The Use of the Pyridostigmine Growth Hormone-Releasing Hormone Stimulation Test to Detect Growth Hormone Deficiency in Patients With Pituitary Adenomas

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The pyridostigmine (PD)/growth hormone-releasing hormone (GHRH) stimulation test was used to determine growth hormone (GH) secretion in patients with pituitary adenomas prior to (n = 55) and after (n = 72) transsphenoidal adenomectomy, as well as in 98 controls. In controls, maximum concentrations of GH showed a strong negative relationship both with body mass index (BMI) and age. Having calculated the 95% confidence intervals for maximum GH concentrations to be expected for any given age and BMI according to a statistical model, we compared these individually preedicted ranges to GH concentrations actually observed in patients with pituitary disease during PD/GHRH stimulation. Preoperatively and postoperatively, a maximum GH concentration below the calculated confidence intervals was seen in 29 of 55 (52%) and in 57 of 72 (79%) of these patients, respectively. In the remaining patients, maximum GH concentrations were in or above the range defined by these confidence intervals. Our results indicate that maximum concentrations of GH during the PD/GHRH test depend to a large extent on the individuals' age and BMI. The results obtained with the PD/GHRH stimulation must, in each individual patient, be compared with a large control group taking into account both age and BMI. In individuals older then 55 years and with a BMI greater than 35 kg/^{2}, the diagnosis of GH deficiency cannot safely be made, at least not with this test.

IN THE ABSENCE OF clear-cut clinical characteristics of acquired growth hormone (GH) deficiency in adults, the definition of this disorder has to rely on biochemical criteria. To this end, dynamic tests of GH secretion are still considered mandatory. Unfortunately, some stimulation tests, such as the administration of growth hormone-releasing hormone (GHRH) are simple to perform and well tolerated, but are of little value in the definition of adult GH deficiency.[1] On the other hand, the insulin-tolerance test (ITT),[2], the hitherto most widely used procedure, is a frequently unpleasant and potentially hazardous maneuver[3] of only moderate reproducability.[4] Furthermore, even cut-off levels defined by the ITT are arbitrary and hence controversial.[5]

More recently, the combination of pyridostigmine (PD) and GHRH[6-8] was presented as a safe, potent, reproducible, and reliable[8] tool to investigate GH secretory reserve. We have evaluated this newer method taking into account that many patients with potential GH deficiency due to pituitary tumors are, of course, neither young nor nonobese. Because GH secretion decreases with age[9] and body weight,[10] the relevant question in clinical terms is not whether a group of patients with established pituitary insufficiency presents with a reduced secretion of GH as compared with a healthy, lean control group,[5,11-14] but rather whether GH secretory capacity can be reliably assessed in an individual patient of a certain age and body weight who does not have established pituitary insufficiency.[1] Indeed, any study reporting on adult GH-deficient patients should calibrate its data at least in terms of age and body mass index (BMI) and possibly even for additional factors, such as sex,[15] the phase of the patients' menstrual cycle,[16] his or her psychic condition,[17] and smoking habits.[18] Until these influences have convincingly been ruled out for any of the available stimulatory tests, it remains uncertain whether GH deficiency in adults can be defined by biochemical criteria at all.

MATERIALS AND METHODS

Controls

A PD/GHRH stimulation test was performed in 98 nonselected persons who had been referred for evaluation of various suspected nonpituitary diseases (eg, nodular goiter, hypertension, gynecomastia). None of these patients suffered from disorders or received medication known to interfere with GH secretion. The control group was of a similar age and BMI as the patients with pituitary tumors (Table 1). Both groups comprised individuals with a broad range of age and BMI.

All individuals were investigated in the fasting state. They first received PD (Mestinon; Roche, Basel, Switzerland; 120 mg) orally with a glass of water and 100 μg GHRH intravenously (IV) 60 minutes thereafter. Serum concentrations of GH were determined before and 60, 90, and 120 minutes after IV GHRH.

Patients With Pituitary Tumors

In patients with pituitary adenomas, GH release was determined in an analogous fashion either before (n = 55) or after (n = 72) transsphenoidal, selective adenomectomy (Table 1). There was no indication of childhood-onset GH deficiency in any of these patients. Seven patients of the preoperative group and 25 patients of the postoperative group needed a substitution therapy with either thyroxine, glucocorticoids, or testosterone. This medication represented replacement therapy in each case.

