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Spontaneous Nocturnal Growth Hormone Secretion in Anorexia Nervosa

MASSIMO SCACCHI, ANGELA I. PINCELLI, ANDREA CAUMO, PAOLO TOMASI, GIUSEPPE DELITALA, GABRIELLA BALDI, AND FRANCESCO CAVAGNINI

Second Chair of Endocrinology, University of Milan, Istituto Scientifico Ospedale San Luca (M.S., A.I.P., G.B., F.C.), and Istituto Scientifico Ospedale San Raffaele (A.C.), Milan; and Ospedale San Giovanni di Dio, Olbia (P.T.), and Chair of Endocrinology, University of Sassari (G.D.), Sassari, Italy

ABSTRACT

In anorexia nervosa, serum GH levels are increased under basal conditions and respond abnormally to provocative stimuli. We report here, for the first time, an analysis of pulsatile GH secretion in these patients performed by Cluster algorithm. Seven anorectic and six normal weight, healthy women underwent serial blood sampling at 20-min intervals from 2030–0830 h for GH estimation. The total area under the curve (AUC; micrograms per L/min) was elevated 4-fold in anorectic patients compared to controls (4743.0 ± 1520.09 *vs.* 1148.6 ± 519.27; $P < 0.01$), largely due to an increase in the nonpulsatile fraction (3212.5 ± 990.45 *vs.* 378.7 ± 123.27; $P < 0.01$). Accordingly, the valley mean value was higher in anorectic than in control subjects (5.9 ± 2.25 *vs.* 1.0 ± 1.30 μg/L; $P < 0.01$). Furthermore, pulsatile AUC was also greater in anorectic patients (1530.4 ± 654.72 *vs.* 769.8 ± 404.02; $P < 0.01$) due to a significant increase in GH peak frequency (5.0 ± 0.81 *vs.* 3.0 ± 0.89; $P < 0.01$). No correlations were observed in these patients between body mass index and any of the parameters of spontaneous GH release, whereas a positive correlation was found between insulin-like growth factor I levels and pulsatile AUC ($r^2 = 0.583$; $P < 0.05$), peak height ($r^2 = 0.743$; $P = 0.01$), peak increment ($r^2 = 0.801$; $P < 0.01$), and GH valley mean ($r^2 = 0.576$; $P < 0.05$).

In conclusion, it appears that the enhanced GH secretion in anorexia nervosa is the result of an increased frequency of secretory pulses superimposed on enhanced tonic GH secretion. Although this latter is consistent with a reduction of hypothalamic SRIH tone, the former may be accounted for by an increased number of GHRH discharges. Considering that in normal weight and obese subjects parameters of GH release are negatively correlated with adiposity indexes, the lack of such a negative correlation in our patients suggests that the enhancement of spontaneous GH release in anorectic patients is not merely the consequence of malnutrition-dependent impairment of insulin-like growth factor I production, but reflects a more complex hypothalamic dysregulation of GH release. (*J Clin Endocrinol Metab* **82:** 3225–3229, 1997)

THE PATHOPHYSIOLOGY of the GH-insulin-like growth factor I (IGF-I) axis in anorexia nervosa is not fully clarified. In this clinical condition, low IGF-I plasma levels (1) are associated with high baseline GH concentrations (2) and altered GH responsiveness to several provocative stimuli. Based on the abnormal GH release after pharmacological manipulations (3–8), an impairment of central SRIH tone has been postulated in anorexia nervosa. This hypothesis, however, is challenged by the normal somatotropin responsiveness to arginine infusion (9) and to a double GHRH iv bolus (6). Hypersomatotropism with low IGF-I levels is observed in other diseases associated with malnutrition. Both chronic undernutrition (marasmus and kwashiorkor) and acute dietary restriction (fasting) induce a marked reduction of plasma IGF-I levels (10–13). Fasted humans display increased 24-h integrated GH concentrations due to augmented pulse frequency and amplitude (14). Low IGF-I levels have also been described in critically ill, hypercatabolic patients (15, 16) displaying progressive protein store wasting despite high calorie parenteral nutrition. In these patients, however, pulsatile GH release appears to be limited, displaying low values of mean serum concentration, mean secretion rate, amount per secretory burst, and secretory burst amplitude (16). Anorexia nervosa is a model of particular interest, because fasting and protein depletion coexist (17). For this reason, we elected to analyze, by means of a pulse detection algorithm, the pattern of spontaneous nocturnal GH release in this clinical condition, an issue addressed only using chronobiological approaches to date (18).

