REVIEW

Diagnosis and testing for growth hormone deficiency across the ages: a global view of the accuracy, caveats, and cut-offs for diagnosis

Kevin C J Yuen[1], Gudmundur Johannsson[2], Ken K Y Ho[3], Bradley S Miller[4], Ignacio Bergada[5] and Alan D Rogol[6]

[1]Departments of Neuroendocrinology and Neurosurgery, Barrow Neurological Institute, University of Arizona College of Medicine and Creighton School of Medicine, Phoenix, Arizona, United States
[2]Department of Endocrinology, Sahlgrenska University Hospital and Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[3]The Garvan Institute of Medical Research and the Faculty of Medicine, University of New South Wales, Sydney, Australia
[4]Pediatric Endocrinology, University of Minnesota Medical School, M Health Fairview Masonic Children's Hospital, Minneapolis, Minnesota, United States
[5]Centro de Investigaciones Endocrinológicas "Dr César Bergadá" (CEDIE), CONICET-FEI-División de Endocrinología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina
[6]Pediatric Diabetes and Endocrinology, University of Virginia, Charlottesville, Virginia, United States

Correspondence should be addressed to K C J Yuen: kevin.yuen@dignityhealth.org

Abstract

Growth hormone deficiency (GHD) is a clinical syndrome that can manifest either as isolated or associated with additional pituitary hormone deficiencies. Although diminished height velocity and short stature are useful and important clinical markers to consider testing for GHD in children, the signs and symptoms of GHD are not always so apparent in adults. Quality of life and metabolic health are often impacted in patients with GHD; thus, making an accurate diagnosis is important so that appropriate growth hormone (GH) replacement therapy can be offered to these patients. Screening and testing for GHD require sound clinical judgment that follows after obtaining a complete medical history of patients with a hypothalamic–pituitary disorder and a thorough physical examination with specific features for each period of life, while targeted biochemical testing and imaging are required to confirm the diagnosis. Random measurements of serum GH levels are not recommended to screen for GHD (except in neonates) as endogenous GH secretion is episodic and pulsatile throughout the lifespan. One or more GH stimulation tests may be required, but existing methods of testing might be inaccurate, difficult to perform, and can be imprecise. Furthermore, there are multiple caveats when interpreting test results including individual patient factors, differences in peak GH cut-offs (by age and test), testing time points, and heterogeneity of GH and insulin-like growth factor 1 assays. In this article, we provide a global overview of the accuracy and cut-offs for diagnosis of GHD in children and adults and discuss the caveats in conducting and interpreting these tests.

Key words

- growth hormone deficiency
- children
- adults
- diagnosis
- testing
- insulin tolerance test
- glucagon
- macimorelin
- clonidine

Endocrine Connections (2023) **12**, **e220504**

Introduction

Growth hormone deficiency (GHD) is a clinical syndrome that can present either as isolated GHD or associated with additional pituitary hormone deficiencies in children and adults. Childhood-onset GHD (CO-GHD) is often idiopathic but may also be triggered by organic causes. To distinguish between transient and persistent GHD in the adolescent patient transitioning into adulthood requires re-testing in the transition phase after the completion of linear growth (1, 2, 3). The exceptions when re-testing is generally not required are in patients with

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0504

genetic/congenital defects, irreversible hypothalamic–pituitary lesions, and in those with panhypopituitarism and low serum insulin-like growth factor 1 (IGF1) levels (4, 5). Conversely, the causes of adult-onset GHD (AO-GHD) are mainly due to hypothalamic–pituitary lesions and their associated treatment by surgery and/or radiotherapy (6), and performing diagnostic testing should only be limited to patients who are at risk for GHD (4, 5, 7) (Table 1).

Through the ages, the range of growth hormone (GH)/IGF axis defects represents a spectrum ranging from severe GHD to severe GH resistance (8). Thus, establishing the diagnosis of GHD can be challenging in children and adults. Although short stature and growth faltering are useful clinical markers for GHD in children, signs and symptoms of adult subjects with GHD are generally more subtle and less apparent. Thus, it is imperative to accurately identify GHD so that appropriate GH replacement therapy can be offered; equally, it is essential to avoid over-diagnosing patients as having GHD, since the administration of recombinant human GH therapy in GH-sufficient patients may potentially lead to adverse events, unjustified excess cost, and minimal to no therapeutic benefits. Confirming the diagnosis of GHD requires sound clinical judgment followed by the utilization of appropriate biochemical testing and imaging modalities. Because the diagnosis of GHD cannot be established in most patients by random measurements of serum GH (except in neonates), IGF1 or insulin-like growth factor-binding protein 3 (IGFBP3) levels, GH stimulation test/s are often required (4, 5, 7, 9, 10, 11). In patients with a high pre-test probability of GHD, one GH stimulation test is usually sufficient, whereas, for patients with a low pre-test probability (e.g. idiopathic CO-GHD),

Table 1 Causes of GHD and types of GHD that requires testing for adult GHD.

Testing for GHD is required	Testing for adult GHD is not required
Acquired	Pituitary hormone deficiencies ≥3 and low IGF-I
Tumors of the skull base and/or their treatment	Congenital
Pituitary adenoma	Genetic
Craniopharyngioma	Transcription factor defects (PIT-1, PROP-1, LHX3/4, HESX-1, PITX-2)
Rathke's cleft cyst	GHRH receptor gene defects
Meningioma	GH gene defects
Glioma/astrocytoma	GH receptor/post-receptor defects
Neoplastic sellar and parasellar lesions	Associated with brain structural defects
Chordoma	Single central incisor
Lymphoma	Cleft lip/palate
Metastases/hematological malignancy	Acquired causes
Other	Perinatal insults
Brain injury	
Traumatic brain injury	
Sports-related head trauma	
Blast injury	
Infiltrative/granulomatous disease	
Langerhans cell histiocytosis	
Autoimmune hypophysitis (primary, secondary)	
Sarcoidosis	
Tuberculosis	
Wegener's granulomatosis	
Amyloidosis	
Surgery of the pituitary or hypothalamus	
Cranial irradiation	
Central nervous system infections	
Bacterial, viral, fungal, parasital	
Infarction/hemorrhage	
Apoplexy	
Sheehan's syndrome	
Subarachnoid hemorrhage	
Ischemic stroke	
Snake bite	
Empty sella	
Hydrocephalus	
Idiopathic	

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0504

two different GH stimulation tests are recommended (4, 5). Conversely, testing for adult GHD is not recommended in patients presenting with generalized, nonspecific symptoms of weakness, fatigue, frailty, or obesity without a history of organic hypothalamic/pituitary disease, as such patients are unlikely to benefit from GH therapy (4, 5, 7). These considerations are important when deciding which patients to test for possible adult GHD.

Nevertheless, testing and diagnosis for GHD are not without their caveats. Although IGF1 levels reflect the integrated levels of GH, there remain substantial problems when used as a screening test as these levels tend to be low early in life, rise in adolescence, and decline throughout adulthood that may overlap with the range of adults with increasing age and adults with GHD (12, 13). Low serum IGF1 levels may also be observed in conditions other than GHD, such as malnutrition, renal failure, and type 1 diabetes mellitus (14), and IGFBP3 levels may be a better screening biomarker than IGF1 in children under the age of 3 years (15).

Physiology of GH secretion

GH is synthesized and secreted episodically in a pulsatile manner by the anterior pituitary somatotrophs throughout the lifespan that declines with age (16). Slow-wave sleep triggers nocturnal GH secretion (17), which contributes to a small fraction of the total daily GH secretion in women, but the majority in men (18). Fasting and exercise increase, whereas feeding decreases GH secretion (11). In men, estrogens derived from paracrine aromatization of androgens are responsible for driving the central secretion of GH, independent of the androgen receptor, whereas in women, the evidence supporting a role for estrogen in the central regulation of GH secretion is less consistent (19). During puberty, a three-fold increase in pulsatile GH secretion occurs that peaks in mid-puberty in both boys and girls (20). These factors are important when selecting patients for testing of possible GHD and result interpretation.

Secretion of GH from the pituitary is primarily controlled by the stimulatory effects of growth hormone-releasing hormone (GHRH) (16) and ghrelin (21) and the inhibitory effects of somatostatin (16). Amino acids stimulate GH (16), whereas IGF1 mediates GH action and inhibits GH secretion in a negative feedback loop (22). Because circulating IGF1 has a longer half-life than GH, it provides the basis for screening patients for possible GHD (4, 5, 7). Like GH, serum IGF1 levels rise during puberty and decline with aging (23) and tend to be low early in life and in patients with obesity (24) that may overlap with GH-deficient patients. Thyroid hormone and sex steroids also may exert positive influences (16), whereas glucocorticoids tend to inhibit GH secretion (25). Hence, it is important that other pituitary hormone deficiencies are identified and optimally replaced before testing for GHD.

Causes of GHD

CO-GHD is often idiopathic and isolated, without other pituitary hormone deficiencies. Other less common causes of CO-GHD include congenital, structural, and acquired causes (4, 5, 7) (Table 1). Importantly, childhood cancer survivors especially those who had received cranial irradiation and/or intrathecal methotrexate are at higher risk for GHD that may develop years after treatment (26).

Conversely, AO-GHD is most commonly acquired from hypothalamic–pituitary tumors and/or their treatment by pituitary surgery or radiotherapy (4, 5, 7), with pituitary adenomas and craniopharyngiomas accounting for over half the prevalence of adult GHD (27). Other less common conditions include infiltrative diseases of the hypothalamus and stalk, autoimmune hypophysitis, and meningoencephalitis (28, 29). In the last decade, non-tumoral causes of hypopituitarism associated with GHD (e.g. traumatic brain injury, stroke, and subarachnoid hemorrhage) have also been increasingly recognized (30, 31, 32, 33) (Table 1).

Diagnosis of GHD

Children

GHD exists on a spectrum from complete (e.g. deficiency of the *GH1* gene) to partial. However, most of the testing for GHD in children has been binary of either deficient or sufficient. In children, the ontogeny of GH secretion significantly varies across the pediatric age range. Dynamic secretion is modulated strongly by age, gender, body composition, and sexual maturation (16). After birth, high serum GH levels are noted during the neonatal period that progressively decrease through infancy and childhood (34). Throughout pubertal maturation, serum GH concentration peak amplitude increases two- to three-fold in the mid- to later stages of puberty (35). Therefore, selecting the most appropriate diagnostic tests to evaluate for pediatric GHD has to be addressed, based on the age and stage of pubertal maturation of the child. Pharmacological stimulation tests established for the diagnostic evaluation

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0504

of CO-GH are not approved in newborns or infants. During the first week of life, a random serum GH cutoff value above 7 μg/L has been reported to reliably exclude GHD (36). When spontaneous hypoglycemia ('critical sample of <40 mg/dL') occurs in a child, increased GH secretion as part of the counter-regulatory hormone response ensues, and the demonstration of GH levels <10 μg/L is also likely to establish the diagnosis of GHD (11). Thereafter, GH provocative tests using GH cut-offs between 4.7 and 6.5 μg/L are likely to establish the diagnosis of GHD, although the diagnostic threshold varies by the GH assays used and region (37). Within the peripubertal years, due to the physiological reduction of GH secretion, the use of primed (estrogens/testosterone) GH provocative tests to reduce false-positive results has been proposed (38).

Recently, LUM-201 (ibutamoren, formerly MK-0677), an orally administered GH-releasing peptide (GHRP) under development for the treatment of pediatric GHD (NCT 04614337), has put an interesting wrinkle into the concept of diagnosing partial GHD. A single, oral test with LUM-201 can prospectively identify children with GHD, defined by historical and auxological criteria and the diagnosis of GHD by subnormal GH release to two GH stimulation tests, who may respond to chronic therapy with LUM with increased height velocity and increased adult height. The children in the group of interest should meet two additional criteria, GH peak response ≥5 μg/L and baseline IGF1 level >30 μg/L (assay dependent), and these are referred to as predictive enrichment markers (PEM). These criteria were derived from an entire group of GHD children, because striking differences have been observed in GH responses between the GH secretagogue test and standard GH stimulation tests, especially at the higher end of peak GH responses of 5–10 μg/L. At the lower end of the peak GH response to the stimulation tests, there were no differences between the two tests. In a similar manner, the differences between tests were quite small below an IGF1 of 40 μg/L but ranged to about 75 μg/L above that IGF1 level (39). Thus, the GH response to a single low-dose LUM-201 test may have additional utility in denoting children who might not respond to a clinical trial with the secretagogue and would likely have an even greater than average response to GH therapy. Previous data (40) have also indicated that PEM-negative children with GHD would grow more robustly to treatment with GH with little additional growth with LUM-201 (41). However, PEM-positive children with GHD showed a lesser growth response to GH and a greater growth response to LUM-201 than those PEM-negative children.

Transition

Reassessment of GH secretion in children with GHD is necessary upon completion of linear growth to determine whether continuing GH therapy is indicated. GH therapy does not provide any height benefit after the attainment of skeletal maturity due to epiphyseal closure (bone age ~15 years in females, ~17 years in males). However, continued GH therapy during the transition period is important for the attainment of normal bone mineral density, body composition, muscle mass and strength, exercise recovery, and maintaining the quality of life (42). GH stimulation testing during the transition period typically utilizes provocative agents and diagnostic thresholds that are used in adults. A substantial proportion of patients (25–100%) with CO-GHD, especially children with isolated GHD and normal or small pituitary on MRI, have normalization of GH secretion following re-testing (2). Professional societies recommend at least 4 weeks after discontinuation of childhood GH replacement for re-testing (4, 5). Not all subjects require re-testing, and this includes patients with ≥3 pituitary hormone deficiencies, isolated GHD associated with an identified mutation and or a specific pituitary/hypothalamic structural defect, except for an ectopic posterior pituitary, and the presence of a transcription-factor mutation (4, 5). Re-testing should also be carried out in the following conditions: idiopathic isolated GHD with or without a small pituitary/ectopic posterior pituitary, GHD associated with only one other pituitary hormone deficiency, and patients previously treated with cranial irradiation (Table 1).

