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Dynamic Tests in Pituitary Endocrinology: Pitfalls in Interpretation during Aging

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Abstract
Aging and age-related diseases represent hot topics of current research. Progressive damage in morphology and function of cells and tissue characterizes the normal process of aging that is influenced by both genetic and environmental factors. The ability of each individual to adapt to these stressors defines the type of aging and the onset of age-related diseases (i.e., metabolic syndrome, inflammatory disorders, cancer, and neurodegenerative diseases). The endocrine system plays a critical role in this process because of its complex relationships with brain, immune system, and skeletal muscle; thus, alterations in hormonal networks occur during aging to maintain homeostasis, with consequent under- or overactivity of specific hypothalamic-pituitary-peripheral hormone axes. On the other hand, the increase in life expectancy has led to increasing incidence of age-related diseases, including endocrine disorders, which may prompt assessment of endocrine function in aging individuals. In this context, there is growing awareness that natural changes of endocrine physiology and physiopathology occurring with increasing age may necessitate age-driven diagnostic cutoffs requiring validation in the elderly. This review aims to analyze the available literature on the hormone response to the most important dynamic tests currently used in the clinical practice for the screening of anterior pituitary-related diseases to underline pitfalls in interpretation during aging.

Introduction

The assessment of altered pituitary function in the elderly is a potentially complex task. The increased life expectancy in the general population is associated with a higher incidence and prevalence of many endocrine diseases with age [1], including pituitary adenomas or hypopituitarism. At least in part, the higher rates can be due to the greater availability of newer and highly sensitive diagnostic techniques (magnetic resonance imaging, positron emission tomography, and multi-slice TC), as well as automated hormonal assays, which have increased the diagnostic ability/accuracy of endocrine disorders as well as

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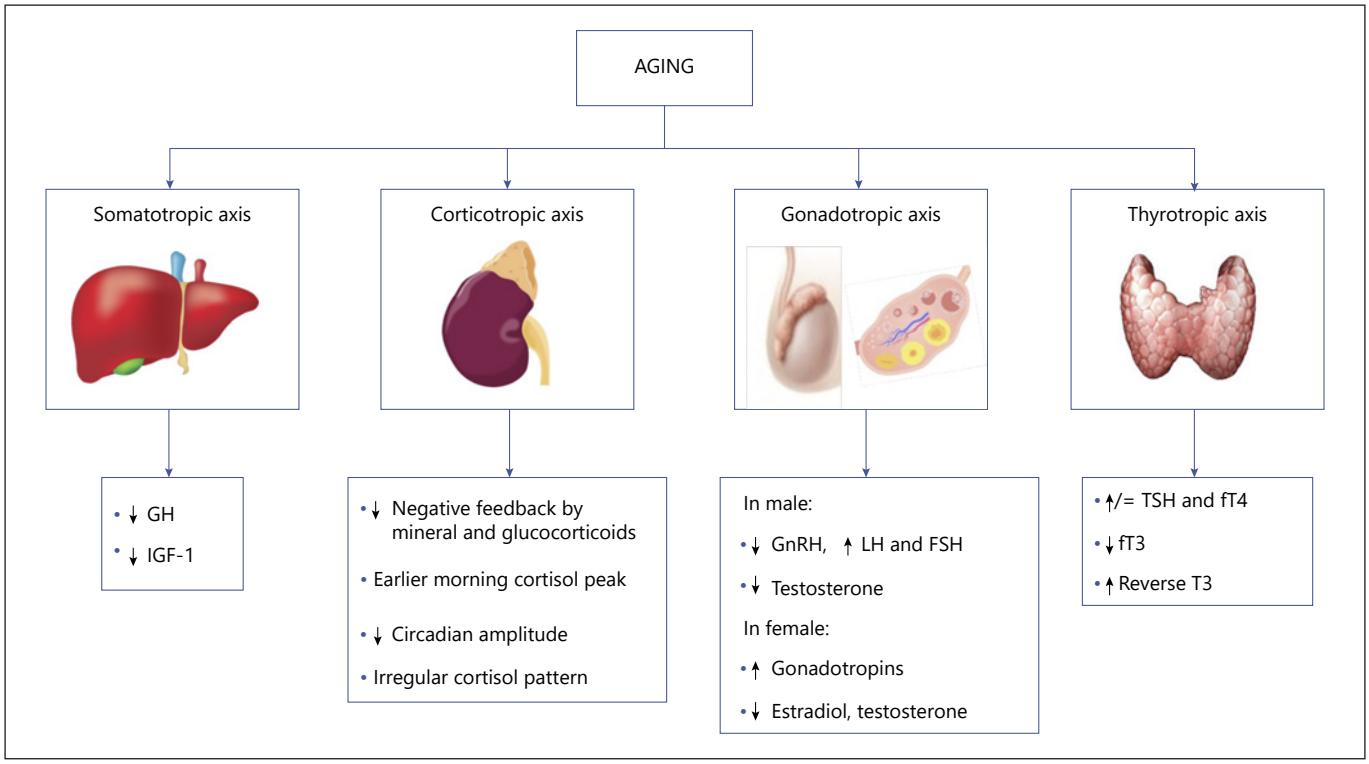

Fig. 1. Main changes in the hormonal axis associated with aging.

incidental diagnoses. Deciphering pathology from physiological adaptation to aging is a challenge.

Human aging is a dynamic physiological process characterized by changes in cells and tissues that result from of a continuous adaptation of the organism to exposure to internal and external stressors [2]. This complex orchestration leads to various aging phenotypes depending on the ability of everyone to respond to stressors [3]. However, an appropriate definition of human aging is difficult to achieve since it constitutes a physiological and a dynamic process developing with time. The process is complex and individualized. Hence, the influence of biological, psychological, and social determinants, in association with environmental stimuli, is involved in defining the time of onset of the neuroendocrinological changes associated with aging.

