point during testing are diagnostic of adult GHD.
CAUTION: Clinical suspicion of pre-test probability should be taken into consideration when interpreting GST results in patients > 70 years of age and in patients with impaired glucose tolerance and poorly controlled diabetes mellitus, as no peak GH responses have been studied in these patients.

IM: intramuscular, IV: intravenous.

[1]Serum GH: peak GH levels tend to occur between 120-180 mins; [2]blood glucose: usually peaks around 90 mins and then gradually declines (not a requirement to interpret the test).

Macimorelin Test

Growth hormone secretagogues (GHSs) are peptidyl (GH-releasing peptide [GHRP]) and nonpeptidyl molecules that exert strong dose-dependent and specific stimulatory effects on the animal and human somatotrope secretion (59). These agents act as functional somatostatin antagonists by binding to their specific GH secretagogue receptor-1a in the hypothalamus and pituitary. The natural ligand for this receptor is the gut peptide ghrelin (60). Growth hormone secretagogues are now considered as ghrelin mimetic agents and can be administered

parenterally (e.g., GHRP-2, GHRP-6, hexarelin) or orally (e.g., MK-677 and macimorelin).

Macimorelin (formerly known as AEZS-130, ARD-07, and EP-01572) is a novel GH secretagogue that binds the GHS-R1a receptor and to pituitary and hypothalamic extracts with a similar affinity to ghrelin (61). In healthy volunteers, it is readily absorbed with good stability and oral bioavailability, and effectively stimulates endogenous GH secretion (61). An open-label, crossover, multicenter trial examined the diagnostic accuracy of a single oral dose of macimorelin (0.5 mg/kg) compared to GHRH plus arginine in adults with GHD and healthy matched controls (62). Peak GH levels were 2.36 ± 5.69 and 17.71 ± 19.11 μg/L in adults with GHD and healthy controls, respectively, with optimal GH cut-points ranging between 2.7 and 5.2 μg/L (62). Macimorelin showed good discrimination comparable to GHRH plus arginine, with peak GH levels that were inversely associated with BMI in controls. In a recent multicenter, open-label, randomized, two-way crossover study, oral macimorelin was compared to the ITT to validate its use for the diagnosis of adult GHD (63). The GH cut-point levels of 2.8 μg/L for macimorelin and 5.1 μg/L for ITT provided 95.4% (95% CI, 87% to 99%) negative agreement, 74.3% (95% CI, 63% to 84%) positive agreement, 87% sensitivity, and 96% specificity. In both studies (62, 63), macimorelin was well-tolerated, reproducible, and safe. In December 2017, the United States FDA approved macimorelin for use as a diagnostic test for adult GHD and mandated the GH cut-point of 2.8 μg/L to be used to differentiate patients with normal GH secretion from those with GHD. However, in the study by Garcia *et al*. (63), when the GH cut-point was increased to 5.1 μg/L for both macimorelin and ITT, negative agreement and specificity was unchanged at 94% (95% CI, 85% to 98%) and 96%, respectively, but interestingly, positive agreement and sensitivity was higher at 82% (95% CI, 72% to 90%) and 92%. Because measured serum GH levels are dependent on the GH assays used, using the GH cut-point of 5.1 μg/L for macrimorelin that is identical to the cut-point accepted for the ITT could be considered in patients with peak serum GH levels between 2.8 μg/L to 5.1 μg/L, especially if the patient has a high pre-test probability, e.g., history of surgery on a sellar/parasellar mass with 1-2 other pituitary hormone deficiencies. It is important to note that this test is not affected by age, BMI, or sex indicating its robustness for diagnosing adult GHD (64).

Main advantages of macimorelin are that the drug is orally administered, unlike the ITT, GHRH plus arginine or GST, that requires intravenous or intramuscular administration, and no risk of causing hypoglycemia. In addition, the test only lasts 90 minutes with 3-4 blood sample collections required, in contrast to more blood sample collections over 2 hours for the ITT and 3-4 hours for the GST. The most commonly reported side effect was mild dysgeusia, which did not require any intervention and resolved spontaneously (63). One drug-related serious adverse event was reported; that was in a subject with an asymptomatic QT interval prolongation on the electrocardiogram that resolved spontaneously within 24 h (62). Thus, careful assessment of the patient's concurrent medications is recommended as well as discontinuation of strong CYP3A4 inducers, provided this is considered safe by the prescribing physician and with sufficient washout time prior to testing.

However, in August 2022, a press announcement stated that Novo Nordisk Healthcare AG provided a 270-day notice period to terminate the amended development and commercialization license agreement for macimorelin (Macrilen®) in the United States (65). This means that as of May 23, 2023, Aerterna Zentaris regained its full rights in the United States and Canada to macimorelin but because it has yet to find a partner in the United States to market

macimorelin, it was further announced that sales of macimorelin will be temporarily discontinued and use of the agent beyond May 2023 will continue until its supplies in the United States runs out (66).

