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## **Acromegaly : treatment and follow-up : the Leiden studies**

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## **Octreotide represses secretory-burst mass and nonpulsatile secretion but does not restore event frequency or orderly GH secretion in acromegaly**

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## ABSTRACT

Octreotide is a potent somatostatin analog that inhibits growth hormone (GH) release and restricts somatotrope cell growth. The long-acting octreotide formulation Sandostatin LAR is effective clinically in ~60% of patients with acromegaly. Tumoral GH secretion in this disorder is characterized by increases in pulse amplitude and frequency, nonpulsatile (basal) release, and irregularity. Whether sustained blockade by octreotide can restore physiological secretion patterns in this setting is unknown. To address this question, we studied seven patients with GH-secreting tumors during chronic receptor agonism. Responses were monitored by sampling blood at 10-min intervals for 24 h, followed by analyses of secretion and regularity by multiparameter deconvolution and approximate entropy (ApEn). The somatostatin agonist suppressed GH secretory-burst mass, nonpulsatile (basal) GH release, and pulsatile secretion, thereby decreasing total GH secretion by 86% (range 70–96%). ApEn decreased from  $1.203 \pm 0.129$  to  $0.804 \pm 0.141$  ( $P = 0.032$ ), denoting greater regularity. None of GH pulse frequency, basal GH secretion rates, or ApEn normalized. In summary, chronic somatostatin agonism is able to repress amplitude-dependent measures of excessive GH secretion in acromegaly. Presumptive tumoral autonomy is inferred by continued elevations of event frequency, overall pattern disruption (irregularity), and nonsuppressible basal GH secretion.

## INTRODUCTION

GROWTH HORMONE (GH) IS SECRETED in a pulsatile fashion in healthy individuals. In the daytime and fed state, GH is released in diminutive bursts, whereas in fasting and during sleep, GH outflow unfolds in volley-like episodes. Primary secretagogues are hypothalamic GH-releasing hormone (GHRH) and, possibly, ghrelin. Autoinhibition is imposed by somatostatin, which mediates central feedback actions of GH and IGF-I. GHRH and somatostatin also influence somatotrope cell growth chronically (1). Acromegaly is a disease of excessive and autonomous GH secretion, associated with an increased mass (number and size) of somatotrope cells. Most often, a pituitary adenoma can be visualized and removed surgically. Investigations of tumoral GH secretion in active acromegaly have revealed increases in pulse-event frequency, basal (nonpulsatile) secretion, irregularity, and (absolute) diurnal rhythmicity (2–5). In patients with noninvasive adenomas, transsphenoidal surgery can normalize each attribute (6,7).

Octreotide is a potent somatostatin agonist that effectually suppresses GH hypersecretion in ~60% of patients with active acromegaly. The rationale for using this agent is to block GH release and limit somatotrope cell growth specifically, without the risk implicit in radiotherapy of inducing subsequent pituitary insufficiency (8–12). Octreotide therapy is used when curative surgery is not attainable. The basis of repression of mean serum GH concentrations by these agents is not clear (13). However, somatostatin agonists strongly suppress GH burst mass and nonpulsatile GH release and enforce regularity in normal young men and women, older men, and postmenopausal women (14–17). We tested the clinical postulate that prolonged receptor occupancy achieved by the depot form of octreotide could reverse selected facets of autonomous tumoral GH secretion in patients with active acromegalic disease.

## PATIENTS AND METHODS

### Patients and Control Subjects

Seven patients (4 men and 3 women, mean age 55 yr, range 39–75 yr), with clinically active acromegaly, and 18 healthy control subjects (9 males and 9 females, mean age 51 yr, range 39–69 yr) were recruited for this study. Body mass index ( $\text{wt}/\text{ht}^2$ ) was comparable at  $25.6 \pm 1.5 \text{ kg}/\text{m}^2$  in patients and  $23.9 \pm 0.72 \text{ kg}/\text{m}^2$  in control subjects. The diagnosis of acromegaly was based on progressive acral growth, incomplete suppression of GH during oral glucose loading (nadir concentration  $>5 \text{ mU}/\text{l}$ ), and an elevated serum IGF-I concentration interpreted by age. Five patients had previous transsphenoidal surgery without normalization of GH excess.