Adults

Because serum IGF1 levels rise in adolescence and decline throughout adulthood, there is a greater overlap in serum IGF1 levels between normal and GH-deficient subjects with increasing age (12). Consequently, the diagnostic sensitivity of serum IGF1 level is more reliable in adolescents and young adults, but less so in adults >40 years old (43). Serum IGF1 levels may lack specificity in patients with hepatic resistance to GH, who may have low IGF1 levels despite being GH-sufficient. This situation is caused by a range of conditions, including malnutrition, anorexia nervosa, starvation, type 1 diabetes mellitus, chronic liver disease, renal disease, severe hypothyroidism, and oral estrogen therapy (14). Conversely, patients with ≥3 pituitary hormone deficiencies, low serum IGF1 levels (specifically IGF1 SDS <−2), and a history of sellar mass lesion, pituitary surgery, or radiation therapy are likely

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0504

(>95%) to have GHD and these patients can forego GH stimulation testing (4, 5). In general, adults at risk for GHD but do not meet these criteria require GH stimulation testing to establish the diagnosis of GHD (Table 1).

Types of GH stimulation tests

All GH stimulation tests are based on the concept that a pharmacological 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. Currently, no ideal GH stimulation test exists and the decision to consider performing a test must factor in individual patient characteristics, the validity of the chosen test and its different GH cut-offs (Table 2), and the availability of local resources and expertise.

Growth hormone stimulation tests

Insulin tolerance test

The insulin tolerance test (ITT) remains the accepted 'gold standard' test for assessment of GHD in children and adults, with a GH cut-off of 3–5 μg/L (adults) when adequate hypoglycemia (blood glucose <40 mg/dL or 2.2 mmol/L) is achieved (4, 5, 7). The ITT demonstrates good sensitivity and, when conducted properly, is generally safe. Advantages of the ITT include that it can be used to assess patients with a history of cranial irradiation and allows for simultaneous assessment of the hypothalamic–pituitary–adrenal axis (44). Disadvantages include the requirement of close medical supervision, poor reproducibility (45), unpleasant symptoms of hypoglycemia, and contraindication in the elderly and in patients at risk of cardio-/cerebrovascular disease and seizures. 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 prolonging the test and time required for medical supervision. Due to potential safety concerns, the utilization of ITT has been decreasing in the United States (47), but not in Europe (48, 49).

Glucagon stimulation test

The mechanism/s of glucagon-induced GH stimulation remain/s unclear. Advantages of the glucagon stimulation test (GST) include its reproducibility, safety, and lack of influence by gender (50, 51). Disadvantages include the lengthy test duration (3–4 h) and the requirement for an intramuscular injection. Side effects include nausea, vomiting, and headaches and seem to be more pronounced in elderly subjects (52, 53). It is unclear whether hyperglycemia influences peak GH responses to glucagon stimulation as none of the earlier studies included patients with uncontrolled diabetes mellitus (defined as hemoglobin A1c >8%). Furthermore, no peak GH responses have been studied using the GST in normal controls >70 years of age, independent of central adiposity. Stratification of GH responsiveness by the degree of glycemia would be helpful to clinicians in interpreting the GST results in patients with glucose intolerance. However, because these data are unavailable, caution should be exercised when interpreting abnormal GST results in those with uncontrolled diabetes mellitus.

Previous studies have assessed the GST in identifying GHD in adult patients with pituitary disorders and reported that GH cut-offs between 2.5 and 3 μg/L provided good sensitivity and specificity (54, 55, 56). There have also been other studies that suggest that using a GH cut-off of 3 μg/L overdiagnoses adult GHD in overweight/obese subjects (57, 58). Lowering the GH cut-off from 3 to 1 μg/L has been shown to reduce misclassifying adult GHD in overweight (body mass index (BMI) 25–30 kg/m^2) patients with a low pre-test probability and in obese (BMI >30 kg/m^2) patients (57, 59, 60, 61). This lower peak GH level is now accepted as the GH cut-off in overweight/obese subjects by the American Association of Clinic Endocrinology (5, 63).

GH-releasing peptide tests

The use of GHRPs (e.g. GHRP-6, GHRP-2, hexarelin, and macimorelin) has previously been investigated as diagnostic tests for GHD, either alone or in combination with GHRH (65, 64). These peptides are synthetic secretagogues that elicit a dose-dependent and specific GH release response by binding to a specific receptor, for which ghrelin has been shown to be the natural ligand (65). The GH-releasing effect of GHRPs does not depend on gender but undergoes age-related variations; increases from birth to puberty, persists at a similar level in adulthood, and decreases by the sixth decade of life (65).

Early studies utilized orally administered GHRP-6 in short normal children and demonstrated GH responses that were comparable with intravenously administered GHRH (66), and GHRP-2 in children with GH insufficiency, whereas idiopathic short stature (ISS) showed that the GH release was dependent on the presence of endogenous or

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0504

Table 2 Accepted GH cut-offs for GH stimulation tests used in the United States and Europe to diagnose adult GHD.

Types of GH stimulation tests	GH cut-offs (μg/L)	Comments
ITT	<3.0–5.0	Requires close medical supervision throughout the test due to concerns for hypoglycemia (46)
		May be unpleasant and cautioned in some patients because of potential side effects (e.g. seizures or loss of consciousness resulting from neuroglycopenia) and contraindicated in the elderly and patients at risk for cardio/cerebrovascular disease (5)
		Patients with insulin resistance may fail to achieve adequate hypoglycemia because of underlying insulin resistance, requiring the use of higher insulin doses (0.15–0.2 IU/kg), thus increasing the risk of delayed hypoglycemia (47)
		Although the ITT demonstrates good sensitivity can be used to simultaneously test for secondary adrenal insufficiency (45), its reproducibility is a limitation (46)
Glucagon		Advantages include reproducibility, safety, and lack of influence by gender (51, 52)
BMI < 25 kg/m^2	≤3.0	Disadvantages include the long duration of the test (3–4 h), intramuscular injection, and relatively common side effects that include nausea, vomiting, and headaches ranging from <10% to 34% (51, 53, 54)
BMI 25–30 kg/m^2	≤1.0	Cautioned in the elderly, where severe symptomatic hypotension, hypoglycemia, and seizures have been reported (53, 54)
BMI ≥ 30 kg/m^2	≤1.0	
GH-releasing peptides 2 and 6	Ranges from 3.5 to 15.0 depending on which test	Advantages include not being affected by gender (68), safe, well tolerated, convenient, sensitive, reproducible (66, 67, 72), can be combined with GHRH (64, 65), can be used to simultaneously test for secondary adrenal insufficiency (75, 76, 77), and can be administered by several routes (e.g. intravenous, subcutaneous, intranasal, and oral) (64, 65, 71, 70, 73, 74, 73, 74, 75, 78, 79)
		Disadvantages include some tests that cannot discriminate with acceptable sensitivity and specificity between healthy children (66) and GH-deficient patients and limited accessibility (only accessible in Japan) (72)
Macimorelin	≤2.8	First oral GH secretagogue to be approved as a diagnostic test for adult GHD (80, 81)
		Approved for use as a diagnostic test in the United States (78) and Europe (79)
		Showed good discrimination comparable to GHRH plus arginine and ITT (77)
		Simple, highly reproducible, well-tolerated, and safe (76, 77)
		The United States Food and Drug Administration selected a low GH cut-off of 2.8 μg/L (78), but using a higher GH cut-off of 5.1 μg/L was still able to correctly identify all GH-deficient patients without misclassifying those that were GH-sufficient (76)
		Performance not dependent on age, BMI, or sex (76, 77)
Clonidine	≤6.8	Used more commonly in children, but not in adults (83, 84)
	≤0.4	Relatively short over 90 min (85)
		Side effects may be prolonged over several hours (81, 82)
L-dopa	<6.0	High incidence of side effects, hence rarely used (89)
		Lower sensitivity but similar specificity compared to the clonidine test (87)
Arginine	≤6.5 for children and adolescents and ≤0.4 for adults	Weak GH secretagogue, requiring very low GH cut-off (5)
		Side effects uncommon, but 5–10% of subjects reported paresthesias, dry mouth, and headache (81)
		Still used in children but no longer recommended for use in adults unless no other GH stimulation tests are available (5)
GHRH-arginine		Transient facial flushing may occur after administration of recombinant GHRH (99)
BMI < 25 kg/m^2	<11.0	Recombinant GHRH is now not available in the United States, but still available and used in Europe (49)
BMI 25–30 kg/m^2	<8.0	
BMI ≥ 30 kg/m^2	<4.0	

BMI, body mass index; FDA, Food and Drug Administration; GH, growth hormone; GHD, growth hormone deficiency; GHRH, growth hormone-releasing hormone; ITT, insulin tolerance test.

exogenous GHRH (69). When GHRP-6 was compared with the ITT for the diagnosis of adult GHD, the peak GH cut-off of 3.5 μg/L provided 80% sensitivity and 95% specificity (70). Studies using the combination of GHRP-2 (64) and GHRP-6 (64, 69) with GHRH have been shown to be safe, well tolerated, convenient, and sensitive in diagnosing GHD, but cannot discriminate with acceptable sensitivity and specificity between healthy children and GH-deficient

patients (except possibly during puberty). The GHRP-2 test, which requires intravenous administration, demonstrated favorable reproducibility and tolerability and diagnosed severe adult GHD with high reliability using a GH cut-off of 15 μg/L at 60 min (70). When combined with arginine, GHRP-2 provided sensitivities and specificities of 93.8/81.3% and 85.5/94.5%, respectively, when compared to the ITT for the diagnosis of severe adult GHD (71). Additionally, GHRP-2 stimulates corticotrophs, thus allowing for the added advantage of concurrently assessing the hypothalamic–pituitary–adrenal axis (72, 73, 74). However, some of these tests cannot discriminate with acceptable sensitivity and specificity between healthy children and GH-deficient patients. Furthermore, these peptides are not widely available with its use limited to Japan only, where it is commercially available.

Macimorelin, a novel orally administered GHRP, binds to the GH secretagogue receptor 1a which mediates the actions of ghrelin on pituitary somatotrophs (75, 76). 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 (76). 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-offs ranging between 2.7 and 5.2 μg/L (76). In a multicenter, open-label, randomized, two-way crossover study, oral macimorelin was compared to the ITT in adults with GHD (75). The GH cut-offs of 2.8 μg/L for macimorelin and 5.1 μg/L for ITT provided 95.4% (95% CI, 87–99%) negative agreement, 74.3% (95% CI, 63–84%) positive agreement, 87% sensitivity, and 96% specificity. In December 2017 and January 2019, the United States Food and Drug Administration and European Medicines Agency, respectively, approved macimorelin as a diagnostic test for adult GHD (77, 78). Advantages are that it is orally administered, well-tolerated, reproducible, safe, short duration (only 90 min), and its performance is unaffected by age, BMI, or sex. Mild dysgeusia was the most common side effect, which did not require any intervention and resolved spontaneously (75, 76). One drug-related serious adverse event was reported in a subject with an asymptomatic QT interval prolongation on the electrocardiogram that resolved spontaneously (75). It is noteworthy that this subject was taking citalopram, a serotonin selective uptake inhibitor known to be associated with QT prolongation. Disadvantages include its lack or limited availability in many countries, high cost (mainly in the United States), and its potential for drug-to-drug interaction.

Recently, macimorelin has been studied as a GH stimulation test for the diagnosis of GHD in children. Pharmacokinetics, pharmacodynamics, safety, and tolerability of escalating single doses of macimorelin (0.25, 0.50, and 1.0 mg/kg) are assessed in children between the ages of 4 and 15 years (79). Currently, a global multi-center phase 3 clinical trial of 1.0 mg/kg macimorelin for the diagnosis of GHD in children is underway (NCT04786873), where the GH response to macimorelin is compared to clonidine and arginine stimulation tests.

Clonidine test

Clonidine is an alpha 2-adrenergic agonist that increases GHRH and inhibits somatostatin secretion. This test, used almost exclusively in children, is relatively short (90 min) and can cause hypotension and drowsiness. Drowsiness may prolong fasting and induce unwanted hypoglycemia, so patients are encouraged to eat or drink soon after the test is complete. The GH cut-off of 6.8 μg/L has been proposed by some investigators to diagnose GHD in children (82), with a GH cut-off of 3.0 μg/L measured by immunochemiluminescent assay indicating normal GH responsiveness to stimulation, independent of gender, BMI, and pubertal maturational stage (81). Some investigators have suggested using this test first to screen GH status to diagnose GHD in short children with suspected GHD due to its efficiency (82) and reliability (83).

