Growth hormone response to growth hormone-releasing hormone varies with the hypothalamic-pituitary abnormalities

Mohamad Maghnie, Antonia Moretta, Anice Valtorta, Daniela Larizza, Mariam Sayegh, Anna Maria Greco, Enio Castoldi and Francesca Severi

Department of Pediatrics, University of Pavia, Istituto di Ricovero e Cura a Carattere Scientifico Policlinico S. Matteo, Pavia, Italy


We determined growth hormone (GH) and insulin-like growth factor I (IGF-I) levels after a 3 h infusion of escalating doses of growth hormone-releasing hormone (GHRH(1-29)) followed by a bolus injection in hypopituitary patients with marked differences in pituitary features at magnetic resonance imaging (MRI) in order to evaluate further the contribution of MRI in the definition of pituitary GH reserve in GH-deficient patients. Twenty-nine patients (mean age 14.5 ± 4.0 years) were studied. Group I comprised 13 patients: seven with isolated GH deficiency (IGHD) (group Ia) and six with multiple pituitary hormone deficiency (MPHD) (group Ib) who had anterior pituitary hypoplasia, unidentified pituitary stalk and ectopic posterior pituitary at MRI. Group II consisted of eight patients with IGHD and small anterior pituitary/empty sella, while in group III eight had IGHD and normal morphology of the pituitary gland. Growth hormone and IGF-I levels were measured during saline infusion at 08.30–09.00 h, as well as after infusion of GHRH(1–29) at escalating doses for 3 h: 0.2 μg/kg at 09.00–10.00 h, 0.4 μg/kg at 10.00–11.00 h, 0.6 μg/kg at 11.00–12.00 h and an intravenous bolus of 2 μg/kg at 12.00 h. In the group I patients, the peak GH response to GHRH(1–29) was delayed (135–180 min) and extremely low (median 2 mU/l). In group II it was delayed (135–180 min), high (median 34.8 mU/l) and persistent (median 37.4 mU/l at 185–210 min). In group III the peak response was high (median 30.8 mU/l) and relatively early (75–120 min) but it declined rapidly (median 14.4 mU/l at 185–210 min). In one group I patient, GH response increased to 34.6 mU/l. The mean basal value of IGF-I levels was significantly lower in group I (0.23 ± 0.05 U/ml) than in groups II (0.39 ± 0.13 U/ml, p < 0.01) and III (1.54 ± 0.46 U/ml, p < 0.001) and did not vary significantly during the GHRH(1–29) infusion. The present study demonstrates that the impaired GH response to 3 h of continuous infusion of escalating doses of GHRH(1–29) was strikingly indicative for pituitary stalk abnormality, strengthening the case for use of GHRH in the differential diagnosis of GH deficiency. The low GH response, more severe in MPHD patients, might be dependent on the residual somatotrope cells, while the better response (34.6 mU/l) in the group Ia patients might suggest that prolonged GHRH infusion could help in evaluating the amount of residual GH pituitary tissue. Pituitary GH reserve, given the GH response to GHRH infusion in GH-deficient patients with small anterior pituitary/empty sella, seems to be maintained.

M Maghnie, Department of Pediatrics, University of Pavia, IRCCS Policlinico S Matteo, 1-27100 Pavia, Italy

Growth hormone-releasing hormone (GHRH) infusion has been employed with different modalities for evaluation of GH response in normal or GH-deficient subjects (1–5). Responses were, however, extremely variable and, particularly, much lower in patients with severe than in those with partial GH deficiency (6–9). In all these studies the diagnosis of GH deficiency was made without any pituitary morphological documentation; it was based on clinical features and biochemical evidence of inadequate GH responses of less than 15–20 mU/l to at least two provocative tests. The wide variation in GH response to GHRH has been ascribed to the biological variability of different neuroregulatory functions, differences of pituitary GH reserve and other factors because of the heterogeneous condition of GH deficiency. Now, magnetic resonance imaging (MRI) adds further information on the etiology of idiopathic hypopituitarism by identifying markedly different pituitary morphologies as distinct hypothalamic–pituitary entities (10–12). Our MRI finding of a normal pituitary gland in one idiopathic hypopituitary patient with combined hormone deficiency was suggestive of a Pit-1 gene defect (13), indicating that MRI could be helpful in revising our classification schemes in hypopituitarism. The GH response to bolus GHRH(1–40) was much lower in the cases with anterior pituitary hypoplasia, ectopic posterior pituitary and unidentified pituitary stalk than in those with small or normal pituitary gland (11).