Patients were studied before and during sustained octreotide treatment (20-mg injections of Sandostatin LAR at 4-wk intervals). They were selected from 11 acromegalics on the basis

of clinical responsiveness to octreotide and normal IGF-I concentration. None of the patients had clinical signs or biochemical evidence of hypopituitarism. The study was approved by the ethical committee of the Leiden University Medical Center, and written informed consent was obtained from all patients and control subjects.

#### Sampling Protocol

Patients were hospitalized on the evening before sampling. On the following morning, an indwelling intravenous cannula was inserted in a forearm vein. Blood samples were withdrawn at 10-min intervals for 24 h starting at 0900. Serum was stored at  $-20^{\circ}\text{C}$  until assayed. A slow intravenous infusion of heparin (1 U/ml) was used to maintain access. Patients were free to move about but not to sleep during daytime. Meals were served at 0800, 1230, and 1730. Lights were turned off between 2200 and 2400. No sleep monitoring was carried out.

GH was measured in samples collected every 10 min, and octreotide in sera was obtained every hour. At the start of the protocol (0900, fasting), an extra blood sample was obtained for screening safety data and measurements of IGF-I, IGF-binding protein-3, free thyroxine, cortisol, testosterone, estradiol, and LH.

Sampling was carried out 12–16 days after intramuscular injection with octreotide and after the patients had received injections for  $\geq 3$  mo (range 3–18, median 12). At that time IGF-I had normalized, and this measure remained stable during subsequent follow-up at the same dosing schedule.

#### Assays

GH concentrations were measured with a sensitive time-resolved fluoroimmunoassay (Wallac, Turku, Finland) specific for the 22-kDa GH protein. The assay uses recombinant human (rh)GH as standard (Genotropin; Pharmacia & Upjohn, Uppsala, Sweden), which is calibrated against the World Health Organization First International Reference Preparation (no. 80-505). The limit of detection is 0.03 mU/l, or 0.01  $\mu\text{g/l}$ . Intra-assay coefficients of variation (CVs) were 1.6–8.4% in the concentration range of 0.26–47 mU/l.

The total serum IGF-I concentration was determined by RIA after extraction and purification on ODS-silica columns (Incstar, Stillwater, MN). The interassay CV was  $<11\%$ . The detection limit was 11  $\mu\text{g/l}$ . Age-related normative data were determined in the same laboratory.

Serum octreotide concentrations were measured by RIA in the research laboratory of Novartis Pharma (Basel, Switzerland). The limit of detection was 50 pg/ml, and intra-assay CVs were 6–10%.

#### Analyses

Deconvolution analysis. Multiparameter deconvolution analysis was used to quantitate basal and pulsatile GH secretion and the GH half-life (18). This waveform-specific technique estimates the rate of basal release, the number and mass of randomly ordered secretory bursts,

and the subject-specific (monoexponential) half-life (19). The daily pulsatile GH secretion rate is the product of secretory-burst frequency and the mean mass of GH released per event. Total GH secretion is the sum of basal and pulsatile secretion (18,19).

**Approximate entropy.** Approximate entropy (ApEn) was used as a scale- and model-independent regularity statistic to quantitate the orderliness or regularity of serial GH concentrations over 24 h. Normalized ApEn parameters of  $m = 1$  (test range) and  $r = 20\%$  (threshold) of the intraseries SD were used, as described previously (17). Hence, this member of the ApEn family is designated ApEn(1, 20%). The ApEn metric evaluates the consistency of recurrent subordinate (nonpulsatile) patterns in a time series and thus yields information distinct from and complementary to cosinor and deconvolution (pulse) analyses (20). Higher absolute ApEn values denote greater relative randomness of hormone patterns, such as observed for tumoral secretion of ACTH, GH, and prolactin (3,5,21). Data are presented as absolute ApEn values and normalized ApEn ratios, defined by the mean ratio of absolute ApEn to that of 1,000 randomly shuffled versions of the same series (20).