L-dopa test

L-dopa stimulates the GH secretion through dopaminergic and alpha-adrenergic pathways (86). The accuracy of this test is 81% at a GH cut-off of 6 μg/L and 56% at a GH cut-off of 7 μg/L (87). The L-dopa test is currently used infrequently because of its side-effect profile (e.g. nausea, vomiting, vertigo) and high incidence of false-negative results. This test has a lower sensitivity but similar specificity compared to the clonidine test, and when combined with arginine, has been shown to improve its accuracy (85, 88, 87).

Arginine test

Arginine inhibits somatostatin release and enhances the GH response to exogenous GHRH (90, 91). This test is used more commonly in children but is now no longer advocated in adults, lasts for 120 min, and can cause nausea and vomiting. Guzzetti *et al.* (80) reported that a GH cut-off of 6.5 μg/L provided the best sensitivity and specificity

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0504

in children and adolescents. Side effects are uncommon, with 5–10% of subjects reporting paresthesias, dry mouth, and headache. Painless hematuria is a rare, but alarming side effect of arginine administration. For adults, a lower GH cut-off of 0.4 point μg/L was proposed because it is a weak GH secretagogue; however, this test is currently no longer recommended for use, unless no other GH stimulation tests are available (5), because of its low sensitivity and specificity.

GHRH-arginine test

The GHRH-arginine test is essentially the arginine test combined with a potent priming agent (a GHRH analog) in one test. Recombinant GHRH analog stimulates pituitary GH synthesis and release (92), whereas arginine potentiates the stimulatory effects of GHRH by inhibiting hypothalamic somatostatin release (93). The peak GH response in this test is neither age- nor gender-dependent but is dependent on BMI, central adiposity, and the presence of non-alcoholic fatty liver disease (94, 95). Because of its convenience, reproducibility, and high discriminatory power (96, 97), this test was widely accepted in the United States until EMD Serono in 2008 discontinued the production of the GHRH analog (Geref™) (51). Disadvantages of this test are that it can cause uncomfortable transient facial flushing, stimulates both the hypothalamus and the pituitary, and may yield false-negative results in hypothalamic GHD (e.g. patients with previous cranial irradiation or hypothalamic tumors) (98). The GH cut-offs for this test are BMI-dependent, with peak serum GH levels ≤11.0 μg/L, ≤8.0 μg/L, and ≤4.0 g/μL at every time point during testing in patients with BMIs <25 kg/m^2, between 25 and 30 kg/m^2, and >30 kg/m^2, respectively, diagnostic of adult GHD (97, 100).

Diagnostic test factors

Accuracy of GH and IGF1 assays

Accurate measurement of serum GH and IGF1 levels is critical for making the correct diagnosis of GHD. Specific GH cut-offs for GH stimulation tests must be interpreted in the context of the analytical method used. Endogenous GH in serum exists in numerous isoforms with the majority being the isoform of 22 kDa molecular weight. Approximately 10% circulates as the 20 kDa isoform and other isoforms and GH fragments circulate in smaller proportions (101). Serum GH level has been historically measured by a variety of approaches including bioassays, radio receptor assays, immunoassays, and mass spectrometry (102), and currently, immunoassays are used most frequently. However, diagnostic errors may still be introduced by the large inter-assay variability of commercial and 'in-house' GH assays. A study comparing 96 laboratories in the United Kingdom (UK) found an inter-laboratory agreement of 25% geometric coefficient of variation. By inference, this would translate to a range between 5 and 10 μg/L for a mean GH concentration of 7 μg/L (103).

To standardize across isoforms, professional guidelines recommend assay calibration with a highly purified preparation of the 22 kDa recombinant human GH isoform of GH (11, 101). Additionally, GH immunoassays transitioned from using polyclonal antibodies that targeted multiple epitopes on varying GH isoforms to monoclonal antibodies targeting one isoform (100). With these changes, current assays have a narrower target. All assay manufacturers should specify the validation of their product, which should include specification of the GH isoforms detected (20 kDa GH, 22 kDa GH, and other isoforms), the analytes being measured, the specificities of the antibodies used, and the presence or absence of GH-binding protein interference.

Differences in IGF1 assay performance should also be taken into account when evaluating GHD and monitoring GH replacement. A robust reference population is necessary, with details provided by the laboratory. To demonstrate the potential discrepancies among GH and IGF1 assays, samples of identical concentrations were sent to laboratories in the UK as part of the UK National External Quality assessment service (104). These identical samples were analyzed by 104 centers for the GH sample and 23 centers for the IGF1 sample utilizing 14 distinct GH assay techniques and 6 IGF1 assay techniques. Serum GH and IGF1 levels demonstrated a 2.5-fold difference between the lowest and highest results from the various assays. These data reinforce the importance of validation of each GH and IGF assay, and using the same IGF1 assay for a given patient throughout treatment follow-up, whenever possible.

There have been consensus statements calling to address the measurements of serum GH and IGF1 levels and the need for clinicians to use IGF1 SDS from validated assays and laboratories for the purposes of harmonization and standardization of diagnostic tools and clinical decision-making (101, 105). Laboratory results can be improved with the use of single universally accepted standard preparations for GH and IGF1. International standards for GH and IGF1

are both available. The second international standard for somatropin, which is a recombinant DNA-derived human GH standard 98/574 has been assigned units of 1.95 mg per ampoule and has a conversion of 1 mg to 3 IU, with recommended reporting in mass units (101, 103). International standard 02/254 is the most current World Health Organization (WHO) approved reference standard for IGF1 and has been analyzed in several laboratories for purity, activity, and stability (101, 103).

Implementing certain measures may improve the reliability of serum GH and IGF1 measurements. In addition to using the WHO reference standard 02/254 for IGF1, standard reference samples should be available for quality control. Methods that either eliminate or minimize binding protein interference should be implemented, validated, and communicated as part of the results for each assay.

More recently, IGF1 levels in plasma have been quantitated by LC-MS/MS, either as the intact peptide or as two signal peptides following tryptic digestion (106, 107). The first is an antibody-free method in which the IGF1/IGFBP complexes are dissociated in trifluoroethanol and the non-IGF1 proteins precipitated in acid ethanol before quantitation by LC-MS/MS (105). Results compared favorably to the 'standard' immunoassay method, IDS-iSYS, except at very high concentrations (105). The second method employed a urea-based IGF1/IGFBP complex dissociation directly followed by tryptic digestion. Following solid phase sample clean-up, the digest is quantitated by means of two signal peptides by LC-MS/MS (104). Factors that interfere with serum IGF1 measurement require further study and normative data should include a sufficient sample of individuals across all ages and pubertal stages. Measurement of IGF1 levels by LC-MS/MS methods can be falsely low or low normal in individuals expressing sequence variants (benign and pathologic) of IGF1 (108, 109). This issue may be unique to LC-MS/MS methodology. However, immunoassays are likely to miss these variants entirely.

Caveats

Duration of tests

Several studies of various GH stimulation test protocols have suggested reducing sample numbers and test duration without compromising diagnostic accuracy (110, 111, 112, 113, 114). Data reported from a single-center experience of GH stimulation testing using an identical protocol on 315 patients with short stature and/or growth failure showed peak GH response was reached by 2 h in 98% of those tested, suggesting that the arginine and L-dopa test can be discontinued after 2 h without compromising its diagnostic value based on the GH cut-off of 10 μg/L (87). Other studies in adults with GHD have proposed shortening GSTs to 3 h (108, 110), and evaluating GH levels between 3 and 5 time points (0, 90, 120, 150, and 180 min) as the majority of GH peaks occur between 120 and 180 min (115, 116). However, Yuen *et al.* (50) showed that 3-h GSTs will still misclassify 6.6% of GH peaks and recommend prolonging the GST to 4 h. However, performing a 4-h test is time-consuming, increases costs, and is labor-intensive.

Individual patient considerations

Age

At puberty and adulthood, differences in GH secretion are found between both sexes with integrated GH levels in young menstruating women being higher than in young men (117). Most studies show that GH secretion is higher in women than in men under basal conditions (118, 119) and after stimulation (120, 121). van den Berg *et al.* (117) reported that the daily GH production was three-fold greater in women than in men, largely due to an amplitude-specific divergence in the pulsatile mode of GH secretion. This sex difference is closely related to estrogen secretion and possibly influenced by serum testosterone as well (122). Furthermore, oral estrogen administration increases integrated GH concentration both in healthy pre- and postmenopausal women by virtue of lesser negative feedback due to lowered hepatic IGF1 generation (123). In contrast to healthy men and women, IGF1 levels in GH-deficient adults are lower in women than in men (124). When healthy adults underwent GHRH plus arginine and arginine tests, several investigators found that peak GH levels are higher in females than males within the same BMI category (125, 126). Thus, if sex differences are not taken into account, then males are at greater risk of being overdiagnosed with GHD. It is noteworthy that sex-related GH cut-offs are currently not employed for any GH stimulation tests, and an argument can be made to lower the GH cut-offs for males.

Puberty and sex hormones

Due to the physiologic rise of GH secretion during puberty (24), it has been suggested that prepubertal children

should be 'primed' with sex hormones before undergoing GH stimulation testing to reduce false-positive rates (127, 128, 129). In a study by Marin *et al.* (130), the subset of 11 prepubertal normal children primed with 2 days of estrogen demonstrated peak GH levels that rose to those detected in subjects at pubertal stages 4 and 5. In a study by Muller *et al.* (131) of 26 boys primed with a single dose of testosterone, almost 80% increased their peak GH level to >10 µg/L. In a study by Cohen *et al.* (132) of prepubertal children characterized as GH-deficient or ISS based on GH stimulation testing without sex hormone priming, those with ISS required GH doses nearly 2 times higher to reach an IGF1 of +2 SDS. The stimulated peak GH level response to two pharmacologic stimuli that distinguish between GH-deficient and GH-sufficient patients is unclear and likely exists on a continuum with levels of 5, 7, and 10 µg/L having been proposed, each without sufficient data for substantiation. The currently agreed GH cutoff is 10 µg/L (133, 134). However, with the newer, harmonized assays, this level is approximately 7 µg/L with the appropriate standard. In 2016, the Pediatric Endocrine Society updated their guidelines supporting priming with sex hormones in prepubertal children (boys >11 years old and girls >10 years old) to avoid inappropriate GH treatment of children with constitutional delay of growth and puberty (11). Nevertheless, the practice of priming remains controversial and is not universally accepted yet (135, 136).

It is noteworthy that the hormonal milieu of puberty is not sustained after priming. With supraphysiologic testosterone levels, endogenous GH secretion may be overestimated, but whether these children who respond to exogenous sex hormones can secrete adequate GH at the time of puberty remains unclear. It is also unclear whether peripubertal children who have lower GH peak levels without priming would benefit from exogenous GH therapy and whether children diagnosed with GHD with or without priming would respond similarly to GH treatment. Furthermore, overestimation of GH levels from priming can lead to false-negative results and deny eligible children from receiving GH therapy. An exception where priming may be considered is in patients with constitutional delay of growth and puberty, where the conditions can be difficult to differentiate (137).

Currently, distinct GH cut-offs are only defined for children and adults, and GH cut-offs based on pubertal staging would bridge the continuum. Reassessment of the GH/IGF1 axis when a child treated with GH prepubertally enters puberty has been proposed as an alternative to priming (138), and GH therapy could be paused at the onset of puberty to repeat GH stimulation testing and determine if continued therapy is necessary rather than continuing GH therapy until epiphyseal closure once diagnosed with GHD. Obtaining pubertal hormone levels at the time of GH stimulation testing to correlate GH response to pubertal status and deciding whether to treat with GH or not should be based on the clinician's discretion together with GH stimulation test results.

Nutrition

During fasting, IGF1 levels decrease because of lack of nutrients resulting in portal insulinopenia, both of which are responsible for regulating the hepatic generation of GH-induced IGF1 (139, 140, 141, 142). The low IGF1 levels lead to the removal of pituitary feedback inhibition due to the withdrawal of the inhibitory effects of nutrients and insulin on GH secretion (143), resulting in increased GH secretion which in turn stimulates lipolysis and gluconeogenesis; both of which spare the utilization of protein as an energy source (137, 138, 139, 140). Thus, fasting results in a subacute progressive increase in GH secretion which is then followed by a decrease in IGF1 levels despite sustained elevations in GH secretion (144). As insulin positively regulates hepatic GH receptors (145), fasting causes hepatic GH resistance in generating IGF1, the production of which is also dependent on adequate nutrition (146). The stimulation of GH secretion during fasting serves a metabolic role in sparing protein loss during fasting by enhancing lipolysis and the utilization of lipids (147).

Adults with GHD show abnormal GH responses to fasting. Aimaretti *et al.* demonstrated that after short-term fasting, GH levels were found to be higher in normal subjects than in GH-deficient adults without overlap; this phenomenon occurs before significant changes in IGF1 and IGFBP3 levels are observed (148). Conversely, subjects undergoing refeeding, either after short-term fasting or in recovery from chronic undernutrition, demonstrate suppression of previously enhanced GH secretion and serum IGF1 increase, returning to normal or nearly normal levels (149, 150). Soliman *et al.* (151) measured GH and IGF1 in 51 malnourished children before re-feeding and in the survivors following slow re-feeding. Serum IGF1 levels were severely reduced and returned to normal after re-feeding, while basal GH levels were significantly raised and returned to normal following re-feeding. In another study conducted in non-obese healthy men, overeating suppressed GH secretion before any measurable weight gain was observed but was associated with rapid and

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0504

sustained elevations in plasma insulin levels (152). Due to these important effects of fasting on the GH/IGF1 axis, it is important to standardize fasting in relation to the GH stimulation test; however, to date, this has not been systematically studied.

