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The history of an effective, specific and sensitive diagnostic test: the GHRH test in clinical practice

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Abstract
Growth hormone (GH) secretion is pulsatile, entropic, and nycthemeral and is mainly controlled by the hypothalamus through two neurohormones, the stimulating growth hormone releasing hormone (GHRH) and the inhibiting somatostatin. Shortly after its discovery and synthesis, GHRH was intensely investigated diagnostically to define GH secretion. The nascent enthusiasm for using GHRH as a single diagnostic tool to investigate GH deficiency (GHD) dropped down quickly due to a flawed reproducibility. The subsequent combinatory use of molecules implicated in GH secretion through inhibition of the somatostatinergic tone, such as arginine (ARG), or the synthesis of receptor-orphan pharmaceutical compounds capable of stimulating pituitary somatotrophs to release GH, such as the GH secretagogues (GHSs), improved the reproducibility of GH response to GHRH alone, thus gaining access into the clinical practice by means of different diagnostic approaches. This review will focus on the history of the GHRH test, with main emphasis on GHRH plus ARG as a dynamic testing for the diagnosis of GHD. Our attention will extend crosswise from studies aimed at validating GHRH-based tests for the clinical practice, to address main pitfall conditions capable of affecting per se GH secretion, such as obesity, hypothalamic damage, and ageing. The history of GHRH test has been progressively dismantled due to the cease of its production for business reasons, opening a gap in the diagnostic workup of patients with GHD. In the urgency to seek further robust, safe, and validated diagnostic tests or tools, we hope to stimulate attention on a so important peptide for the health of our patients suffering from pituitary diseases.

Keywords GHRH + arginine · Diagnosis · Accuracy · GH deficiency

1 Introduction: how GHRH physiology influenced the history of GHRH testing in GH deficiency

Growth hormone (GH) secretion is pulsatile, entropic, and nycthemeral and is mainly controlled by the hypothalamus through two neuropeptide hormones, the stimulatory growth hormone releasing hormone (GHRH) and the inhibitory somatostatin. GHRH is synthesized primarily in the arcuate nucleus and stimulates GH synthesis and secretion upon binding its cognate receptors located on pituitary somatotrophs; the antagonistic somatostatin originates from the periventricular region of the hypothalamus and acts to inhibit pituitary GH secretion. GHRH receptor activation also synchronizes spontaneous coactivation of neighbouring cells, being implicated in the secretory pulsatility [1–3].

Evidence suggested, over the years, that the regulation of GH secretion is complex, and several factors in addition to insulin-like growth factor-1 (IGF-1) can subtly contribute to its behavior directly or indirectly, by modulating GHRH and somatostatin tone. As such, subjects with inactivating mutations of the GHRH receptor have been shown to maintain unaltered the rhythmic GH pulsatile secretion, supporting the existence of other potential GH releasing factors besides GHRH [4–6]. Furthermore, studies on rodents documented that around 70% of GH pulses are associated with GHRH

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pulses, while 30% remain apparently dissociated [7]. The dynamic secretion of GH is alsodeeply influenced by gender (as females have higher fasting GH pulses than males), sleep, exercise, blood glucose (hyperglycemia decreases GH release, while hypoglycemia increases GH since it is one of the counter-regulatory hormones), amino acids like arginine (ARG), free fatty acids (FFAs), insulin (through distinct pathways) and insulin resistance, neurotransmitters such NPY and galanin, glucagon, thyroid hormones, and cortisol (by both stimulatory and inhibitory feedback) [5, 6, 8].

The metabolic and hormonal regulation of GH secretion is intriguingly complicated by the evidence that several pharmacological compounds, consequently named GH-releasing peptides (GHRPs) or GH secretagogues (GHSs), stimulate pituitary somatotrophs to release GH, and also act on the arcuate nucleus of the hypothalamus to release GHRH. The cloning of their natural receptor, the GHS-R, and subsequent identification of the natural ligand, ghrelin, reinforced enthusiasm to the topic and gave further insights into the central regulation of GH and GHRH [9, 10]. Large evidence suggested that GHSs potentiate the effects of GHRH and functionally antagonize somatostatin [6, 11]. GHRP-6, hexarelin and other non-classic synthetic GHRPs potently increase GH secretion by synergizing with GHRH [9], and GHSR antagonism results in a decrease in GH pulse amplitude in rodents [12]. Cross-approximate entropy analysis on ghrelin pulsatility found that ghrelin and GH are regularly co-secreted in fasting during nighttime [13, 14]. Furthermore, during feeding ghrelin exerts a positive feedback on GH secretion [14–16]. The GH response to ghrelin bolus in healthy subjects and several pathological conditions is more potent than after GHRH bolus or hexarelin. Although it is synergistic with the GH response to GHRH, pituitary stalk damage or mutations in the GHRH receptor cab blunt the GH response to ghrelin and GHSs, thus suggesting that the integrity of GHRH is necessary to elicit a potent GH release after their administration [9, 17].

Collectively, current evidence suggests that no single peptide and/or factor act in the modulation of the GH release, but the control is multifold and coordinated. For example, GHRH stimulates pituitary ghrelin gene expression; intrahypothalamic somatostatin tone can blunt GHRH secretion; and ghrelin/GHSs synergize with GHRH and antagonize somatostatin action [6, 8–12]. The interactive control is coincidentally modulated by gender, age, time of the day, auto-feedback, stress, physical exercise and external stimuli such as nutrition [3, 5]. Modifications in environmental signals affect the sensitivity or the pattern of episodic secretion of the somatotroph cells, with potential repercussions on the pattern of GH responses observed under varying pathological conditions. Hence, the complex background network governing physiologically the secretion of GH is a likely explanation for the existence of a spectrum of dynamic GH stimulatory tests that have been investigated, and validated, to assess the adequacy of GH secretion in health and pathological conditions involving the pituitary gland, mainly GH deficiency (GHD).

When first discovered, the GHRH became soon a tool to investigate GH secretion for diagnostic purposes. However, the GHRH test alone encountered problems of intra- and inter-individual variability on the grounds that, while the long-lasting pacemaker activity of somatotropes can be induced by relatively short exposures to GHRH thus favoring prolonged GH secretion, this electric activity can occur even without GHRH or GHS-mediated stimuli [2, 3]. Moreover, it was found in healthy adult males that repetitive stimulation bouts with GHRH could reduce the secreted amount of the 22 kDa GH isoform, the main circulating GH isoform, devoid of modifications of the 20 kDa isoform, thus increasing the proportion of the latter in the circulation [18]. These and other caveats dampened the growing enthusiasm for GHRH as a single diagnostic tool for patients with GHD.

