# Pituitary diseases: long-term clinical consequences

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# Chapter 6

## Growth hormone (GH) deficiency in patients irradiated for acromegaly: significance of GH stimulatory tests in relation to the twenty-four hour GH secretion

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## ABSTRACT

### Background

Radiotherapy for pituitary adenomas frequently leads to growth hormone (GH) deficiency. The characteristics of GH secretion in GH deficiency induced by postoperative radiotherapy for acromegaly are not known. Hypothesis: In the long-term, stimulated and spontaneous GH release is not different between patients with GH deficiency treated by postoperative radiotherapy for acromegaly or for other pituitary adenomas.

### Design/subjects

We compared the characteristics of basal and stimulated GH secretion in patients with GH deficiency, who had previously received adjunct radiotherapy after surgery for GH-producing adenomas (n=10) versus for other pituitary adenomas (n =10). All patients had a maximal GH concentration by insulin tolerance test (ITT) of 3 µg/l or less, compatible with severe GH deficiency. Mean time after radiation was 17 and 18.7 years, respectively. Stimulated GH release was also evaluated by infusion of GHRH, GHRH-arginine and arginine, and spontaneous GH by 10 min. blood sampling for 24h. Pulse analyses were done by Cluster and approximate entropy.

### Results

There were no differences between both patient groups in stimulated GH concentrations in any test. Spontaneous GH secretion was not different between both patient groups, including basal GH release, pulsatility and regularity. Pulsatile secretion was lost in 2 acromegalic and 3 non-acromegalic patients. IGF-I was below -2 SD-score in 9 patients in each group.

### Conclusion

Acromegalic patients treated by surgery and postoperative radiotherapy with an impaired response to ITT do not differ in the long-term in GH secretory characteristics from patients treated similarly for other pituitary tumors with an impaired response to ITT. The ITT (or the GHRH-arginine test) is therefore reliable in establishing the diagnosis of GHD in patients treated for acromegaly by surgery and radiotherapy.

## INTRODUCTION

The aim of treatment in acromegaly is first to relief the symptoms of growth hormone (GH) excess and the mass effects of the pituitary tumor. Additional aims are the restoration of the metabolic changes and the reduction of the increased mortality risk associated with active acromegaly (1). Ideally, the therapy should be directed towards the restoration of physiological GH secretion, which is achieved when responses to dynamic stimuli and the 24h GH production are normalized, including restoration of secretory characteristics such as diurnal rhythm and secretory regularity. At the present time only surgery is capable to fulfill these goals in a limited number of patients, even by expert surgery (2-5). Therefore, additional treatment is required frequently, which may be given as pharmacotherapy (e.g. somatostatin analogs, GH-receptor blockade drugs, dopaminergic drugs or combinations there off) or as radiotherapy.

After pituitary irradiation a decline of ~50% in serum GH levels is observed in the first two years and of ~75% after 5 years (6-8). The normalization of GH and IGF-I levels during follow-up after radiotherapy is mainly dependent on the pre-irradiation serum GH concentrations. Many patients with other pituitary adenomas (e.g. non-functioning adenomas, adrenocorticotrope hormone (ACTH) - or prolactin (PRL)-secreting adenomas) develop GH deficiency after pituitary irradiation (9). Therefore, it seems logical to expect GH deficiency in the long-term in acromegaly after such treatment. In accordance, we have documented a decreased response of GH to insulin-induced hypoglycemia in 36% of the patients with acromegaly, during long-term follow-up of postoperative radiotherapy (7).

In acromegalic patients with GH deficiency after radiotherapy, little information is available on spontaneous 24h GH secretion and other GH-stimulation tests in relation to the insulin tolerance test (ITT), being the most widely used and recommended GH provocative measure for the diagnosis of GH deficiency (10). Therefore, we specifically wished to address whether basal and stimulated GH secretion differed between patients with GH deficiency, who had previously received adjunct radiotherapy after surgery for GH-producing adenomas (n=10) and those who had received adjunct radiotherapy for other pituitary adenomas (n=10). In both groups GH deficiency was defined as a maximal GH concentration during insulin tolerance test (ITT) of 3 µg/l, compatible with severe GH deficiency. We therefore explored various aspects of GH pathophysiology, including spontaneous 24h GH secretion, GH provocative tests aimed at the pituitary gland or acting indirectly, prevailing IGF-I concentrations and the mutual interrelations between these parameters.