**Nyctohemeral (24-h) rhythmicity.** Diurnal variations in GH and octreotide concentrations were appraised by cosinor analysis, as reported earlier (22). Ninety-five percent statistical confidence intervals were determined for the 24-h cosine amplitude (50% of the zenith-nadir difference), mesor (mean), and acrophase (time of the maximal value).

#### Statistical Analysis

Data are presented as means  $\pm$  SE, unless otherwise noted. Statistical analyses were carried out via ANOVA and linear regression. Logarithmic transformation was used to limit heterogeneity of variance.  $P < 0.05$  was considered significant.

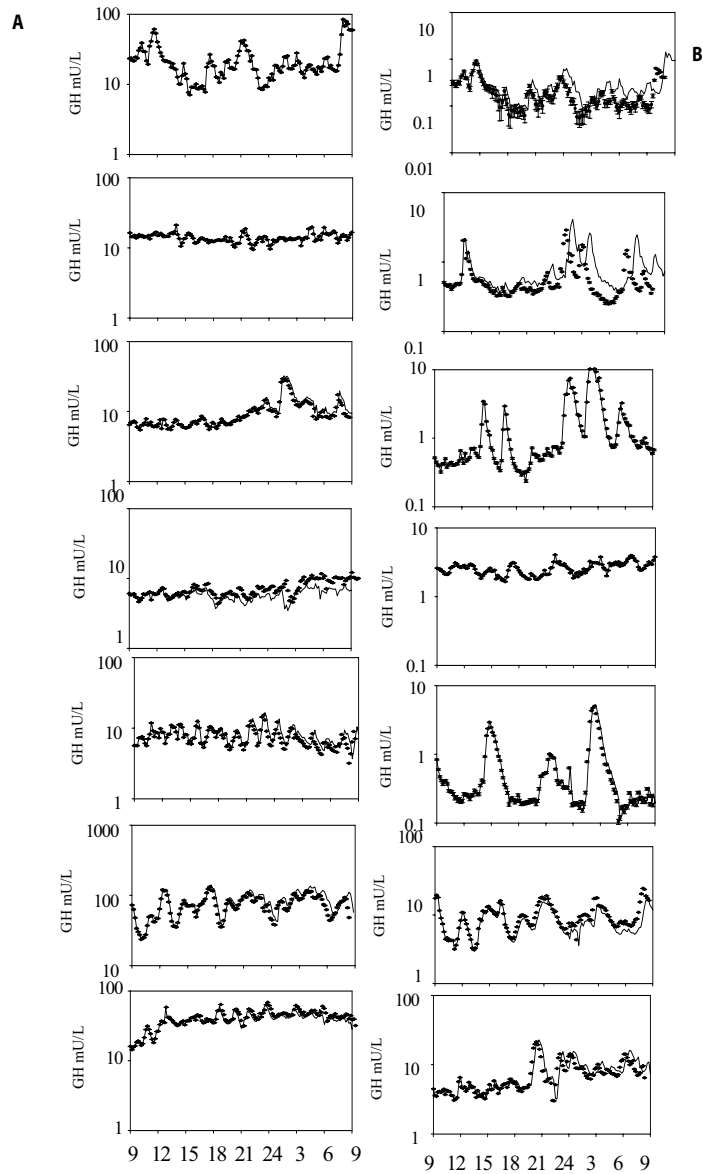
## RESULTS

#### Biochemical and Clinical Outcomes

During octreotide intervention, IGF-I concentrations decreased from  $424 \pm 76$  to  $143 \pm 7.6$   $\mu\text{g/l}$  (range 122 – 168  $\mu\text{g/l}$ ) and normalized in each subject ( $P = 0.01$ ). All patients experienced clinical improvement concurrently. Illustrative GH time series are shown in Fig. 1.

#### Deconvolution Analysis

Octreotide inhibited basal GH secretion by more than ninefold and pulsatile secretion by sevenfold. The latter was explained by a sixfold fall in burst mass, with no reduction in event frequency or GH half-life (Table 1). However, octreotide did not normalize either basal secretion rate or burst frequency. The fraction of basal to total GH secretion also remained elevated ( $P < 0.001$ ). Octreotide significantly repressed the sum of pulsatile and basal GH secretion (total daily GH production) by  $86 \pm 3\%$  (range 70 – 96%) (Fig. 2).



