How (Not) to Diagnose Growth Hormone Deficiency in Adults: Stimulated Serum Concentrations of Growth Hormone in Healthy Subjects and in Patients With Pituitary Macroadenomas

H. Vierhapper, P. Nowotny, T. Czech, C. Bieglmayer, W. Raber, and W. Waldhäusl

The secretion of growth hormone (GH) stimulated by GH-releasing hormone ([GHRH] 100 μg intravenously [IV]) was determined in 33 patients with nonfunctioning pituitary macroadenomas before and after transsphenoidal adenomectomy and in 28 controls. Patients who needed substitution therapy for at least one additional pituitary hormone presented with lower GH secretion than the remaining patients with pituitary tumors. However, there was a marked overlap of stimulated GH secretion between these two groups (3.2 ± 4.3 ng/mL and 7.2 ± 6.6 ng/mL, respectively) and between either group with the control group (7.1 ± 5.5 ng/mL). In an independent investigation, the effect of IV GHRH (100 μg) on the secretion of GH in seven healthy volunteers was shown to be comparable to that seen during an insulin tolerance test ([ITT] 0.1 U/kg IV). Thus, the GHRH stimulation test, a simple and comparatively unharmful procedure, is a useful alternative to the ITT in patients with potential pituitary defects. However, the pronounced overlap of stimulated serum GH concentrations in patients with pituitary macroadenomas and those estimated in healthy subjects and in patients with nonpituitary diseases underlines the difficulty in biochemically defining acquired GH deficiency in adults. We suggest that GH therapy in adults should primarily be instituted in patients with additional defects in anterior pituitary function.

WHEREAS LIMITED availability of natural human growth hormone (GH) had previously precluded its replacement in adults, the advent of biosynthetic GH has made this therapeutic option widely obtainable. Considerable efforts have been made to define acquired GH deficiency in adults by biochemical criteria, since the costs of a potentially lifelong therapy with GH and some definite[1-3] or hypothetical[4,5] side effects must be balanced against its potential benefits with regard to quality of life,[6,7] metabolism,[8-10] and perhaps life expectancy.[11] Dynamic tests of GH secretion are superior not only to the one-time determination of GH itself but also to that of insulin-like growth factor-I (IGF-I) and/or IGF binding protein-3, since these parameters show both considerable intraindividual variability and overlap with healthy control groups.[12-14] Since even cutoff levels defined by the insulin tolerance test (ITT) are arbitrary and hence controversial,[13] it remains uncertain at present whether GH deficiency in adults can be defined by biochemical criteria at all. In a pragmatic approach to this question, we thus first compared stimulated GH secretion during an ITT with that induced by intravenous (IV) arginine and by IV GH-releasing hormone (GHRH) in a group of healthy volunteers. Subsequently, we attempted to define, by means of the GHRH stimulation test, the prevalence of GH deficiency in patients with nonfunctioning pituitary macroadenomas before and after transsphenoidal surgery, since this group of patients has been suggested to include a large percentage of potential candidates for GH replacement therapy.[15,16]

From the Clinical Division of Endocrinology and Metabolism, Clinic for Internal Medicine III, University of Vienna, Vienna, Austria.

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Address reprint requests to H. Vierhapper, MD, Clinical Division of Endocrinology and Metabolism, University Clinic for Internal Medicine III, Währinger Gürtel 18-20, A-1090 Wien, Austria.

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SUBJECTS AND METHODS

Healthy Volunteers

Changes in serum concentrations of GH were investigated following stimulation of secretion by insulin, arginine, and GHRH in seven healthy volunteers (three men and four women aged 29 to 41 years). The experimental protocol and potential side effects were carefully explained to each volunteer, and written consent was obtained in each case. Blood samples for determination of GH were obtained before (−15 and 0 minutes) and after (10, 20, 30, 45, 60, 75, 90, and 120 minutes) IV administration of regular insulin (0.1 U/kg body weight), arginine (30 g/30 min, t = 30 minutes), and GHRH (100 μg Somatobiss; Chemie Bissendorf, Germany), respectively. Blood glucose was determined at identical time intervals only following administration of insulin. The three stimulation tests were performed in randomized sequence at weekly intervals with the volunteers in the supine position after an overnight fast.

