Testing Pituitary Function in Aging Individuals

Roberta Giordano, MD a, Gianluca Aimaretti, MD, PhD a, Fabio Lanfranco, MD a, Mario Bo, MD b, Matteo Baldi, MD a, Fabio Broglio, MD, PhD a, Roberto Baldelli, MD a, Silvia Grottoli, MD a, Ezio Ghigo, MD a, Emanuela Arvat, MD a,*

a Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Turin, Italy
b Section of Geriatrics, Department of Medical and Surgical Disciplines, University of Turin, Turin, Italy

Changes in endocrine function in aging individuals often reflect age-related impairment in animal and human neuroendocrine regulation of pituitary function. Growth hormone/insulin-like growth factor (GH/IGF)-I hypofunction in the elderly is a clear example of decreased activity as a function of age-related changes in the neural control of somatotroph cells [1–4]. GH secretion undergoes clear variations during an individual’s lifespan: its secretion is maximal at birth, strongly increased during puberty, and progressively decreased thereafter showing very low secretion in the aged [5]. Mechanisms underlying the age-related changes of GH secretion involve peripheral influences (ie, gonadal steroids and adiposity), but age-related changes in hypothalamic neuropeptide and neurotransmitter release, mainly GHRH and somatostatin (SS), play a major role. GHRH and SS are likely to reflect age-dependent changes in suprathyroidal functions [1–4]. Animal and human studies indicate that age-related changes in neurotransmitter regulation leading to GHRH

This article is based on the personal studies of the authors supported by the Ministero Istruzione Università e Ricerca (MIUR, Rome, Italy) and the University of Turin and SMEM Foundation of Turin (Turin, Italy).

* Corresponding author. Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Corso Dogliotti 14, 10126 Turin, Italy.
E-mail address: emanuela.arvat@unito.it (E. Arvat).
hypoactivity and absolute or relative SS hyperactivity mainly account for the reduction of spontaneous and stimulated GH secretion in the elderly [1–4].

Cholinergic impairment in the aging brain involves hypothalamic pathways and contributes to the disrupted GHRH/SS interplay that underlies GH/IGF-I hypossecretion [6]. Also, age-related variations in the ghrelin system (ghrelin being a gastric hormone discovered as a natural GH secretagogue [GHS] acting within the central nervous system [CNS] and the hypothalamus) could play a role in the decreased GH secretion that connotes aging [7–9].

From a clinical point of view, aging is associated with changes in body composition, metabolism, and structural functions and results in reduced lean mass, increased adiposity, decreased bone mass, and protein synthesis [1,10]. These alterations are similar to those in young adults with GH deficiency (GHD) [11,12], and this evidence, together with demonstrated age-related declining activity of the GH/IGF-I axis, has led to the neologism somatopause, which indicates the potential link between age-related decline in GH and IGF-I levels and frailty in the aging [13].

Taking into account the clinical and hormonal features of somatopause, diagnosis of GHD in the aging is the subject of much controversy. GHD must be carefully distinguished from the reduction in GH secretion that accompanies normal aging and obesity. To avoid overtreatment, GH replacement is warranted only for severe GHD [14]. There is consensus that very low IGF-I levels may be representative of adult GHD, although normal IGF-I levels do not rule out the presence of severe GHD [15–18]. As total IGF-I levels are often within the normal age-related limits, subjects suspected of having adult GHD often undergo provocative testing of GH secretion, which determines whether somatotroph function is impaired or normal [14,18,19]. The importance of accurate diagnosis of GHD in the elderly is based on evidence that elderly patients who have GHD do benefit from replacement treatment with recombinant human GH (rhGH), while the efficacy of rejuvenating the GH/IGF-I axis in somatopause has not been proven.