Followingly, evidence was collected that the combinatory administration of molecules that are per se implicated in GH secretion through inhibition of the somatostatinergic tone, namely pyridostigmine (PD) and arginine (ARG), could improve the reproducibility of GH response to the administration of GHRH as a single agent. This combined approach was explored more so to avoid the drawbacks associated with other dynamic tests, mainly the insulin tolerance test (ITT). PD is a cholinergic agonist (cholinesterase inhibitor) that increases the effectiveness of endogenous acetylcholine to reduce somatostatin neuronal activity. Cholinesterase inhibitors also increase GHRH release from the hypothalamus [19]. ARG, like other amino acids in mammals, induces a reduction in somatostatin tone and an increase in secretory GH dynamics. It also directly increases the calcium flux into anterior pituitary cells, thus amplifying the pacemaker activity and the response to GHRH [2]. Both GHRH + PD and GHRH + ARG were fully explored over the years for diagnosis purposes in patients suspected of GHD, with establishment of different cut-offs relating to age (from pediatric to elderly age) and body mass index (BMI) (from normal weight to overweight and obesity) [20, 21]. On this account, GHRH + ARG widely entered into the clinical practice of GHD diagnosis by virtue of low risks of adverse events, and the need for fewer blood samples during the stimulatory curve [22–25]. Contemporarily, increasing knowledge around the role and function of synthetic GHSs and ghrelin in relation to GH stimulation provided evidence of their reliability as a provocative test for the diagnosis of GHD. BothGHRP-6 and hexarelin as single agents potently stimulate GH release [9, 10]. When combined with GHRH,

GHRP-6 or hexarelin are probably the most potent GH stimuli known so far, with a likelihood ratio of having a positive test of 10.1 and 16.9, respectively [20]. However, GHRH combined with ARG, PD, or GHSs have an increased risk of false-negative results due to decreased sensitivity in patients with hypothalamic or stalk lesion, in particular after radiotherapy [6, 23–26]. Despite that, an orally active GHS, macimorelin, has been tested in patients with GHD in comparison to the GHRH + ARG test and subsequently approved, but its use for diagnostic purposes is burdened by high costs and by temporary discontinuation in 2022 in the commercial market of the US [27].

After so many years of efforts in physiology knowledge, the history of GHRH has been progressively darkened by the cease in its production for business reasons in 2008 in the US and 2023 in Europe, opening a gap in the diagnostic workup of GHD, mainly when ITT or glucagon are contraindicated [28]. In the urgency to seek further alternative tests, we hope to stimulate the maintenance of the production of a so important peptide for the health of our patients. In this view, this review will focus on the main pillars of the history of the GHRH + ARG test. The main characteristics of GHRH-based provocative tests for the diagnosis of GHD are summarized in Table 1.

2 General efficacy of GHRH + ARG test

As mentioned above, GHRH alone is not reliable in assessing the somatotroph function, while the combination of GHRH with other substances capable of inhibiting the hypothalamic somatostatin release, such as ARG, considerably enhances its GH-releasing effect, yielding one of the most powerful and reproducible stimuli of GH secretion [2, 29]. Mean GH peak recorded after the GHRH + ARG is frequently higher than the GH peak response to other provocative tests, with good inter- and intraindividual reproducibility both in normal individuals and in patients with GHD [29–34]. Moreover, the GH response to GHRH + ARG is fundamentally unaffected by age and gender [34–37], as well as it is refractory to the negative feedback exerted by IGF-1 and other metabolic factors (e.g., blood glucose, FFAs), which can negatively influence GH secretion [21, 29]. Furthermore, the degree of the GH response to GHRH + ARG was found to be positively correlated with IGF-1 levels and parallels the clinical status, being positively associated with bone turnover markers and bone mineral density, while being negatively associated with unfavorable changes in lipid profile in GHD patients [38, 39]. This evidence, combined with the lack of significant side effects (mainly transient facial

Table 1 Main characteristics of GH provocative tests based on the administration of GHRH combined with ARG or other GHSs for the diagnosis of GHD [20–24, 29]

GH stimulation test	Procedure	Peak GH cutoffs	Side effects	Contraindications
GHRH + PD	PD 60/120 mg (children/adults) *per os* at -60 min + GHRH 1 ug/kg, iv bolus at + 0 min Blood samples for GH at 0, + 15,+ 30, + 60, (+ 75) and + 90 min	Children: < 20 µg/L Adults: <12.8–17.7 µg/L	Cholinergic side effects (e.g. gastrointestinal symptoms, excessive salivation, bradycardia, muscle cramps)	Seizure, arrhythmias or other unstable cardiovascular conditions, asthma and chronic lung diseases, elderly subjects
GHRH + ARG	GHRH 1 ug/kg, iv bolus at 0 min + ARG 0.5 g/kg (max 30 g) i.v. from 0 to + 30 min Blood sample for GH at 0, + 30, +45, + 60, +75, + 90, +105, and + 120 min Short procedure: 0, + 30, +45, + 60 min	Children: < 20 µg/L Transition age-patients: < 19 µg/L Adults: BMI < 25 kg/m^2:: < 11.5 µg/L 25 < BMI < 30 kg/m^2: < 8 µg/L BMI > 30 kg/m^2:: < 4.2 µg/L	Transient facial flushing (GHRH), nausea and vomiting (ARG)	Chronic kidney failure
GHRH + GHRP-6	GHRH 1 ug/kg, iv bolus + GHRP-6 1 µg/kg i.v.at 0 min Blood sample for GH at 0, + 15, +30, + 45, +60, + 90, and + 120 min	GHD: ≤ 10.0 µg/L Normal: > 20 µg/L	None	None
GHRH + GHRP-2	GHRH 1 ug/kg, iv bolus + GHRP-2 0.1 µg/kg i.v.at 0 min Blood sample for GH at 0, + 15, +30, + 45, +60, + 90 min	≤ 5.6 µg/L	None	None
GHRH + Hexarelin	GHRH 1 ug/kg, iv bolus + Hexarelin 0.25 µg/kg i.v.at 0' Blood sample for GH at 0, + 15, +30, + 45, +60, + 90 min	< 51.2 µg/L	Transient facial flushing	None
GHRH + MET	GHRH 1 ug/kg, iv bolus + MET 0.2 g/kg i.v. from 0 to + 30 min	Children: < 26 µg/L	Transient facial flushing	None

GH: growth hormone; GHRH: GH releasing hormone; PD: pyridostigmine; ARG: arginine; GHRP-6: Growth hormone-releasing peptide 6; GHRP-2: Growth hormone-releasing peptide 2; MET: methionine; GHD: GH deficiency; i.v. intravenous

flushing induced by GHRH) and contraindications (except for chronic renal failure in respect to ARG load), provided the rationale for considering the GHRH + ARG test as a valuable alternative to ITT for the diagnosis of GHD across all ages [23, 29, 40]. The GHRH + ARG test protocol is presented in Fig. 1.

Evidence on the diagnostic reliability of the GHRH + ARG test for GHD diagnosis of patients with pituitary disease first originated from pivotal studies achieving similar results in terms of sensitivity and specificity compared with the gold-standard ITT [22, 32, 34, 41]. In a study by Aimaretti et al. on a large population of hypopituitary adults, the majority of whom suffered form acquired adult-onset GHD caused by pituitary tumors and/or their treatment (surgery or radiotherapy), the authors showed that the GHRH + ARG test is at least as sensitive as the ITT when appropriate GH serum cut-offs are considered (i.e., peak GH response < 9 ug/L) [32]. The same group demonstrated that the GH response to GHRH + ARG progressively decreases with the increasing number of pituitary hormone deficiencies, thus showing different degrees of GHD better than ITT [22]. Moreover, they showed that the standard GHRH + ARG test can be usefully shortened (i.e., by performing no more than 3 or 4 blood samplings) without compromising its diagnostic accuracy, thus resulting in a less time- and cost-consuming procedure [42]. In a multicomparative study evaluating the relative performance of 6 different GH stimulation tests in patients with adult-onset hypothalamic-pituitary disease and in control subjects matched for age, sex, and BMI, the GHRH + ARG test also performed equally well compared to ITT, providing high sensitivity (95%) and specificity (SP 91%) at a peak GH cut-off of 4.1 µg/L [41]. Of note, the average BMI of this study population was in the obese range (i.e., > 30 kg/m2) [41]. Indeed, obesity and overweight are associated with blunted somatotroph responses to well-known GH stimuli as well as to classic provocative tests [5, 29, 43, 44], and the GH response to GHRH + ARG was shown to be negatively associated with BMI [29, 43, 45]. In line with this evidence, appropriate BMI-dependent cut-offs were established for the diagnosis of adult GHD, namely peak serum GH < 11.5 ug/l (sensitivity/specificity 98.7/83.7%), < 8 ug/l (sensitivity/specificity 96.7/83.7%), and < 4.2 ug/l (sensitivity/specificity 93.5/78.3%) in lean, overweight, and obese subjects, respectively [23, 46].