Patients With Pituitary Tumors

In 33 patients (median age, 47 years) with nonfunctioning pituitary macroadenomas (maximum tumor diameter, >1.0 cm), GH release was determined both before and within 4 to 6 weeks after transsphenoidal selective adenomectomy. There was no indication of childhood-onset GH deficiency in any of these patients. Pituitary tumors were deemed nonfunctioning by clinical and serological evaluation. Specifically, acromegaly, Cushing's disease, hyperthyroidism, and hyperprolactinemia were excluded in each case. Eleven of these patients needed substitution therapy with either thyroxine, glucocorticoids, or testosterone before neurosurgical intervention. In one additional patient, such replacement had to be instituted postoperatively. Serum concentrations of GH were determined in the fasting state before IV administration of 100 μg GHRH and 60, 90, and 120 minutes thereafter. Patients with diabetes mellitus or those taking drugs with a potential influence on GH secretion were excluded.

Finally, a GHRH stimulation test was performed in an analogous fashion in 25 nonselected persons who had been referred for evaluation of various suspected nonpituitary diseases that had been excluded by appropriate investigations. None of these patients had disorders or received medication known to interfere with GH secretion. This group was comparable to the patients with pituitary tumors in terms of age

(controls, 17 to 76 years; patients, 28 to 71 years), height (controls, 171 ± 9 cm; patients, 170 ± 7 cm), and weight (controls, 79 ± 20 kg; patients, 75 ± 14 kg).

GH was determined with a time-resolved fluoroimmunoassay kit (Delfia; Wallac, Turku, Finland) using an automated AutoDelfia system. This kit is calibrated against the World Health Organization First International Reference Preparation 80/205 (1 ng/mL = 2.6 mU/mL). Interassay and intraassay coefficients of variation were less than 5%. The minimal detection limit of this assay was 0.01 ng/mL. Data are presented as the mean ± SD. Student's *t* test and Duncan's multiple-range test[17] were used for statistical evaluation to compare two or several groups of data, respectively. Statistical significance was defined by a *P* value less than .05.

RESULTS

Healthy Volunteers

GHRH, arginine, and insulin induced the expected increase in serum GH concentrations. The mean nadir of blood glucose (22 ± 8 mg/dL; range, 15 to 37) during the ITT was reached after 20 to 30 minutes. Maximum concentrations of GH (17.8 ± 8.3 ng/mL; range, 3.5 to 31.5) were seen after 60 to 90 minutes and were similar ($P > .05$) to those induced by GHRH (17.5 ± 10.8 ng/mL; range, 6.3 to 36.4), although peak values occurred 30 to 60 minutes sooner after GHRH as compared with the ITT (Fig 1). In this group of healthy volunteers, arginine proved to be the weakest stimulus for GH secretion (maximum concentration, 8.3 ± 5.1 ng/mL; range, 3.5 to 16.9).

Patients

Maximum GHRH-stimulated serum concentrations of GH both in the small group of healthy volunteers just described and in the additional larger and older control group (7.1 ± 5.5 ng/mL) showed a substantial overlap ($P > .05$) with values seen in patients with pituitary macroadenomas both before (5.7 ± 6.1 ng/mL) and after (5.9 ± 10.3 ng/mL) pituitary surgery. Although stimulated concentrations of GH were different from basal values in each member of this larger control group, 13

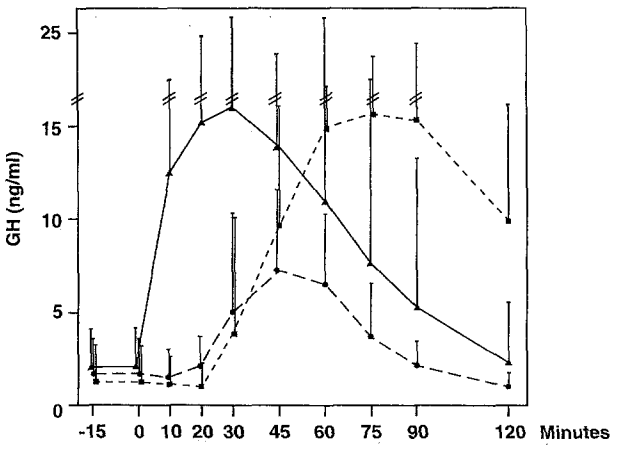

Fig 1. Effect of arginine (30 g/30 min IV, ●-----●), insulin (0.1 U/kg body weight IV, ■--■), and GHRH (100 μg IV, ◆—◆) on serum concentrations of GH (ng/mL) in healthy volunteers (n = 7, mean ± SD).