Profound tests for somatotroph release as a function of age

*Insulin-induced hypoglycemia: insulin tolerance test*

The insulin tolerance test (ITT) is considered the gold standard test to investigate somatotroph function. Hypoglycemia stimulates GH secretion by means of multifactorial central mechanisms representing the hypothalamic neuroendocrine response to a stressful condition and includes activation of the hypothalamus-pituitary-adrenal axis. An increase in endogenous GHRH activity with a concomitant decrease in hypothalamic SS release, and an increase in catecholamine release with α-adrenergic activation seem to be the most significant mechanisms that lead to an increase in GH. GH response to ITT (0.05 to 0.15 IU regular insulin intravenously)
undergoes age-related variations, increasing from childhood to adulthood without significant change in the aging. GH response to ITT does not appear to be affected by sex, but is reduced in obesity, where it shows poor intraindividual reproducibility, implying low specificity. It is generally contraindicated and risky when CNS or cardiovascular diseases are present or suspected [19–26].

**Arginine and ornithine**

The mechanisms of action of arginine are not unlike those of ornithine, an arginine metabolite, but other amino acids (eg, methyonine) act by means of other unknown mechanisms. Arginine likely acts primarily by means of negative modulation of hypothalamic SS release, although this assumption is based on indirect evidence only [8]. The arginine-induced GH response (0.5 g/kg infused for 30 minutes intravenously) is independent of age, but not sex (more prevalent in women than in men as a function of the positive influence of estrogens), and reduced in obesity. Poor intraindividual reproducibility of GH response to this test has been demonstrated, which implies low specificity. It is generally well-tolerated, although vomiting can occur as function of overdose or rapid infusion [24–29].

**Glucagon**

Intramuscular or subcutaneous, but not intravenous, administration of glucagon is followed by a marked increase in GH and corticotropin (ACTH)/cortisol. Thus, glucagon is not a true stimulus of somatotroph secretion. The mechanisms of action are likely related to products of its degradation. There is no confirmed data about GH response to intramuscular glucagon (1.0 mg) as a function of age and sex, although glucagon’s stimulatory effect is reduced obesity. Poor intraindividual reproducibility of GH response to this test has been demonstrated, which implies low specificity. It is generally well-tolerated, although vomiting can occur as a function of overdose or rapid infusion [19,26,30–32].

**Levodopa**

Levodopa, another one of the oldest provocative tests, stimulates GH by means of dopaminergic activation, which, in turn, likely inhibits hypothalamic SS release or stimulates endogenous GHRH activity. There is no confirmed data about GH response to levodopa as a function of age, sex, and body weight. Levodopa (125, 250, and 500 mg by mouth for a body weight less than 15 kg, between 15 and 30 kg, and more than 30 kg, respectively) is a stimulus weaker than ITT, arginine, and intramuscular glucagon. Very poor intraindividual reproducibility of the response to this test has been demonstrated, implying low specificity. Levodopa administration often induces nausea and vomiting [25,26,33].
**Clonidine**

Clonidine, an $\alpha_2$-adrenergic agonist, displays the GH-releasing effect in agreement with the positive influence of $\alpha$-adrenergic receptors on somatotroph function. The stimulatory effect of clonidine comprises concomitant stimulation of GHRH–secreting neurons and inhibition of SS-secreting neurons. There is no definitive data supporting the GH-releasing effect of clonidine as a function of age and sex, although its effect is reduced obesity. It is assumed that clonidine represents a stimulus weaker than ITT, arginine, and intramuscular glucagon. An assumption based on low oral doses (0.15 mg/m$^2$) which are usually administered to avoid more marked vascular adverse effects observed after intravenous administration. Very poor intraindividual reproducibility of the response to this test has been demonstrated, implying low specificity [26,33,34].