Intrinsic mechanisms relating to aging include cellular alterations associated with oxidative stress [4] and epigenetic modifications involving DNA methylation and acetylation, changes in telomere length, and the number and function of stem cells [5]. A milestone review by Lopez-Otin et al. [6] identified 9 molecular and cellular hallmarks of aging in connection with the pathophysiological core of aging, which intervene to determine individual longevity, frailty, or susceptibility to disease. A role for lifestyle and environmental factors is emerging in this cross talk [7], also in relation to the processing of environmental stimuli by the neuroendocrine-immune network, which responds to immune senescence with changes in neuroendocrine-immune network involving both cellular and organ systems [8]. From an endocrine viewpoint, in fact, changes occur in the hypothalamic-pituitary axes relating both to the secretory capacity of glands and the feedback mechanisms of gland receptors. The decline in homeostatic and regenerative capacities of aging tissues has been attributed to degenerative alterations in tissue-specific stem cells, stem cell niches, and systemic signals regulating stem cell functions [9]. The master endocrine mechanisms of aging are characterized by a progressive reduction in the synthesis and/or peripheral action of anabolic hormones, such as growth hormone (GH) and gonadal hormones, and the increase in the synthesis of hormones with catabolic properties such as cortisol, as shown in Figure 1. These adaptations go in parallel with an increase in pituitary-related diseases due to the higher life expectancy. In the literature, the age-related regulation of the endocrine system is a relevant topic that is mainly investigated for the GH/insulin-like growth fac-

tor (IGF)-I axis and the gonadal axis in relation to neonatal age, infancy, adolescence, the transition period, and adulthood in reproductive and post-reproductive stages. Age-driven cutoffs for the GH response to dynamic tests have been extensively explored and validated throughout different life phases [10, 11], although the period of senescence has been so far overlooked. Taking into appropriate account the effect of aging as a potential confounding factor for the diagnostic accuracy of dynamic hypothalamic-pituitary tests is of paramount importance for decision-making diagnostic processes.

In this review, we will summarize the physiopathological aspects of aging in relation to dynamic testing for anterior pituitary axes; in particular, we will focus on the first step inhibition and stimulation tests for corticotropic and somatotropic axes used in clinical practice discussing the relevance of validated cutoff values in aging individuals. Dynamic pituitary tests for the thyrotropic and gonadal axes are poorly used in the elderly and therefore have not been included in the present review.

Corticotropic Axis

The hypothalamic-pituitary-adrenal (HPA) axis shows a clear circadian rhythm. The 24-h secretory profiles of adrenocorticotropic hormone (ACTH) and cortisol in peripheral blood are parallel and characterized by higher levels in the early morning (acrophase), which progressively decline during the day (quiescence), then reach a nadir around midnight, and, accordingly, show a fast increase until the early morning [12, 13].

In healthy subjects, the average 24-h levels of cortisol show a progressive increase from the second to the eighth decade of life, with a lower amplitude of the circadian oscillations [14]. In the elderly, circadian rhythm of ACTH and cortisol is reduced in amplitude due to a high nadir of cortisol around midnight. Moreover, both the peak and nadir of cortisol secretion occur approximately 2 h earlier in the morning than younger counterparts [15, 16].

Studies on animal models showed that these changes reflect a blunted negative feedback, resulting from the decreased expression of glucocorticoid and mineralocorticoid receptors in the hippocampus as well as from the activation of hypothalamic neurons secreting corticotropin-releasing hormone and vasopressin [17]. Interestingly, some studies associated the functional hyperactivity of the HPA axis in the elderly with some psychiatric disorders, including depression [13, 18, 19].

A gender difference exists in aging-related changes of the HPA axis, since all these adaptations are more pronounced in female than in male individuals. Moreover, diabetes mellitus, chronic inflammation, arterial hypertension, obstructive sleep apnea, and genetic polymorphisms can also increase ACTH and cortisol release [17]. Whether changes in cortisol secretion patterns are due to aging per se or rather reflect other senescence-related effects (i.e., low-grade inflammation, altered sleep characteristics, or switches in social or emotional status) remain to be clarified.

Somatotropic Axis

The activity of the GH/IGF-I axis presents age-related modifications during the life span and typically declines with aging. The spontaneous GH secretion is higher at birth and subsequently decreases reaching levels that are at plateau until the beginning of puberty. During puberty, a new increase in GH levels occurs, due to the increased amplitude of the secretory peaks. In adults, the daily GH release undergoes a constant decline, which is more pronounced in male than female individuals, likely due to the role of sex hormones and their age-related changes [20]. In the elderly, a further decrease in circadian GH secretion occurs (about 14% *per decade*), reflecting a concomitant decrease in the frequency and amplitude of the GH peaks, contributing to overall low circulating GH concentrations [20, 21].

The reduced GH secretion observed in aging humans mainly reflects variations in the hypothalamic control of somatotropic secretion, that is, the increase in somatostatinergic activity and the decreased activity of GH-releasing hormone (GHRH), rather than a reduced secretory capacity [20, 22]. Likewise, aging reduces endogenous signaling and secretion of ghrelin, the endogenous ligand for the GH secretagogue (GHS) receptor [23]. In addition, a decrease in GH-binding protein (BP) levels has been observed together with an altered expression or clearance of the GH receptor [24]. Therefore, the average 24-h GH concentration, together with IGF-I and IGFBP-3 levels, is lower in older subjects than in young subjects [20].