The safety and reliability of the GHRH + ARG test have been also demonstrated in children and adolescents [30, 31, 47–52]. In a study evaluating the GH response to 8 different provocative tests (ITT, ARG, clonidine, levodopa,

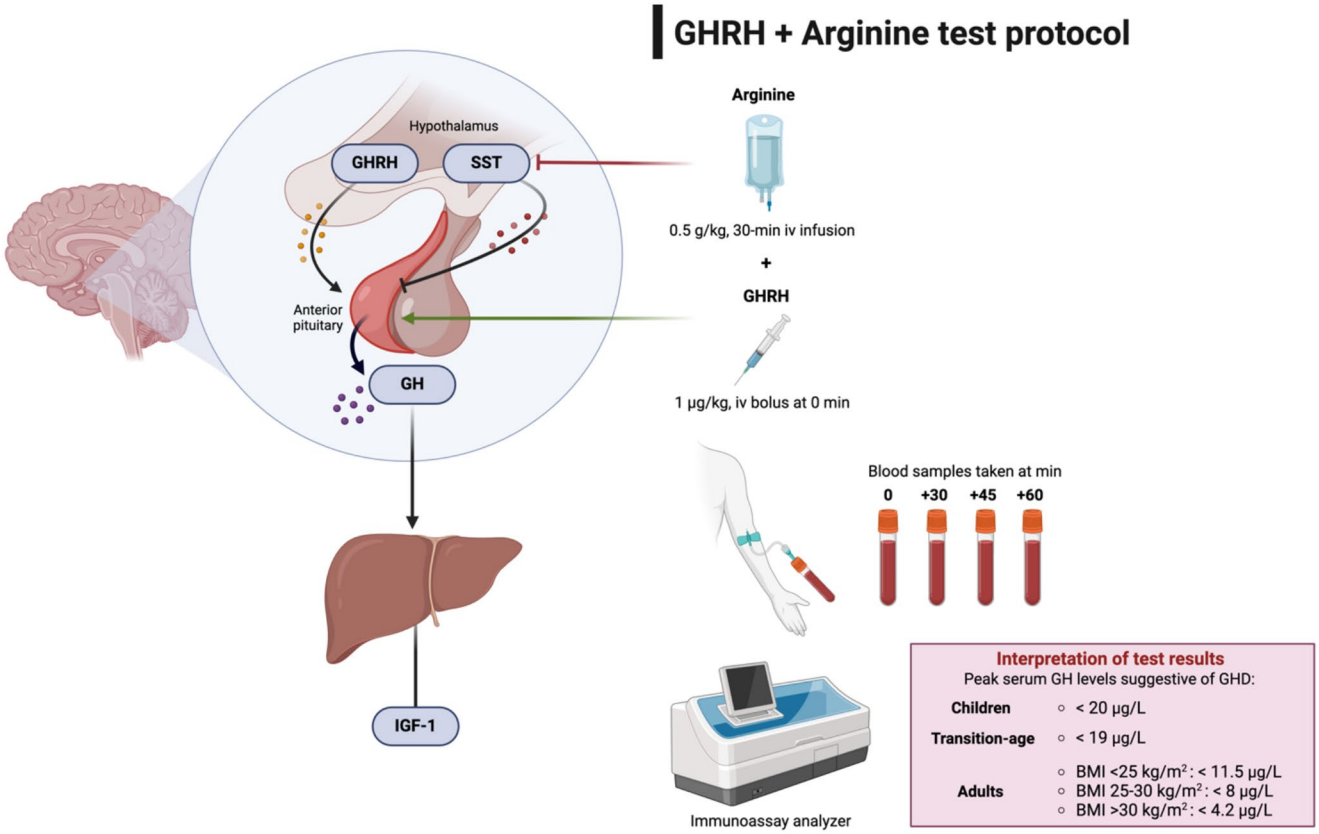

Fig. 1 GHRH + ARG test protocol and mechanism of action. GHRH: growth hormone releasing hormone; SST: somatostatin; GH: Growth hormone; IGF-1: insulin-like growth factor-1; GHD: GH deficiency; BMI: body mass index. Created with Biorender.com

glucagon, GHRH alone and combined with PD or ARG) in a large population of normally growing children, the lowest normal GH response to GHRH + ARG was found to be at 19.4 µg/L or > 20 µg/L considering a 95% confidence interval, with no false positive results (defined as peak GH levels < 7 µg/L or < 10 µg/L) in contrast to other classical tests [31]. In later studies, the 20 µg/L cut-off was confirmed as accurate to diagnose GHD in children with either acquired or congenital hypopituitarism but in the case of children with primary hypothalamic involvement or severe impairment of the pituitary stalk [48, 49]. Similarly, studies in transition-phase patients with childhood-onset GHD undergoing GH re-testing suggested a cut-off limit ≥ 19 ug/L for the normal GH response to GHRH + ARG, having the highest pair of sensitivity (100%) and specificity (97%) comparable to the ITT cut-off [50–52]. Of note, no significant puberty-related differences were observed in the peak GH response to GHRH + ARG [30, 31], provided that priming peripubertal children with sex hormones before GH testing remains a matter of debate [53, 54]. Also in children, however, a negative correlation exists between peak GH response to GHRH + ARG and BMI SDS [55–57], while the suggested GH cut-off values of < 20 µg/L or < 19 µg/L in children and late adolescents, respectively, were established in lean subjects [31, 50, 52]. Unlike adult GHD, specific GH cut-offs adjusted for BMI are still lacking in pediatric and transition-age populations [24, 55].

Characteristics of studies investigating the accuracy of GHRH + ARG test and the proposed cut-offs for the diagnosis of GHD in adults and in children and transition-age patients are summarized in Tables 2 and 3, respectively.