Body mass index

Obesity is associated with decreased spontaneous and stimulated GH secretion in both adults (153, 154) and children (155, 156). The mechanism causing the blunted GH response in obesity remains unknown. Both spontaneous and stimulated GH secretion normalize after significant weight loss (157, 158), thus proving that somatotroph insufficiency in obesity is reversible and probably reflects peripheral hormone, metabolic, and neuroendocrine perturbations in a state of chronic overnutrition. For children, the normal range for BMI changes as children grow and mature over the years. Obesity in children is defined as a BMI > 95th percentile (or +1.6 SDS) (159). In a meta-analysis by Abawi *et al.* (160), they noted a negative correlation between BMI SDS and peak GH responses to a GH secretagogue, with each increase in BMI-SDS of 1 unit associated with a decrease in GH_{max} of ~12%. Thieme *et al.* (161) sought to further study that correlation to verify it in a larger cohort of children below the 10th percentile for height for age. In a single center with almost 1000 patients, they showed consistently negative associations between BMI-SDS and the maximal GH level with effect size of about $\beta = -1.1$. However, only 31% of the variance of GH_{max} was accounted for by adjustment for BMI-SDS. Because most of the variance in GH secretion cannot be fully explained by adjustment for BMI-SDS, it remains unclear how to adjust for BMI-SDS when testing for GHD in children. Therefore, due to a lack of adequate evidence, BMI, BMI-SDS, and obesity classification (BMI > 95th percentile) are not currently considered in the interpretation of the peak GH response in children having undergone GH stimulation testing.

Brain MRI findings

Imaging of the brain with MRI is an important component in the diagnostic process of GHD. In CO-GHD, brain MRI may show congenital pituitary abnormalities such as anterior pituitary dysplasia/hypoplasia, pituitary stalk interruption syndrome, developmental cyst, and tumoral lesions. Bozzola *et al.* (162) and Kornreich *et al.* (163) demonstrated that MRI findings may help predict the pattern and severity of hypopituitarism in patients with GHD. However, the severity of GHD based on peak GH levels on GH stimulation tests was not predictive of the presence or absence of brain MRI abnormalities, although severe GHD was more strongly associated with more advanced brain MRI abnormalities (164). Furthermore, MRI may be helpful in differentiating those with moderate or mild GHD. Findings of pituitary abnormalities support decisions on GH treatment in children with moderate GHD (peak GH of 7–10 µg/L), as GHD is expected to evolve. Kessler *et al.* (165) noted varying pituitary volumes in children with isolated GHD, ISS, or normal controls. The pituitary volume differed significantly among the three groups: 231 ± 146 (idiopathic GHD) to 287 ± 108 (ISS) to 344 ± 146 mm^3 (normal controls). In the former two groups, an increase in pituitary volume with age (sexual maturation) was noted. It is possible that the relative pituitary hypoplasia may be responsible for some decreased GH section at puberty in patients with ISS.

Growth hormone cut-offs

The variability of GH stimulation test practices during the past three decades is reflected by a change in peak-stimulated GH cut-offs and the type of GH stimulating agents used that requires validation against normative data, which may not be obtainable in certain populations (e.g. as in children and elderly). Traditionally, the interpretation of GH stimulation testing results has been binary with the adherence to pass/fail diagnostic peak GH cut-offs. Although guidelines have been updated over the last decades resulting in changes in peak GH cut-offs, these GH cut-offs remain mostly arbitrary, especially in children. Perhaps, instead, the results should be interpreted on a continuum that spans severe GHD requiring GH therapy to moderate to mild GHD for which alternative therapies and further monitoring of growth, symptoms, and periodic testing should be performed (136).

There is increasing evidence supporting the need to revisit GH cut-offs after stimulation based on the adoption of newer GH assays (166, 167). In children, the GH cut-off has been accepted traditionally as <10 µg/L, although this cut-off has been proposed to be lowered to <7 µg/L by some because of the standardization of the GH assay (37, 165). In adults, the GH cut-off <3 µg/L has been defined to indicate severe GHD. In mid-adolescence, when GH levels peak, the choice of a mid-point is intuitive. In a study of adolescents aged 17–19 years, a value of 6 µg/L (in response to an ITT) identified a group at high risk of GHD (168). However, previous consensus statements have favored

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0504

using the adult criteria (ITT-induced level of <3 μg/L) in adolescents (4, 5), and uniform GH cut-offs are still commonly used in clinical practice without performing test-specific adaptations.

The use of BMI to increase the accuracy for the GST has also been proposed, with adult GHD defined by a GH cut-off <3 μg/L in normal weight (BMI <25 kg/m^2) or overweight (25–30 kg/m^2 patients with a high pre-test probability, and <1 μg/L in overweight patients with a low pre-test probability and those who are obese (BMI >30 kg/m^2) (5, 63). In children with congenital GHD, but less severe impairment of the pituitary stalk, the GH response to stimulation may be sufficient, but pituitary GH reserve deteriorates with a GH response of <10 ug/L after 20 years of age (169).

As for the macimorelin test, when the GH cut-off was increased to 5.1 μg/L, negative agreement and specificity remained unchanged at 94% (95% CI, 85–98%) and 96%, respectively, but positive agreement and sensitivity increased at 82% (95% CI, 72–90%) and 92% when compared with the ITT (75). Because measured serum GH levels are dependent on GH assays used, using the GH cut-off of 5.1 μg/L for macrimorelin (identical to the ITT) may be reasonable in patients with peak serum GH levels between 2.8 and 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) (5). Thus, future real-world research assessments should be based on factors such as radiation and/or intrathecal chemotherapy exposure, MRI appearance, baseline IGF1, and pre-existing multiple pituitary hormone deficiencies to gauge the strength of these factors in aiding diagnostic accuracy.

Limitations and caveats when interpreting GH stimulation tests

Caution needs to be applied to the interpretation of GH stimulation testing, including the non-physiological nature of the tests, intra-individual variability of the responses to different GH stimulation tests, GH and IGF1 assay heterogeneity, sex hormone levels, and arbitrary GH cut-offs deemed as 'normal' that depend on the potency of the specific provocative agent. For the ITT and GST, peak GH cut-offs advocated by previous professional societies were 3–5 and 2.5–3μg/L, respectively (4, 5). Other GH stimulatory agents such as clonidine, L-dopa, and arginine are weaker GH secretagogues and require lower GH cut-offs with the utilization of sensitive GH assays to achieve adequate specificity (e.g. arginine of 0.4μg/L) (170). Additionally, the concept of priming using sex steroids prior to conducting the GH stimulation test is used to test for peripubertal GHD to reduce the chance of a false-positive result. Some tests (e.g. macimorelin test) are costly and inaccessible in some countries, prolonged (e.g. GST), require close medical supervision with available emergency facilities (e.g. ITT), uncomfortable for the patient (e.g. ITT and GST), can carry some risks (e.g. ITT), and test reproducibility that has yet to be adequately validated with multiple studies reporting poor reproducibility (e.g. ITT) that results in different interpretations of the results. Other important limitations include the lack of validated normative data based on age, sex, glycemic status, and the paucity of data for specific etiologies of adult GHD (e.g. head injury, subarachnoid hemorrhage, ischemic stroke, and central nervous system infections) (28, 29). Due to the limitations of the GHST, the Pediatric Endocrine Society guidelines recommend against reliance on GH stimulation test results as the sole diagnostic criterion of GHD in children (11).

Another caveat in interpreting the results of GH stimulation tests is that adult GHD itself is complicated by an increased susceptibility to central obesity. Obesity *per se* is a state of relative GHD (151, 152, 153, 154), 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 (171, 172, 173). Conversely, serum IGF1 levels are unaffected, or even increased, and this discordance is likely due to the increased hepatic GH responsiveness from increased hepatic insulin exposure (174). The decreased serum GH levels in obesity result in the upregulation of GH receptor and sensitivity. Thus, these data suggest that BMI-specific cut-off is an important consideration when testing patients with GHD.

Conclusions

The decision to perform GH stimulation tests should be based on the clinical suspicion of the treating endocrinologist. Whether or not to proceed with GH stimulation testing should only be reached after careful consideration and only when the result will significantly contribute to the diagnostic process. For pediatric patients, if one combines GH stimulation testing results with the patient's anthropometric measurements, height velocity, physical findings, screening tests, and IGF1 and IGFBP3 levels, a more complete clinical picture should be obtained that allows proper individualized diagnostic evaluation and treatment. If the diagnosis is still unclear,

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0504

additional observation for growth failure and repeat GH stimulation testing at a future date may be considered. For adults, if the clinical suspicion is high, such as in a patient with a history of surgery for a sellar mass, concurrent 1–2 other pituitary hormone deficiencies, and a low (<−2 SDS) or low-normal (<0 SDS) serum IGF1 level, then performing one GH stimulation test is sufficient. If the clinical suspicion is low, such as in cases where there is no suggestive history of hypothalamic–pituitary disease, surgery, or radiation therapy, then testing for GHD should not be performed.

The ITT remains the gold standard test, and the GHRH plus arginine test, GST, clonidine, and macimorelin 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 has been shown to misclassify some patients, the utilization of GH cut-offs of the GST is now based on the clinician's level of suspicion of the patient's pre-test probability and underlying BMI. Macimorelin administered orally is attractive because it is easy to conduct with high reproducibility, safe, and has a diagnostic accuracy comparable to the ITT and GHRH plus arginine test. Factors that hinder its wider use include its high cost and inaccessibility in some countries, the potential for drug-to-drug interactions, and the lack of data in children and elderly patients >70 years of age.

For pediatric patients, if one combines GH stimulation testing results with the patient's anthropometric measurements, height velocity, physical findings, screening tests, and IGF1 and IGFBP3 levels, a more complete clinical picture is obtained to allow proper individualized diagnostic evaluation and treatment. If the diagnosis is still unclear, additional observation for growth failure and repeat GH stimulation testing at a future date may be considered.

Substantial progress has been made in refining the diagnostic process of patients suspected of GHD. There remains an ongoing need for GH and IGF1 assay harmonization and standardization using international reference standards (101, 103). The introduction of newer assay technologies may improve the diagnostic accuracy of the tests used. Further studies in populations that make up substantial proportions of patients with GHD, such as children, adolescents, the elderly, and those with obesity, diabetes mellitus, and traumatic brain injury, are needed to refine the GH cut-offs and improve the diagnostic tests used. A systematic, practical, and structured approach to the diagnosis of GHD is essential to accurately identify patients with GHD and appropriately offer GH replacement.

Declaration of interest

KCJY has received research grants as Principal Investigator to Barrow Neurological Institute from Crinetics, Ascendis, Corcept, and Amryt, has served as an occasional advisory board member for Novo Nordisk, Ascendis, Sandoz, Corcept, Ipsen, Amryt, Strongbridge, Crinetics, Recordati and Xeris, and has received lecture fees from Novo Nordisk, Recordati and Corcept. GJ has served as consultant for Novo Nordisk and AstraZeneca and has received lecture fees from Ascendis Pharma, Ipsen, Novartis, Novo Nordisk and Pfizer. KKYH is a consultant for NovoNordisk, Debiopharm and Diurnal. BSM is a consultant for Abbvie, Ascendis Pharma, BioMarin, Bristol Myers Squibb, EMD Serono, Endo Pharmaceuticals, Novo Nordisk, Orchard Therapeutics, Pfizer and Tolmar and has received research support from Alexion, Abbvie, Aeterna Zentaris, Amgen, Amicus, Lumos Pharma, Lysogene, Novo Nordisk, OPKO Health, Pfizer, Prevail Therapeutics and Sangamo Therapeutics. IB has served as consultant for Novo Nordisk, Biosidus and Pfizer, and has received lecture fees from Merck, Novo Nordisk, Pfizer, Sandoz and Biosidus. ADR is a consultant for Antares Pharma, Ascendis Pharma, BioMarin, Pharmaceutical, Lumos Pharma, Tolmar Pharma, and United States Anti-doping Agency.