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Address all correspondence and requests for reprints to: Prof. Francesco Cavagnini, Second Chair of Endocrinology, University of Milan, Istituto Scientifico Ospedale San Luca, via Spagnoletto 3, 20149 Milan, Italy.

Subjects and Methods

Subjects

Seven women suffering from anorexia nervosa (age, 21.2 ± 4.85 yr; mean ± SD) and six normal weight healthy women of comparable age (21.6 ± 3.26 yr) were investigated. All patients met the diagnostic criteria for anorexia nervosa, subgroup severe food restriction, according to the DSM-IV (19). Clinical characteristics of the patients and controls are shown in Table 1. The mean body mass index (BMI), defined as weight divided by the square of the height, was 13.4 ± 1.13 kg/m^2. All anorectic women exhibited a marked food refusal, with an estimated caloric intake of 600–800 Cal daily. None of them presented bingeing or vomiting behavior. Secondary amenorrhea was a constant feature. At the time of the study, weight was stable, and none of the patients had been taking medications for at least 12 months. Control subjects (BMI, 21.5 ± 1.37 kg/m^2) were in good general health, were nonsmokers, and had not undertaken any transmeridian travel for at least 4 weeks. A medical evaluation, including history, physical examination, and routine blood chemistry, revealed no abnormalities. Their weight had been stable during the previous 3 months. All of them were eumenorrheic and were evaluated during the early follicular phase (second to fifth day) of the menstrual cycle.

TABLE 1. Clinical characteristics of anorectic patients and control subjects

Subject no.	Age (yr)	Wt (kg)	Ht (cm)	Duration of disease (months)
Anorectic				
1	15	33.1	158	13
2	18	28.5	146	24
3	21	37.2	164	61
4	17	35.2	164	15
5	28	34.4	173	121
6	25	39.3	161	72
7	25	43.1	168	114
Mean ± SD	21.2 ± 4.85	35.6 ± 4.62	162 ± 8.6	60 ± 45.2
Control				
1	23	52.5	158	
2	26	59.3	164	
3	24	67.9	168	
4	21	45.4	147	
5	18	53.2	163	
6	18	63	173	
Mean ± SD	21.6 ± 3.26	56.8 ± 8.11	162 ± 8.9	

Experimental protocol

All subjects were studied as in-patients in our department. Control subjects were admitted to the metabolic ward the day before the study, whereas anorectic patients had been hospitalized for at least 3 days. Healthy controls were instructed to consume a weight-maintaining diet with 50% carbohydrate for the 3 days preceding the investigation. Anorectic patients were fed a standardized diet (750 Cal/day) containing 50% carbohydrate for 3 days until the study night. Informed consent to participate in this study was obtained from normal subjects and from patients or their parents after full explanation of the experimental nature of the study, which was approved by the ethical committee of our institution.

Blood samples for evaluation of plasma IGF-I and serum estradiol (E_2) levels were collected in the morning after an overnight fast in all patients and controls. An indwelling heparin lock catheter was inserted into an antecubital vein at 1800 h, and serial blood sampling for GH estimation was initiated at 2030 h and continued at 20-min intervals for 12 h. All subjects were instructed not to eat during the night and had a normal nocturnal sleep pattern. To prevent sleep disruptions during the night, both patients and controls slept in their hospital rooms, and a curtain was used to separate investigators from the sleeping subjects when taking samples. Blood was collected into prechilled glass tubes, and samples were immediately centrifuged at 2300 × *g* for 15 min at 4 C; serum was removed and stored at −80 C until assayed.

