New approach to the diagnosis of growth hormone deficiency in adults

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The diagnosis of growth hormone (GH) deficiency (GHD) in childhood is based on auxological and hormonal investigations, whereas this diagnosis in adults is based on hormonal investigations only, i.e. the assessment of serum insulin-like growth factor I (IGF-I) and serum IGF binding protein 3 (IGFBP-3) and the testing of GH secretion after pharmacological stimuli, spontaneous GH secretion or a combination of both. However, there is no consensus as to which test or combination of tests should be used to assess GH status.

The ready availability of recombinant human GH has led to many studies of the biological consequences of GH deficiency and the benefits of treating adults with this drug. To date, several studies in GH-deficient adults report a beneficial effect of GH treatment on body composition and cardiovascular and musculoskeletal function (1–5). Thus, accurate diagnosis of GH deficiency becomes imperative to select adults who are truly GH lacking.

Recently, the value of stimulated GH release, spontaneous secretion, IGF-I and IGF-BP3 measurement in diagnosing GH deficiency has been investigated in adults (6). Of the four tests used to assess GH secretory status, the insulin tolerance test (ITT) was the only one able to differentiate patients with organic hypothalamic/pituitary disease from their matched normal counterparts. Although ITT provided 100% diagnostic accuracy in defining GH deficiency, this test has potential dangers. In addition, ITT is contraindicated in patients at risk of epilepsy in major neurosurgery. On the other hand, the risk of inducing profound hypoglycaemia is higher in patients with GH deficiency because they have an enhanced sensitivity to the action of insulin.

We have reported previously (7–9) that pyridostigmine (PD), a muscarinic cholinergic agonist, and arginine (ARG) clearly increase the growth hormone (GH) response to growth hormone-releasing hormone (GHRH) in man. The current study was undertaken to investigate the value and safety of PD + GHRH and ARG + GHRH tests as well as the measurement of serum insulin-like growth factor I (IGF-I) in diagnosing GH deficiency in adults. Fifty-four patients considered GH deficient from extensive organic or idiopathic pituitary disease and 326 healthy adults were studied. The IGF-I concentrations were lower than the 3rd percentile of normal values in only 31 of the 54 (57.4%) patients with hypopituitarism. However, the IGF-I levels in hypopituitary patients and in normal subjects overlapped more frequently between 41 and 60 years (50%) and between 61 and 80 years (92.3%) as opposed to between 20 and 40 years (8.6%). In contrast to the IGF-I measurement, the ranges of peak GH responses to PD + GHRH and ARG + GHRH tests were clearly differentiated between the hypopituitary (0.2–6.8 and 0.1–9.5 μg/l, respectively) and normal subjects (17.7–114 and 16.1–119 μg/l, respectively). However, the PD + GHRH test was reliable only in subjects of 20–40 years of age. In conclusion, IGF-I measurement had no value in the diagnosis of GH deficiency in adults aged over 40 years, but is reliable enough when young adults of 20–40 years of age are considered. Both PD + GHRH and ARG + GHRH testing should be considered more reliable biochemical measurements of GH deficiency. In contrast to the PD + GHRH test, the ARG + GHRH test is reliable throughout the adult lifespan and appears to be the most appropriate for patient compliance and safety.