Funding

This work did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

References

1 Clayton PE, Cuneo RC, Juul A, Monson JP, Shalet SM, Tauber M & European Society of Paediatric Endocrinology. Consensus statement on the management of the GH-treated adolescent in the transition to adult care. *European Journal of Endocrinology* 2005 **152** 165–170. (https://doi.org/10.1530/eje.1.01829)

2 Laurer E, Sirovina A, Blaschitz A, Tischlinger K, Montero-Lopez R, Hortenhuber T, Wimleitner M & Hogler W. The landscape of retesting in childhood-onset idiopathic growth hormone deficiency and its reversibility: a systematic review and meta-analysis. *European Journal of Endocrinology* 2022 **187** 265–278. (https://doi.org/10.1530/EJE-21-1179)

3 Penta L, Cofini M, Lucchetti L, Zenzeri L, Leonardi A, Lanciotti L, Galeazzi D, Verrotti A & Esposito S. Growth hormone (GH) therapy during the transition period: should we think about early retesting in patients with idiopathic and isolated GH deficiency? *International Journal of Environmental Research and Public Health* 2019 **16**. (https://doi.org/10.3390/ijerph16030307)

4 Molitch ME, Clemmons DR, Malozowski S, Merriam GR, Shalet SM, Vance ML, Endocrine Society's Clinical Guidelines Subcommittee & Stephens PA. Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society Clinical Practice Guideline. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 1621–1634. (https://doi.org/10.1210/jc.2005-2227)

5 Yuen KCJ, Biller BMK, Radovick S, Carmichael JD, Jasim S, Pantalone KM & Hoffman AR. American Association of Clinical Endocrinologists and American college of endocrinology guidelines for management of growth hormone deficiency in adults and patients transitioning from pediatric to adult care. *Endocrine Practice* 2019 **25** 1191–1232. (https://doi.org/10.4158/GL-2019-0405)

6 Ratku B, Sebestyen V, Erdei A, Nagy EV, Szabo Z & Somodi S. Effects of adult growth hormone deficiency and replacement therapy on the cardiometabolic risk profile. *Pituitary* 2022 **25** 211–228. (https://doi.org/10.1007/s11102-022-01207-1)

7 Ho KK & 2007 GH Deficiency Consensus Workshop Participants. Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: a statement of the GH Research Society in association with the European Society for Pediatric Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society,

and Endocrine Society of Australia. *European Journal of Endocrinology* 2007 **157** 695–700. (https://doi.org/10.1530/EJE-07-0631)
8 Savage MO, Burren CP & Rosenfeld RG. The continuum of growth hormone-IGF-I axis defects causing short stature: diagnostic and therapeutic challenges. *Clinical Endocrinology* 2010 **72** 721–728. (https://doi.org/10.1111/j.1365-2265.2009.03775.x)
9 Chesover AD & Dattani MT. Evaluation of growth hormone stimulation testing in children. *Clinical Endocrinology* 2016 **84** 708–714. (https://doi.org/10.1111/cen.13035)
10 Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, Chernausek SD, Savage MO, Wit JM & 2007 ISS Consensus Workshop participants. Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 4210–4217. (https://doi.org/10.1210/jc.2008-0509)
11 Grimberg A, DiVall SA, Polychronakos C, Allen DB, Cohen LE, Quintos JB, Rossi WC, Feudtner C, Murad MH & Drug. Guidelines for growth hormone and insulin-like growth factor-I treatment in children and adolescents: growth hormone deficiency, idiopathic short stature, and primary insulin-like growth factor-I deficiency. *Hormone Research in Paediatrics* 2016 **86** 361–397. (https://doi.org/10.1159/000452150)
12 Hoffman DM, O'Sullivan AJ, Baxter RC & Ho KK. Diagnosis of growth-hormone deficiency in adults. *Lancet* 1994 **343** 1064–1068. (https://doi.org/10.1016/s0140-6736(94)90181-3)
13 Iwayama H, Kitagawa S, Sada J, Miyamoto R, Hayakawa T, Kuroyanagi Y, Muto T, Kurahashi H, Ohashi W, Takagi J, *et al.* Insulin-like growth factor-1 level is a poor diagnostic indicator of growth hormone deficiency. *Scientific Reports* 2021 **11** 16159. (https://doi.org/10.1038/s41598-021-95632-0)
14 Puche JE & Castilla-Cortazar I. Human conditions of insulin-like growth factor-I (IGF-I) deficiency. *Journal of Translational Medicine* 2012 **10** 224. (https://doi.org/10.1186/1479-5876-10-224)
15 Buscher AK, Buscher R, Pridzun L, Langkamp M, Wachendorfer N, Hoyer PF, Ranke MB & Hauffa BP. Functional and total IGFBP3 for the assessment of disorders of the GH/IGF1 axis in children with chronic kidney disease, GH deficiency, or short stature after SGA status at birth. *European Journal of Endocrinology* 2012 **166** 923–931. (https://doi.org/10.1530/EJE-11-0923)
16 Giustina A & Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. *Endocrine Reviews* 1998 **19** 717–797. (https://doi.org/10.1210/edrv.19.6.0353)
17 Born J, Muth S & Fehm HL. The significance of sleep onset and slow wave sleep for nocturnal release of growth hormone (GH) and cortisol. *Psychoneuroendocrinology* 1988 **13** 233–243. (https://doi.org/10.1016/0306-4530(88)90021-2)
18 Jaffe CA, Ocampo-Lim B, Guo W, Krueger K, Sugahara I, DeMott-Friberg R, Bermann M & Barkan AL. Regulatory mechanisms of growth hormone secretion are sexually dimorphic. *Journal of Clinical Investigation* 1998 **102** 153–164. (https://doi.org/10.1172/JCI2908)
19 Birzniece V & Ho KKY. Mechanisms in endocrinology: paracrine and endocrine control of the growth hormone axis by estrogen. *European Journal of Endocrinology* 2021 **184** R269–R278. (https://doi.org/10.1530/EJE-21-0155)
20 Leung KC, Johannsson G, Leong GM & Ho KK. Estrogen regulation of growth hormone action. *Endocrine Reviews* 2004 **25** 693–721. (https://doi.org/10.1210/er.2003-0035)
21 Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, Mori K, Komatsu Y, Usui T, Shimatsu A, *et al.* Ghrelin strongly stimulates growth hormone release in humans. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 4908–4911. (https://doi.org/10.1210/jcem.85.12.7167)
22 Gahete MD, Cordoba-Chacon J, Lin Q, Bruning JC, Kahn CR, Castano JP, Christian H, Luque RM & Kineman RD. Insulin and IGF-I inhibit GH synthesis and release in vitro and in vivo by separate mechanisms. *Endocrinology* 2013 **154** 2410–2420. (https://doi.org/10.1210/en.2013-1261)
23 Lamberts SW, van den Beld AW & van der Lely AJ. The endocrinology of aging. *Science* 1997 **278** 419–424. (https://doi.org/10.1126/science.278.5337.419)
24 Ghigo E, Arvat E, Gianotti L, Lanfranco F, Broglio F, Aimaretti G, Maccario M & Camanni F. Hypothalamic growth hormone-insulin-like growth factor-I axis across the human life span. *Journal of Pediatric Endocrinology and Metabolism* 2000 **13**(Supplement 6) 1493–1502. (https://doi.org/10.1515/jpem-2000-s624)
25 Giustina A & Wehrenberg WB. The role of glucocorticoids in the regulation of growth hormone secretion: mechanisms and clinical significance. *Trends in Endocrinology and Metabolism* 1992 **3** 306–311. (https://doi.org/10.1016/1043-2760(92)90142-n)
26 Sklar CA, Antal Z, Chemaitilly W, Cohen LE, Follin C, Meacham LR & Murad MH. Hypothalamic-pituitary and growth disorders in survivors of childhood cancer: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* 2018 **103** 2761–2784. (https://doi.org/10.1210/jc.2018-01175)
27 Webb SM, Strasburger CJ, Mo D, Hartman ML, Melmed S, Jung H, Blum WF, Attanasio AF & HypoCCS International Advisory Board. Changing patterns of the adult growth hormone deficiency diagnosis documented in a decade-long global surveillance database. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 392–399. (https://doi.org/10.1210/jc.2008-0713)
28 Pekic S & Popovic V. Diagnosis of endocrine disease: expanding the cause of hypopituitarism. *European Journal of Endocrinology* 2017 **176** R269–R282. (https://doi.org/10.1530/EJE-16-1065)
29 Tanriverdi F & Kelestimur F. Classical and non-classical causes of GH deficiency in adults. *Best Practice and Research. Clinical Endocrinology and Metabolism* 2017 **31** 3–11. (https://doi.org/10.1016/j.beem.2017.02.001)
30 Booij HA, Gaykema WDC, Kuijpers KAJ, Pouwels MJM & den Hertog HM. Pituitary dysfunction and association with fatigue in stroke and other acute brain injury. *Endocrine Connections* 2018 **7** R223–R237. (https://doi.org/10.1530/EC-18-0147)
31 Dassa Y, Crosnier H, Chevignard M, Viaud M, Personnier C, Flechtner I, Meyer P, Puget S, Boddaert N, Breton S, *et al.* Pituitary deficiency and precocious puberty after childhood severe traumatic brain injury: a long-term follow-up prospective study. *European Journal of Endocrinology* 2019 **180** 281–290. (https://doi.org/10.1530/EJE-19-0034)
32 Ioachimescu AG, Hampstead BM, Moore A, Burgess E & Phillips LS. Growth hormone deficiency after mild combat-related traumatic brain injury. *Pituitary* 2015 **18** 535–541. (https://doi.org/10.1007/s11102-014-0606-5)
33 Lillicrap T, Garcia-Esperon C, Walker FR, Ong LK, Nilsson M, Spratt N, Levi CR, Parsons M, Isgaard J & Bivard A. Growth hormone deficiency is frequent after recent stroke. *Frontiers in Neurology* 2018 **9** 713. (https://doi.org/10.3389/fneur.2018.00713)
34 Ogilvy-Stuart AL. Growth hormone deficiency (GHD) from birth to 2 years of age: diagnostic specifics of GHD during the early phase of life. *Hormone Research* 2003 **60**(Supplement 1) 2–9. (https://doi.org/10.1159/000071219)
35 Veldhuis JD, Roemmich JN & Rogol AD. Gender and sexual maturation-dependent contrasts in the neuroregulation of growth hormone secretion in prepubertal and late adolescent males and females--a general clinical research center-based study. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 2385–2394. (https://doi.org/10.1210/jcem.85.7.6697)
36 Binder G, Weidenkeller M, Blumenstock G, Langkamp M, Weber K & Franz AR. Rational approach to the diagnosis of severe growth hormone deficiency in the newborn. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 2219–2226. (https://doi.org/10.1210/jc.2009-2692)
37 Collett-Solberg PF, Ambler G, Backeljauw PF, Bidlingmaier M, Biller BMK, Boguszewski MCS, Cheung PT, Choong CSY, Cohen LE,