The term *somatopause* has been proposed to define the clinical alterations related to aging, including sarcopenia, osteopenia, and increased visceral adiposity with insulin resistance, which could be caused by the decreased GH secretion [20, 25–27]. This evidence led to the definition of ranges of IGF-I levels normalized by age, favoring the

Table 1. Stimulation tests for suspected hypocortisolism and GHD in adulthood and elderly [36]

Dynamic test	Procedure	Interpretation (normal response)	Contraindications in the elderly
ACTH test 250 μg	Administer ACTH 1–24 (cosyntropin) 250 μg i.v. o i.m. Blood sample for cortisol at 0, 30, and 60 min	Cortisol peak >18–20 μg/dL (500–550 nmol/L) at 30 or 60 min	None
ACTH test 1 μg	Administer ACTH 1–24 (cosyntropin) 1 μg i.v. Blood sample for cortisol at 0 and 30 min	Cortisol peak >18 μg/dL (500 nmol/L) at 30 min	None
ITT	Administer bolus of regular insulin i.v., 0.05–0.15 U/kg Blood sample for GH and/or ACTH and/or cortisol at −30, 0, 30, 60, and 120 min	Glucose should drop <40 mg/dL (2.2 mmol/L) GH peak >3–5 μg/L Cortisol peak >18–20 μg/dL (500–550 nmol/L)	Ischemic heart disease, cerebrovascular disease, and epilepsy Risk in the elderly
GHRH + ARG	Administer bolus of GHRH i.v. followed by ARG infusion i.v. over 30 min GHRH dose: 1 μg/kg (max 100 μg) ARG dose: 0.5 g/kg (max di 30 g) Blood sample for GH at 0, 30, 45, 60, 75, 90, 105, and 120 min	GH peak >4 μg/L Cutoff BMI related: BMI <25 kg/mq, GH peak >11.5 μg/L 25 <BMI <30 kg/mq, GH peak >8 μg/L BMI >30 kg/mq, GH peak >4.2 μg/L	Active oncologic disease, diabetic retinopathy, not controlled type 1 or type 2 diabetes mellitus, chronic kidney failure False-negative: cranial irradiation
Glucagon	Administer glucagon 1 mg (1.5 mg if body weight >90 kg) i.m. Blood sample for GH and glucose at 0, 30, 60, 90, 120, 150, 180, 210, and 240 min	GH peak >3 μg/L GH response should be correlated to BMI (obesity should blind GH response to stimulation) Cutoff BMI related GH peak >3 mg/L for normal-weight and overweight patients with a high pretest probability GH peak >1 mg/L for obese and overweight patients with a low pretest probability	Malnutrition and fasting glucose >180 mg/dL (10 mmol/L) Risk in the elderly
Macimorelin	Administer macimorelin per os (0.5 mg/kg) Blood sample for GH at 0, 45, 60, and 90 min	GH peak >2.8 μg/L Possibility to use a GH cutoff of 5.1 μg/L if the probability of GHD is high	Remarks: drugs that may interact with macimorelin and cause prolongation of the QT interval or reduce plasma macimorelin concentrations leading to false-positive test results (i.e., CYP3A4 inducers)[a]

GH, growth hormone; ACTH, adrenocorticotropic hormone; GHRH, GH-releasing hormone; ITT, insulin tolerance test; GHD, GH deficiency; ARG, arginine. [a] One reported asymptomatic QT interval prolongation on ECG resolved spontaneously in an individual taking citalopram.

interpretation of the hormonal disorders of the GH-IGF-I axis during life span.

The decreased activity of the somatotropic axis in the elderly could also be related to changes in lifestyle, including reduced physical exercise and energy intake [28]. In the condition of caloric restriction, an increase in GH secretion was shown, representing the combined effect of changes in both the frequency and the amplitude of secretory peaks. Conversely, IGF-I levels are reduced. Thus, insufficient caloric supply determines a reduced response of peripheral organs to GH action, a marked decrease in the synthesis and release of IGF-I and a loss of normal IGF-I/GH negative feedback mechanisms [20, 29–31]. This cascade of events leads to a vicious circle since generalized protein-energy malnutrition, protein depletion, or specific micronutrient deficiency (e.g., zinc), as frequently observed in the elderly, may delay or impair normal tissue regeneration and healing, in association with reduced production of somatomedin that mediate many of the key processes required for normal tissue growth and repair [32]. Because exercise is a potent stimulant of GH secretion by yet unclear neuroendocrine and metabolic mechanisms [33], the decline in voluntary motor activity, physical fitness, and resistance to exercise observed could further contribute to lower GH generation observed with aging [34].

Table 2. Demographic features of patients enrolled in studies designed to calculate diagnostic accuracy of the high-dose (250 μg) ACTH stimulation tests in the diagnosis of secondary adrenal insufficiency