**Figure 1.** Serum growth hormone (GH) concentration time series in 7 acromegalic patients before (A) and during sustained octreotide therapy (B). Data reflect sampling of blood every 10 min for 24 h. *y*-Axis scales are logarithmic and differ before and during intervention to aid in visualization.

**Table 1.** Deconvolution analysis of daily GH concentration time series.

Measure	Untreated	Treated	P-value, Treated vs Untreated	Controls	P-value, Treated vs Control subjects
Secretary-burst half duration (min)	23.4 ± 1.9	24.6 ± 2.1	0.600	25.8 ± 1.1	0.60
Half-life (min)	14.2 ± 1.0	16.9 ± 0.9	0.104	17.0 ± 0.6	0.97
Number of secretary bursts / 24 h	34.3 ± 2.2	29.4 ± 3.6	0.180	13.3 ± 0.7	0.0037
Interburst interval (min)	42.5 ± 3.0	52.5 ± 6.5	0.135	105 ± 6	0.00002
Secretary-burst amplitude (mU/L/min)	1.04 ± 0.39	0.16 ± 0.05	0.0002	0.22 ± 0.04	0.35
Burst mass (mU/L)	27.2 ± 10.6	4.25 ± 1.29	0.00009	5.73 ± 1.17	0.35
Basal secretion (mU/L/24 h)	675 ± 137	69 ± 24	0.00076	6.0 ± 0.97	0.0012
Pulsatile secretion (mU/L/24 h)	970 ± 415	141 ± 51	0.00003	73 ± 14	0.38
Total secretion (mU/L/24 h)	1645 ± 540	210 ± 72	0.00017	80 ± 14	0.10

Values are means ± SE. GH, growth hormone. Data are from 7 acromegalic patients studied before and during octreotide intervention. Statistical comparisons were made with the two-tailed Student's t-test for paired data. Logarithmic transformation of the data was done where required.

#### Diurnal GH Characteristics

Cosinor data are depicted in Table 2. Octreotide lowered the mesor (mean 24-h GH concentration about which the calculated rhythm varies) and amplitude (50% of nadir to zenith difference) sixfold. The acrophase (time of maximum) occurred ~3 h after midnight in both the patient and control cohorts and was unaffected by suppressive therapy.

**Table 2.** Cosinor analysis of the 24-hr GH concentrations in acromegalic patients before and during intervention with octreotide.

	Mesor (mU/L)	Amplitude (mU/L)	Acrophase (hr)
Untreated Patients	20.7 ± 7.3	6.1 ± 2.6	0245 (2149-0741)
Treated Patients	3.5 ± 1.4 <sup>2</sup>	0.98 ± 0.51 <sup>1</sup>	0233 (2349-0517)
Controls	0.51 ± 0.06 <sup>3</sup>	0.35 ± 0.04	0302 (0112-0452)

Data for the mesor and amplitude are means ± SE and those for the acrophase are means and 95% confidence intervals. <sup>1</sup> treated vs untreated, P=0.014; <sup>2</sup> treated vs untreated, P=0.007; <sup>3</sup> treated vs control, P=0.002.