Cohen P, *et al.* Diagnosis, genetics, and therapy of short stature in children: a Growth Hormone Research Society international perspective. *Hormone Research in Paediatrics* 2019 **92** 1–14. (https://doi.org/10.1159/000502231)
38 Martinez AS, Domene HM, Ropelato MG, Jasper HG, Pennisi PA, Escobar ME & Heinrich JJ. Estrogen priming effect on growth hormone (GH) provocative test: a useful tool for the diagnosis of GH deficiency. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 4168–4172. (https://doi.org/10.1210/jcem.85.11.6928)
39 Bright GM & Thorner MO. A GH secretagogue receptor agonist (LUM-201) elicits greater GH responses than standard GH secretagogues in subjects of a pediatric GH deficiency trial. *Hormone Research in Paediatrics* 2022 **95** 76–81. (https://doi.org/10.1159/000524244)
40 Bright GM, Do MT, McKew JC, Blum WF & Thorner MO. Development of a predictive enrichment marker for the oral GH secretagogue LUM-201 in pediatric growth hormone deficiency. *Journal of the Endocrine Society* 2021 **5** bvab030. (https://doi.org/10.1210/jendso/bvab030)
41 Blum WF, Bright GM, Do MT, McKew JC, Chen H & Thorner MO. Corroboration of height velocity prediction markers for rhGH with an oral GH secretagogue treatment in children with GHD. *Journal of the Endocrine Society* 2021 **5** bvab029. (https://doi.org/10.1210/jendso/bvab029)
42 Hulthen L, Bengtsson BA, Sunnerhagen KS, Hallberg L, Grimby G & Johannsson G. Growth hormone is needed for the maturation of muscle mass and strength in adolescents. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 4765–4770. (https://doi.org/10.1210/jcem.86.10.7897)
43 Hilding A, Hall K, Wivall-Helleryd IL, Saaf M, Melin AL & Thoren M. Serum levels of insulin-like growth factor I in 152 patients with growth hormone deficiency, aged 19-82 years, in relation to those in healthy subjects. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 2013–2019. (https://doi.org/10.1210/jcem.84.6.5793)
44 Lee P, Greenfield JR & Ho KK. Factors determining inadequate hypoglycaemia during insulin tolerance testing (ITT) after pituitary surgery. *Clinical Endocrinology* 2009 **71** 82–85. (https://doi.org/10.1111/j.1365-2265.2008.03466.x)
45 Gordon MB, Levy RA, Gut R & Germak J. Trends in growth hormone stimulation testing and growth hormone dosing in adult growth hormone deficiency patients: results from the ANSWER Program. *Endocrine Practice* 2016 **22** 396–405. (https://doi.org/10.4158/EP14603.OR)
46 Hoeck HC, Vestergaard P, Jakobsen PE & Laurberg P. Test of growth hormone secretion in adults: poor reproducibility of the insulin tolerance test. *European Journal of Endocrinology* 1995 **133** 305–312. (https://doi.org/10.1530/eje.0.1330305)
47 Yuen KC, Biller BM, Molitch ME & Cook DM. Clinical review: is lack of recombinant growth hormone (GH)-releasing hormone in the United States a setback or time to consider glucagon testing for adult GH deficiency? *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 2702–2707. (https://doi.org/10.1210/jc.2009-0299)
48 Tavares AB, Seixas-da-Silva IA, Silvestre DH, Paixao CM, Jr, Vaisman M & Conceicao FL. Potential risks of glucagon stimulation test in elderly people. *Growth Hormone and IGF Research* 2015 **25** 53–56. (https://doi.org/10.1016/j.ghir.2014.11.002)
49 Tavares ABW, Seixas-da-Silva IA, Silvestre DHS, Pinheiro MFC, Vaisman M & Conceicao FL. Growth hormone and cortisol secretion in the elderly evaluated using the glucagon stimulation test. *Endocrine* 2017 **56** 317–324. (https://doi.org/10.1007/s12020-017-1275-8)
50 Berg C, Meinel T, Lahner H, Yuece A, Mann K & Petersenn S. Diagnostic utility of the glucagon stimulation test in comparison to the insulin tolerance test in patients following pituitary surgery. *European Journal of Endocrinology* 2010 **162** 477–482. (https://doi.org/10.1530/EJE-09-0824)
51 Bellone J, Ghizzoni L, Aimaretti G, Volta C, Boghen MF, Bernasconi S & Ghigo E. Growth hormone-releasing effect of oral growth hormone-releasing peptide 6 (GHRP-6) administration in children with short stature. *European Journal of Endocrinology* 1995 **133** 425–429. (https://doi.org/10.1530/eje.0.1330425)
52 Mahajan T & Lightman SL. A simple test for growth hormone deficiency in adults. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 1473–1476. (https://doi.org/10.1210/jcem.85.4.6545)
53 Ghigo E, Arvat E, Muccioli G & Camanni F. Growth hormone-releasing peptides. *European Journal of Endocrinology* 1997 **136** 445–460. (https://doi.org/10.1530/eje.0.1360445)
54 Chihara K, Shimatsu A, Hizuka N, Tanaka T, Seino Y, Katofor Y & KP-102 Study Group. A simple diagnostic test using GH-releasing peptide-2 in adult GH deficiency. *European Journal of Endocrinology* 2007 **157** 19–27. (https://doi.org/10.1530/EJE-07-0066)
55 Kano T, Sugihara H, Sudo M, Nagao M, Harada T, Ishizaki A, Nakajima Y, Tanimura K, Okajima F, Tamura H, *et al.* Comparison of pituitary-adrenal responsiveness between insulin tolerance test and growth hormone-releasing peptide-2 test: a pilot study. *Peptides* 2010 **31** 657–661. (https://doi.org/10.1016/j.peptides.2009.12.023)
56 Suzuki S, Ruike Y, Ishiwata K, Naito K, Igarashi K, Ishida A, Fujimoto M, Koide H, Horiguchi K, Tatsuno I, *et al.* Clinical usefulness of the growth hormone-releasing Peptide-2 test for hypothalamic-pituitary disorder. *Journal of the Endocrine Society* 2022 **6** bvac088. (https://doi.org/10.1210/jendso/bvac088)
57 Garcia JM, Biller BMK, Korbonits M, Popovic V, Luger A, Strasburger CJ, Chanson P, Medic-Stojanoska M, Schopohl J, Zakrzewska A, *et al.* Macimorelin as a diagnostic test for adult GH deficiency. *Journal of Clinical Endocrinology and Metabolism* 2018 **103** 3083–3093. (https://doi.org/10.1210/jc.2018-00665)
58 Spiliotis BE, Papadimitriou DT, Alexandrides TK, Karystianos C, Nikiforidis G, Vassilakos P, Beratis NG & Casanueva FF. Combined growth hormone-releasing hormone and growth hormone-releasing peptide-6 test for the evaluation of growth hormone secretion in children with growth hormone deficiency and growth hormone neurosecretory dysfunction. *Hormone Research* 2008 **70** 215–223. (https://doi.org/10.1159/000151593)
59 Kinoshita Y, Tominaga A, Usui S, Arita K, Sakoguchi T, Sugiyama K & Kurisu K. The arginine and GHRP-2 tests as alternatives to the insulin tolerance test for the diagnosis of adult GH deficiency in Japanese patients: a comparison. *Endocrine Journal* 2013 **60** 97–105. (https://doi.org/10.1507/endocrj.ej12-0230)
60 Kageyama K, Nigawara T, Sakihara S, Takayasu S, Terui K, Moriyama T & Suda T. Diagnostic usefulness of the growth hormone-releasing peptide-2 test as a substitute for the insulin tolerance test in hypopituitarism. *Endocrine Journal* 2008 **55** 777–783. (https://doi.org/10.1507/endocrj.k07e-168)
61 Garcia JM, Swerdloff R, Wang C, Kyle M, Kipnes M, Biller BM, Cook D, Yuen KC, Bonert V, Dobs A, *et al.* Macimorelin (AEZS-130)-stimulated growth hormone (GH) test: validation of a novel oral stimulation test for the diagnosis of adult GH deficiency. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 2422–2429. (https://doi.org/10.1210/jc.2013-1157)
62 Drugs.com. Macrilen (macimorelin) FDA approval history. Silver Spring, MD, USA: Food and Drug Administration, 2017. (available at: https://www.drugs.com/history/macrilen.html#)
63 EMA. Ghryvelin (previously Macimorelin Aeterna Zentaris). Amsterdam, The Netherlands: European Medicines Agency (EMA), 2021. (available at: https://www.ema.europa.eu/en/medicines/human/EPAR/ghryvelin-previously-macimorelin-aeterna-zentaris#:~:text=GHRYVELIN%20(previously%20Macimorelin%20Aeterna%20Zentaris)%20is%20a%20medicine%20used%-20to,treat%20patients%20with%20the%20condition)
64 Csakvary V, Ammer N, Bagci EB, Bolshova OV, Damholt BB, Katanic D, Mikhailova E, Muzsnai Á, Raduk D, Senatorova G, *et al.* Safety, tolerability, pharmacokinetics, and pharmacodynamics of macimorelin in children with suspected growth hormone deficiency: an open-label, group comparison, dose-escalation trial. *Hormone Research in Paediatrics* 2021 **94** 239–250. (https://doi.org/10.1159/000519232)

65 Borges Mde F, Teixeira FC, Feltrin AK, Ribeiro KA, Nascentes GA, Resende EA, Ferreira BP, Silva AP & Palhares HM. Clonidine-stimulated growth hormone concentrations (cut-off values) measured by immunochemiluminescent assay (ICMA) in children and adolescents with short stature. *Clinics (Sao Paulo)* 2016 **71** 226–231. (https://doi.org/10.6061/clinics/2016(04)09)

66 Yackobovitch-Gavan M, Lazar L, Diamant R, Phillip M & Oron T. Diagnosis of growth hormone Deficiency in Children: the Efficacy of glucagon versus clonidine Stimulation Test. *Hormone Research in Paediatrics* 2020 **93** 470–476. (https://doi.org/10.1159/000513393)

67 Ibba A, Guzzetti C, Casula L, Salerno M, Di Iorgi N, Allegri AME, Cappa M, Maghnie M & Loche S. Reliability of clonidine testing for the diagnosis of growth hormone deficiency in children and adolescents. *Clinical Endocrinology* 2018 **89** 765–770. (https://doi.org/10.1111/cen.13845)

68 Yau M, Chacko E, Regelmann MO, Annunziato R, Wallach EJ, Chia D & Rapaport R. Peak growth hormone response to combined stimulation test in 315 children and correlations with metabolic parameters. *Hormone Research in Paediatrics* 2019 **92** 36–44. (https://doi.org/10.1159/000502308)

69 Corneli G, Di Somma C, Baldelli R, Rovere S, Gasco V, Croce CG, Grottoli S, Maccario M, Colao A, Lombardi G, *et al.* The cut-off limits of the GH response to GH-releasing hormone-arginine test related to body mass index. *European Journal of Endocrinology* 2005 **153** 257–264. (https://doi.org/10.1530/eje.1.01967)

70 van Nieuwpoort IC, van Bunderen CC, Arwert LI, Franken AA, Koppeschaar HP, van der Lelij AJ, Twisk JW, Boers M & Drent ML. Dutch National Registry of GH Treatment in Adults: patient characteristics and diagnostic test procedures. *European Journal of Endocrinology* 2011 **164** 491–497. (https://doi.org/10.1530/EJE-10-0914)

71 Plumpton FS & Besser GM. The adrenocortical response to surgery and insulin-induced hypoglycaemia in corticosteroid-treated and normal subjects. *British Journal of Surgery* 1969 **56** 216–219. (https://doi.org/10.1002/bjs.1800560315)

72 Brabant G, Poll EM, Jonsson P, Polydorou D & Kreitschmann-Andermahr I. Etiology, baseline characteristics, and biochemical diagnosis of GH deficiency in the adult: are there regional variations? *European Journal of Endocrinology* 2009 **161**(Supplement 1) S25–S31. (https://doi.org/10.1530/EJE-09-0273)

73 Yuen KC, Biller BM, Katznelson L, Rhoads SA, Gurel MH, Chu O, Corazzini V, Spiller K, Gordon MB, Salvatori R, *et al.* Clinical characteristics, timing of peak responses and safety aspects of two dosing regimens of the glucagon stimulation test in evaluating growth hormone and cortisol secretion in adults. *Pituitary* 2013 **16** 220–230. (https://doi.org/10.1007/s11102-012-0407-7)

74 Conceicao FL, da Costa e Silva A, Leal Costa AJ & Vaisman M. Glucagon stimulation test for the diagnosis of GH deficiency in adults. *Journal of Endocrinological Investigation* 2003 **26** 1065–1070. (https://doi.org/10.1007/BF03345251)

75 Gomez JM, Espadero RM, Escobar-Jimenez F, Hawkins F, Pico A, Herrera-Pombo JL, Vilardell E, Duran A, Mesa J, Faure E, *et al.* Growth hormone release after glucagon as a reliable test of growth hormone assessment in adults. *Clinical Endocrinology* 2002 **56** 329–334. (https://doi.org/10.1046/j.1365-2265.2002.01472.x)

76 Dichtel LE, Yuen KC, Bredella MA, Gerweck AV, Russell BM, Riccio AD, Gurel MH, Sluss PM, Biller BM & Miller KK. Overweight/Obese adults with pituitary disorders require lower peak growth hormone cutoff values on glucagon stimulation testing to avoid overdiagnosis of growth hormone deficiency. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 4712–4719. (https://doi.org/10.1210/jc.2014-2830)

77 Toogood A, Brabant G, Maiter D, Jonsson B, Feldt-Rasmussen U, Koltowska-Haggstrom M, Rasmussen AK, Buchfelder M, Saller B & Biller BM. Similar clinical features among patients with severe adult growth hormone deficiency diagnosed with insulin tolerance test or arginine or glucagon stimulation tests. *Endocrine Practice* 2012 **18** 325–334. (https://doi.org/10.4158/EP11146.OR)

78 Diri H, Karaca Z, Simsek Y, Tanriverdi F, Unluhizarci K, Selcuklu A & Kelestimur F. Can a glucagon stimulation test characterized by lower GH cut-off value be used for the diagnosis of growth hormone deficiency in adults? *Pituitary* 2015 **18** 884–892. (https://doi.org/10.1007/s11102-015-0666-1)

79 Hamrahian AH, Yuen KC, Gordon MB, Pulaski-Liebert KJ, Bena J & Biller BM. Revised GH and cortisol cut-points for the glucagon stimulation test in the evaluation of GH and hypothalamic-pituitary-adrenal axes in adults: results from a prospective randomized multicenter study. *Pituitary* 2016 **19** 332–341. (https://doi.org/10.1007/s11102-016-0712-7)

80 Wilson JR, Utz AL & Devin JK. Effects of gender, body weight, and blood glucose dynamics on the growth hormone response to the glucagon stimulation test in patients with pituitary disease. *Growth Hormone and IGF Research* 2016 **26** 24–31. (https://doi.org/10.1016/j.ghir.2015.12.005)

81 Yuen KC, Tritos NA, Samson SL, Hoffman AR & Katznelson L. American Association of Clinical Endocrinologists and American college of endocrinology disease state clinical review: update on growth hormone stimulation testing and proposed revised cut-point for the glucagon stimulation test in the diagnosis of adult growth hormone deficiency. *Endocrine Practice* 2016 **22** 1235–1244. (https://doi.org/10.4158/EP161407.DSCR)

82 Leal A, Lage M, Popovic V, Torres E, Koppeschaar HP, Paramo C, Micic D, Garcia-Mayor RV, Dieguez C & Casanueva FF. A single growth hormone (GH) determination is sufficient for the diagnosis of GH-deficiency in adult patients using the growth hormone releasing hormone plus growth hormone releasing peptide-6 test. *Clinical Endocrinology* 2002 **57** 377–384. (https://doi.org/10.1046/j.1365-2265.2002.01623.x)

83 Tuilpakov AN, Bulatov AA, Peterkova VA, Elizarova GP, Volevodz NN & Bowers CY. Growth hormone (GH)-releasing effects of synthetic peptide GH-releasing peptide-2 and GH-releasing hormone (1–29NH2) in children with GH insufficiency and idiopathic short stature. *Metabolism: Clinical and Experimental* 1995 **44** 1199–1204. (https://doi.org/10.1016/0026-0495(95)90016-0)

84 Alaioubi B, Mann K & Petersenn S. Diagnosis of growth hormone deficiency in adults: provocative testing with GHRP6 in comparison to the insulin tolerance test. *Hormone and Metabolic Research* 2009 **41** 238–243. (https://doi.org/10.1055/s-0028-1093350)

85 Guzzetti C, Ibba A, Pilia S, Beltrami N, Di Iorgi N, Rollo A, Fratangeli N, Radetti G, Zucchini S, Maghnie M, *et al.* Cut-off limits of the peak GH response to stimulation tests for the diagnosis of GH deficiency in children and adolescents: study in patients with organic GHD. *European Journal of Endocrinology* 2016 **175** 41–47. (https://doi.org/10.1530/EJE-16-0105)

86 Boyd AE, 3rd, Lebovitz HE & Pfeiffer JB. Stimulation of human-growth-hormone secretion by L-dopa. *New England Journal of Medicine* 1970 **283** 1425–1429. (https://doi.org/10.1056/NEJM197012242832602)