Author, year	*N*	Mean age (yr ± SD)	Age range, years	Sensitivity, %	Specificity, %	Cutoff
Abdu et al. [39]	64	47.5±11.5	28–70	100	90.0	500 nmol/L
Ammari et al. [40]	30	43.0±12.0	19–66	na	na	400–550 nmol/L
Cho et al. [41]	182	40.0±13	14–69	75.9	99.0	17.4 μg/dL
Courtney et al. [42]	41	52.0 (median age)	23–73	81.8	100	650 nmol/L
Ferrante et al. [43]	55	40.5±10.6	na	64	64	500 nmol/L
Giordano et al. [44]	31	45.8±2.4	28–72	71.4	82.4	582.1 nmol/L
Kehlet et al. [45]	25	43.0±15.8	13–64	na	na	na
Maghnie et al. [46]	24	18.1±5.6	4.2–31	na	na	550 nmol/L
Mukherjee et al. [47]	21	45.0±2.5	23–67	na	na	580 nmol/L
Nye et al. [48]	42	46.0	20–81	na	na	500 nmol/L
Orme et al. [49]	16	43.69±3.72	21–64	60	82	500–580 nmol/L
Rasmuson et al. [50]	27	50.0±13.1	19–68	na	na	500 nmol/L
Stewart et al. [51]	58	46.2±13.9	na	na	na	550 nmol/L
Talwar et al. [52]	32	37.0	16–60	na	na	550 nmol/L
Tordjman et al. [53]	89	48.0	na	12.5	100	500 nmol/L
Dokmetas et al. [54]	19	51.04±2.9	24–69	100	17	550 nmol/L

na, not available; ACTH, adrenocorticotropic hormone.

Stimulation Tests

The biochemical diagnosis of hypopituitarism in elderly individuals is based on identical laboratory procedures as those used in adults. However, aging per se or via its associated diseases can impact the correct interpretation of endocrine tests for diagnostic purposes. Although clinical experience and awareness of age-related pituitary changes could aid the diagnostic workup, the lack of elderly related normal cutoffs as well as the intrinsic difficulty in generating reliable age-related ranges constitutes diagnostic drawbacks. The following sections will describe the pitfalls in the diagnosis of ACTH deficiency and GH deficiency (GHD). The tests used in the clinical practice and recommended by the guidelines are summarized in Table 1.

ACTH Deficiency

Secondary adrenal insufficiency is characterized by the failure of the adrenal cortex in the production of cortisol due to pituitary deficiency of ACTH. Although a timely diagnosis is key to prevent adrenal crisis [35], adrenal symptoms of ACTH deficiency (fatigue, inappetence, weight loss, hypotension, and nausea) can be unspecific at any age, and in the elderly, they could be undistinguishable from those developing in highly prevalent diseases in the elderly, such as neoplastic diseases, depression, and aging-associated malnutrition and cachexia.

When there is a clinical suspicion of adrenal insufficiency, biochemical tests are needed to confirm the diagnosis. The assessment of basal serum cortisol in the morning (8:00–9:00 a.m.) is the first diagnostic step: basal circulating cortisol levels <3 μg/dL are suggestive of hypocortisolism, while levels >15 μg/dL exclude the diagnosis. If cortisol levels are between 3 and 15 μg/dL, then a dynamic test is necessary to confirm or exclude diagnosis [36].

The insulin tolerance test (ITT) is considered the gold standard [36] according to high sensitivity and specificity. However, because symptomatic hypoglycemia is needed (<40–45 mg/dL), this test is not recommended in the elderly, due to high neurological (seizure) and cardiologic risks (arrhythmias and hearth attack). Therefore, the ACTH test, proposed as an alternative to the ITT, should be considered the election procedure for the diagnosis of secondary hypoadrenalism in geriatric age [37]. The Endocrine Society guidelines suggest the low-dose (1 μg) or the standard-dose (250 μg) ACTH test (Table 1) [36]. This recommendation is based on a recent meta-analysis of 1,209 adult patients showing similar diagnostic accuracy for the 2 tests, which was moderate due to low sensitivity [38]. Notably, analyzing features of the population included in the meta-analysis [39–57], mean age of patients ranged from 37 to 51 years (Tables 2, 3), so the results are not easily generalizable in the elderly population.

Table 3. Demographic features of patients enrolled in studies designed to calculate diagnostic accuracy of the low-dose (1 μg) ACTH stimulation tests in the diagnosis of secondary adrenal insufficiency

Author, year	*N*	Mean age (yr ± SD)	Age range, years	Sensitivity, %	Specificity, %	Cutoff
Abdu et al. [39]	64	47.5 6±11.5	28–70	100	93.3	500 nmol/L
Ambrosi et al. [55]	57	na	19–73	71.0	93.0	500 nmol/L
Cho et al. [41]	182	40.0	14–69	83.1	82.8	5.8 μg/dL
Choi et al. [56]	72	46.0	28–74	97	78	550 nmol/L
Courtney et al. [42]	41	52.0 (median age)	23–73	81.8	86.7	650 nmol/L
Giordano et al. [44]	31	45.8±2.4	28–72	73.0	80.0	477.3 nmol/L
Maghnie et al. [46]	24	18.1±5.6	4.2–31	na	na	550 nmol/L
Nye et al. [48]	42	46.0	20–81	na	na	378 nmol/L
Rasmuson et al. [50]	27	50.0±13.1	19–68	na	na	500 nmol/L
Soule et al. [57]	86	35.0	14–79	83	58	600 nmol/L
Talwar et al. [52]	32	37.0	16–60	na	na	550 nmol/L
Tordjman et al. [53]	89	48.0	na	94.7	90.0	500 nmol/L
Dokmetas et al. [54]	19	51.04±2.9	24–69	100	66	550 nmol/L

na, not available; ACTH, adrenocorticotropic hormone.

A few studies examined cortisol responses to stressful stimuli according to patients' age [58, 59]. Giordano et al. [58] demonstrated that in normal elderly subjects (age 63–75 years), the cortisol response to tetracosactin was preserved after supramaximal (250 μg) and submaximal (0.5 μg) doses, but it was absent after administration of a very low ACTH dose (0.06 μg), in agreement with other studies [60, 61], suggesting a possible reduced sensitivity to ACTH of the adrenal fasciculata zone occurring with aging. In line with this hypothesis, a meta-analysis of 45 studies on cortisol responses to different stimuli found an increased cortisol response to challenge in older (mean age 69 ± 6 years) as compared to young (mean age 28 ± 5 years) subjects [59].