Table 2 Demographic, anthropometric, and clinical features of patients enrolled in studies investigating GHRH + ARG test accuracy and proposed GH cutoffs for the diagnosis of adult GHD

Author, year	Study population	Age, years (mean ± SD)	BMI, Kg/m2 (mean ± SD)	Comparative tests	Sensitivity	Specificity	Peak GH cutoffs
Aimaretti et al. 1998 [32]	40 patients: 21 acquired adult-onset GHD 19 COGHD	36.4 ± 2.1	NA	ITT	NA	NA	Severe GHD:9 µg/L Partial GHD: 16.5 µg/L
Biller et al. 2002 [41]	94 patients with adult-onset HPA disease 39 MPHD 21 0–1 PHD 34 controls	MPHD: 48.9 ± 11.1 PHD: 48.2 ± 11.3 Controls: 47.2 ± 11.3	MPHD: 30.5 ± 6.1 PHD: 29.2 ± 8.3 Controls: 30.3 ± 5.8	ITT, GHRH, ARG, L-DOPA ± ARG,	95%	91%	4.1 µg/L
Corneli et al. 2005 [46]	322 patients with organic HPA disease: 211 TPHD, 111 0–2 PHD 318 controls	TPHD: 47.6 ± 1.1 0–2 PHD: 48 ± 1.3 Controls: 39.9 ± 0.8	NA	NA	98.7% 96.7% 93.5%	83.7% 75.5% 78.3%	Lean 11.5 µg/L Overweight 8 µg/L Obese 4.2 µg/L
Chanson et al. 2010 [34]	69 subjects: Group C (low probability of GHD): 15 Group B (high probability of GHD): 38 Group A (controls): 16	Group C: 35.4 ± 14.1 Group B: 42.2 ± 11.7 Group A: 37.8 ± 6.9	Group C: 24.9 ± 5 Group B: 27.3 ± 5.2 Group A: 25.1 ± 1.9	ITT	79% 84%	100% 87%	3.67 µg/L 7.89 µg/L
Gasco et al. 2023 [59]	322 patients with HPA disease	45.1 ± 17.2	27.2 ± 5.8	NA	Lean: 95% Overweight (high/low pre-test probability of GHD): 97.3/68.5% Obese (high/low pre-test probability of GHD): 84.3/70%	Lean: 100% Overweight (high/low pre-test probability of GHD): 82.8/96.6% Obese (high/low pre-test probability of GHD): 91.7/97.2%	Lean: 8.0 µg/l Overweight (high/low pre-test probability of GHD): 7.0/2.6 µg/l Obese (high/low pre-test probability of GHD): 2.8/1.75 µg/l

GHD: GH deficiency; COGHD: childhood-onset GH deficiency; HPA: hypothalamic-pituitary disease; TPHD: total pituitary hormone deficits; PHD: pituitary hormone deficits; MPHD multiple pituitary hormone deficits; ITT: insulin tolerance test; ARG: arginine; L-DOPA: levodopa; NA: not available

Table 3 Demographic, anthropometric, and clinical features of patients enrolled in studies investigating GHRH + ARG test accuracy and proposed GH cutoffs for the diagnosis of GHD in children and transition-age patients

Author, year	Study population	Age, years (mean ± SD)	BMI, Kg/m2 (mean ± SD)	Comparative tests	Sensitivity	Specificity	Peak GH cutoffs
Ghigo et al.; 1996 [31]	472 normally growing children	11.2 ± 0.1	NA	ITT, ARG, PD, GHRH ± PD, clonidine, L-DOPA, glucagon	NA	NA	19.4 µg/L
Maghnie et al. 2002 [49]	36 patients with acquired GHD	9.6 ± 3.1	19 ± 3.5	ITT, ARG	75%	96.4%	20 µg/L
Donau-bauer et al.; 2003 [50]	43 patients: 28 organic COGHD, 15 idiopathic COGHD 40 controls	Patients: 20.4 ± 2.5 Controls: 21.3 ± 2.4	Patients: 23.5 ± 4.6 Controls: 21.6 ± 2.4	ITT, PD + GHRH	100%	97.5%	15.1–20.3 µg/L
Corneli et al. 2007 [52]	152 patients with organic HPA disease: Group A (panhypopituitary patients): 15 Group B (1–2 PHD): 18 Group C (0 PHD): 99 201 controls	Patients: 19.2 ± 0.2 Group A: 21.2 ± 0.4 Group B: 20.2 ± 0.9 Group C: 18.2 ± 0.2 Controls: 20.7 ± 0.2	Patients: NA Controls: 21.3 ± 0.2	NA	100%	97%	19 µg/L
Patti et al. 2019 [57]	88 COGHD: 29 idiopathic GHD, 44 cancer survivors, 15 CGHD; 34 MPHD, 54 isolated GHD	Total: 17.2 (range 16-18.8) GHD: 17.9 (range 16.3–19.1) Cancer survivors: 16.9 (range 15.6–18.2) CGHD: 17.6 (range 16-20.2) MPHD: 17.3 (range 15.6–18.6) Isolated GHD: 17.2 (range 16.1–18.8)	22.7 (range 20.1–25.5) Idiopathic GHD: 20.2 (range 19.2–23) Cancer survivors: 24.1 (range 21-26.6) CGHD: 23.5 (range 19.8–27.9) MPHD: 24.6 (range 22.1–26.7) Isolated GHD: 21.4 (range 19.3–24.1)	ITT	CGHD: 93.3% Cancer survivors: 70.5% MPHD: 79.4%	CGHD: 86.2% Cancer survivors: 100% MPHD: 80.5%	CGHD: 23.5 µg/L Cancer survivors: 15.7 µg/L MPHD: 13.8 µg/L

GHD: GH deficiency; COGHD: childhood-onset GH deficiency; HPA: hypothalamic-pituitary disease; CGHD: congenital GHD; PHD: pituitary hormone deficits; MPHD multiple pituitary hormone deficits; ITT: insulin tolerance test; ARG: arginine; PD: pyridostigmine; L-DOPA: levodopa; NA: not available

3 Pitfalls of GHRH + ARG test

Compelling evidence supports that the GHRH + ARG test is a generally effective and widely accepted method for evaluating the somatotroph function, being a potent, safe, convenient, reproducible, and reliable test with good sensitivity and specificity independently of age and gender, provided that appropriate BMI-based cut-offs are assumed within an appropriate clinical context. However, although generally effective, testing with GHRH + ARG is not devoid of potential pitfalls and limitations. Indeed, several factors can influence the diagnostic accuracy of GH provocative tests, including the lack of standardization of GH assays, which may lead to inconsistencies in GH measurements between different laboratories, as well as patients' characteristics and pretest probability of GHD [20, 24, 54]. Moreover, it should be considered that comparing patients with a pre-established diagnosis of GHD versus normal individuals as well as comparing an index test with a different one assumed as the reference standard may yield the possible risk of either over- or underestimating the test accuracy [20, 58, 59]. In this view, by studying a large group of adult patients with hypothalamic–pituitary disease and using a clinical gold standard (i.e., patients' pituitary function) for the diagnosis of GHD, some authors have recently proposed that the BMI-related cut-offs of the GH response to GHRH + ARG may need to be revised, suggesting different diagnostic thresholds also according to the pretest probability of GHD (i.e., 8.0 µg/l, 7.0/2.6 µg/l, and 2.8/1.75 µg/l, in lean, overweight and obese subjects with high/low pretest probability, respectively) [59]. Furthermore, the peak GH response may change according to the etiology of GHD. For instance, the GHRH + ARG test may fail to recognize patients with GHD due to primary hypothalamic dysfunction, which may show falsely "normal" GH responses based on recommended cut-offs [26, 57, 60, 61].