87 Sizonenko PC, Clayton PE, Cohen P, Hintz RL, Tanaka T & Laron Z. Diagnosis and management of growth hormone deficiency in childhood and adolescence. Part 1: diagnosis of growth hormone deficiency. *Growth Hormone and IGF Research* 2001 **11** 137–165. (https://doi.org/10.1054/ghir.2001.0203)

88 Weldon VV, Gupta SK, Klingensmith G, Clarke WL, Duck SC, Haymond MW & Pagliara AS. Evaluation of growth hormone release in children using arginine and L-dopa in combination. *Journal of Pediatrics* 1975 **87** 540–544. (https://doi.org/10.1016/s0022-3476(75)80816-x)

89 Ghigo E, Aimaretti G, Gianotti L, Bellone J, Arvat E & Camanni F. New approach to the diagnosis of growth hormone deficiency in adults. *European Journal of Endocrinology* 1996 **134** 352–356. (https://doi.org/10.1530/eje.0.1340352)

90 Rochiccioli P, Enjaume C, Tauber MT & Pienkowski C. Statistical study of 5473 results of nine pharmacological stimulation tests: a proposed weighting index. *Acta Paediatrica* 1993 **82** 245–248. (https://doi.org/10.1111/j.1651-2227.1993.tb12652.x)

91 Barinaga M, Bilezikjian LM, Vale WW, Rosenfeld MG & Evans RM. Independent effects of growth hormone releasing factor on growth

hormone release and gene transcription. *Nature* 1985 **314** 279–281. (https://doi.org/10.1038/314279a0)
92 Alba-Roth J, Muller OA, Schopohl J & von Werder K. Arginine stimulates growth hormone secretion by suppressing endogenous somatostatin secretion. *Journal of Clinical Endocrinology and Metabolism* 1988 **67** 1186–1189. (https://doi.org/10.1210/jcem-67-6-1186)
93 Dichtel LE, Corey KE, Haines MS, Chicote ML, Kimball A, Colling C, Simon TG, Long MT, Husseini J, Bredella MA, *et al.* The GH/IGF-1 axis is associated with intrahepatic lipid content and hepatocellular damage in overweight/obesity. *Journal of Clinical Endocrinology and Metabolism* 2022 **107** e3624–e3632. (https://doi.org/10.1210/clinem/dgac405)
94 Makimura H, Stanley T, Mun D, You SM & Grinspoon S. The effects of central adiposity on growth hormone (GH) response to GH-releasing hormone-arginine stimulation testing in men. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 4254–4260. (https://doi.org/10.1210/jc.2008-1333)
95 Aimaretti G, Corneli G, Razzore P, Bellone S, Baffoni C, Arvat E, Camanni F & Ghigo E. Comparison between insulin-induced hypoglycemia and growth hormone (GH)-releasing hormone + arginine as provocative tests for the diagnosis of GH deficiency in adults. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1615–1618. (https://doi.org/10.1210/jcem.83.5.4837)
96 Ghigo E, Aimaretti G, Arvat E & Camanni F. Growth hormone-releasing hormone combined with arginine or growth hormone secretagogues for the diagnosis of growth hormone deficiency in adults. *Endocrine* 2001 **15** 29–38. (https://doi.org/10.1385/ENDO:15:1:029)
97 Darzy KH, Thorner MO & Shalet SM. Cranially irradiated adult cancer survivors may have normal spontaneous GH secretion in the presence of discordant peak GH responses to stimulation tests (compensated GH deficiency). *Clinical Endocrinology* 2009 **70** 287–293. (https://doi.org/10.1111/j.1365-2265.2008.03359.x)
98 Qu XD, Gaw Gonzalo IT, Al Sayed MY, Cohan P, Christenson PD, Swerdloff RS, Kelly DF & Wang C. Influence of body mass index and gender on growth hormone (GH) responses to GH-releasing hormone plus arginine and insulin tolerance tests. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 1563–1569. (https://doi.org/10.1210/jc.2004-1450)
99 Baumann GP. Growth hormone isoforms. *Growth Hormone and IGF Research* 2009 **19** 333–340. (https://doi.org/10.1016/j.ghir.2009.04.011)
100 Ribeiro de Oliveira Longo Schweizer J, Ribeiro-Oliveira A, Jr & Bidlingmaier M. Growth hormone: isoforms, clinical aspects and assays interference. *Clinical Diabetes and Endocrinology* 2018 **4** 18. (https://doi.org/10.1186/s40842-018-0068-1)
101 Clemmons DR. Consensus statement on the standardization and evaluation of growth hormone and insulin-like growth factor assays. *Clinical Chemistry* 2011 **57** 555–559. (https://doi.org/10.1373/clinchem.2010.150631)
102 Pokrajac A, Wark G, Ellis AR, Wear J, Wieringa GE & Trainer PJ. Variation in GH and IGF-I assays limits the applicability of international consensus criteria to local practice. *Clinical Endocrinology* 2007 **67** 65–70. (https://doi.org/10.1111/j.1365-2265.2007.02836.x)
103 Junnila RK, Strasburger CJ & Bidlingmaier M. Pitfalls of insulin-like growth factor-I and growth hormone assays. *Endocrinology and Metabolism Clinics of North America* 2015 **44** 27–34. (https://doi.org/10.1016/j.ecl.2014.10.003)
104 Bronsema KJ, Klont F, Schalk FB, Bischoff R, Kema IP & van de Merbel NC. A quantitative LC-MS/MS method for insulin-like growth factor 1 in human plasma. *Clinical Chemistry and Laboratory Medicine* 2018 **56** 1905–1912. (https://doi.org/10.1515/cclm-2017-1042)
105 Pratt MS, van Faassen M, Remmelts N, Bischoff R & Kema IP. An antibody-free LC-MS/MS method for the quantification of intact insulin-like growth factors 1 and 2 in human plasma. *Analytical and Bioanalytical Chemistry* 2021 **413** 2035–2044. (https://doi.org/10.1007/s00216-021-03185-y)
106 Hines J, Milosevic D, Ketha H, Taylor R, Algeciras-Schimnich A, Grebe SK & Singh RJ. Detection of IGF-1 protein variants by use of LC-MS with high-resolution accurate mass in routine clinical analysis. *Clinical Chemistry* 2015 **61** 990–991. (https://doi.org/10.1373/clinchem.2014.234799)
107 Walenkamp MJ, Karperien M, Pereira AM, Hilhorst-Hofstee Y, van Doorn J, Chen JW, Mohan S, Denley A, Forbes B, van Duyvenvoorde HA, *et al.* Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 2855–2864. (https://doi.org/10.1210/jc.2004-1254)
108 Cain JP, Williams GH & Dluhy RG. Glucagon-initiated human growth hormone release: a comparative study. *Canadian Medical Association Journal* 1972 **107** 617–622.
109 Jaruratanasirikul S, Leethanaporn K & Sriplung H. Should the duration of the insulin tolerance test be shortened to 90 minutes? *Journal of Pediatric Endocrinology and Metabolism* 2004 **17** 1105–1109. (https://doi.org/10.1515/jpem.2004.17.8.1105)
110 Mitchell ML, Byrne MJ, Sanchez Y & Sawin CT. Detection of growth-hormone deficiency. *New England Journal of Medicine* 1970 **282** 539–541. (https://doi.org/10.1056/NEJM197003052821005)
111 Muster L, Zangen DH, Nesher R, Hirsch HJ, Muster Z & Gillis D. Arginine and clonidine stimulation tests for growth hormone deficiency revisited--do we really need so many samples? *Journal of Pediatric Endocrinology and Metabolism* 2009 **22** 215–223. (https://doi.org/10.1515/jpem.2009.22.3.215)
112 Strich D, Terespolsky N & Gillis D. Glucagon stimulation test for childhood growth hormone deficiency: timing of the peak is important. *Journal of Pediatrics* 2009 **154** 415–419. (https://doi.org/10.1016/j.jpeds.2008.08.044)
113 Leong KS, Walker AB, Martin I, Wile D, Wilding J & MacFarlane IA. An audit of 500 subcutaneous glucagon stimulation tests to assess growth hormone and ACTH secretion in patients with hypothalamic-pituitary disease. *Clinical Endocrinology* 2001 **54** 463–468. (https://doi.org/10.1046/j.1365-2265.2001.01169.x)
114 Orme SM, Price A, Weetman AP & Ross RJ. Comparison of the diagnostic utility of the simplified and standard i.m. glucagon stimulation test (IMGST). *Clinical Endocrinology* 1998 **49** 773–778. (https://doi.org/10.1046/j.1365-2265.1998.00610.x)
115 Ho KY, Evans WS, Blizzard RM, Veldhuis JD, Merriam GR, Samojlik E, Furlanetto R, Rogol AD, Kaiser DL & Thorner MO. Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. *Journal of Clinical Endocrinology and Metabolism* 1987 **64** 51–58. (https://doi.org/10.1210/jcem-64-1-51)
116 Frantz AG & Rabkin MT. Effects of estrogen and sex difference on secretion of human growth hormone. *Journal of Clinical Endocrinology and Metabolism* 1965 **25** 1470–1480. (https://doi.org/10.1210/jcem-25-11-1470)
117 van den Berg G, Veldhuis JD, Frolich M & Roelfsema F. An amplitude-specific divergence in the pulsatile mode of growth hormone (GH) secretion underlies the gender difference in mean GH concentrations in men and premenopausal women. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 2460–2467. (https://doi.org/10.1210/jcem.81.7.8675561)
118 Dawson-Hughes B, Stern D, Goldman J & Reichlin S. Regulation of growth hormone and somatomedin-C secretion in postmenopausal women: effect of physiological estrogen replacement. *Journal of Clinical Endocrinology and Metabolism* 1986 **63** 424–432. (https://doi.org/10.1210/jcem-63-2-424)
119 Diamond MP, Jones T, Caprio S, Hallarman L, Diamond MC, Addabbo M, Tamborlane WV & Sherwin RS. Gender influences counterregulatory hormone responses to hypoglycemia. *Metabolism: Clinical and Experimental* 1993 **42** 1568–1572. (https://doi.org/10.1016/0026-0495(93)90152-e)
120 Kerrigan JR & Rogol AD. The impact of gonadal steroid hormone action on growth hormone secretion during childhood and adolescence. *Endocrine Reviews* 1992 **13** 281–298. (https://doi.org/10.1210/edrv-13-2-281)
121 Bellantoni MF, Vittone J, Campfield AT, Bass KM, Harman SM & Blackman MR. Effects of oral versus transdermal estrogen on the