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Subjects and methods
Fifty-four patients with hypopituitarism (30 women and 24 men aged 20–80 years) and 326 healthy adults (228 women and 98 men aged 20–80 years), all within ±15% of ideal body weight, were studied. The body mass index in hypopituitary patients ranged from 18.2 to 27.1 kg/m². After an explanation of the protocol and the test procedures, all subjects gave their consent to participate. The protocol had been approved by our Ethical Committee. Patients with a history of a structural lesion of the pituitary or hypothalamus frequently treated with surgery and/or radiotherapy (12 craniohypophyseal, 17 non-functioning pituitary adenomas, five prolactinomas, two Sheehan’s syndromes, one cyst of arachnoid, one meningioma, one hystiocitosis and one cholesteatoma) or, in the absence of a structural lesion, with a history of idiopathic hypopituitarism (N = 7) were considered to be GH deficient. Out of these 47 patients, five (10.6%) were gonadotrophin- and ACTH-deficient and three (6.4%) were ACTH- and TSH-deficient; in the remaining 39 patients (83.0%), three anterior pituitary hormone (gonadotrophin, ACTH and TSH) deficiencies were shown. The duration of hypopituitarism ranged from 2 to 12 years. The seven patients previously diagnosed as having isolated GH deficiency for short stature (< 3sd) had already received GH replacement therapy during childhood. Hypopituitary subjects received standard substitutive therapy for thyroid, adrenal or gonadal hypofunction, which included 75–100 μg of thyroxine per day, 25–37.5 mg of cortisone acetate per day, ester association of 250 mg of testosterone intramuscularly once a month for men and standard cyclical oestrogen replacement for women.

Measurement of serum IGF-I was performed on all controls and hypopituitary subjects. Eighty-four controls (46 females and 38 males) and 27 hypopituitary subjects underwent a PD + GHRH test and 73 controls (34 females and 39 males) and 24 hypopituitary subjects underwent an ARG + GHRH test. Sixteen of the hypopituitary patients underwent both tests. Nineteen hypopituitary patients also underwent an insulin tolerance test (ITT).

After overnight fasting, tests were carried out starting at 09.00 h, 30 min after an indwelling catheter was inserted into an antecubital vein, kept patien with a slow infusion of 0.9% saline.

Pyridostigmine + GHRH test
Pyridostigmine (120 mg; Mestinon, Hoffmann-Laroche, Basel, Switzerland) was administered orally 60 min before GHRH (1 μg/kg as iv bolus).

Arginine + GHRH test
Arginine (30 g in 100 ml) was infused from 0 to 30 min and GHRH (1 μg/kg) was injected as an iv bolus at 0 min.

Insulin tolerance test
Soluble insulin (0.1 IU/kg Actrapid; Novo-Nordisk, Copenhagen, Denmark) was given intravenously at 0 min. Blood samples were taken at 15-min intervals, starting at −15 to 120 min for GH measurement. During ITT, glucose measurement was performed and a minimum plasma glucose level of 2.2 nmol/l or less was detected.

Serum GH levels were measured in duplicate by immunoradiometric assay (HGH-CTK, Sorin, Italy). All samples from an individual subject were analysed together. The sensitivity of the assay was 0.15 μg/l. The inter- and intra-assay coefficients of variation were 4.9–6.5% and 1.5–2.9%, respectively. The IGF-I levels were measured in duplicate by radioimmunoassay (Nichols Institute Diagnostics, USA). To avoid interference by binding proteins, all plasma samples were treated with acid-ethanol. The sensitivity of the assay was 0.1 μg/l. The inter- and intra-assay coefficients of variation were 10.1–15.7% and 7.6–15.5%, respectively.

Statistical methods
Shapiro–Wilks’ w test was applied to test the normality of the population distribution. When the distribution of the population was not normal, percentiles were calculated with conventional methods. The results were expressed by median values and ranges. The Kruskall–Wallis ANOVA test was used to test the sex difference. Linear correlations were calculated using Spearman’s rank correlation test.

Results
Insulin-like growth factor 1
Serum IGF-I levels in normal subjects decreased significantly (r = −0.60, p < 0.0001) during their lifespan, with no significant sex difference (Fig. 1). The IGF-I concentrations were lower than the 3rd percentile of normal values in only 31 of the 54 (57.4%) patients with hypopituitarism. However, the IGF-I levels in hypopituitary patients and in normal subjects overlapped more frequently between the ages of 41–60 years (50%) and 61–80 years (92.3%), as opposed to 20–40 years (8.6%).