Assays

Serum GH and plasma IGF-I concentrations were measured by specific RIA [Technogenetics, Recordati (Milan, Italy) and Nichols Institute Diagnostics (San Juan Capistrano, CA), respectively]. IGF-I levels were determined without prior serum extraction. Detection limits are 0.2 μg/L for GH (double incubation procedure increasing assay sensitivity) and 19 ng/mL for IGF-I, respectively. Each assay comprised samples obtained from controls and anorectic subjects. The mean intraassay coefficients of variation (CVs) at mean GH concentrations of 0.48, 0.88, 2.0, 4.9, 7.9, 9.7, 13.2, and 31.6 μg/L were 24.7%, 17.7%, 9.9%, 5.0%, 5.7%, 2.9%, 8.3%, and 9.1%, respectively, and the interassay CVs at mean GH concentrations of 2.0, 4.9, 9.7, 13.2, and 31.6 μg/L were 9.9%, 6.6%, 2.9%, 8.7%, and 9.8%, respectively. Intra- and interassay CVs for IGF-I were 5.1% and 10.1%, respectively. In our laboratory, the normal range for plasma IGF-I in 20-yr-old female subjects is 86–420 ng/mL.

Serum E_2 levels were measured by a competitive chemiluminescent immunoassay (Ciba Corning Diagnostics Corp., Medfield, MA). The sensitivity of the assay was 37 pmol/L. Intra- and interassay coefficients of variation were 9.4% and 9.8%, respectively. The normal range for the follicular phase of the menstrual cycle is 70–300 pmol/L.

Calculations

The secretory GH profiles were analyzed by the Cluster pulse detection algorithm (20), which defines significant hormonal excursions in relation to the actual experimental variance; a peak is defined as a statistically significant increase in a cluster of GH values followed by a statistically significant decrease in a second cluster of values. The undetectable samples (<0.2 μg/L) were assigned an arbitrary fixed value of 0.1 μg/L, which is half the detection limit of our assay. A 1 × 1 cluster configuration (one sample in the test nadir and one in the test peak) was used with a *t* statistic of 2.32 for both the significant up-strokes and down-strokes. Using these parameters, the probability of the false positive error rate is constrained to less than 5%. The minimum threshold for a peak was set at 0.6 μg/L, *i.e.* 3 SD above the detection limit of GH assay. Cluster was used to calculate peak frequency (number of significant GH peaks per 12 h), interpeak interval (time in minutes separating consecutive peak maxima), peak duration in minutes, peak height (maximal GH concentration in a peak), incremental peak height (the difference between the largest peak value and the preceding nadir), interpeak valley value (defined as regions between the significant down-strokes and up-strokes), area under the curve (AUC), and pulse area (integrated concentration under a peak in excess of the mean pre- and postpeak nadirs). The areas of GH release were calculated using the trapezoidal rule. The proportion of the total AUC contained within GH concentration pulses (pulsatile AUC) was calculated as the product of the pulse frequency and the mean pulse area. The difference between the total AUC and the pulsatile AUC was denoted the basal AUC.

The mean nocturnal GH concentration was calculated as the mean of GH concentrations recorded over the 12-h sampling period.

Statistical analysis

Statistical evaluation was performed by nonparametric Mann-Whitney rank sum test to compare the significance of differences between groups and by the Wilcoxon signed rank test to assess changes within the same group.

Linear regressions of BMI and IGF-I on GH peak parameters were performed in anorectic patients to determine the relationships between measures of GH release and these parameters. A computer program was used for all statistical calculations (StatView IV, Abacus Concepts, Berkeley, CA). All results are expressed as the mean ± SD. The level of statistical significance was set at $P < 0.05$.

Results

Figure 1 shows nocturnal serum GH levels in two representative anorectic and control subjects. In normal women, GH was released in large bursts separated by periods of secretory quiescence, with baseline GH concentrations frequently approaching assay sensitivity. Only 17.5% of all samples obtained from normal women had undetectable GH concentrations. The mean nocturnal GH concentration was 1.5 ± 0.71 μg/L (range, 0.4–2.4 μg/L). In contrast, in anorectic patients, the episodes of GH release were superimposed on sustained baseline hormone levels; in fact, in none of the samples drawn from these patients were GH levels below the detection limit of the assay. In these patients the mean nocturnal GH concentration was significantly increased compared to that in the control subjects (6.5 ± 2.10 μg/L; range, 4.4–9.6 μg/L; $P < 0.01$ *vs.* controls).