## METHODS

### Patients

Ten previously operated and irradiated patients with a GH-secreting macroadenoma and 10 identically treated patients with a non-functioning pituitary macroadenoma (n=8) or an ACTH-producing microadenoma (n=2), matched for gender and age, were enrolled (pituitary adenoma control group: PT controls). The acromegalic patients were chosen from a cohort of clinically inactive acromegalic patients, previously (>10 years) treated by transsphenoidal surgery and, because of persisting postoperative GH excess by postoperatively conventional radiotherapy (40-45Gy). This cohort of acromegalic patients has previously been described extensively (7). The inclusion criterion was a subnormal GH response to the ITT (short-acting insulin 0.05-0.1 U/kg body weight, blood samples drawn at 0, 20, 30, 45, 60 and 90 min; glucose levels were required to drop below 2.2 mmol / l). The increase in GH concentrations was considered insufficient, when peak GH response was below 3 µg/l (10). The number of pituitary deficiencies other than that of GH were similar for both groups (P = 0.25). Three deficient anterior pituitary functions were established in 6 acromegalic patients and in 7 of the control patient group. Replacement treatment for secondary hypocortisolism was given to 7 acromegaly patients and to 9 control patients (NS), thyroid hormone therapy was given to 7 and 8 patients, respectively (NS), and treatment for secondary hypogonadism to 4 patients of each group (NS). Three of the 10 acromegalic patients and 4 of the 10 control patients used lipid-lowering drugs. None of the patients used dopamine agonists, but 2 of the patients in the PT control group used inhalation $\beta_2$-sympathicomimetics and 2 of the patients in the acromegalic group used β-adrenoreceptor blocking medication. The purpose, nature, and possible risks of the study were explained to all subjects and written informed consent was obtained. The study protocol was approved by the ethics committee of the Leiden University Medical Center.



### Clinical Protocol

First the GH secretory reserve was assessed by three stimulation tests in addition to the ITT, and subsequently spontaneous GH secretion was measured during 24h with blood sampling intervals of 10 minutes.

The GH stimulation tests were carried out in random order on separate days (during a two-week period) in the fasting condition. The following tests were performed: the GHRH test (Ferring, Hoofddorp, The Netherlands: 1 µg/kg body weight by i.v. bolus injection, blood samples drawn at 0, 20, 30, 45, 60 and 90 min), the l-arginine infusion test (500 mg/kg body weight with a maximum of 30 g, infusion during 30 minutes, blood samples drawn at 0, 30, 45, 60, 90 and 120 min), and the combined GHRH-arginine test as an i.v. bolus injection of GHRH (1 µg/kg body weight) after which l-arginine (500 mg/kg body weight with a maximum of 30 g) was infused during 30 minutes, blood samples drawn at 0, 30, 45, 60, 90 and 120 min. The peak serum response of GH was used as the primary variable for analysis of stimulation tests.

For the 24h sampling study, the patients were admitted to the Clinical Research Center in the morning. An indwelling i.v. cannula was inserted in a forearm vein at least 60 min before sampling began. Blood samples were withdrawn at 10 min. intervals for 24h, starting at 09.00h. A slow infusion of 0.9% NaCl and heparin (1 U/ml) was used to maintain patency of the i.v. catheter. The subjects were not allowed to sleep during the daytime. Meals were served at 09.00, 12.30 and 17.30h. Lights were turned off between 22.00-24.00h. Plasma samples for GH measurements were collected, centrifuged at 4°C for 7 minutes, and stored at –20°C until later analysis.