Hypothalamic damage may impair the normal regulation of GH release for two main reasons: (i) altered GHRH secretion: hypothalamic damage reduces the production and release of GHRH, which is essential to stimulate GH secretion from the pituitary gland. In the absence of adequate stimulation by GHRH, GH release becomes insufficient, contributing to the condition of GHD. (ii) Paradoxical response of somatostatin: normally, somatostatin acts to inhibit GH release in a regulated cycle. However, hypothalamic damage can disrupt this balance, leading to dysfunctional regulation of somatostatin. In some patients with hypothalamic damage, an excessive or paradoxical response of somatostatin is observed, which continues to inhibit GH even when increased secretion is needed. This exaggerated inhibitory mechanism of somatostatin worsens the condition of GHD. As discussed before, the GHRH + ARG stimulation test has significant limitations in patients with damage to the hypothalamic-pituitary axis. This is because it acts directly on the GH-secreting cells of the pituitary gland, bypassing the central mechanisms of GH release regulation mediated by the hypothalamus. Previous studies using a GHRH stimulation test demonstrated the preservation of somatotroph responsiveness in the presence of severe GHD in hypothalamic dysfunction, for instance in irradiated patients [62–64]. If there is hypothalamic injury or dysfunction, in fact, the pituitary gland may respond to the GHRH administered during the test, even if the central GH control circuits are altered. This is secondary to a low or absent somatostatin negative feedback. This condition conveys the test less reliably to identify deficits at the hypothalamic level, hence it may lead to false-negative results when a central deficit goes unnoticed. For this reason, it is preferable to use other tests (such as ITT) that stimulate GH secretion through central mechanisms [57, 60].

Hypothalamic-pituitary damage has different aetiologies: anatomical and/or genetic causes, alterations due to cranial irradiation, traumatic brain injury or subarachnoid haemorrhage or local surgical treatments are the main investigated causes [57].

The role of the pituitary stalk is crucial, especially when using GHRH as a diagnostic or therapeutic trigger. The pituitary stalk is the anatomical structure that connects the hypothalamus to the pituitary gland, transmitting hormonal signals and neurotransmitters critical for endocrine regulation. If this structure is injured or compromised, like it can occur in the case of brain cancer, trauma, or other central diseases, the signals that control GH release may be altered. If the pituitary stalk is damaged, the response to GHRH may be reduced or absent, as the damage may impair the ability of the pituitary gland to respond to several hormonal stimuli [65].

Hypothalamic mutations (*GHRH*, *PROKR2*, *KISS1R*, *LEPR*, *GLI2* among the most frequent ones) that can cause GHD are relatively rare and involve genes implicated in the regulation and production of GHRH and other hypothalamic factors. Some of these genes directly affect GHRH synthesis and secretion, while others may affect the development and general functioning of the hypothalamus, indirectly altering GH production. Exogenous administration of GHRH can, in many cases, induce a normal or near-normal response depending on the involved gene [66–70]. Mutations in the *GHRH* gene lead to reduced or absent endogenous GHRH production. However, since the pituitary gland and somatotropic cells remain functional, patients with GHRH gene mutations respond well to exogenous GHRH administration, achieving near-normal GH levels [69]. Mutations in the *PROKR2* and *KISS1R* genes, involved in hypothalamic regulation of GHRH production, can also result in insufficient or irregular GHRH production. In such cases, the response to the GHRH stimulation test is often positive, indicating that the pituitary somatotropic cells are capable of responding to external GHRH stimulation [70]. *GLI2* gene is crucial for the proper development of the hypothalamus and pituitary gland. If the defect primarily affects the hypothalamus, the pituitary gland may be structurally normal and retain the ability to respond to exogenous GHRH, with GH levels reaching values that are close to normal. If there is severe structural and functional impairment of the pituitary gland, however, the test response is more limited [69, 70]. Interestingly, a recent paper on retesting in the transition age has shown that the diagnostic accuracy of GHRH + ARG differ in patients with childhood-onset GHD from different genetic aetiologies and tumoral GHD. These results probably reflect the heterogeneity of genes implicated in childhood-onset GHD and the different GH answer to GHRH we discussed formerly [57].

Considering radiotherapy, the hypothalamus is more sensitive to irradiation than the anterior pituitary [71] and GHD is usually the first and often the only established endocrine sequel of radiation therapy. The hypothalamic site of damage is believed to be responsible for the phenomenon of radiation-induced GH neurosecretory dysfunction [72, 73], which occurs after having receiving > 18 Gy radiation doses [26]. Specifically, radiation doses between 18 and 24 Gy carry a 30–50% risk of GHD. This risk increases with higher doses; at 25 to 29 Gy, the incidence of GHD can reach 50–60%. At doses of 30 Gy or more, the incidence rises significantly to 70–90%, and at extremely high doses (≥ 50 Gy), the risk approaches 100% [74]. More recently, the presence of antipituitary and antihypothalamus antibodies has been demonstrated in a cohort of pediatric patients treated for brain cancers. Their presence was associated with the type of tumours and radiotheraphy, as well as with

pituitary deficiency including GHD [75]. These findings open new perspectives to an involvement of autoimmunity in the diagnostic accuracy of GHRH + ARG in this specific condition. In line with this hypothesis, in an animal model of experimental autoimmune encephalomyelitis the *GH*, *GHRHR* and *GHR* genes were upregulated, whereas the gene expression of *GHRH* and *SST* in the hypothalamus did not change. Furthermore, GHRH KO mice were resistant to the development of experimental autoimmune encephalomyelitis [76, 77]. All these findings suggest that brain tumour, radiotheraphy, and local autoimmunity or immunity dysregulation could influence the GH response to GHRH + ARG.

The recent Clinical Practice Guideline from the Endocrine Society offers essential guidance on addressing hypothalamic-pituitary dysfunction in survivors of childhood cancer, a group particularly prone to growth and hormonal irregularities [26]. These guidelines advise against using GHRH, whether alone or combined with ARG, for diagnosing GHD in childhood cancer survivors who have received radiation targeting the hypothalamic-pituitary axis. This recommendation reflects the limited diagnostic accuracy of these tests, mainly GHRH + ARG, in patients who have previously undergone radiation in the hypothalamic-pituitary area [26]. Furthermore, the guideline identifies a gap in diagnostic recommendations for assessing GHD in individuals who have reached adult stature or in those with childhood-onset GHD in whom hypothalamic-pituitary dysfunction persists. This lack of clear guidance highlights the need for more research and for a stronger clinical consensus on managing these cases effectively [26, 57]. Overall, these insights emphasize the necessity of a customized approach when diagnosing and treating GHD in childhood cancer survivors. Both the level of radiation exposure and the appropriateness of various diagnostic methods should be carefully considered, especially in patients with significant damage to the hypothalamic-pituitary axis.

Darzy et al. conducted a comparative study to explore this evolving context, evaluating the responses to two different stimuli GHRH + ARG and the ITT in a large cohort of skull-irradiated patients with a presumptive diagnosis of GHD [62]. The findings revealed that the peak GH response to the ITT decreased significantly, but with minimal change between the 5 and 10-year marks post-irradiation. In contrast, the peak GH response to GHRH + ARG changed notably within the first 5 years after irradiation, but then showed a marked decline over the subsequent 10 years. This indicated that the evolution of GH responsiveness to the two different stimuli followed distinct trajectories, resulting in a significantly increased discordance ratio between the two tests in the first 5 years post-irradiation, which then tended to normalize over the following 10 years [62]. Indeed, hypothalamic dysfunction is an early complication of radiation damage, which presents with early discordance in the GH response to different tests. GH dysfunction, indicated by the attenuation of GH responses to GHRH + ARG, is a delayed complication, becoming more prominent more than 5 years after irradiation. The delayed somatotrophic dysfunction could be attributed to secondary atrophy of somatotrophs due to ongoing GHRH deficiency or to delayed direct damage to the pituitary gland caused by radiation [62]. Additionally, the high rate of false negatives observed after the GHRH + ARG test makes it an unreliable tool in the early years following cranial irradiation, as it may fail to detect early hypothalamic damage [62]. Thus, careful selection of diagnostic tests and their timing is crucial for accurately diagnosing GHD in this population, as the complex interplay between hypothalamic and pituitary damage evolves over time.