GH was determined with a time-resolved fluoroimmunoassay kit (Delfia, Wallac Oy, Turku, Finland) using an automated AutoDelfia system. This kit is calibrated against the World Health Organization (WHO) 1st International Reference Preparation 80/205; 1 ng/mL = 2.6 mU/mL). Inter- and intrassay coefficients of variation were less than 5%. Minimal detection limit of this assay was 0.01 ng/mL.

Statistical Methods

Means and standard deviations (SD) describe continuous variables, and distributions are given for categorical ones. Comparison between 2

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Table 1. Age, Sex, BMI, and Concentrations of GH (basal and following stimulation with PD/GHRH) in Patients With Pituitary Tumors and in Controls

	Patients With Pituitary Tumors		
	Before Surgery	After Surgery	Controls
No.	55	72	98
M/F	25/30	33/39	60/38
Age (yr)	46 (21-78)	47 (23-73)	41 (14-87)
BMI (kg/m^2)	27 (20-43)	26 (18-38)	29 (16-61)
<25 n	25	30	40
25-30 n	21	32	26
30-35 n	7	9	11
>35 n	2	1	21
Additional medication	7	25	—
GH (ng/mL)			
Basal	1.7 ± 2.3	1.1 ± 2.0	1.8 ± 2.8
Max	9.5 ± 6.7*	7.0 ± 9.1*	14.7 ± 12.4

*P *v* controls < .01.

groups is based on Student's *t* test. An analysis of covariance model was fitted to the observed data of controls subjects, assuming logarithm of maximum GH concentration as dependent variable, age, BMI, and sex as covariates. Sex was removed as a covariate from the final model (Table 2), its effect being not significant. To show for our model the shape of the relationship among logarithm of maximum GH concentration, age, and BMI, a 3-dimensional plot was drawn based on interpolated predicted values for maximum GH concentration. Based on this fitted model, 95% predicted confidence intervals for logarithm of maximum GH concentration were computed from age and BMI of patients with pituitary disease and compared with the observed values.

RESULTS

In controls, there was a pronounced negative relationship (Table 2) between maximum GH concentrations during the PD/GHRH test and, both, age and BMI (Figs 1 and 2). When these relationships are depicted as a 3-dimensional plot (Fig 3), it becomes apparent that in individuals beyond 55 years of age and with a BMI of greater than 35 kg/m^2, PD/GHRH-stimulated GH release is minute or nonexistent. This excludes the possibility to diagnose GH deficiency in this group of patients.

Having thus calculated the 95% confidence intervals for maximum growth hormone concentrations to be expected from our statistical model for any given age and BMI, we compared these predicted values to GH concentrations actually observed in patients with pituitary disease during PD/GHRH stimulation. Preoperatively and postoperatively, a maximum GH concentration below the calculated confidence intervals was seen in 29 of 55 (52%) and in 57 of 72 (79%) of these patients, respectively. In the remaining patients, maximum GH concentrations were

Table 2. Fitted Model on Controls

Variable		Parameter Estimate	Standard Error	*P* Value (*t* test)
Intercept	β_0	5.324	0.279	<.0001
BMI	β_1	−0.073	0.008	<.0001
Age	β_2	−0.022	0.003	<.0001

NOTE. Ln_GHmax (control subject i) = $y_i = \beta_0 + \beta_1 BMI_i + \beta_2 Age_i +$ error.

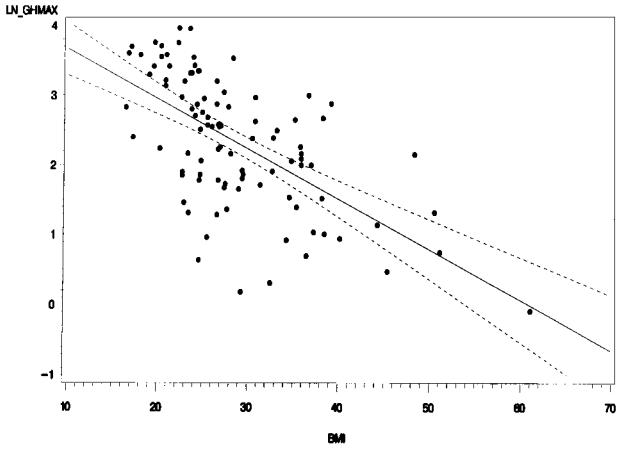

Fig 1. Maximum concentrations of GH (GH; ng/mL) *v* BMI in controls. The solid line indicates a marginal linear regression. The dotted lines refer to 95% confidence intervals of expected values.

in or above the range defined by these confidence intervals (Table 3).