Recently, Le et al. [62] investigated the cortisol response to ACTH 250 μg in a cohort of 51 old women aged 85–96 years. They demonstrated similar pre-ACTH stimulation levels of cortisol among frail, pre-frail, and non-frail participants; on the other hand, after ACTH administration, a prolonged cortisol response to the stimulus was shown, suggesting an inadequate negative feedback. These data could imply an exaggerated cortisol response to the ACTH stimulus in aging or, alternatively, mechanisms related to diminished ACTH metabolism or enhanced cortisol clearance. Data in larger series of aging patients are warranted since the current literature is repeatedly referred to small samples of patients who rarely are older than 70 years. Considering the reported sensitivity and specificity, the sample size, and the age of patients included in the studies (Tables 2, 3), the low-dose ACTH test (1 μg) should be considered for the diagnosis of secondary hypoadrenalism in elderly individuals taking into account the cost effectiveness, even if no accuracy in the targeted studies is still available in the geriatric population.

GH Deficiency

GHD in adults is the potential consequence of several etiological causes and may originate from 2 different clinical situations: GHD occurring in adulthood or GHD occurring at the pediatric age [63–67]. Adult patients affected by GHD may present peculiar signs and symptoms, such as a relative increase in abdominal fat mass, sarcopenia, a low basal metabolic rate, lipid abnormalities and decreased bone density, self-isolation, and a reduced quality of life [68, 69]. These features are nonspecific and significantly overlap other characteristics of the senile age. Since the accuracy of the dynamic tests strongly depends on the pretest probability of disease, the endocrine workup of GHD in geriatric patients should be started only in particular categories of subjects with a high clinical suspicion, such as (i) subjects presenting a history or signs and symptoms of hypothalamic-pituitary diseases (i.e., pituitary and nonpituitary tumors, empty sella, genetic disorders, infiltrative diseases, and infections), (ii) subjects who underwent cranial irradiation or other treatments for intracranial cancer, (iii) those with a history of traumatic brain injuries or aneurysmal subarachnoid

Table 4. Demographic features of patients studied to compare accuracy of ITT versus GHRH + ARG tests in the diagnosis of GHD

Author, year	*N*	Mean age (yr ± SD)	Age range, years	Sensitivity	Specificity	Cutoff
Aimaretti et al. [79]	40	36.4±2.1	na	na	na	ITT 5 μg/L GHRH + ARG 16.5 μg/L
Biller et al. [80]	94	48.9±11.1 (MPHD) 48.2±11.3 (0–1 PHD) 47.2±11.3 (controls)	na	ITT 96% GHRH + ARG 95%	ITT 92% GHRH + ARG 91%	ITT 5.1 μg/L GHRH + ARG 4.1 μg/L
Chanson et al. [81]	69	37.8±6.9 (healthy subjects) 42.2±11.7 (high probability of GHD) 35.4±14.1 (low probability of GHD)	18–60	ITT 89% ITT 95% GHRH + ARG 79% GHRH + ARG 84%	ITT 100% ITT 100% GHRH + ARG 100% GHRH + ARG 87%	ITT 3.0 μg/L ITT 5.17 μg/L GHRH + ARG 3.67 μg/L GHRH + ARG 7.89 μg/L

ARG, arginine; ITT, insulin tolerance test; GHD, growth hormone deficiency; GHRH, growth hormone-releasing hormone; MPHD, multiple pituitary hormone deficiency; na, not available; PHD, pituitary hormone deficiency.

hemorrhage [70–72], and (iv) patients treated with immunotherapies capable of altering hypothalamic-pituitary axes [73]. Therefore, the diagnosis of GHD in aging individuals is based on the demonstration of a reduced response of the GH stimulation test in the appropriate clinical context [63, 70–72]. IGF-I levels, as well as IGFBP-3, cannot distinguish normal subjects from GHD patients, especially in the overweight and underweight population where IGF-I secretion is mostly driven by nutritional factors [70–72]. GHD must be carefully distinguished from the reduction of GH secretion accompanying the normal aging process, obesity, and protein malnutrition. An accurate and correct diagnosis is fundamental, especially in elderly subjects, to avoid unnecessary therapies in potentially already multi-treated patients.

According to several guidelines, like for ACTH deficiency, the ITT represents the gold standard for the diagnosis of GHD in adulthood [11, 72], but it is not recommended in elderly patients due to the risk of serious adverse events [11]. Moreover, the mechanisms that lead to the increase in GH during this test are driven essentially by the increase in the endogenous activity of GHRH with the concomitant reduction in the hypothalamic release of somatostatin and the increase in the release of catecholamines following alpha-adrenergic activation; thus, the GH response to ITT is influenced by age-related changes in the somatotropic axis, as discussed above [74].

An alternative test is the GHRH + arginine (ARG) test which is well tolerated and has good sensitivity and specificity [72]. The GHRH + ARG test has been shown to distinguish adult patients with GHD from normal subjects with a sensitivity comparable to the ITT, considering appropriate BMI-related cutoffs [69, 75]. Indeed, the GH response to GHRH + ARG is negatively associated with BMI (Table 1), as also recently demonstrated for the ITT [76]. Thus, the GHRH + ARG test could be the first choice in the elderly for its safety profile and the lack of significant contraindications, except for chronic renal failure [77]. However, since the combined tests stimulate both the hypothalamus and the pituitary gland, GHD due to hypothalamic disease can remain undiagnosed. This is exemplified by studies on patients treated with cranial irradiation, in which the ITT shows the highest sensitivity and specificity in the first 5 years after radiotherapy treatment; if GH peak after the GHRH + ARG test is normal in this category of patients, the ITT or glucagon test (GST) should be used [11, 70, 72, 78].