4 GHRH + ARG test: clinical consideration in obesity

Obesity is a state of functional GHD, being characterized by reduced spontaneous GH secretion as well as low somatotroph response to all provocative stimuli [78, 79]. Thus, obesity represents the most important confounding factor for the diagnosis of GHD, and these considerations should be taken into account in clinical practice for the reliability and interpretation of the GHRH + ARG test.

In obesity, reduction in the half-life of GH [80] as well as a significant decrease in the production and secretion of GH have been reported [81]. In particular, obesity is characterized by decreased spontaneous secretion, pulses, and half-life of GH, and BMI has a negative correlation with GH half-life and amplitude of GH secretory pulses. Likewise, body fat is inversely related with daily GH concentration, GH amount per secretory pulses and GH half-life [78].

Among potential neuroendocrine causes of the impairment in spontaneous and stimulated GH secretion, GHRH hypoactivity has been described, as well as alterations in the modulation of ghrelin, neuropeptide Y and/or leptin [82], and the alterations in neuroendocrine control could be related to metabolic alterations, as elevation of circulating FFAs and hyperinsulinism, characterizing obesity [83]. In fact, in obesity, an increase in circulating levels of FFA goes in parallel to a decrease in GH secretion. FFAs have an important inhibitory effect on GH secretion [5], with different mechanisms, i.e., directly by suppression of GH release from somatotrophs and indirectly by inhibition of somatotrophs at the hypothalamic level, as demonstrated by studies in humans and animals using a lipid infusion [84]. As a demonstration of fact, the pharmacological suppression

of FFA by acipomox, a well-known anti-lipolytic agent, is followed by a recovery of GH secretion in obese subjects. Thus, it was hypothesized that the reduction of GH secretion observed in obesity should be mainly an effect of elevated levels of circulating FFAs. However, a meta-analysis demonstrated stable levels of plasmatic FFA regardless of the severity of obesity, and whether alterations in circulating levels of FFAs observed in obesity are directly the cause of reduced GH secretion remains unknown [85]. Likewise, animal models with body weight gain induced by diet show a reduction of GH secretion without an elevation of FFAs concentrations [86]. Thus, the direct correlation between the reduction of GH secretion in the early stage of obesity and the elevation of FFAs has not been definitively demonstrated [84]. Another hypothesis investigated the role of insulin, considering that impaired GH secretion in obesity should be correlated to the insulin resistance and hyperinsulinism observed in obesity, rather than in response to an elevation in circulating levels of FFAs. Following this assumption, the recovery of GH secretion after acute treatment with acipimox should also occur as a consequence of altered insulin feedback, considering that acipimox also reduces circulating levels of insulin in obese subjects [84].

The role of insulin resistance could be investigated by the human model of GHRH resistance (i.e., Itabaianinha syndrome, caused by inactivating mutation GHRH receptor gene). These patients are characterized by visceral adiposity, but increased insulin sensitivity, accompanied by high serum adiponectin, suggesting that a threshold of GH secretion is necessary for visceral adiposity to impair insulin sensitivity [87].

Another hypothetical pathogenetic cause of GH insufficiency in obesity is the enhanced negative IGF-1 feedback at hypothalamic level. In obesity, total IGF-1 levels have been reported to be increased, normal or decreased, but free IGF-1 levels have been found to be increased, assuming the possibility of hypoactivity of GH/IGF-1 axis. The elevated free IGF-1 levels in obesity could be explained by the increase of GH-binding protein (GHBP) levels and enhanced GH receptor (GHR) sensitivity related to hyperinsulinism of obesity. In turn, increased free IGF-1 levels could exert an increased negative feedback action on somatotroph cells [88].

Taking into account these drawbacks, the diagnostic approach for GHD in obese patients ramains insidious, since these patients show low GH responses to all provocative stimuli for GH secretion [79]. Particularly, the somatotropic response to all provocative stimuli is negatively correlated to the patient's BMI, such that the GH response in obese subjects can result impaired as it would in hypopituitary GHD patients. It is also worth noting that there is a high prevalence of overweight and obesity among patients affected by acquired hypothalamic-pituitary diseases [89].

Investigating GH secretion in patients with obesity by GHRH, glucagon, levodopa, physical exercise, slow wave sleep, ARG, ITT, as well as the GHRH + ARG test has shown a variable degree of impaired GH response. Therefore, considerable care is needed for diagnosing GHD in obesity, as the accepted cut-offs for many of these tests should be not applicable to obese subjects.

These evidences drove the researchers to define BMI-related cut-off limits of the GH response to provocative tests for the diagnosis of adult GHD. Corneli et al. (2005) calculated the cut-off limits of peak GH response to the GHRH-ARG to reduce the misclassification of GHD [46]. This study enrolled 322 patients with organic hypothalamic-pituitary disease and 318 control subjects. Patients were divided according to the number of pituitary deficient hormones: (a) total pituitary hormone deficit (TPHD), assumed to be GHD; (b) no more than two pituitary hormone deficits (PHD), including subjects with either normal or impaired GH secretion. The diagnostic cut-off points were calculated for normal weight (BMI < 25 kg/m2), overweight (BMI 25–30 kg/m2) and obese (BMI > 30 kg/m2) subjects, according to ROC analysis. In the lean population, the best sensitivity (98.7%) and specificity (83.7%) was calculated using a peak GH cut-off point of 11.5 µg/L. In overweight subjects, the best pair of values (sensitivity 96.7% and specificity 75.5%) was found using a peak GH cut-off point of 8.0 µg/L. In the obese population, the best pair of values (sensitivity 93.5 and specificity 78.3%) was found using a peak GH cut-off point of 4.2 µg/L [46].

This cutoff value was comparable with the one provided by Biller et al. in 2002 (4.1 µg/L) in an obese population [41], and were confirmed the study by Colao et al. in 2009, who demonstrated that waist circumference was the best predictor of GH peak at GHRH + ARG, followed by BMI and age [45].

While BMI-based normal limits in adult patients have been well described, BMI-related cut offs in young adults who achieved final height await classification. In fact, in transition patients the current validated cut-off limit for GH response to GHRH + ARG is more than 19 µg/L, but this cutpoint value was originally obtained in a study performed on a large cohort of patients with normal BMI [52]. BecauseBMI has benn shown to exert a strong negative influence on GH response to stimulation tests, new GH cut-off limits appropriate for BMI to GHRH + ARG should be established during transition phase, The same principle applies to pediatric subjests suspected to harbor GHD, due tothe high prevalence of overweight and obesity in childhood, especially if affected by GHD. In the study of Dreismann et al. transition-age patients who presented

a BMI comprised between −1 and 0 SDS showed higher GH peaks compared to those with BMI > 1 SDS [56], These data were recently confirmed by Patti et al. who reported a significant inverse correlation between BMI SDS and GH peak responses to GHRH + ARG in patients undergoing retesting during transition [57]. Thus, the impact of BMI during transition should be better clarified, also considering that puberty is a time of considerable metabolic and hormonal change and it is associated with a decrease in insulin sensitivity [90].