DISCUSSION

The combination of PD and GHRH[6-8] has been suggested to be a safe, potent, reproducible, and reliable[8] tool to investigate peak GH levels. Because the symptoms of acquired GH deficiency are not easily separated from the normal aging process, which in combination with progressive obesity, diminishes GH secretion,[9,10] a test with these qualities would be highly welcome. This might be considered a minor problem if therapy with GH would be cheap and without risks. Unfortunately, it is neither[19-24] and should ideally be based on clear-cut criteria.

Similar to other stimulation tests, such as the ITT[2,4] or the GHRH test,[1] the PD/GHRH test can be used to document reduced secretion of GH in patients with pituitary disease as a group and set them apart from the mean levels of a control group. In addition, the results of the present study indicate that the PD/GHRH test may be of use to identify individual patients

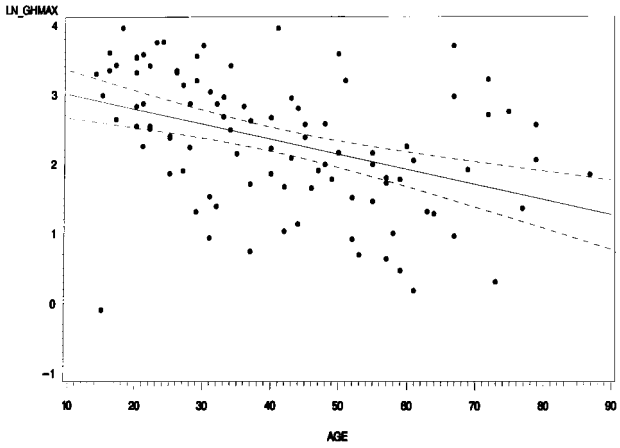

Fig 2. Maximum concentrations of GH (GH; ng/mL) *v* age in controls.

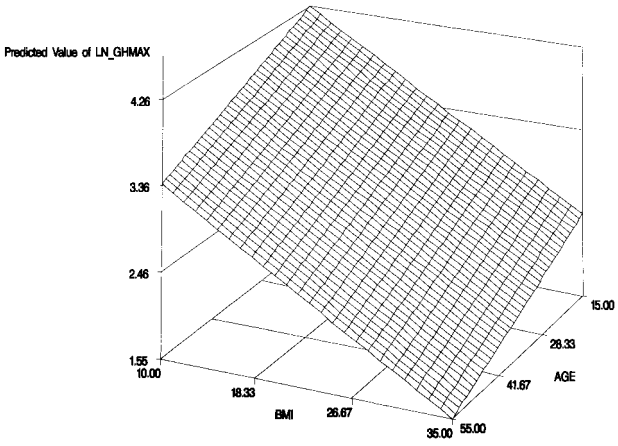

Fig 3. Interpolated predicted maximum concentrations of GH (GH; ng/mL) *v* BMI and age in controls. Confidence intervals are not shown.

with lower than normal secretion of GH. However, because the results obtained with PD/GHRH stimulation and, persumably with any other GH stimulation test, depend to a large extent on age and BMI, the results obtained in each individual patient must be compared with a large control group taking into account the impact of these covariates. In individuals older than 55 years and with a BMI greater than 35 kg/m^2, the diagnosis of GH deficiency cannot be made, at least not with the PD/GHRH test. Whether this holds true for other tests currently used to evaluate GH secretion in adults remains to be investigated. In any case, these other maneuvers should be evaluated and subsequently used in an analogous fashion.

Table 3. Predicted Result of the PD/GHRH Test in Patients With Pituitary Adenomas Before and After Neurosurgical Intervention Based on the Individual Patients' Age and BMI and on the Relationship of Maximum GH Concentrations, BMI, and Age Calculated in a Control Group

	Lower Than Predicted	As Predicted	Higher Than Predicted	Total
Before surgery	n = 29	n = 14	n = 12	n = 55
After surgery	n = 57	n = 6	n = 9	n = 72

The considerable percentage of our patients presenting with below-normal GH secretion is in keeping with previous reports about a high prevelance of GH deficiency among patients with pituitary adenomas.[12,25,26-28] The individualized interpretation of PD/GHRH-stimulated GH concentrations will help to decide whether GH therapy should be instituted in these patients.

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