As continuous or repetitive GHRH infusion has been reported to be more informative on the pituitary GH
reserve (14, 15), the present study was undertaken in order to clarify further the contribution of MRI in the definition of pituitary GH reserve after short-term GHRH infusion at escalating doses in GH-deficient patients with different pituitary characteristics.

Patients and methods

Study subjects

Twenty-nine patients with hypopituitarism (20 males and nine females aged 4.8–23 years, mean 14.5 ± 4.0 years) were studied. Twenty-three had isolated GH deficiency (IGHD) and six had multiple pituitary hormone deficiency (MPHD). All these had hypothryoidism and hypogonadism, and all but one had hyposurrenalism; PRL levels were high in four of them. Growth hormone deficiency was not due to gross structural abnormalities of the GHRH gene (16). In all patients antipituitary antibodies were absent (17). On the basis of MRI findings, the patients were subdivided into three groups. Group I comprised 13 patients with multiple pituitary abnormalities (anterior pituitary hypoplasia, unidentified pituitary stalk and ectopic posterior pituitary) that, associated in some with congenital brain anomalies (18), suggested a prenatal disturbance of morphogenesis; seven had IGHD (group Ia) and six had MPHD (group Ib). In group II eight patients had small anterior pituitary/empty sella, normal pituitary stalk and posterior pituitary and IGHD, while in group III eight had normal morphology of the pituitary gland and IGHD. The term pituitary hypoplasia was preferred for patients of group I with GHD of congenital origin. At the time of diagnosis, the GH peak levels following insulin-induced hypoglycemia or arginine or t-dopa were 1–5.4 mU/l in group I (median peak 2.1 mU/l), 4.6–19 mU/l in group II (median peak 7 mU/l) and 4.2–19.8 mU/l in group III (median peak 14.4 mU/l, p = 0.005). Both IGHD and MPHD were diagnosed according to previously reported criteria (11). Bolus GHRH(1–40) or GHRH(1–29) was given in three patients from group Ia (8.2 ± 3.4 mU/l), four from group Ib (5.2 ± 2.1 mU/l), five from group II (65.6 ± 10.2 mU/l) and four from group III (57.8 ± 12.8 mU/l) at the time of presentation. In the absence of pituitary stalk, the low GH response to bolus GHRH(1–40), the delayed TSH response to TRH and the low FSH and LH responses to GnRH and hyperprolactinemia all suggest both pituitary and hypothalamic disorders (11). The MPHD patients were receiving appropriate additional replacement therapy. Sellar and pituitary volumes evaluated according to Di Chiro and Nelson (19) did not differ significantly between groups I and II but they were significantly higher in group III (Table 1). The pituitary volumes were strikingly abnormal and less than 150 mm³ in both groups I and II, whatever the formula used. Details of the MRI technique and images have been reported previously (10, 11). At the time of the study the mean age was 16.1 ± 5.1 years in group I, 12.6 ± 2.5 years in group II and 13.5 ± 1.5 years in group III. The body mass index (BMI) for each patient, calculated from weight/height², was 15–22 kg/m² (mean 18.0 ± 2.5) in group I, 13–26 kg/m² (mean 17.1 ± 4.4) in group II and 15–23 kg/m² (mean 18.7 ± 2.7) in group III.

Study design

The study protocol was approved by the Institutional Review Board of the Pediatric Department of the University of Pavia. Informed consent was obtained from the parents and, when appropriate, from the patients. The study was performed 1 month after r-GH withdrawal in 27 patients, while one patient had stopped GH treatment 3 years before evaluation and one 4.8-year old was evaluated before the beginning of GH treatment. The duration of GH treatment before evaluation was 3.3–14.3 years in group I, 1.6–9.7 years in group II and 2.9–8.9 years in group III.