Considering other GH stimulating agents able to maximize the GH response to GHRH, the multicentric study on 542 subjects published in 2006 [91] demonstrated that the historically reported cut-off values of the GHRH + GHRP-6 test for the diagnosis of GHD (i.e., GH peak ≤ 10 µg/L) were appropriate in subjects with a BMI ≤ 35 Kg/m^2, but the thresholds should be reduced by 5 µg/L (i.e., GH peak ≤ 5 µg/L for GHD diagnosis) in subjects affected by severe obesity,. The relative statistical weight of excess adiposity on the overall secretion of GH elicited by this test was around 25% [91].

These insights on GHRH + ARG test have been receipted by international guidelines on the diagnosis and management of GHD [24, 92], even if the most recent guidelines do not consider this test considering the lack of GHRH [24].

5 GHRH + ARG test: the impact of age

It is well established that the activity of the somatotropic axis presents age-related changes during the life span and progressively declines with aging. In older adults, a 14% reduction in circadian GH secretion per decade has been reported, indicating a parallel decline in both the frequency and amplitude of the GH peaks, which contributes to determine lower circulating GH levels [80, 93].

In this setting, the physiological decrease of GH secretions reflects the changes in the hypothalamic regulation of somatotropic secretion, specifically an increase in the somatostatinergic activity and a decline in the activity of GHRH [80, 94], rather than a decrease in the secretory ability. Similarly, aging process reduces endogenous signaling and secretion of ghrelin, the endogenous ligand for the GH secretagogue receptor [9, 12, 95]. Also, a reduction in GH-binding protein (BP) levels has been documented in elderly alongside a changing of expression or clearance of the GH receptor [96]. Therefore, the mean 24-h GH concentrations, together with IGF-1 and insulin-like growth factor-binding protein 3 (IGFBP-3) levels, are reduced in elderly compared to younger individuals [80].

Several clinical alterations related to the decrease of GH secretion, the so-called somatopause, has been extensively documented including osteosarcopenia, and increased visceral adiposity together with insulin resistance. The reduced activity of the somatotropic axis in the elderly could be associated to a decrease in energy intake and physical activity [97]. Insufficient energy intake leads to a reduced response of peripheral tissues to GH, a decrease in the production and secretion of IGF-1 and a loss of IGF-1/GH negative feedback mechanisms [97–99]. Reduced level of physical exercise could further contribute to lower GH secretion in elderly. Moreover, aging-related decrease in GH secretion and pulse amplitude lead to a lack of day-night GH rhythm with a loss of nocturnal sleep-related GH pulses [101].

In this context, GHD must be accurately distinguished from the physiological decrease of GH secretion related to the aging process, protein malnutrition and sarcopenic obesity, to avoid unnecessary treatments in pluricomorbid patients [37].

The ITT represents the most widely used dynamic tests for GHD diagnosis, but are generally avoided in elderly, because of their potential adverse events in pluricomorbid patients [24, 102]. Conversely, the GHRH + ARG test seems to achieve the best accuracy/safety ratio in aging [23, 103]. Also, as previously stated, it is well established that the GHRH + ARG test is able to discriminate adult patients with GHD from normal subjects with a sensitivity comparable to ITT, considering BMI related cut-off [46, 104].

When GHRH is combined with ARG, it provides a stimulus to GH secretion which is more potent than all the classical provocative tests to evaluate the maximal secretory capacity of somatotrope cells and shows good within-subject reproducibility [29, 30, 105]. Moreover, the potentiating effect of ARG on the GHRH-induced GH response is fully preserved in aging [29], as it is able to differentiate between patients with GHD and normal subjects from young adulthood to the elderly [29, 30, 32].

The 3rd and 1st centile limits of the peak GH response to GHRH + ARG were defined in a population of 157 normal subjects (79 males), with a BMI ranging from 19 to 26 kg/m2 and aged 20–80 years (72 subjects aged 20–40 years, 39 subjects aged 41–65 years and 49 over 65 years). In this population, the 3rd centile limit of the peak was 16.5 µg/l, while the 1st centile limit was 9 µg/1 [29].

The specificity of GHRH + ARG test was 98.7% above this latter cutoff value. Only 2 of 157 normal subjects with a normal body weight had a GH peak below 9 g/L, and 6 of 157 had a GH peak below 16.5 g/L. The mean peak GH response to GHRH + ARG in this population was 63.1 µg/l and no significant gender- or age-related differences were observed [29, 32].

The intraindividual variability of the GH response to GHRH + ARG in two testing sessions was evaluated in both adult and elderly normal subjects [30, 32]. The

intraindividual reproducibility of the peak GH response to GHRH + ARG was good in both adult and elderly subjects who showed similar mean peak GH response to this test [30, 32].

On these bases, the GHRH + ARG test could be considered the gold standard test for GHD diagnosis in the elderly for its safety profile and the lack of significant contraindications, except for chronic renal failure [29, 106]. However, in the geriatric population, no-adjusted cut-off were established for interpretation of GHRH + ARG and the other dynamic tests, despite the well-known physiological decrease of GH and IGF-1 levels with aging [107, 108]. Even though the progressive aging-related increase in body weight suggests a potential use of BMI-related cutoffs previously mentioned, elderly individuals could harbor an even apparently normal BMI with a concomitant excess in visceral adipose and a decrease of lean body mass [37]. In fact, BMI does not represent an indicator for the distribution of body mass and weight excess in the geriatric population. Further studies should be done to identify specific cut-off values during aging in order to correctly interpret the dynamic tests results and support an accurate diagnosis in the elderly. However, the shortage of GHRH has abruptly interrupted this line of research.

Obesity- and age-related changes of the somatotropic axis are summarized in Fig. 2.

6 Present and future of GH testing

Since GHRH analog became unavailable in US and Europe due to the company's decision to withdraw production, clinical experience has increased on alternative diagnostic tools for GHD, given the caveats regarding IGF-1 measurement in terms of accuracy, reproducibility, standardization, performance and methodology [54].

Up to now, the cornerstone diagnostic method for GHD remains the ITT, which allows for the simultaneous evaluation of the somatotropic and hypothalamic-pituitary-adrenal (HPA) axis, independent of previous cranial irradiation. Current guidelines recommend an ITT-related GH cut-off of 3–5 µg/L in adults, provided that hypoglycaemia (blood glucose < 40 mg/dL) is achieved [23, 24, 109]. In Europe, most centres adopt a GH cut-off of < 3 µg/L [25]. Even so, GH responsiveness to ITT seems to depend on BMI, and GH cutpoints of 3.5 µg/L in lean, 1.3 µg/L in overweight and 2.2 µg/L in obese subjects have been proposed [44]. Along with it, patients with high BMI or insulin resistance often require higher insulin doses, e.g. 1.5-2 IU/kg instead of 1 IU/kg dose, making them vulnerable to delayed hypoglycemia after test completion (110). Other disadvantages include the need for medical supervision, hypoglycemia-related discomfort, contraindications in persons with ischaemic heart disease and epilepsy, as well as unsuitability for children and elderlies [23, 24, 45, 109].