The results of the Cluster analysis are summarized in Table 2. The mean 12-h AUC was 4-fold higher in anorectic patients than in normal women due to an increase in both basal and pulsatile AUC. The valley mean, an index of basal GH release, was also elevated in anorectic compared to normal women. Anorectic patients displayed an increased peak frequency with consequently decreased interpeak interval. Accordingly, the mean pulsatile AUC was significantly greater

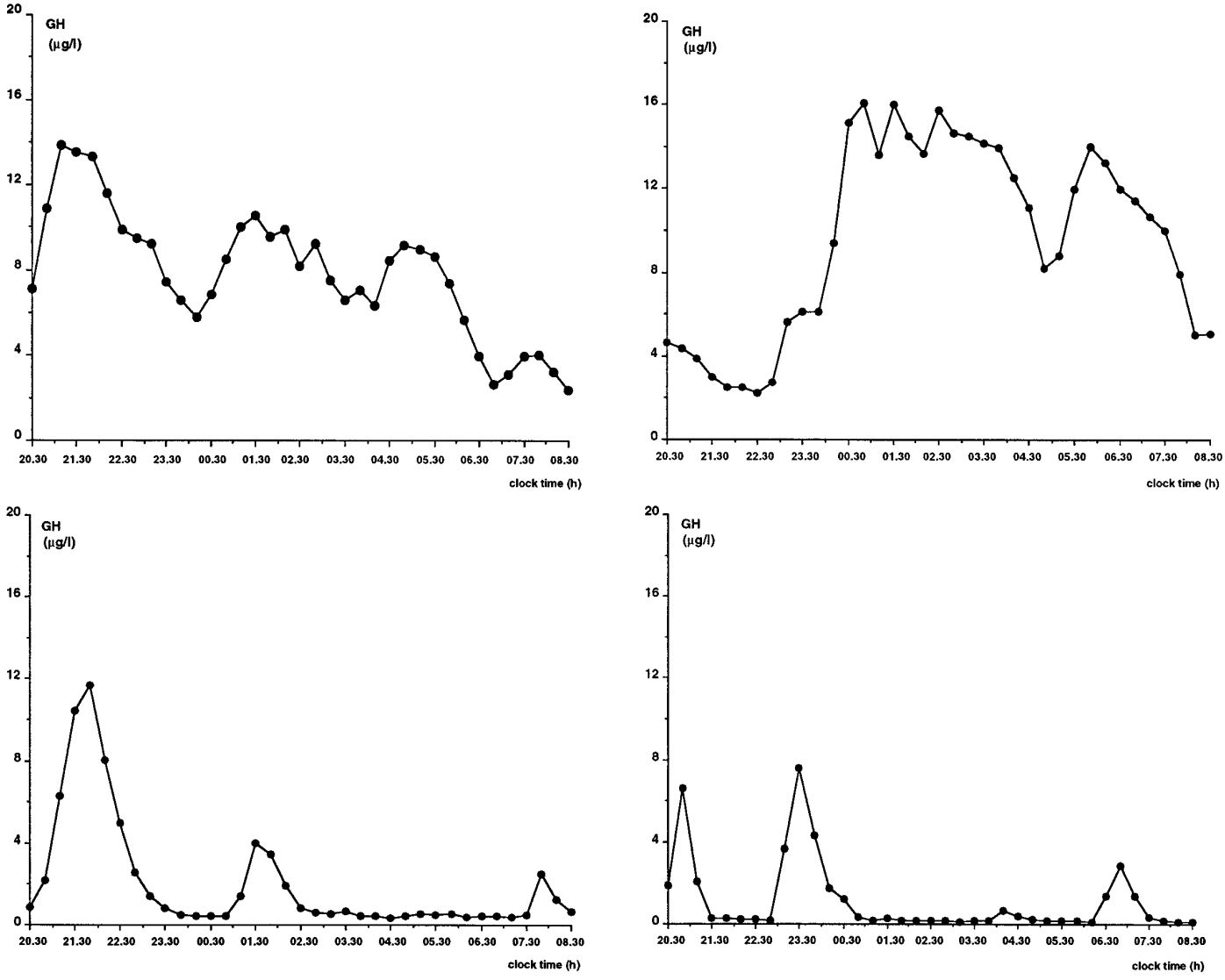

FIG. 1. Serum GH profiles in two representative anorectic patients (*upper panel*) and in two control women (*lower panel*).