**Other neuroactive substances used as stimuli of growth hormone secretion**

In agreement with the evidence that acetylcholine plays a major role in the neural regulation of GH secretion, through the negative modulation of hypothalamic SS release, cholinergic agonists such as pyridostigmine stimulate GH secretion. Although several cholinergic agonists have been shown to significantly increase GH secretion, pyridostigmine has been the most studied in people. The GH response to pyridostigmine in children and adults is similar, although it is reduced in elderly subjects. It seems independent of sex but influenced by body weight, being decreased in obesity. Pyridostigmine (60 mg by mouth in children and 120 mg by mouth in adults) elicits a GH response lower than that recorded after ITT, arginine, and glucagon. Cholinergic adverse effects (fasciculations, abdominal pain) often are observed after drug administration [24,29,33,35].

**Galanin**

Galanin is a neuropeptide that stimulates GH secretion by means of concomitant GHRH increase and SS inhibition. Its effect depends on age (less marked in the aging) and sex (more marked in women) and decreased obesity. Galanin (80 pmol/kg/min intravenously) has been investigated as a potentially new provocative test for GH secretion, but it represents a stimulus weaker than ITT, arginine, and glucagon and cannot be used in current clinical practice [33].

**Steroids**

Glucocorticoids play a dual role in GH secretion. Besides long-term inhibition following overexposure to natural and synthetic glucocorticoids,
acute administrations of synthetic glucocorticoids stimulate GH secretion, probably by inhibiting hypothalamic SS release. Synthetic glucocorticoids have been proposed as new provocative test, but their advantage over other classical provocative tests has not yet been demonstrated [36].

Although gonadal steroids are devoid of a direct stimulatory effect on GH secretion, they have been often used to sensitize GH response to classical provocative stimuli in children. In fact, pretreatment with androgens and estrogens has been shown to increase GH responsiveness to stimulatory agents in boys and girls. This procedure was popular in past years, but never validated, and there is no consensus about usefulness of priming with sex steroids before provocative tests. Gonadal steroids also enhance spontaneous and stimulated GH secretion in the aging. Somatotroph pulsatility is, in fact, amplified by both estrogens and androgens in elderly women and men, respectively. Moreover, estrogen pretreatment significantly increased GHRH-induced GH release in postmenopausal women. Priming with gonadal steroids, however, has never been proposed in clinical practice for evaluating somatotrope function in aging [5,37,38].

**Growth hormone-releasing hormone**

By activating pituitary GHRH receptors, GHRH is a neurohormone that specifically stimulates somatotroph cell proliferation, GH synthesis, and release. Its tight interaction with SS is needed to generate GH pulsatility. Although originally considered a more useful stimulus for GH secretion in the diagnosis of childhood and adult GHD in comparison with classical tests, GHRH has poor reproducibility and reliability as a provocative test. This variability likely reflects variations in endogenous somatostatinergic tone and makes it difficult to define reliable normative limits for this test, the specificity of which is very low. It remains debatable whether GH response to GHRH (1 μg/kg intravenously) is sex-dependent (having been reported to be higher in women and enhanced by exposure to estrogens). GH response to GHRH is influenced by body weight and less effective obesity. It has been demonstrated that the GH-releasing effect of GHRH changes during an individual’s lifespan. It is at its peak in newborns, similar in children and young adults, slightly higher than that seen after ITT, and markedly decreased in the elderly, likely a consequence of hypothalamic SS hyperactivity. In about 30% of elderly subjects, however, a normal GH response to GHRH can be detected, which is in agreement with evidence in animals showing that pituitary somatotroph capacity is preserved with advancing age. As a consequence of the age-related decline in the GH-releasing activity of GHRH, testing with GHRH is not useful in the diagnosis of GHD in elderly people, unless combined with other neuroactive substances that inhibit SS release. GHRH administration does not induce significant adverse effects, except for mild and transient facial flushing [19,24–26,33].
Natural and synthetic growth hormone secretagogues

Synthetic GHS and the natural ligand of GHS receptors (ie, ghrelin) strongly stimulate GH secretion by directly stimulating the pituitary gland and primarily activating GHRH-secreting neurons in the hypothalamus. Their action as functional antagonists of somatostatin also has demonstrated [9].