### Assays

GH concentrations in the samples of the stimulation tests were measured by time resolved immunofluorometric assay (Wallac, Inc, Turku, Finland). Reference values, listed in the tables and main text were obtained with the same assay. Human biosynthetic GH (Pharmacia and Upjohn, Inc, Uppsala, Sweden) was used as standard, calibrated against WHO-IRP 80-505 and the detection limit of this GH assay is 0.01 µg/l with an interassay coefficient of variation of 1.6-8.4%, between 0.1 and 15 µg/l (1 µg/l = 2.6 mU/l). GH concentration in the serum samples of 24h profiles of the patients in this study were measured with the more sensitive automatic immunochemiluminescence assay (Nichols Diagnostics Institute, San Clemente, CA), using 22 kDa rhGH as standard. Cross reactivity with 20kDa GH was 30%. Assay sensitivity (defined as 3 SD above the zero dose level) was 0.005 µg/l. Median intra- and interassay coefficients of variation were 5.2 and 8.3%, respectively. GH concentration was measured in every sample in duplicate. All samples from a single subject were assayed together to eliminate interassay variability,

Serum IGF-I concentrations were determined by Immulite 2000 (DPC, Los Angeles, CA). The assay is calibrated with WHO 2nd International Standard 87/518. Sensitivity of the assay is 20 µg/l. The intra-assay precision is 2.6-4.3 % over the adult operating range. All serial samples in this study were run in the same assay.

### Calculations and Statistics

#### *Cluster analysis*

For the detection of discrete GH peaks Cluster analysis was used (11). This computerized pulse algorithm is largely model-free, and identifies statistically significant pulses in relation to dose-dependent measurement error in the hormone time series. For the present analysis a 2x1 test cluster configuration was used, two data points for the test nadir and one for the test peak, and a t-statistic of 2.0 for the up- and down-strokes, which minimizes both false positive and false negative peaks. The locations and widths of all significant concentration peaks were identified, the total number of peaks was counted, and the mean peak interval was calculated in minutes. In addition, the following pulse parameters were determined: peak height (highest

value attained within the peak), incremental peak amplitude (the difference between peak height and pre-peak nadir), and area under the peak. Interpulse valleys were identified as regions embracing nadirs with no intervening up-strokes. The total area under the curve was also calculated, as well as the summed pulse areas.

### *Approximate Entropy*

ApEn was used as a scale- and model-independent regularity statistic to quantitate the orderliness or regularity of serial GH serum concentrations over 24h. Normalized ApEn parameters of m = 1 (test range) and r = 20% (threshold) of the intra-series SD were used, as described previously (12). The ApEn metric evaluates the consistency of recurrent subordinate (nonpulsatile) patterns in successive data, and thus yields information distinct from and complimentary to cosinor and deconvolution (pulse) analyses (13). Higher absolute ApEn values denote greater relative randomness of hormone patterns; e.g. as observed for ACTH in Cushing's disease, GH in acromegaly, and PRL in prolactinomas (14-16). Normalized ApEn ratios of observed to 1000 randomly shuffled data series are reported.

### *Statistical analysis*

Results are presented as the mean and 95% confidence interval, unless stated otherwise. Statistical analyses were carried out with the Kolmogorov-Smirnov test. Comparisons between the GH stimulation tests were carried out with General Linear Model (GLM) with appropriate post-hoc contrasts. Associations between variables were quantified by the Spearman's rho test. Statistical calculations were performed with Systat, version 11 (Systat Software, Inc, Richmond, CA) or SPSS version 11 (SPSS Inc, Chicago, Ill). $P<0.05$ was considered significant.



## RESULTS

### Patients

The age of the patients treated for acromegaly was 56 ± 12 years and of PT control group 62 ± 12 years (p=0.30, Table 1). Body mass index (BMI) of the acromegalic patients was 29.1 ± 2.9 kg/m$^2$ and 28.3 ± 4.5 kg/m$^2$ in the PT control group (p=0.66). Conventional radiation therapy with a 8 MeV linear accelerator, with a total tumor dose of 40-45 Gy and fractionated in at least 20 sessions, was given 17.0 ± 7.0 years prior to testing in the acromegalic group, and 18.7 ± 7.6 years in the PT control group (p=0.67).

### Stimulation Tests

In Figure 1 the GH response to the 4 stimulation tests are displayed. The peak values reached during these tests are shown in Table 2. No significant differences in GH responses between the two groups were present for any test.