Three studies compared the performance of the ITT versus the GHRH + ARG test in the diagnosis of GHD according to the pretest probability; the tests showed comparable results in terms of sensitivity and specificity. At odds with the large number of studies performed in pediatric and transition-age populations, only a few studies thus far focused on patients aged 65 years or older [79–81] (Table 4).

Another recommended test to explore GH secretory status is the GST, considering the 3 μg/L cutoff as the best combination of sensitivity and specificity [72]. The secretory effect of glucagon is reduced in obesity, as for the tests discussed before; thus, 2019 AACE Growth Hormone task force recommended GH cutoffs related to BMI (Table 1) [11]. Recently, Tavares et al. [82] recruited 41 elderly volunteers from a geriatric ambulatory unit (age range 67–88 years, mean age 77.3 ± 5.2 years) who were subjected to the GST. The study population had common

morbidities related to their age, including arterial hypertension, diabetes mellitus, Parkinson's disease, early stages of senile dementia, and osteoporosis. Median GH peak was 5.99 μg/L, and GH peak was >3 μg/L in 73.2% of subjects, and considering lower cutoffs recently proposed (1.0 μg/L for overweight subjects) [11], only 2 patients showed a GH peak below this value. No difference in GH peak was found after stratifying subjects by age (younger or older than 80 years). Thus, according to the new proposed cutoff points, they found 95% of normal GH responses in elderly subjects. Of note, the authors described adverse events in 21.4% of this series, including 4 cases of severe symptomatic hypotension, dizziness, and sweating [83]. In fact, although the GST is generally well tolerated, it can cause vomit after rapid infusion and late hypoglycemia. The test is contraindicated in malnutrition or in hyperglycemic subjects (fasting blood glucose >180 mg / dL). Especially, the risk of hypoglycemia could be a contraindication, like for the ITT, and it may not be a good alternative in this setting.

Another potentially useful test is the GHRH + GH-releasing peptide-6 (GHRP-6). GHRP-6 is a synthetic GHS, which is able to stimulate hypothalamic and pituitary GH secretion and acts as a functional somatostatin antagonist [84]. In adults, a GH peak ≥20.0 μg/L after GHRH + GHRP-6 administration is considered as normal, while a GH peak ≤10.0 μg/L suggests the presence of GHD [84]. Patients at high risk for hypopituitarism showing a GH peak between 10 and 20 μg/L should perform a second GH stimulation test to receive a definitive diagnosis of GHD [84]. The effect of GH after stimulation with GHRH combined with a GHS such as GHRP-6 or ghrelin, the natural GHS, is highly dependent on age, showing an important reduced activity by aging [85]. This probably reflects age-related changes in primary regulation of GH secretion, which involves impaired cholinergic activity and somatostatinergic hypertonicity. Therefore, the response to this test does not appear to be reliable in the elderly since age-appropriate cutoffs are still warranted.

Among the GHRPs, also macimorelin has been recently approved by the EMA [86]. Like GHRP-6, macimorelin binds to the GHS-R1a receptor. The advantages of macimorelin are the oral administration (0.5 mg/kg), a shorter test duration (90 min), and the small number of samples needed (at baseline and after 45, 60, and 90 min) [11]. Macimorelin testing shows an accuracy comparable to the GHRH + ARG and ITT [87]. The GH peak after macimorelin is inversely proportional to the BMI (Table 1). As with other GHSs, however, experience with macimorelin in the elderly is limited and should be used with caution in patients with pro-arrhythmic manifestations.

In summary, the GHRH + ARG test with specific BMI cutoffs seems to be the safest and most reliable dynamic test for the diagnosis of GHD, even in elderly individuals [71]. Although the progressive aging-related increase in body weight advises toward the use of BMI-related cutoffs, elderly subjects could present an even apparently normal BMI while harboring an excess in visceral adipose tissue associated with insulin resistance, due to the loss of lean body mass. In fact, BMI is not a suggested indicator for the distribution of body mass and weight excess in the geriatric population. Therefore, the aim for future research could be to assess whether GHRH + ARG cutoffs related to waist circumference [88], rather than BMI, as well as the age-related GH response after GHSs. Furthermore, the modulation by sex steroids in sex-specific GH cutoffs, particularly in this population, should be considered.

Inhibition Tests

The diagnosis of endocrine diseases caused by hormonal hypersecretion in the elderly is based on the same procedures used in younger patients. Nevertheless, while age-related cutoffs have been established for IGF-I at least, clear reference values for the diagnostics of HPA axis in the elderly have not been defined yet.

Cortisol Hypersecretion

Cushing syndrome (CS) results from chronic exposure to an excess of glucocorticoids and recognizes exogenous (iatrogenic) and endogenous causes. The former due to the prolonged use of corticosteroid drugs are the most frequent. The latter is determined by spontaneous hypersecretion of cortisol by the adrenal cortex or ACTH by pituitary tumors or by ectopic secretion. Overall, they are characterized by the loss of negative feedback mechanism and alterations in the circadian rhythm of cortisol [89].