in anorectic patients than in control women. In patients, the mean peak height was increased; however, the incremental peak height did not differ significantly from that in normal subjects. The pulse area was comparable in the two groups. The GH peaks in anorectic patients and in normal subjects were also of comparable widths. The ratio between pulsatile and total AUC was decreased in anorectic patients compared to that in control women.

As expected, fasting baseline plasma IGF-I levels were below the normal range in anorectic patients and were normal in control subjects [49.4 ± 7.84 ng/mL (range, 36.3–63.1 ng/mL) *vs.* 183.5 ± 8.91 ng/mL (range, 164.1–197.0 ng/mL); $P < 0.05$]. Serum E_2 concentrations were lower in anorectic patients than in control subjects (174 ± 86.4 *vs.* 283 ± 18.6 pmol/L; $P < 0.01$, respectively).

In anorectic patients, no correlations were observed between BMI and any of the parameters of GH release, whereas a positive correlation was found between IGF-I levels and pulsatile AUC ($r^2 = 0.583$; $P < 0.05$), peak height ($r^2 = 0.743$; $P = 0.01$), peak increment ($r^2 = 0.801$; $P < 0.01$), and GH valley mean ($r^2 = 0.576$; $P < 0.05$).

Discussion

In the present study the pulse detection algorithm Cluster has been applied for the first time to analysis of the spontaneous GH release in anorexia nervosa. In anorectic patients, compared to normal subjects, the nocturnal GH secretion is enhanced and characterized by an increase in peak frequency and interpeak hormonal concentrations. The enhanced hormonal release is largely due to an increase in its nonpulsatile component. Indeed, a clear-cut increase in the nonpulsatile GH area was observed in anorectic patients. Although this component constituted less than 40% of GH release in normal subjects (a figure probably overestimated by the RIA), it represented up to 70% of GH secretion in

TABLE 2. Mean pulse parameters of anorectic patients and normal subjects

	Anorectic patients	Normal women	*P*
Peak frequency (no./12 h)	5.0 ± 0.81	3.0 ± 0.89	<0.01
Peak width (min)	108.2 ± 16.71	101.6 ± 36.84	NS
Peak ht (μg/L)	8.6 ± 3.37	5.1 ± 2.00	<0.05
Incremental peak ht (μg/L)	3.0 ± 1.39	4.2 ± 1.71	NS
Interpeak interval (min)	114.1 ± 27.78	297.2 ± 121.08	<0.01
Valley mean (μg/L)	5.9 ± 2.25	1.0 ± 1.30	<0.01
Pulse area (μg/L·min)	323.9 ± 182.74	261.2 ± 149.16	NS
Total AUC (μg/L·min)	4743.0 ± 1520.09	1148.6 ± 519.27	<0.01
Pulsatile AUC (μg/L·min)	1530.4 ± 654.72	769.8 ± 404.02	<0.05
Basal AUC (μg/L·min)	3212.5 ± 990.45	378.7 ± 123.27	<0.01
Pulsatile/total AUC	0.31 ± 0.06	0.62 ± 0.16	<0.05

anorectic women. However, although some GH peaks may have been missed in normal women due to the small, but not negligible, percentage of undetectable samples, the pulsatile component of GH release was also significantly enhanced in the patients due to the higher number of secretory episodes and despite the comparable amount of GH released per burst. Of note, spontaneous GH release was enhanced in our patients despite their lower levels of E_2, which is a positive determinant of somatotropin secretion in normal women (21).