**Table 6/1:** Clinical characteristics of the patient groups

| | Patients treated for acromegaly | Pituitary tumour control patients | P-value |
|---|---|---|---|
| Age (years) | 56 (47-65) | 62 (53-71) | 0.30 |
| Sex (males / females) | 4/ 6 | 5/ 5 | 0.65 |
| BMI (kg/m$^2$) | 29.1 (27.1-31.2) | 28.3 (25.1-31.5) | 0.66 |
| IGF-I (µg/l) | 59 (36-82) | 56 (41-71) | 0.96 |
| Interval between stimulation tests and radiotherapy (years) | 17.0 (7-25) | 18.7 (11-28) | 0.67 |

Data are shown as the mean and the 95% confidence interval. Statistical comparisons were performed with the Kolmogorov-Smirnov test.

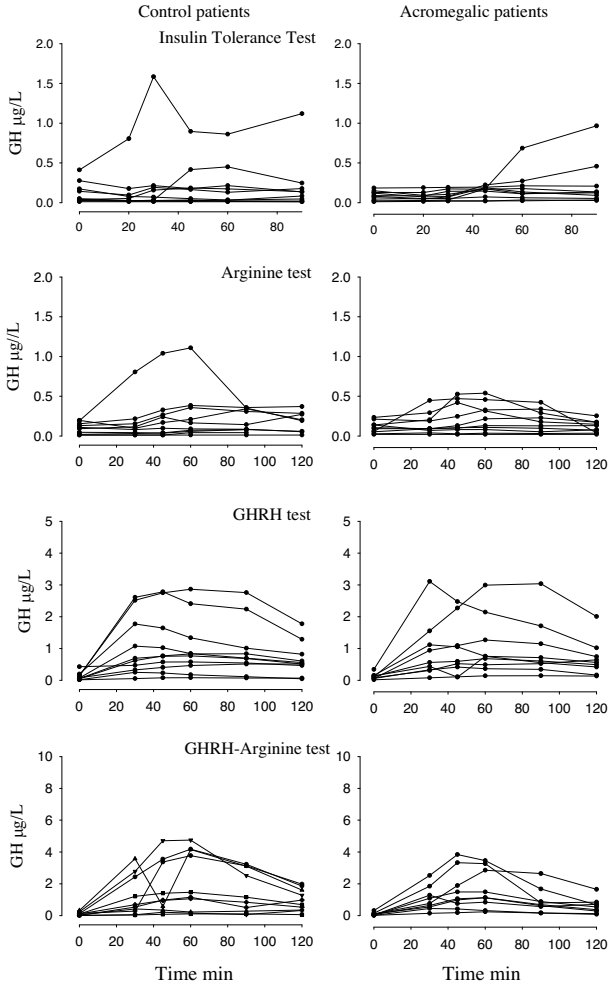


**Figure 6/1:** GH response to the ITT, arginine, GHRH and the combined GHRH-arginine tests.

**Table 6/2:** Peak growth hormone response during stimulation tests in patients with acromegaly and pituitary tumor control patients.

| | Patients treated for acromegaly | PT control patients | P-value |
|---|---|---|---|
| Insulin tolerance test (µg/l) | 0.18 (0.03-0.92) | 0.18 (0.01-1.58) | 1.00 |
| GHRH test (µg/l) | 0.75 (0.15-3.10) | 0.80 (0.08-2.86) | 0.96 |
| GHRH-arginine test (µg/l) | 1.28 (0.24-3.80) | 1.30 (0.11-4.75) | 0.77 |
| Arginine test (µg/l) | 0.28 (0.02-1.36) | 0.19 (0.01-1.10) | 0.66 |

Data are shown as median and data limits in parentheses. Statistical comparisons were performed with the Kolmogorov-Smirnov test. Reference values in healthy adults for our laboratory are ITT: > 3 µg/l, GHRH > 3 µg/l; arginine test > 2.9 µg/l, GHRH-arginine test > 8 µg/l.

However, the figure clearly demonstrates the differences in magnitude of GH responses between the tests. Indeed, the univariate ANOVA of the GH peak values applied to the combined patient groups was highly significant ($P < 0.001$). Post-hoc analyses revealed that the GH response to the insulin tolerance test was not different from the arginine test (p=0.39). The GH response in the GHRH and the combined GHRH-arginine tests were significantly higher than in the ITT (p<0.001, Figure 1). The GH response to insulin correlated significantly with that to the combined GHRH-arginine test (R=0.64, p=0.003), and arginine alone (R=0.63, p=0.003), but not with the GH response to GHRH (R=0.42, p=0.07).