During chronic hypercortisolism, clinical manifestations characterizing metabolic syndrome such as central obesity, high blood pressure, hyperglycemia, and dyslipidemia occur. The clinical picture is characterized by other comorbidities, including disorders of neurological and cognitive sphere: anxious-depressive syndrome, psychosis, cognitive and memory deficits, and sleep disorders, common in these patients especially during aging. There-

Table 5. Inhibition tests for suspected hypercortisolism [90] and GH hypersecretion [106]

Dynamic test	Procedure	Interpretation (normal response)	Contraindications in the elderly
Nugent test	Administer dexamethasone 1 mg between 23:00 and 24:00 h; the following morning blood sample for cortisol between 08:00 and 9:00 h	Cortisol <1.8 μg/dL (50 nmol/L)	Remarks Drugs that may interfere with dexamethasone concentration Drugs that may increase CBG levels
Liddle I test	Administer dexamethasone 0.5 mg for 48 h, beginning at 09:00 h on day 1 at 6-h intervals (i.e., 09:00–15:00–21:00–03:00); blood sample for cortisol level at 09:00 h of the day 3, 6 h after the last dose of dexamethasone	Cortisol <1.8 μg/dL (50 nmol/L)	Remarks Drugs that may interfere with dexamethasone concentration[a] Drugs that may increase CBG levels[b]
OGTT (75 g) for GH	Administer glucose 75 g per os; blood sample for GH at 0, 30, 60, 90, and 120 min	GH nadir <1 μg/L	Diabetes mellitus

Drugs that impair dexamethasone metabolism by inhibition of CYP 3A4: aprepitant/fosaprepitant, itraconazole, ritonavir, fluoxetine, diltiazem, and cimetidine; CBG, cortisol-binding globulin. GH, growth hormone; OGTT, oral glucose tolerance test. [a] Drugs that accelerate dexamethasone metabolism by induction of CYP 3A4 phenobarbital, phenytoin, carbamazepine, primidone, rifampin, rifapentine, ethosuximide, and pioglitazone. [b] Estrogens and mitotane.

fore, symptoms of hypercortisolism in elderly patients can overlap those originating from other conditions associated with aging, such as diabetes mellitus, hypertension, osteoporosis, cognitive changes, and sleep disturbances.

Laboratory data of endogenous hypercortisolism should always take the clinical context into appropriate account. The first diagnostic step includes screening tests, such as the dexamethasone suppression test (1 mg overnight or Nugent test; 2 mg/day for 2 days or Liddle I test) (Table 5). Failure to suppress cortisol secretion (>1.8 μg/dL) is considered diagnostic for hypercortisolism [90]. In interpreting the response to the dexamethasone test in an older population, clinicians should primarily consider that (1) some drugs frequently taken by elderly patients could interfere with dexamethasone absorption or metabolism by acting as CYP3A4 modulators; (2) clinical conditions associated with an increase in circulating cortisol-binding globulin levels can cause false-positive values [90]; (3) the clearance rate of dexamethasone can be influenced by changes in kidney or liver function (more frequent in the elderly) [90]; (4) disturbance of HPA axis functioning under basal and challenged conditions is related to visceral fat accumulation [91]; and (5) dexamethasone metabolism can be influenced by liver function in male and BMI in female subjects [92]. Noteworthy, studies that established criteria for interpretation of serum cortisol concentrations during the dexamethasone suppression test in the diagnosis of CS included a prevalent young adult population with mean age between 29 and 39 years [93, 94]. In general, the results of dexamethasone suppression tests showed higher cortisol levels in elderly subjects than in young ones, regardless of their clinical condition [95].

Considering diagnostic pitfalls during aging, no changes in cortisol-binding globulin levels were found in normal subjects during life span; in a longitudinal study in healthy elderly subjects (mean age ranging from 60 to 90 years), changes in total cortisol levels reflected changes in free cortisol level only [96]. On the contrary, a significant correlation between post-dexamethasone cortisol levels and age has been demonstrated [96]; however, not all the studies showed this association, but the difference in the results seems to be strongly influenced by mean age of the population analyzed.

To explain the apparent loss of feedback in elderly subjects, some authors have hypothesized a possible role of an inadequate blood concentration of dexamethasone, although this evidence does not seem to be supported by in vivo studies [97]; another proposed explanation is a possible alteration in the central glucocorticoid receptor sensitivity. In particular, numerous glucocorticoid receptors are located in the hippocampus, and resistance to dexamethasone could be linked to age-related cell loss in this cerebral area.

Despite of this evidence, age-related cutoffs have not been validated so far for dexamethasone suppression tests. Therefore, results in the geriatric population should be interpreted after integration between laboratory and clinical data upon considering the potential effect of interfering drugs (phenobarbital, phenytoin, and carbamazepine), the role of malabsorption, and the underlying presence of psychiatric disorders (depression).

Table 6. Demographic features of patients enrolled in studies designed to calculate diagnostic accuracy of the dexamethasone-CRH test for differential diagnosis of CS or PC