Pyridostigmine + GHRH test
Figures 2 and 3 report a peak GH response to the PD + GHRH test in 84 normal subjects and in 27 patients with hypopituitarism and IGF-I levels ranging
from 7 to 196 µg/l. In normal subjects, the PD + GHRH test induced peak GH responses that ranged from 3.7 to 114.0 µg/l (median 27.2 µg/l). No significant sex difference was highlighted, but the median GH peak was lower in elderly subjects (60–80 years of age) than in young adults (20–40 years of age) (14.5 vs 54.5 µg/l; p < 0.05). The GH peak occurred at 15–90 min after GHRH administration. Peak GH responses between normal and hypopituitary subjects overlapped, with 18.5% (five out of 27) of hypopituitary patients reporting values within the range of normal subjects. However, if only young adults aged between 20 to 40 years of age are considered, a clear differentiation in the range of peak GH responses between the two groups is highlighted, with the hypopituitary group reporting the highest value of 6.8 µg/l as opposed to a range of 17.7–114.0 µg/l (3rd and 97th percentiles of 17.8 and 109.0 µg/l, respectively) in normal subjects. The sensitivity and specificity of the test were 100% and 94%, respectively.

In 11 hypopituitary patients of 20–40 years of age, the peak GH response to the PD + GHRH test (median 2.6 µg/l; range 0.2–6.8 µg/l) did not differ significantly

Fig. 1. Age distribution of serum IGF-I levels in 326 healthy adults (20–80 years old) of both sexes (upper panel) and in 54 hypopituitary patients aged 20–80 years (lower panel). Solid triangles are IGF-I levels in idiopathic GH deficiency. The shaded area depicts the 95% confidence levels in normal subjects per decade of age.

Fig. 2. Peak GH responses to pyridostigmine (PD) + GHRH or arginine (ARG) + GHRH tests in normal (○) and hypopituitary (●) subjects. Solid triangles are GH levels in idiopathic GH deficiency.

Fig. 3. Peak GH responses to pyridostigmine (PD) + GHRH or arginine (ARG) + GHRH tests in relation to age in normal (○) and hypopituitary (●) subjects. Solid triangles are GH levels in idiopathic GH deficiency.

Fig. 4. Comparison in the same hypopituitary subjects between peak GH responses to the pyridostigmine (PD) + GHRH test (aged 20–40 years) or to the arginine (ARG) + GHRH test (aged 20–80 years) and the insulin tolerance test (ITT). Solid triangles are GH levels in idiopathic GH deficiency.
from those to insulin-induced hypoglycaemia (median 1.2 µg/l; range 0.4–3.1 µg/l) (Fig. 4).

The responses to the PD + GHRH test were also not different in hypopituitary and isolated GH-deficient patients and were not correlated with the duration of GH deficiency.

**Arginine + GHRH test**

Figures 2 and 3 also report peak GH responses to the ARG + GHRH test in 73 normal subjects and in 24 patients with hypopituitarism and IGF-I levels ranging from 14 to 196 µg/l.

In normal subjects, the ARG + GHRH test induced peak GH responses that ranged from 16.1 to 119.0 µg/l (median 49.5 µg/l; 3rd and 97th percentiles of 16.4 and 113.0 µg/l, respectively), with no significant sex difference. The GH peak occurred at 15–90 min after GHRH administration. Contrary to the PD + GHRH test, the median GH peak to ARG + GHRH did not differ significantly in elderly and young subjects (47.6 vs 57.0 µg/l). There was a clear differentiation in the range of peak GH responses between normal and hypopituitary subjects (including the five patients who had overlapping “normal” peak GH levels following PD + GHRH), with the highest value in the hypopituitary group reported at 9.5 µg/l as opposed to a range of 16.1–119.0 µg/l in normal subjects. The sensitivity and specificity of the test were 100% and 95.8%, respectively.

In 11 hypopituitary patients, the peak GH responses to the ARG + GHRH test (median 3.1 µg/l; range 0.1–9.5 µg/l) did not differ significantly from those to insulin-induced hypoglycaemia (median 1.2 µg/l; range 0.2–5.9 µg/l) (Fig. 4).