The GH response to both ghrelin and synthetic GHS (1 or 2 µg/kg intravenously) is independent of sex, although it is decreased in ratio of increasing body weight. The GH-releasing activity of ghrelin and synthetic GHS undergoes marked age-related variations, different from those recorded after GHRH. In fact, as opposed to GHRH, GH response to GHS is low at birth, significantly increased during puberty, and progressively decreased with advancing age, likely a consequence of age-dependent changes in the hypothalamic neuroendocrine regulation of somatotroph function (ie, GHRH hypoactivity and SS hyperactivity). Even in the elderly, however, ghrelin and GHS are more potent than GHRH in stimulating GH secretion. Good intraindividual reproducibility of the GH response to GHS has been demonstrated, but normative limits, which should be age-related, have not been defined. The administration of ghrelin and GHS does not induce relevant adverse effects, except for mild, transient facial flushing and hunger [7–9,39].

Growth hormone-releasing hormone in combination with arginine, pyridostigmine, or growth hormone secretagogues

GHRH has been shown to be one of the most powerful and reproducible stimuli of GH secretion when given in combination with substances with the capacity to increase its GH-releasing activity by counteracting the SS tone (Figs. 1 and 2). Several neuroactive substances are able to strengthen GH
response to GHRH: propranolol, pyridostigmine, arginine, galanin, ghrelin, and synthetic GHS. By inhibiting SS release or antagonizing its inhibitory activity on somatotroph cells, these molecules allow GHRH to fully express its GH-releasing action and assess the maximal releasable pool of GH. This, in turn, reflects the appropriate function of hypothalamic mechanisms regulating GH synthesis and secretion. Particularly, GHRH + pyridostigmine, GHRH + arginine, and GHRH + GHRP-6 or hexarelin, two synthetic GHS, have been proposed as provocative tests for diagnosing GHD. GH response to these tests does not seem to be dependent on sex, although it is inversely associated with body mass.

As far as the utility of these provocative stimulations in the elderly is concerned, the GH-releasing effect of GHRH combined with pyridostigmine or GHS is strongly age-dependent, showing reduced activity with advancing age. This likely reflects age-related changes in the primary regulation of GH secretion, involving impairment of cholinergic activity and SS hypertone. Thus, they are not as reliable in the aged as in young subjects to distinguish normal from GHD-aged subjects. Conversely, GHRH when combined with arginine, does not show age-related GH responses, demonstrating a similar GH response during an individual’s life span. This evidence implies at least two major considerations:

1. The maximal somatotroph capacity is preserved in human aging, in agreement with animal studies, indicating that the age-related GH decline mostly reflects central variations in neuroendocrine control of pituitary function.
2. The normal cut-off limit of the GH response to this test can be assumed to be the same in all ages, making this a good provocative test for diagnosing GHD through the life span.
Very good diagnostic reliability of GHRH + arginine test has been shown in the period from childhood to the elderly years. Thus, this testing procedure is considered as an alternative provocative test for diagnosing adult GHD when ITT is contraindicated. This procedure is brief (30, 45, and 60 minutes), which reduces testing time and costs. The adverse effects recorded during this test are negligible, and similar to those reported after GHRH or arginine alone [24,26,39–44].

**Diagnosis of growth hormone deficiency in the aging individual**

Following the Consensus Guidelines of the Growth Hormone Research Society, it is recommended that the diagnosis of GHD in adulthood be established biochemically by provocative testing of GH secretion [14]. An evaluation for GHD should be considered only in patients for whom there is high suspicion for acquired hypothalamic-pituitary disease or in patients with childhood-onset GHD [14,24,45].