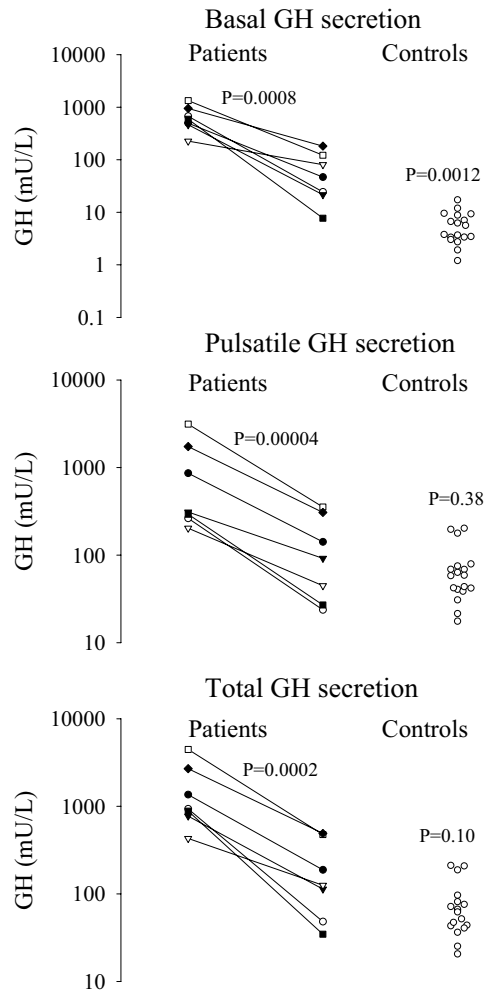
#### Octreotide and GH

Octreotide concentrations did not vary significantly over 24 h. The overall mean 24-h concentration (pg/ml) was 900 (range 530 – 1,540) pg/ml, and the coefficient of variation was 13% (range 10 – 17.2%).

#### ApEn

The octreotide intervention reduced ApEn of the GH concentration time series significantly (P = 0.032). A decrease denotes feedback repression (7). However, values failed to normalize (P = 0.003; Fig. 3). The latter distinction was not attributable to amplitude differences,



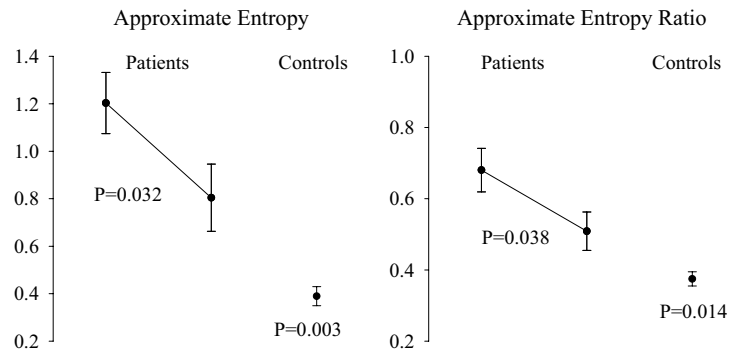


**Figure 2.** Twenty-four-hour GH secretion rates in 7 acromegalic patients before and during chronic octreotide treatment and in 18 healthy sex- and age-matched control subjects. Daily GH secretion is partitioned analytically into basal (nonpulsatile), pulsatile, and total (sum of basal and pulsatile) secretion. *P* values denote the effect of octreotide (patients) and the contrast with normal volunteers (controls).

because the normalized ApEn ratio (ratio in ApEn of observed to randomly shuffled cognate series) exhibited comparable changes ( $P = 0.014$  vs. control).

## DISCUSSION

Sustained ( $\geq 3$  mo) exposure to a potent and selective somatostatin receptor agonist (octreotide) significantly repressed basal, pulsatile, and more irregular patterns of GH secretion by somatotrope tumors. Comparable response mechanisms are inferrable in healthy individuals



**Figure 3.** Approximate entropy (ApEn) of GH concentration time series in 7 acromegalic patients before and during octreotide therapy and 18 sex- and age-matched controls. A: absolute ApEn (1, 20%), the right normalized ApEn ratios (observed to random). An ApEn ratio of 1.0 is not distinguishable from that of randomly shuffled data series. *P* values are represented as described in Fig. 2.

exposed to somatostatin (1,15,17). In contradistinction, octreotide failed to normalize the elevated GH secretion, the more disorderly GH release patterns, and the increased GH pulse frequency associated with tumoral GH secretion. Biomathematical simulation studies indicate that accelerated pulse frequency, but not elevated interpulse hormone concentrations, contribute statistically to heightened irregularity (23). Some, but not all, surgical series report normalization of rapid GH pulsatility after surgery (24). Discrepancies among studies may reflect choice of GH assay, definitions of cure, patient selection, sampling frequency, and duration and peak-detection methods (2–4,6,7). Radiotherapy tends to normalize total IGF-I and glucose-suppressed GH concentrations (25,26) but not dynamic secretory characteristics. For example, irregular patterns of GH release (elevated ApEn), rapid event frequency, and high basal GH secretion may persist after irradiation (27). The pathophysiology of anomalous GH-secretory control in the latter setting could include hypothalamic or pituitary injury due to prior therapeutic radiation and/or partially nonsuppressible continuing secretion by residual tumoral cells (27,28). Normal somatotropes would predictively secrete little if any GH under the profound inhibitory effects of octreotide (1).