growth hormone/insulin-like growth factor I axis in younger and older postmenopausal women: a clinical research center study. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 2848–2853. (https://doi.org/10.1210/jcem.81.8.8768841)
122 Fisker S, Jorgensen JO, Vahl N, Orskov H & Christiansen JS. Impact of gender and androgen status on IGF-I levels in normal and GH-deficient adults. *European Journal of Endocrinology* 1999 **141** 601–608. (https://doi.org/10.1530/eje.0.1410601)
123 Deutschbein T, Bidlingmaier M, Schopohl J, Strasburger CJ & Petersenn S. Anthropometric factors have significant influence on the outcome of the GHRH-arginine test: establishment of normative data for an automated immunoassay specifically measuring 22 kDa human growth hormone. *European Journal of Endocrinology* 2017 **176** 273–281. (https://doi.org/10.1530/EJE-16-0668)
124 Markkanen HM, Pekkarinen T, Hamalainen E, Valimaki MJ, Alfthan H & Stenman UH. Gender has to be taken into account in diagnosing adult growth hormone deficiency by the GHRH plus arginine test. *Growth Hormone and IGF Research* 2017 **35** 52–56. (https://doi.org/10.1016/j.ghir.2017.07.002)
125 Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH research society. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 3990–3993. (https://doi.org/10.1210/jcem.85.11.6984)
126 Murray PG, Dattani MT & Clayton PE. Controversies in the diagnosis and management of growth hormone deficiency in childhood and adolescence. *Archives of Disease in Childhood* 2016 **101** 96–100. (https://doi.org/10.1136/archdischild-2014-307228)
127 Sato T, Kusakawa M, Ichihashi Y, Ishii T & Hasegawa T. Testosterone priming increased growth hormone peak levels in the stimulation test and suppressed gonadotropin secretion in three Japanese adolescent boys. *Clinical Pediatric Endocrinology* 2020 **29** 119–121. (https://doi.org/10.1297/cpe.29.119)
128 Marin G, Domene HM, Barnes KM, Blackwell BJ, Cassorla FG & Cutler GB, Jr. The effects of estrogen priming and puberty on the growth hormone response to standardized treadmill exercise and arginine-insulin in normal girls and boys. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 537–541. (https://doi.org/10.1210/jcem.79.2.8045974)
129 Muller G, Keller A, Reich A, Hoepffner W, Kratzsch J, Buckler JM, Kiess W & Keller E. Priming with testosterone enhances stimulated growth hormone secretion in boys with delayed puberty. *Journal of Pediatric Endocrinology and Metabolism* 2004 **17** 77–83. (https://doi.org/10.1515/jpem.2004.17.1.77)
130 Cohen P, Germak J, Rogol AD, Weng W, Kappelgaard AM, Rosenfeld RG & American Norditropin Study Group. Variable degree of growth hormone (GH) and insulin-like growth factor (IGF) sensitivity in children with idiopathic short stature compared with GH-deficient patients: evidence from an IGF-based dosing study of short children. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 2089–2098. (https://doi.org/10.1210/jc.2009-2139)
131 Gandrud LM & Wilson DM. Is growth hormone stimulation testing in children still appropriate? *Growth Hormone and IGF Research* 2004 **14** 185–194. (https://doi.org/10.1016/j.ghir.2003.11.003)
132 van Vught AJ, Nieuwenhuizen AG, Gerver WJ, Veldhorst MA, Brummer RJ & Westerterp-Plantenga MS. Pharmacological and physiological growth hormone stimulation tests to predict successful GH therapy in children. *Journal of Pediatric Endocrinology and Metabolism* 2009 **22** 679–694. (https://doi.org/10.1515/jpem.2009.22.8.679)
133 Binder G, Reinehr T, Ibanez L, Thiele S, Linglart A, Woelfle J, Saenger P, Bettendorf M, Zachurzok A, Gohlke B, *et al.* GHD diagnostics in Europe and the US: an audit of National guidelines and practice. *Hormone Research in Paediatrics* 2019 **92** 150–156. (https://doi.org/10.1159/000503783)
134 Hage C, Gan HW, Ibba A, Patti G, Dattani M, Loche S, Maghnie M & Salvatori R. Advances in differential diagnosis and management of growth hormone deficiency in children. *Nature Reviews. Endocrinology* 2021 **17** 608–624. (https://doi.org/10.1038/s41574-021-00539-5)
135 Lazar L & Phillip M. Is sex hormone priming in peripubertal children prior to growth hormone stimulation tests still appropriate? *Hormone Research in Paediatrics* 2010 **73** 299–302. (https://doi.org/10.1159/000284396)
136 Allen DB. Diagnosis of growth hormone deficiency remains a judgment call - and that is good. *Hormone Research in Paediatrics* 2021 **94** 406–409. (https://doi.org/10.1159/000521628)
137 Caputo M, Pigni S, Agosti E, Daffara T, Ferrero A, Filigheddu N & Prodam F. Regulation of GH and GH signaling by nutrients. *Cells* 2021 **10**. (https://doi.org/10.3390/cells10061376)
138 Clemmons DR, Klibanski A, Underwood LE, McArthur JW, Ridgway EC, Beitins IZ & Van Wyk JJ. Reduction of plasma immunoreactive somatomedin C during fasting in humans. *Journal of Clinical Endocrinology and Metabolism* 1981 **53** 1247–1250. (https://doi.org/10.1210/jcem-53-6-1247)
139 Hartman ML, Clayton PE, Johnson ML, Celniker A, Perlman AJ, Alberti KG & Thorner MO. A low dose euglycemic infusion of recombinant human insulin-like growth factor I rapidly suppresses fasting-enhanced pulsatile growth hormone secretion in humans. *Journal of Clinical Investigation* 1993 **91** 2453–2462. (https://doi.org/10.1172/JCI116480)
140 Merimee TJ, Zapf J & Froesch ER. Insulin-like growth factors in the fed and fasted states. *Journal of Clinical Endocrinology and Metabolism* 1982 **55** 999–1002. (https://doi.org/10.1210/jcem-55-5-999)
141 Yamashita S & Melmed S. Insulin regulation of rat growth hormone gene transcription. *Journal of Clinical Investigation* 1986 **78** 1008–1014. (https://doi.org/10.1172/JCI112654)
142 Ho KY, Veldhuis JD, Johnson ML, Furlanetto R, Evans WS, Alberti KG & Thorner MO. Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. *Journal of Clinical Investigation* 1988 **81** 968–975. (https://doi.org/10.1172/JCI113450)
143 Leung KC, Doyle N, Ballesteros M, Waters MJ & Ho KK. Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 4712–4720. (https://doi.org/10.1210/jcem.85.12.7017)
144 Thissen JP, Ketelslegers JM & Underwood LE. Nutritional regulation of the insulin-like growth factors. *Endocrine Reviews* 1994 **15** 80–101. (https://doi.org/10.1210/edrv-15-1-80)
145 Norrelund H, Nair KS, Nielsen S, Frystyk J, Ivarsen P, Jorgensen JO, Christiansen JS & Moller N. The decisive role of free fatty acids for protein conservation during fasting in humans with and without growth hormone. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 4371–4378. (https://doi.org/10.1210/jc.2003-030267)
146 Aimaretti G, Colao A, Corneli G, Pivonello R, MacCario M, Morrison K, Pflaum CD, Strasburger CJ, Lombardi G & Ghigo E. The study of spontaneous GH secretion after 36-h fasting distinguishes between GH-deficient and normal adults. *Clinical Endocrinology* 1999 **51** 771–777. (https://doi.org/10.1046/j.1365-2265.1999.00885.x)
147 Argente J, Caballo N, Barrios V, Munoz MT, Pozo J, Chowen JA, Morande G & Hernandez M. Multiple endocrine abnormalities of the growth hormone and insulin-like growth factor axis in patients with anorexia nervosa: effect of short- and long-term weight recuperation. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 2084–2092. (https://doi.org/10.1210/jcem.82.7.4090)
148 Counts DR, Gwirtsman H, Carlsson LM, Lesem M & Cutler GB, Jr. The effect of anorexia nervosa and refeeding on growth hormone-binding protein, the insulin-like growth factors (IGFs), and the IGF-binding proteins. *Journal of Clinical Endocrinology and Metabolism* 1992 **75** 762–767. (https://doi.org/10.1210/jcem.75.3.1381372)
149 Soliman AT, Hassan AE, Aref MK, Hintz RL, Rosenfeld RG & Rogol AD. Serum insulin-like growth factors I and II concentrations and growth hormone and insulin responses to arginine infusion in children

with protein-energy malnutrition before and after nutritional rehabilitation. *Pediatric Research* 1986 **20** 1122–1130. (https://doi.org/10.1203/00006450-198611000-00012)
150 Cornford AS, Barkan AL & Horowitz JF. Rapid suppression of growth hormone concentration by overeating: potential mediation by hyperinsulinemia. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 824–830. (https://doi.org/10.1210/jc.2010-1895)
151 Iranmanesh A, Lizarralde G & Veldhuis JD. Age and relative adiposity are specific negative determinants of the frequency and amplitude of growth hormone (GH) secretory bursts and the half-life of endogenous GH in healthy men. *Journal of Clinical Endocrinology and Metabolism* 1991 **73** 1081–1088. (https://doi.org/10.1210/jcem-73-5-1081)
152 Makimura H, Feldpausch MN, Rope AM, Hemphill LC, Torriani M, Lee H & Grinspoon SK. Metabolic effects of a growth hormone-releasing factor in obese subjects with reduced growth hormone secretion: a randomized controlled trial. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 4769–4779. (https://doi.org/10.1210/jc.2012-2794)
153 Loche S, Guzzetti C, Pilia S, Ibba A, Civolani P, Porcu M, Minerba L & Casini MR. Effect of body mass index on the growth hormone response to clonidine stimulation testing in children with short stature. *Clinical Endocrinology* 2011 **74** 726–731. (https://doi.org/10.1111/j.1365-2265.2011.03988.x)
154 Radack JA, White PC, Adams-Huet B & Oden JD. Stimulated growth hormone concentrations in obese pediatric patients with mild and severe insulin resistance: a pilot study. *Journal of Pediatric Endocrinology and Metabolism* 2010 **23** 355–361. (https://doi.org/10.1515/jpem.2010.056)
155 De Marinis L, Bianchi A, Mancini A, Gentilella R, Perrelli M, Giampietro A, Porcelli T, Tilaro L, Fusco A, Valle D, *et al.* Growth hormone secretion and leptin in morbid obesity before and after biliopancreatic diversion: relationships with insulin and body composition. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 174–180. (https://doi.org/10.1210/jc.2002-021308)
156 Rasmussen MH, Hvidberg A, Juul A, Main KM, Gotfredsen A, Skakkebaek NE, Hilsted J & Skakkebae NE. Massive weight loss restores 24-hour growth hormone release profiles and serum insulin-like growth factor-I levels in obese subjects. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 1407–1415. (https://doi.org/10.1210/jcem.80.4.7536210)
157 CDC. Defining child BMI categories. Atlanta, GA, USA: Centers for Disease Control and Prevention (CDC), 2023. (available at: https://www.cdc.gov/obesity/basics/childhood-defining.html)
158 Abawi O, Augustijn D, Hoeks SE, de Rijke YB & van den Akker ELT. Impact of body mass index on growth hormone stimulation tests in children and adolescents: a systematic review and meta-analysis. *Critical Reviews in Clinical Laboratory Sciences* 2021 **58** 576–595. (https://doi.org/10.1080/10408363.2021.1956423)
159 Thieme F, Vogel M, Gausche R, Beger C, Vasilakis IA, Kratzsch J, Korner A, Kiess W & Pfaffle RW. The influence of body mass index on the growth hormone peak response regarding growth hormone stimulation tests in children. *Hormone Research in Paediatrics* 2022 **95** 452–460. (https://doi.org/10.1159/000526240)
160 Bozzola M, Adamsbaum C, Biscaldi I, Zecca M, Cisternino M, Genovese E, Richard I, Kalifa G & Chaussain JL. Role of magnetic resonance imaging in the diagnosis and prognosis of growth hormone deficiency. *Clinical Endocrinology* 1996 **45** 21–26.
161 Kornreich L, Horev G, Lazar L, Schwarz M, Sulkes J & Pertzelan A. MR findings in growth hormone deficiency: correlation with severity of hypopituitarism. *AJNR. American Journal of Neuroradiology* 1998 **19** 1495–1499.
162 Alba P, Tsai S & Mitre N. The severity of growth hormone deficiency does not predict the presence or absence of brain magnetic resonance imaging abnormalities: a retrospective review. *European Endocrinology* 2020 **16** 60–64. (https://doi.org/10.17925/EE.2020.16.1.60)
163 Kessler M, Tenner M, Frey M & Noto R. Pituitary volume in children with growth hormone deficiency, idiopathic short stature and controls. *Journal of Pediatric Endocrinology and Metabolism* 2016 **29** 1195–1200. (https://doi.org/10.1515/jpem-2015-0404)
164 Lotierzo M, Olaru-Soare F, Dupuy AM, Plawecki M, Paris F & Cristol JP. Comparative study of human growth hormone measurements: impact on clinical interpretation. *Clinical Chemistry and Laboratory Medicine* 2022 **60** 191–197. (https://doi.org/10.1515/cclm-2021-1109)
165 Wagner IV, Paetzold C, Gausche R, Vogel M, Koerner A, Thiery J, Arsene CG, Henrion A, Guettler B, Keller E, *et al.* Clinical evidence-based cutoff limits for GH stimulation tests in children with a backup of results with reference to mass spectrometry. *European Journal of Endocrinology* 2014 **171** 389–397. (https://doi.org/10.1530/EJE-14-0165)
166 Secco A, di Iorgi N, Napoli F, Calandra E, Calcagno A, Ghezzi M, Frassinetti C, Fratangeli N, Parodi S, Benassai M, *et al.* Reassessment of the growth hormone status in young adults with childhood-onset growth hormone deficiency: reappraisal of insulin tolerance testing. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 4195–4204. (https://doi.org/10.1210/jc.2009-0602)
167 Maghnie M, Salati B, Bianchi S, Rallo M, Tinelli C, Autelli M, Aimaretti G & Ghigo E. Relationship between the morphological evaluation of the pituitary and the growth hormone (GH) response to GH-releasing hormone Plus arginine in children and adults with congenital hypopituitarism. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 1574–1579. (https://doi.org/10.1210/jcem.86.4.7394)
168 Biller BM, Samuels MH, Zagar A, Cook DM, Arafah BM, Bonert V, Stavrou S, Kleinberg DL, Chipman JJ & Hartman ML. Sensitivity and specificity of six tests for the diagnosis of adult GH deficiency. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 2067–2079. (https://doi.org/10.1210/jcem.87.5.8509)
169 Langendonk JG, Meinders AE, Burggraaf J, Frolich M, Roelen CA, Schoemaker RC, Cohen AF & Pijl H. Influence of obesity and body fat distribution on growth hormone kinetics in humans. *American Journal of Physiology* 1999 **277** E824–E829. (https://doi.org/10.1152/ajpendo.1999.277.5.E824)
170 Magiakou MA, Mastorakos G, Gomez MT, Rose SR & Chrousos GP. Suppressed spontaneous and stimulated growth hormone secretion in patients with Cushing's disease before and after surgical cure. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 131–137. (https://doi.org/10.1210/jcem.78.1.7507118)
171 Veldhuis JD, Iranmanesh A, Ho KK, Waters MJ, Johnson ML & Lizarralde G. Dual defects in pulsatile growth hormone secretion and clearance subserve the hyposomatotropism of obesity in man. *Journal of Clinical Endocrinology and Metabolism* 1991 **72** 51–59. (https://doi.org/10.1210/jcem-72-1-51)
172 Yuen KC, Cook DM, Rumbaugh EE, Cook MB & Dunger DB. Individual IGF-I responsiveness to a fixed regimen of low-dose growth hormone replacement is increased with less variability in obese compared to non-obese adults with severe growth hormone deficiency. *Hormone Research* 2006 **65** 6–13. (https://doi.org/10.1159/000090121)

Received 3 March 2023
Accepted 13 April 2023
Available online 13 April 2023
Version of Record published 12 June 2023