An increase in the GH peak frequency and valley hormonal concentrations has also been described in acromegalic patients (22) and fasted volunteers (14). Acromegaly is characterized by a marked enhancement of nonpulsatile GH release (22, 23) associated with a moderate increase in the pulsatile secretory component (22). On the contrary, fasted volunteers display a significant enhancement of pulsatile GH release (14, 24), whereas their basal GH secretion, which was found to be increased in earlier studies (14), was more recently reported to be unchanged (23, 24).

Among the syndromes of acquired GH resistance, the pattern of spontaneous GH secretion in anorexia nervosa appears to be different from that described in critically ill patients (16), who display low values of secretory burst amplitude and GH amount per secretory burst. This discrepancy may reflect pathophysiological differences, because anorexia, even when associated with important malnutrition and reduction of total body protein (17), is not characterized by the markedly increased protein catabolic rate, causing vital tissue wasting with fat depot preservation, typically observed in long lasting critical illness.

Abnormalities of spontaneous GH secretion similar to those observed in our anorectic patients, *i.e.* increased peak frequency and interpeak GH concentration with unchanged individual pulse area, are observed in another condition of cellular fasting, poorly controlled diabetes mellitus (25).

The alteration in spontaneous GH secretion described in this paper in addition to the abnormalities of somatotropin responsiveness to pharmacological challenges reported by us (8) and others (3–7) point to a hypothalamic dysregulation of GH release in anorexia nervosa. Although the elevated interpulse GH levels are compatible with a reduction of hypothalamic SRIH tone, the increase in GH pulse frequency might be accounted for by an increased frequency of GHRH discharges. The former possibility is indirectly supported by the impaired GH responses to stimuli thought to act via inhibition of SRIH secretion (7, 8) and is directly strengthened by the observation of low SRIH levels in the cerebrospinal fluid of these patients (26). However, the reduction of SRIH tone does not seem to be absolute, as a release of the neuropeptide is probably elicited by adequate stimuli. This is suggested by the lack of GH response to the second of two consecutive GHRH boluses observed in anorexia nervosa (6), indicating that the SRIH-mediated negative GH autofeedback is preserved in these patients.

Although no changes in GH half-life have been reported in fasted volunteers (24), the possibility that a decreased metabolic clearance of the hormone contributes to the high interpulse GH levels in anorexia nervosa cannot be excluded.

The integrity of the IGF-I negative feedback on GH secretion in anorexia nervosa has not been investigated to date. However, in fasted humans the infusion of recombinant human IGF-I suppresses the enhanced pulsatile GH secretion with a similar time course as refeeding, suggesting that changes in plasma IGF-I concentrations mediate the effects of nutrition on GH release (27). The lack of a negative correlation between IGF-I levels and the parameters of spontaneous GH secretion observed in our patients merges with the absence of a correlation between somatomedin concentrations and magnitude of the GH response to GHRH reported by others (28) in suggesting that factors other than or in addition to the malnutrition-induced lowering of IGF-I are responsible for the altered GH secretion in anorexia nervosa. Along the same line is the absence of a negative correlation between BMI and spontaneous GH release seen in our patients. This is in sharp contrast with the negative correlation between BMI or other ponderal indexes and parameters of spontaneous GH release consistently found in normal weight (29) and obese subjects (30). We found a positive correlation between IGF-I levels and pulsatile AUC, peak height, peak increment, and interpulse GH concentrations. This finding, if confirmed by an IGF-I assay performed after IGF-binding protein extraction, would indicate that in anorexia nervosa high GH levels can partially overcome the peripheral resistance to the hormone.

In conclusion, this study has shown that spontaneous nocturnal GH secretion is enhanced in anorexia nervosa due to an increase in both nonpulsatile and pulsatile components. This finding together with the lack of negative correlation between parameters of GH release and nutritional indexes (BMI and IGF-I concentrations) point to an impairment of the hypothalamic control of GH secretion in these patients, who, however, retain some physiological features of GH release, such as its episodic pattern.

Further studies aimed at better defining GH secretion in anorexia nervosa and other conditions of GH insensitivity are recommended.

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