Mean IGF-I concentration was 56 µg/l (95% confidence interval 41-71 µg/l) in control patients, and 59 µg /L (95% confidence interval 36-82 µg/l) in acromegalic patients (p=0.96). No relevant correlations were found within the limited range of IGF-I values. IGF-I was below -2 SD-score in 9 patients in each group.



### Twenty four hour GH profiles

In Figure 2 representative 24h GH profiles of two acromegalic patients and two controls are shown. The results of the Cluster analysis are listed in Table 3. It should be noted that 2 acromegalic patients and 3 PT control patients had no statistically significant GH pulses, although their GH levels were detectable. For the remaining patients no differences could be demonstrated with respect to integrated area, mean GH concentration, mean pulse height, mean pulse area (mass) and nadir concentration.

The integrated area, reflecting 24h GH secretion correlated with the peak GH of the combined GHRH-arginine test (R=0.78, p=0.001), and also with that of the ITT (R=0.54, p=0.036), but not with the other 2 stimulation tests.

Approximate entropy (ApEn (1, 20%) ratio) was not different between acromegalic patients and PT controls (mean 0.76; 95% confidence interval 0.70 – 0.81 and 0.69; 95 % confidence interval 0.57-0.81, respectively, p=0.25). Reference value for these patients groups is 0.41, 95% confidence interval 0.36 – 0.45.

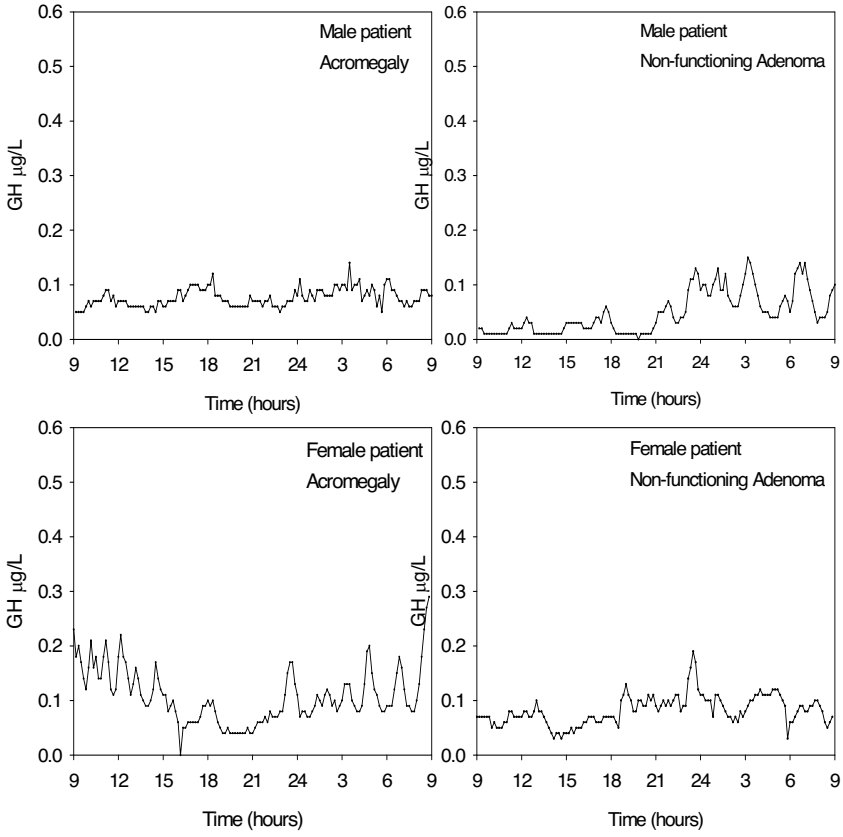


**Figure 6/2:** Representative 24h GH profiles of two acromegalic patients and two PT control patients

**Table 6/3:** Cluster analysis of the 24h serum GH profiles in patients with acromegaly and pituitary tumor control patients.