That the diagnosis of adult GHD requires a clear failure to respond to provocative testing is based on evidence that such tests (eg, insulin-induced hypoglycemia, but not GH surrogates such as IGF-I and IGFBP-3) can distinguish between normal and GHD subjects [21]. In fact, IGF-I levels are often overlapping in normal adults and patients who have GHD, and this is even more evident in aging individuals, based on age-related IGF-I decline [4,16–18].

GH secretion undergoes age-related variations that are generally mirrored by IGF-I levels (the best marker of GH status) in both animals and humans; the notable exception being at birth [1–4]. A progressive fall in 24-hour GH secretory rates occurs during the aging process (~14% per decade) [46,47]. The distinction between normal and GHD subjects based on the amount of spontaneous GH concentration is difficult to determine even in adulthood based on remarkable overlap between normal and GHD subjects on the individual basis and compounded in the elderly [11,19]. Accordingly, although IGF-I levels generally reflect the GH status in aging and in adulthood, IGF-I levels in normal and GHD subjects show remarkable overlap that reduces the diagnostic value of total IGF-I measurement for diagnosing severe GHD. In the absence of nutritional impairment, however, low IGF-I levels strongly point toward possible severe GHD [16–19].

Thus, in aging and in adulthood, the diagnosis of severe GHD must be shown biochemically by single provocative testing, provided that a reproducible test with clear normative limits is available [14,21]. It has been demonstrated that at least some provocative tests distinguish GHD from normal.

As anticipated, ITT is considered the test of choice, and severe GHD, requiring treatment with rhGH, was defined arbitrarily as a GH response below 3 μg/L [14,21]. Based on the ITT contraindications (ischemic heart disease, seizure disorders, and aging), however, this test is unlikely to be that of choice to test elderly subjects suspected for GHD [23]. Alternative
provocative tests with appropriate cut-off limits have been validated. Among classical tests, it has been reported that glucagon is reliable to distinguish normal from GHD subjects, even in aging [19].

GHRH + arginine has been shown to be a reliable means of diagnosing severe GHD, also in the aging individual by distinguishing GHD from normal elderly subjects, while assuming a cut-off point of 9 μg/L GH peak. This reliability reflects the age-independent GH-releasing action of the GHRH and arginine combination [42].

On the other hand, the GHRH, synthetic GHS, and arginine combination represents another potent and reproducible provocative test, which has been shown to distinguish GHD from normal subjects even in the elderly. The cut-off point, below which severe GHD is demonstrated by this test, has been defined as 10 μg/L GH peak. The GH-releasing action has been reported to independent of age [40–42]. The GH response to GHRH in combination with arginine or GHS is likely to depend negatively on BMI; therefore, lower cut-off points should be considered in the presence of obesity [42].

Summary

GH provocative tests remain the only hormonal investigation that provides data on somatotroph function during an individual’s lifespan. Diagnosis of GHD in the elderly is difficult because of age-related GH secretory decline or somatopause. In aging and adulthood, the evaluation of spontaneous GH secretion and IGF-I levels does not provide grounds for distinguishing GHD subjects from normal subjects. Thus, severe GHD must be biochemically demonstrated by provocative testing. Among classical tests, ITT is considered the gold standard, while arginine and glucagon are considered reliable alternatives. ITT, however, has contraindications that are particularly relevant to elderly subjects. GHRH in combination with arginine or synthetic GHS has become the most potent and reproducible stimulus of GH secretion because it explores the maximal secretory capacity of somatotroph cells and is independent of aging. This approach to somatotroph test function shows high specificity and very good sensitivity as it can distinguish between normal and GHD subjects even in the elderly. GHRH in combination with arginine or GHS is safe and has no known contraindications. This profile is therefore relevant in term of availability of a good provocative test for GH secretion in aging individuals. In fact, even in this period of life, diagnosis of GHD may be crucial, based on the evidence that treating GHD patients with rhGH replacement therapy counteracts several clinical symptoms that are more likely associated with GHD than aging.

Acknowledgments

The authors wish to thank Prof. Camanni for his support and suggestions.
References