By way of alternative, growing interest has focused on the glucagon stimulation test (GST), an easily available substitute of ITT that is advantaged by good reproducibility, safety, and lack of influence by gender [54, 111]. Like ITT, GST can be used to explore the integrity of the somatotropic and HPA axis. It is administered intramuscularly or subcutaneously at a fixed-dose of 1 mg in normal-weigh subjects and 1.5 mg in individuals > 90 kg [54, 111–114]. An intranasal glucagon administration has akìlso been tested [115]. The mechanism/s of glucagon-induced GH release are controversial, and potentially engage counterregulatory glycaemic

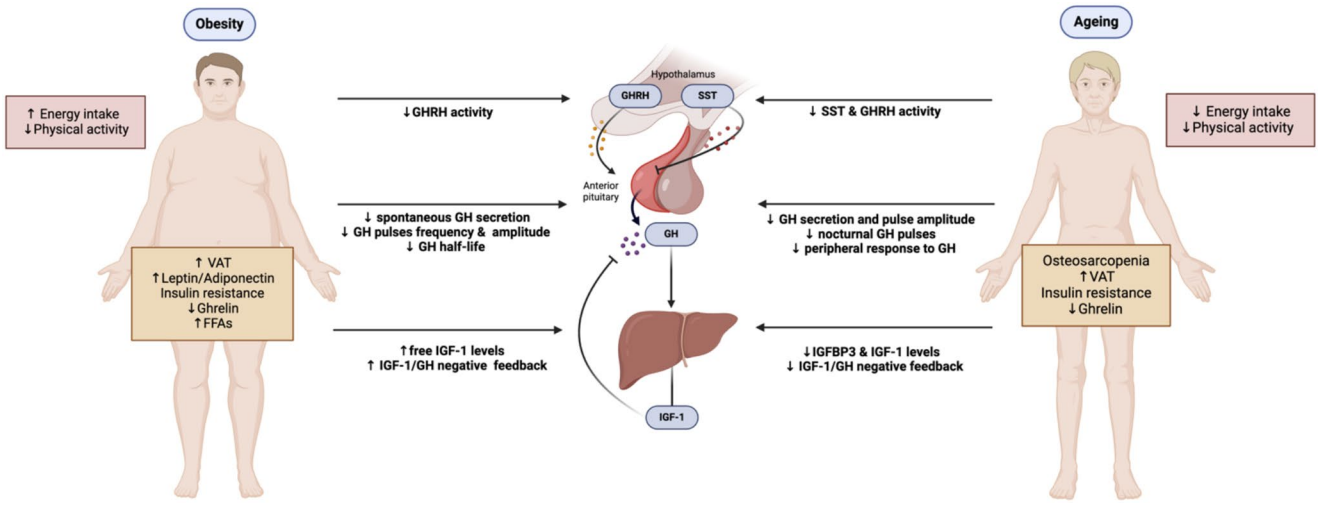

Fig. 2 Obesity- and age-related changes of the somatotropic axis. GHRH: growth hormone releasing hormone; SST: somatostatin; GH: growth hormone; IGF-1: insulin-like growth factor-I; IGFBP3: insulin-like growth factor-binding protein 3; VAT: visceral adipose tissue; FFAs: free fatty acids. Created with Biorender.com

fluctuations, noradrenaline-mediated actions through alpha-adrenergic receptors, modulation of FFA release, and effects relating to the FGF-21 pathway [28] (13). The recommended cut-off for the GH peak, which occurs between 120 and 180 min after glucagon administration, is < 3 ng/mL in normal-weight persons and < 1 ng/mL in overweight/obesity, to avoid overdiagnosis [23, 54, 112]. Aging is negatively correlated with GH peaks during GST in hypopituitary patients [113], hence a < 1 ng/mL threshold has been suggested in persons aged > 70 year to minimize false-positive diagnosis [114]. The magnitude of GH response to GST may depend on the achieved glucose nadir, and some authors proposed adopting a blood glucose threshold of ≤ 60 mg/dl (3.33 mmol/l) as effective. Disadvantages of the GST include testing length (3–4 h), intramuscular injection, and occurrence of nausea/vomiting, which may require antiemetics [114]. Contraindications include malnourishment, prolonged fasting, and known insulinoma or pheochromocytoma [54]. Uncertainty remains on GH responsiveness to GST in uncontrolled diabetes (blood glucose > 180 mg/dl or A1c > 8%), while concerns exist on GST safety in elderlies [28]. Finally, intranasal glucagon administration can promote rapid-onset hypokalemia in 50% of cases [115].

Alternative to the previous, a number of previously described GHSs (e.g. GHRP-6, GHRP-2, hexarelin, and macimorelin) have been inspected for their ability to diagnose GHD alone or in combination with GHRH or GST [54, 116, 117]. These synthetic peptides bind to the endogenous ghrelin receptor, also known as the GHS receptor, and stimulate GH release independent of gender, while being age-dependent [54, 23, 24, 109, 116–118]. Within this class, macimorelin is an oral GHS approved in the US and Europe for diagnosis of adult GHD that has shown diagnostic performance comparable to GHRH + ARG and ITT [54]. The initially determined GH cut-off of 2.8 ng/mL at the 45, 60 and 90 min time-points [119] has been revised and a new GH cut-off of 5.1 ng/mL has been proposed, based on obtained values of specificity (96%), sensitivity (92%), and agreement with the same ITT cut-off point (87%) [120]. Macimorelin testing for GH is reproducible, well-tolerated, and safe, its performance is unaffected by sex, and its relatively age- (up to 65 years) and BMI- (up to 35 kg/m2) independent [120]. Because macimorelin can cause QT prolongation, discontinuation of QT-prolonging drugs is recommended before testing [28]. Unfortunately, its use is burdened by problems of cost, accessibility, potential drug-to-drug interactions, and production discontinuation [28, 121].

7 Conclusions

In conclusion, heterogeneity in age, gender, body mass/composition, nutritional status, glycemic control, health and interfering therapies poses a diagnostic problem with all kinds of GH stimulatory tests. Collectively, the ITT remains a reliable diagnostic tool for diagnosing adult GHD and using BMI-related cut-off limits is expected to curtail overdiagnosis, but it remains cumbersome for both parts with unresolved safety issues.

While present experience with the GST is still narrow, unavailability of GHRH and the long and winding road traveled by macimorelin will probably make this the next most widely used test for GHD when ITT is contraindicated or unfeasible. Establishing diagnostic GH cutoffs with the GST according to individual confounders will advantage the clinical interpretation of GH dynamics, yet questions remain on its diagnostic accuracy and safety in the elderly.

GHRH + ARG has been widely used in the clinical practice, because it is characterized by a low risk of adverse events, diagnostic accuracy as good as ITT when appropriate GH serum cut-offs are considered, and a lower need of blood samples during test, thus resulting in a less time- and cost-consuming procedure. The safety and reliability of the GHRH + ARG test have been also demonstrated in children and adolescents, even if some pitfalls regarding hypothalamic dysfunction should be considered.

Prospectively, identification of disease-specific molecules could help diagnosis and scale down the impact of confounders. Future technical improvements and assays developments may allow approaching GHD diagnosis without stimuli. Particularly, quantitation of IGF-1 by mass spectrometry [122] and identification of low-molecular weight metabolomic compounds for diagnostic purpose [123] could forecast the introduction of potential tools that may prove to be more reliable, robust and accurate than those currently in use.

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