Table 4. Recommended Protocol for Performing the Macimorelin Test
CONTRAINDICATIONS: Drugs that may increase its plasma levels and prolong QT.
PRECAUTIONS: Dysgeusia.
PROCEDURE: Fast from midnight for 8-10 hours. All morning medications can be taken with water. Weigh patient. Place IV cannula for IV access in one forearm. Dissolve in water 1 (120 ml) or 2 pouches (240 ml) of macimorelin (≤ 120 kg = 1 pouch; > 120 kg = 2 pouches) Calculate macimorelin dose (0.5 mg/kg as a single oral dose) and volume of water required to reconstitute macimorelin solution (patient body weight X kg = X ml macimorelin solution, e.g., patient with a body weight of 70 kg would require 70 mL of reconstituted macimorelin solution) After volume of macimorelin is calculated, stir the solution gently and thoroughly for 2-3 min, and use within 30 min of preparation. Draw the exact macimorelin volume of solution into a needleless syringe, transfer the exact volume of into a drinking glass, and instruct the patient to drink the entire volume of solution within 30 seconds.
SAMPLING AND MEASUREMENTS: Blood is drawn for measurements of serum GH levels at 30, 45, 60 and 90 min.
INTERPRETATION: Peak serum GH levels tend to occur between 45-60 mins. When used according to prescribing package label, peak GH levels ≤ 2.8 µg/L at any time point is diagnostic of adult GHD.
CAUTION: Peak GH levels ≤ 5.1 µg/L at any time point may be considered in patients with a high-pre-test probability to diagnose adult GHD, as this higher GH cut-point limits the risk of a false-positive diagnosis and maintains a high detection rate for GH-deficient patients because of the more potent GH stimulatory effect of macimorelin compared with the ITT. Safety and diagnostic performance in patients < 18 and > 65 years of age, and in patients with impaired glucose tolerance and poorly controlled diabetes mellitus, and BMI-adjusted peak GH cut-points for overweight and obese patients is not established.

Summary of Tests

Table 5 displays a summary of the desirable test characteristics of GH stimulation tests currently available in the United States.

Table 5. Summary of Desirable Test Characteristics of each GH Stimulation Test Currently Available in the United States

Test	Accurate?	Safe?	Tolerability?	Simple?	Quick?	Available?	Cost
ITT	Gold standard	No[2]	No[4]	No	No	Yes	$
GST	Yes[1]	Yes[3]	No[3]	Yes	No	Yes	$
Macimorelin	Yes	Yes	Yes	Yes	Yes	Yes/No	$$$

[1]if appropriate BMI-specific GH cut-points are used; [2]contraindicated in patients with a history hypoglycemia, history of previous seizures, in the elderly (> 65 years of age), and in patients at risk of and/or with a history of cardio-/cerebrovascular disease; [3]caution in patients with propensity for nausea and vomiting, and elderly patients who may be at risk of developing symptomatic hypotension and dizziness (57); [4]patients may not tolerate severe symptomatic hypoglycemia. GST, glucagon stimulation test; ITT, insulin tolerance test.

STANDARDIZATION OF GH ASSAYS

Accurate measurement of GH levels is critical for establishing the diagnosis of adult GHD because the analytical method influences the results of GH stimulation tests, which is dependent on specific GH cut-point levels. However, circulating GH is present in several different isoforms and isomers, including the most common variant of 22 kDa, and other smaller molecules, such as the 20 kDa GH variant. Monoclonal antibodies binding to a specific molecular form of GH are used to limit detection to the 22 kDa GH, but will not detect other GH isoforms. Other molecules similar to GH (e.g., placental GH and prolactin) could potentially cross-react and affect the measurement of GH levels. Growth hormone binding protein, to which approximately 50% of circulating GH is bound, can also cause interference in a GH assay. Furthermore, substantial heterogeneity exists among currently utilized assays due to the use of different standard preparations for calibration of GH immunoassays, and lack of harmonization between various GH assays makes it difficult to directly compare diagnostic cut-points across different published studies. Another source of confusion when interpreting data of GH stimulation tests was that some laboratories reported GH levels in activity (mU/L), whereas others used mass units (μg/L) (67).

Due to the heterogeneity of GH assays, it is important that GH assays utilize a universal GH calibration standard 98/574 (National Institute for Biological Standards and Control), a recombinant pituitary GH preparation of high purity (68). All assay manufacturers should also specify the validation of their assay, which should include specification of the

GH isoforms detected (20 kDa GH, 22 kDa GH, and other isoforms), the analyte being measured, the specificities of the antibodies used, and the presence or absence of growth hormone binding protein interference.

CONCLUSIONS

The decision to perform GH stimulation tests should be based on the clinical suspicion of the treating endocrinologist. If the clinical suspicion is high, such as in a patient with history of surgery on a sellar mass, concurrent 1-2 other pituitary hormone deficiencies, and a low (< -2 SDS) or low-normal (< 0 SDS) serum IGF-I level, then performing GH stimulation testing is recommended. If the clinical suspicion is low, such as in cases where there is no suggestive history, such as hypothalamic-pituitary disease, surgery or radiation therapy, head trauma, or childhood-onset GHD, then the diagnosis of adult GHD should not be pursued and GH stimulation testing should not be performed. For now, the ITT remains the gold standard GH stimulation test, and the GST and macimorelin test (where available) are reasonable alternatives to the ITT. As the reliability of the GST GH cut-point of 3 μg/L in overweight/obese subjects and in those with glucose intolerance can misclassify some patients, the utilization of GH cut-points of the GST is now based on the clinician's level of suspicion of the patient's pre-test probability and underlying BMI. Macimorelin, a drug administered orally that was approved by the United States FDA in December 2017 is an attractive test because it is easy to conduct with high reproducibility, safe, and has comparable diagnostic accuracy to the ITT and GHRH plus arginine test. The factors that limit its wider is its high cost (one 60 mg macimorelin packet costs approximately $4,500) (69) and the potential of drug-to-drug interactions that may cause QT prolongation. Following the announcement in August 2022 that macimorelin will be temporarily discontinued in the commercial market effective May 2023, after supplies of macimorelin runs out in the United States, the ITT and GST will only be the two GH stimulation tests available to clinicians, limiting the choices of tests that can be used.

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