Author, year	*N*	Mean age (yr ± SD)	Age range, years	Sensitivity	Specificity	Cutoff
Dexamethasone-CRH test						
Alwani et al. [99]	73	CS (*n* = 53): 45.9±2.0; PC (*n* = 20): 47.7±3.2	na	94%	100%	Cortisol >87 nmol/L
Valassi et al. [100]	116	CS (*n* = 60): 43.9±15.0 PC (*n* = 41): 42.1±15.2	na	88–93%[a]	75–92%[a]	Cortisol >38 nmol/L
Pecori Giraldi et al. [101]	55	CS (*n* = 32): 36.6±2.28 PC (*n* = 23): 32.4±4.38	CS: 12–60 PC: 14–65	100%	93%	Cortisol >38 nmol/L
Gatta et al. [102]	31	CS (*n* = 17): 41.9±2.9 PC (*n* = 14): 39.2±4.2	na	100%	50%	Cortisol >38 nmol/L
Yanovski et al. [103]	58	CS (*n* = 35): 42.9±13.6 PC (*n* = 19): 31.6±11.4 Ectopic CS (*n* = 2): 25.0±2.8 Primary adrenal disease (*n* = 2): 36.0±5.7	na	100%	100%	Cortisol >38 nmol/L
DDAVP test						
Tirabassi et al. [104]	111	CS (*n* = 52): 38.1±1.2 PC (*n* = 28): 35.0±2.2 Control (*n* = 31): 35.6±2.4	na	75% 90%	90% 92%	ACTH >6 pmol/L Cortisol >331 nmol/L and ACTH >4 pmol/L
Pecori Giraldi et al. [101]	55	CS (*n* = 32): 36.6±2.28 PC (*n* = 23): 32.4±4.38	CS: 12–60 PC: 14–65	82%	90%	ACTH >6 pmol/L
Moro et al. [105]	173	CS (*n* = 76): 36.4 PC (*n* = 30): 30.0 Obese (*n* = 36): 34.9 Control (*n* = 31): 28.7	11–75 14–55 16–65 18–60	87%	91%	ACTH >6 pmol/L

CS, Cushing syndrome; PC, pseudo-CS; DDAVP, desmopressin; na, not available; ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone. [a] Analysis divided patients in taking/not taking drugs known to interfere with dexamethasone metabolism.

In this field, differential diagnosis between CS and nonneoplastic hypercortisolism, also known as pseudo-CS, common in chronic alcoholism, CKD, and psychiatric conditions, is still a challenge [98]. Tests proposed to distinguish between the 2 entities are the dexamethasone-corticotropin-releasing hormone test and the desmopressin test. The proposed cutoffs, sample size, and age of subjects included in the published studies are summarized in Table 6 [99–105]. To note, the performance of these tests has been evaluated in young patients, and ad hoc studies in the elderly population are not available to date.

GH Hypersecretion

Given the appropriate clinical and neuroradiological context, the diagnosis of naive or recurrent acromegaly is primarily based on the determination of circulating levels of IGF-I [106]. As mentioned above, IGF-I levels physiologically decrease with age; hence, they should be interpreted according to age-and sex-related cutoffs. Importantly, IGF-I levels are potentially influenced by diabetes mellitus, CKD, liver diseases, and nutritional deficiencies, all of which are more prevalent in older populations. Like in the general population, also in acromegaly, IGF-I levels decline with increasing age [107], and age-dependent IGF-I cutoffs are routinely used to aid diagnosis in older ages. However, there is increasing recognition of patients with acromegaly who show persistent or intermittent discordance between GH and IGF1 in long-term follow-ups of patients who underwent pituitary surgery [108], and age yields a highly significant effect on the serum GH/IGF-I relationship in patients with acromegaly, such that for a given serum GH value, older patients show lower serum IGF-I values [109]. Therefore, clinicians should proceed with caution in using solely IGF-I levels to reveal a diagnosis of acromegaly in the elderly.

High levels of random GH do not allow for the diagnosis of acromegaly due to the pulsatile secretion of the

hormone. Therefore, the evaluation of the GH suppression test after an oral glucose tolerance test (OGTT) as a complementary laboratory investigation in doubtful cases is recommended. During OGTT-induced hyperglycemia, the lack of suppression of GH levels (<1 μg/L) allows to confirm the diagnosis of acromegaly [106]. The introduction of ultrasensitive chemiluminescent assays for GH led to suggest the adoption of lower normal GH threshold, that is, 0.4 μg/L, but this limit is not recommended yet from guidelines due to the variability of the assay methodologies [106].

However, an equivocal GH response to the OGTT can be seen in association with aging. Considering that basal GH levels are lower in the elderly and that this trend is maintained in the acromegalic patients, numerous studies showed that post-OGTT nadir GH negatively correlates with age. In a cohort of naive acromegalic patients (19–77 years), baseline GH and IGF-I levels and GH nadir levels after OGTT were lower in patients with an age >60 years [110]. A potential caveat to consider is that elderly patients show higher incidence of diabetes mellitus and cannot therefore undergo the OGTT; in these cases, the diurnal GH profile could represent an alternative approach, although this test has also raised criticisms. In a cohort of patients undergoing pituitary surgery for acromegaly, the assessment of disease remission by the GH profile showed different cutoffs in older compared to middle-aged patients (1.4 vs. 2.3 μg/L) [111]. Therefore, age appears to be a fundamental factor to be considered in the diagnosis and evaluation of the acromegaly activity. As in normal aging, this phenomenon could result from a reduction in hypothalamic GHRH levels and high plasma steroid concentrations. In addition, older patients usually have higher body weight or excess visceral fat than younger acromegalic patients, which could negatively affect the response of the GH/IGF-I axis to several diagnostic approaches [112].

Conclusions

The management of hypothalamic-pituitary axis diseases during aging represents a clinical-diagnostic challenge. Symptoms relating to a reduced or excessive secretion of one or more pituitary hormones are often nonspecific; hence, they could be not easily distinguishable from the physiological changes occurring with aging. Even if the diagnosis is based on the same procedures and cutoffs used in nonelderly adults, age-related reference parameters should be established for most pituitary tropins. Further studies are needed to evaluate age-related cutoffs to improve the management and treatment of pituitary pathologies in the geriatric population.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.C., C.M., A.F., I.L., and T.D. performed the literature search, reviewed, and extracted data from the papers; M.C., C.M., A.F., I.L., T.D., P.M., and F.P. performed the figure and table designing and the manuscript writing; and P.M., F.P., and G.A. verified the analytical methods and supervised the manuscript drafting. All authors discussed the results and contributed to the final manuscript.

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