An unexplained discrepancy is recognized between the degree of therapy-induced inhibition of GH and IGF-I concentrations (29–33). Transgenic hepatic IGF-I gene-silencing experiments establish that the majority of blood-borne IGF-I is of hepatic origin (namely, 70–80%) (34–36). Other analyses in the rat and human indicate that a continuous basal-like mode of GH secretion (or experimental delivery) preferentially drives hepatic IGF-I synthesis (37,38). Thus the relative admixture of pulsatile and basal GH secretion appears to determine IGF-I concentrations at any mean GH concentration (1,3). In addition, there is variable sensitivity to GH among individuals based on age, gender, and estrogen availability (24,39–43). In this regard, during octreotide intervention, two patients maintained slightly elevated GH concentrations and normal IGF-I concentrations.

Clinical observations support the hypothesis that GH-producing adenomas arise from mutations within the anterior pituitary gland, resulting in variable degrees of secretory autonomy. However, somatostatin and other feedback signals may continue to direct GH secretion in some measure in untreated acromegalic individuals. For example, fasting augments GH release in some patients with this disease (as in healthy subjects) but paradoxically diminishes GH secretion in others despite a fall in IGF-I concentration (44). Conversely, in one study, infusion of rhIGF-I lowered integrated GH concentrations by 25%, consistent with some sensitivity to IGF-I feedback (45). GHRH and GH-releasing peptides stimulate tumoral GH release both acutely and over 24 h (42,46–48). The present responses to constant octreotide suppression are consistent with significant negative feedback on amplitude-specific measures such as GH secretory-burst mass and daily pulsatile GH secretion. The basis for only partial normalization of basal GH release, GH burst frequency, and irregular GH secretion patterns is not known. We hypothesize that these three facets of GH-secretory control are more proximate markers of somatotrope tumoral transformation.

Before octreotide treatment, acromegalic patients maintained a significant diurnal variation of GH release with a normal acrophase. Mechanisms that drive such rhythmicity in normal individuals include activity, food intake, sleep, and internal circadian inputs (1). Octreotide concentrations varied by 13% over 24 h within the target therapeutic range. Under these conditions, octreotide abolished detectable GH rhythmicity in two of seven subjects.

Sample-by-sample regularity (ApEn) mirrors the relative strength of feedback and feedforward signaling in complicated integrated systems, such as neuroendocrine axes. In normal subjects, infusion of somatostatin decreases ApEn, indicating more regular GH release, as expected for an effectual feedback signal on theoretical grounds (49). However, the somatostatin analog failed to restore the control degree of orderly GH release. From a mechanistic point of view, octreotide reduces IGF-I availability for feedback, which could elevate GH secretory irregularity in proportion to the extent of feedback withdrawal (50). On the other hand, a presumptive secondary rise in endogenous GHRH release in response to lower IGF-I concentrations would further drive irregular secretion (50,51). Most plausibly, by analogy with other benign endocrine tumors (3,5,21), properties of transformed somatotrope cells may contribute to irregular (less coordinated) patterns of GH release, as observed here. In this regard, surgical removal of the somatotropinoma normalizes GH ApEn in 70% of patients with operable acromegaly (7).

In conclusion, constant octreotide delivery in patients with acromegaly lowers 24-h GH secretion by 86% through combined inhibition of the mass of individual GH-secretory events and the basal secretion rate. This mode of medical therapy does not normalize accentuated basal secretion, elevated pulse frequency, or disorderly GH release.

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## DISCLOSURES

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