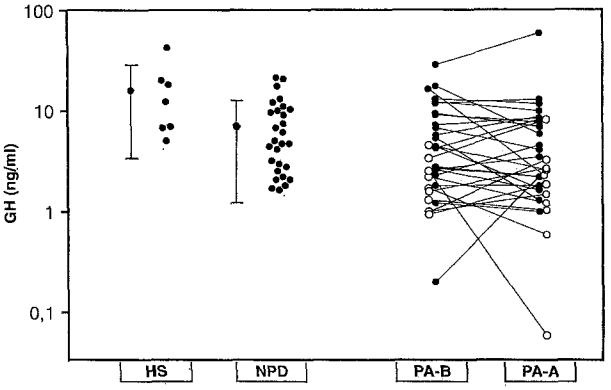

Fig 2. GHRH (100 μg IV)-induced peak concentrations of GH (ng/mL) in healthy subjects ([HS] n = 7), nonselected patients referred to an endocrine outpatient service for evaluation of nonpituitary diseases ([NPD] n = 28), and patients with nonfunctioning pituitary adenomas (n = 33) before (PA-B) and after (PA-A) neurosurgical intervention. (○) Patients on substitution therapy with either thyroxine, testosterone, and/or glucocorticoids; (●) patients not requiring such therapy.

(46%) and five (18%) subjects presented with peak concentrations less than 5 ng/mL and less than 2.5 ng/mL, respectively. On the other hand, peak serum GH concentrations of more than 5 ng/mL and of more than 10 ng/mL were induced in 11 of 33 (33%) and five of 33 (15%) of the patients with pituitary macroadenomas, respectively. Only two patients were completely devoid of any GH response before surgery, although a small increase in GH secretion was seen postoperatively in these two patients (Fig 2).

In patients with pituitary tumors, no difference ($P > .05$) was seen in mean peak serum GH concentrations obtained before and after pituitary surgery. Patients with pituitary adenomas were then subdivided according to whether they did (n = 12) or did not (n = 21) require substitution therapy for one or more other pituitary hormones before surgery. In all but one patient, who was without replacement preoperatively, this was identical with postoperative conditions. The subgroup of 12 patients with partial pituitary insufficiency was characterized by lower ($P < .05$) stimulated GHRH-induced peak concentrations of GH both before (3.2 ± 4.3 ng/mL) and after (2.2 ± 2.1 ng/mL) surgery versus the remaining 21 patients with pituitary adenomas (peak GH concentration before surgery, 7.2 ± 6.6 ng/mL; after surgery, 7.9 ± 12.5 ng/mL), with a substantial overlap between the two groups (Fig 2). None of the patients with such partial pituitary insufficiency had a GHRH-induced serum GH peak more than 5 ng/mL, but an increase of more than 2.5 ng/mL was seen in three of 12 (25%) of these patients.

DISCUSSION

Institution of GH therapy should ideally be based on clear-cut criteria. Regrettably, the lack of a reliable biological parameter—readily available in children—is aggravated by considerable confusion with regard to biochemical markers that may be applied to define GH deficiency in adults. The symptoms of acquired GH deficiency are not easily separated from the normal aging process, which in combination with progressing

obesity diminishes GH secretion.[13] In addition, GH secretion is also influenced by variables as common as the menstrual cycle,[18] psychic disorders,[19] and smoking.[20] Thus, in 1984 it was stated that, "the days when we thought we knew how to use growth hormone are over,"[21] and despite an impressive accumulation of data within the last decade, this statement still applies today.