| | Patients treated for acromegaly | Pituitary adenoma control patients | P-value |
|---|---|---|---|
| Mean 24h GH concentration (µg/l) | 0.12 (0.07-0.16) | 0.20 (0.01-0.40) | 0.82 |
| Integrated area (µg/l/min) | 166 (99-230) | 300 (28-590) | 0.82 |
| Number of GH pulses/ 24h | 15 (11-19) | 11 (9-14) | 0.37 |
| Mean pulse interval (min) | 92 (65-120) | 102 (75-130) | 0.76 |
| Mean pulse amplitude (µg/l) | 0.15 (0.10-0.19) | 0.33 (0.02-0.65) | 0.51 |
| Mean pulse area (µg/l/min) | 2.20 (0.70-3.70) | 9.80 (0-21.0) | 0.16 |
| Valley mean (µg/l) | 0.10 (0.06-0.14) | 0.17 (0.05-0.31) | 0.87 |
| Nadir (µg/l) | 0.09 (0.06-0.13) | 0.15 (0.03-0.27) | 0.87 |

Data are given as the mean and 95 % confidence interval. Statistical comparisons were performed with the Kolmogorov-Smirnov test. Reference values for a comparable group of healthy controls are (mean, and 95% CI): 24h mean GH concentration 0.60 µg/l (0.39-0.80), integrated area 850 µg/l/min (550-1165), number of GH pulses/ 24h 9 (8-11), mean pulse interval 155 min (125-185), mean pulse amplitude 1.40µg/l (0.78-2.05), mean pulse area 70 µg/l/min (35-100), valley mean 0.50 µg/l ( 0.1 -0.90), nadir 0.30 (0.10-0.50).

## DISCUSSION

In this study we compared the characteristics of GH secretion between GH deficient acromegalic patients and GH deficient patients with other pituitary adenomas after long-term follow up of postoperative radiotherapy. We found that patients treated by transsphenoidal surgery and additional radiotherapy for acromegaly with an impaired GH response to ITT did not differ with regard to stimulated and spontaneous GH secretion from patients treated analogously for other pituitary adenomas, who had impaired GH response to ITT.

Hypopituitarism is a well-recognized sequel of radiotherapy for pituitary tumors and GH secretion is usually the first hormone affected (9). The ITT is an effective test to define GH deficiency (10), since responses reflect the functional integrity of the hypothalamic-pituitary-GH axis (17;18). The hypothalamus may be more vulnerable to radiation-induced damage than the pituitary gland, since the pituitary remains responsive to hypothalamic releasing- hormones after radiation (19). GH deficiency after radiotherapy for pituitary tumors may therefore occur due to failure of synthesis and/or delivery of endogenous GHRH (or other putative GH-releasing substances, e.g. hypothalamic ghrelin) to the pituitary (20;21). One could hypothesize that the function of the hypothalamic-pituitary-GH axis in acromegalic patients treated by postoperative radiotherapy, as assessed by the ITT, is impaired due to surgical and radiotherapeutical intervention, whereas tumoral activity may persist, thus preventing (temporarily) the emergence of GH deficiency.



GHRH and combined GHRH-arginine infusions resulted in significantly higher GH peak responses than the ITT in both patient groups. This observation is consistent with results obtained by Aimaretti et al. in hypopituitarism due to various etiologies (22). The generally accepted explanation for this difference in the magnitude of the GH responses is that the GHRH-arginine test combines the somatostatin-suppressing effect of arginine (23) with direct stimulation of the somatotroph cell by exogenous GHRH (24), whereas the ITT requires endogenous GHRH (25). Our finding that the GHRH test alone resulted in a higher GH response compared to the ITT (in both groups of patients) might point to hypothalamic dysfunction with diminished endogenous drive to the pituitary. These observations are also in line with the study by Murray et al., in which they investigated patients treated for acromegaly with the arginine test and the GH secretagogue hexarelin (26). They reported loss of response to the arginine test in patients treated by radiotherapy, although the response to the GH secretagogue was retained in about 50% of these patients (26).

The diagnosis of GHD in adults is established by provocative testing, since IGF-I concentrations and mean 24h GH concentrations overlap in adults considered GH deficient (i.e. due to extensive pituitary disease) and healthy subjects (27). The combined GHRH-arginine test is also considered to be a reliable test to detect GHD (28), provided that appropriate cut-off limits related to BMI are defined (29). However, the GH response to arginine alone is found to be less sensitive to the effects of radiotherapy than the GH response to ITT (30). In accordance with

these inferences, GH responses to arginine and insulin in the present cohorts were identical, although greatly diminished, and comparable to those in patients who received cranial irradiation for non-pituitary diseases (30).