The responses to the ARG + GHRH test were also not different in hypopituitary and isolated GH-deficient patients and were not correlated with the duration of GH deficiency.

**Side effects**

After GHRH administration, transient facial flushing was reported for most of the subjects. Pyridostigmine frequently induced mild abdominal pain and fasciculations, while no side effects were observed after arginine administration.

**Discussion**

The present results confirm previous reports (14, 15) that serum IGF-I concentrations in normal subjects decrease significantly during the lifespan, with no significant sex difference. The increased overlapping between normal and hypopituitary patients over 40 years reduces the diagnostic value of individual age-stratified IGF-I measurements. On the other hand, our results show that IGF-I measurement is reliable enough in young adults aged 20–40 years, with 91.4% of hypopituitary patients having IGF-I concentrations below the 3rd percentile of normal values. These latter results are in accordance with the findings of de Boer et al. (16) and Bates et al. (17).

In contrast to IGF-I measurement, the PD + GHRH and ARG + GHRH tests used to assess GH secretory status were able to differentiate clearly between normal subjects and patients with hypopituitarism. Thus, the subnormal peak GH response to these tests had a diagnostic accuracy of 100% in the differentiation of hypopituitary patients from normal subjects. However, the PD + GHRH test was reliable only in subjects aged 20–40 years. These provocative tests using GHRH are able to probe the real secretory capacity of the somatotrope cells. Possible hypothalamic defects should be verified by the study of spontaneous GH secretion, which seems to be reliable in children but not in adults (6).

In patients with hypopituitarism who underwent both the PD + GHRH (or ARG + GHRH) test and ITT, the peak GH responses overlapped in the two tests. However, in normal subjects the peak GH response to GHRH combined with pyridostigmine or arginine is markedly higher than that to the insulin-induced hypoglycaemia reported by Hoffman et al. (6), the minimum normal GH peak being 17.7 and 16.1 µg/l for the PD + GHRH and ARG + GHRH tests, respectively, and 5.3 µg/l for ITT. Therefore, a clearer differentiation is present in the range of peak GH responses between normal and hypopituitary subjects through PD + GHRH and ARG + GHRH tests than through ITT. As the patients included in this study suffered from severe hypopituitarism, it may be hypothesized that these new tests may also be able to detect minor defects in GH secretion not necessarily revealed by insulin. In agreement with this hypothesis, the GH status of an adult with non-acromegalic pituitary disease has been reported to be related to the degree of hypopituitarism present (18).

In contrast to ITT, GHRH combined with pyridostigmine or arginine is basically a well-tolerated test. However, the possible contraindication to the use of cholinergic agonists and the finding of low GH responses to the PD + GHRH test in elderly subjects indicate ARG + GHRH to be the safest and most reliable test throughout the adult lifespan.

Arginine, but not pyridostigmine, is able to restore totally the reduced GH responsiveness to GHRH in elderly subjects, making it overlap with that in young adults (19, 20). Confirming previous data (21), our results show that the GH response to the PD + GHRH test is lower in the elderly than in the young, while the GH response to the ARG + GHRH test remains unchanged throughout the adult lifespan. Because the GH-releasing effect of both pyridostigmine and arginine is likely to be mediated by inhibition of hypothalamic somatostatin release (22–24), the reduced GH response
to the PD + GHRH test in aging would point to the existence of an impaired tuberoinfundibular cholinergic function in aging. On the other hand, the existence of an impaired function of the cholinergic nervous system in old people has been evidenced by others (25, 26).

In conclusion, IGF-I measurement is sufficiently reliable to diagnose GH deficiency for individuals aged 20–40 years but has no value for those over 40 years. Pyridostigmine + GHRH or ARG + GHRH testing should be considered a very reliable biochemical measurement of GH deficiency. These tests are also able to probe the real secretory capacity of somatotrope cells. However, the PD + GHRH test is reliable only in young adults, whereas the ARG + GHRH test is reliable throughout the adult lifespan and appears to be the most appropriate for patient compliance and safety.

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References

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