Due to its pronounced intraindividual fluctuations,[14,22] determination of basal plasma concentrations of GH is of no value for the diagnosis of GH deficiency, and considerable overlap with healthy control groups precludes the determination of IGF-I and IGF binding protein-3,[12,13,23] analysis of GH pulses,[23] and/or GH determination in pooled serum samples[23] or in urine.[24] Therefore, most investigators agree that stimulation tests are indispensable to define GH deficiency in adults.[12,13,23,25] Although its intraindividual reproducibility is less than desirable,[26] the ITT[27] is the procedure most commonly used. In children, in whom GH deficiency may result from either a hypothalamic or pituitary disorder, this is reasonable, since either defect will be recognized by the ITT but could be overlooked by a procedure directly stimulating GH secretion at the pituitary level such as the GHRH stimulation test. However, in adults with supposed GH deficiency due to pituitary tumors and/or their surgical removal, it is certainly preferable to use a maneuver that is less unpleasant and risky than the artifactual insulin-mediated hypoglycemia, eg, stimulation with arginine[28-30] or with GHRH.[31,32] In our hands, as in the study by Koppeschaar et al,[33] GHRH was a stronger stimulus of GH secretion than arginine. In addition, its mode of administration (IV bolus dose *v* a 30-minute infusion in the case of arginine) argues for its preferential use on a routine, ie, outpatient, basis. Having first demonstrated in healthy volunteers that 100 μg GHRH induced an increase in serum GH concentrations similar to that seen during an ITT, we have subsequently used this procedure to investigate the secretion of GH in patients with nonsecreting pituitary macroadenomas both before and after neurosurgical intervention. The results obtained in healthy volunteers gave us no reason to believe that those subsequently found in patients would have been different had we used a challenge with insulin or arginine rather than with GHRH. Using an ITT, Ho and Hoffman[13] have separated patients with GH deficiency from healthy controls. Our results do not directly refute these data, since an ITT was not performed in our patients with pituitary macroadenomas. However, the demonstration of the equipotency of a GHRH challenge and an ITT in healthy volunteers and the fact that the former in our hands failed to identify patients with GH deficiency represent indirect proof that the conclusions reached by Ho and Hoffman[13] may not necessarily be definitive.

Even when stimulation tests are used, the criteria used for definition of GH deficiency remain unclear, since they have been to a large extent taken from the pediatric literature.[13] It has been suggested that a peak GH response of less than 5 ng/mL in lean adult men is subnormal.[27] However, even in children this criterium, while considered indicative of GH deficiency,[34] is regarded as diagnostic only in the appropriate clinical setting.[21,23] Furthermore, a substantial portion of patients with potential GH deficiency due to pituitary tumors are neither young nor non-obese and thus are potentially subject to other causes of reduced GH release. Therefore, the relevant question in clinical terms is not whether a group of patients with established pituitary insufficiency present with a reduced secretion of GH as compared with a healthy lean control group,[12,13,23-26] but rather whether a patient who does not have established pituitary insufficiency might still suffer from isolated GH deficiency requiring appropriate substitution therapy. Potentially, this could be a comparatively large group of patients, since GH deficiency, supposedly the most common endocrine consequence of pituitary tumors and their therapy,[23] might be present in greater than 80%[15,34-36] of patients even with small pituitary lesions. A deficiency in the secretion of corticotropin and thyrotropin has been reported to occur in 33% and 18% of such patients, respectively.[15] In our hands, 12 of 33 patients with pituitary macroadenomas (maximum tumor diameter, >1.0 cm) had preoperative pituitary insufficiency and required substitution therapy with thyroxine, glucocorticoids, and/or testosterone. In only one additional patient, such therapy had to be instituted postoperatively. GHRH-stimulated secretion of GH was reduced in patients with additional pituitary defects compared with the remaining patients with pituitary tumors, but there was a marked overlap among these two groups and between either group and a control group. Had the decision to institute GH therapy been based exclusively on the determination of stimulated serum GH accepting a cutoff value of either 5 or 2.5 ng/mL,[13] one would face the absurd conclusion that the same therapy would appear to be justified in 48% (or 18%, respectively) of an unselected group of patients referred to an endocrine outpatient unit.

Therefore, in accordance with data published by others using the ITT[25] or the arginine stimulation test,[28] our results indicate that GHRH-stimulated secretion of GH is reduced in a group of patients with pituitary macroadenomas and confirmed partial pituitary insufficiency compared with a control group. However, in an individual patient, this maneuver, even in the presence of partial pituitary insufficiency, may suggest but does not prove an additional GH deficiency. In patients with otherwise intact anterior pituitary function, this is even more evident. In our hands, these are the majority of patients with pituitary tumors. Thus, for the time being and until additional parameters and/or methods are available to assess GH secretion, the therapeutic decision to institute GH substitution therapy in adults remains arbitrary and represents, in part, a cost-benefit analysis.[37] In accordance with others,[38] we therefore suggest that at present GH replacement therapy should only be considered if, in addition to impaired GH secretion,[39] the patients in question present with additional defects in anterior pituitary function and clinical symptoms compatible with lack of GH.

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