There was a moderately positive correlation between peak GH responses to ITT and to the combined GHRH-arginine test and a strongly positive correlation between the peak response to the ITT and arginine. In addition, the peak response to ITT and to combined GHRH-arginine correlated with spontaneous 24h GH secretion. Therefore, from a practical clinical vantage the investigation of GH reserve capacity in acromegalic patients with cardiovascular disease may be equally well explored with the combined test.

GH secretion in active acromegaly is characterized by increased apparent pulse frequency, burst mass and basal (nonpulsatile) secretion (15; 31-32). In contrast, GH burst mass is decreased profoundly in GH deficiency and total 24h secretion is diminished notwithstanding increased pulse frequency (33). In the present study, treated patients with somatotropinomas and other pituitary adenomas clearly fulfilled the criterion of GH deficiency. A remarkable outcome is that there were no differences in mean 24h GH concentration, number of GH pulses per 24h, pulse amplitude or area between the two groups. The spectrum of GH release extended from complete absence of statistically significant GH pulses with low basal concentrations, as observed in 5 patients, to persisting, low amplitude pulsatility (as illustrated in the figures). It is presently unclear, whether residual GH output in treated acromegalic patients is derived from normal somatotrope cells or from tumor remnants.

Peacey et al. described the relationship between 24h GH secretion profiles and IGF-I in cured acromegalic patients (defined as GH levels below 2 µg/l during an oral glucose tolerance test or a GH profile) (34). In that study elevated mean IGF-I concentrations in acromegalic patients treated by radiotherapy compared with healthy controls were inferred to reflect persisting differences in 24h GH secretion (34). The current data extend insights to two more severely compromised groups, in which irrespective of the underlying disorder, IGF-I concentrations were below -2 standard deviations in all but 1 subject and uncorrelated with GH secretory parameters.

Approximate entropy (ApEn), a tool to quantitate the orderliness or regularity of serial GH serum concentrations, did not differ between patients treated for acromegaly and those treated for other pituitary tumors. However, when compared with values reported in normal healthy subjects, ApEn was almost twofold elevated, indicating disorganized GH secretion (5). Elevated ApEn of GH points to increased feed-forward by GHRH or tumoral cells or decreased feedback via somatostatin, GH and IGF-I signaling (35). In active acromegaly disorganized GH secretion is likely attributed to the tumor per se, akin to that of prolactinomas and ACTH-secreting adenomas (14;16). The anatomical substrate for the disorganized secretion might be defective or autonomous cell-cell interactions in the adenoma (36;37). In hypopituitarism of various etiologies, excluding acromegaly, GH secretion is also profoundly irregular and not correlated with pituitary irradiation (33). Such secretory alterations might be attributed to a diminished

somatostatin and/ or GH/IGF-I feedback. Histopathological and controlled feedback studies are required together to elucidate the precise mechanism(s) involved.

We chose to study acromegalic patients with an impaired response to ITT treated by radiotherapy and to compare these patients with those treated likewise without a previous history of acromegaly. The latter group fulfilled the criteria of GH deficiency. Since no differences in spontaneous and stimulated GH secretion between the two groups were found, it is reasonable to conclude that the acromegalic patients became GH-deficient many years after radiation. Another study has suggested that the prevalence of GHD is high in patients treated by surgery alone (38). Direct comparison of those 2 groups would be of interest.

In conclusion, acromegalic patients treated by surgery and postoperative radiotherapy with an impaired response to ITT do not differ in the long-term in GH secretory characteristics from patients treated similarly for other pituitary tumors with an impaired response to ITT. The ITT (or the GHRH-arginine test) is therefore reliable in establishing the diagnosis of GHD in patients treated for acromegaly by surgery and radiotherapy. Irregular GH secretion, low amplitude GH pulses, and reduced IGF-I concentrations thus constitute a final common outcome of combined surgery and radiation treatment of pituitary adenomas.

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