All patients were studied while resting in the supine position after an overnight fast. A heparin-lock cannula was placed in one forearm vein for blood sampling, and a second cannula was placed in a contralateral forearm vein for iv infusion using a constant infusion pump (Life Care Pump Model 4, Abbott/Shaw, USA). The schedule of infusion was as follows: 25 ml of 0.9% saline at 08.30–09.00 h; GHRH (1–29) (Kabi-Pierrel, Stockholm, Sweden) available at a starting concentration of 100 μg/ml was diluted in 50 ml of normal saline at escalating doses for 3 h (0.2 μg/kg at 09.00–10.00 h, 0.4 μg/kg at 10.00–11.00 h and 0.6 μg/kg at 11.00–12.00 h and GHRH(1–29) bolus, 2 μg/kg, at 12.00 h. Blood samples (1.0 ml for GH and 1.0 ml for IGF-I levels) were obtained every 15 min until 12.00 h and then after 5, 10, 15, 20 and 30 min following the GHRH(1–29).

<table>
<thead>
<tr>
<th>Table 1: Magnetic resonance imaging of sella and pituitary volumes in 29 patients with GH deficiency*.</th>
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<tbody>
<tr>
<td>Group I (N = 13)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Sellar volumes (mm³)</td>
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<tr>
<td>Pituitary volume (mm³)</td>
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*Values are means±SEM; *p < 0.001 for group III vs group I and group II.
bolus. Blood pressure, pulse rate and temperature were recorded hourly. After centrifugation at 4°C the plasma was separated and kept frozen at −20°C until used.

Assay procedures

Serum GH levels were measured by RIA using a commercial kit (Pharmacia, Uppsala, Sweden). The intra-and interassay coefficients of variation (CVs) were 10% and 11%, respectively, at the level of 2.2 mU/l, 5% and 8% at the level of 8.4 mU/l, 4% and 6% at the level of 22 mU/l and 9% and 10% at the level of 48 mU/l. Cross-reactivity was less than 1% for PRL and human placental lactogen. The sensitivity of the assay was less than 0.4 mU/l, as determined by the mean of two standard deviations from the zero dose response.

Plasma IGF-I levels were measured by RIA using a commercial kit (Nichols Institute, San Juan Capistrano, CA) after pretreatment of the serum by acid/ethanol extraction. The CVs were 5% and 11.2%, respectively, at the levels of 0.7 U/ml and 0.33 U/ml, and 5.2% and 9.4% at the levels of 1.5 U/ml and 1.3 U/ml. Cross-reactivity was less than 0.01% for GH, porcine glucagon, TSH and LH, less than 0.002% for PRL and porcine insulin and less than 0.03% for porcine proinsulin. The sensitivity of the assay was less than 0.1 U/ml, as determined by the mean of two standard deviations from the zero dose response.

Table 2. Growth hormone response to GHRH(1–29) infusion at escalating doses in 29 patients with GH deficiency.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>30/0 saline</th>
<th>15/60 saline 0.2 µg/kg</th>
<th>75/120 saline 0.4 µg/kg</th>
<th>135/180 saline 0.6 µg/kg</th>
<th>185/210 saline 2 µg/kg</th>
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</thead>
<tbody>
<tr>
<td>GH (mU/l)</td>
<td>0.4 – 5.2</td>
<td>0.4 – 5.2</td>
<td>0.8 – 15.2</td>
<td>0.8 – 34.6</td>
<td>0.8 – 25.6</td>
</tr>
<tr>
<td>GH (mU/l)</td>
<td>0.4 – 5.2</td>
<td>0.8 – 4.4</td>
<td>1.2 – 15.2</td>
<td>0.8 – 34.6</td>
<td>1.2 – 25.6</td>
</tr>
<tr>
<td>GH (mU/l)</td>
<td>0.4 – 1.4</td>
<td>0.8 – 4.4</td>
<td>1.8 – 4.2</td>
<td>0.8 – 3.6</td>
<td>0.6 – 3</td>
</tr>
<tr>
<td>GH (mU/l)</td>
<td>3.4 – 7.6</td>
<td>3.4 – 1.7</td>
<td>6 – 36</td>
<td>34.8 – 37.4</td>
<td>34.8 – 37.4</td>
</tr>
<tr>
<td>GH (mU/l)</td>
<td>6.4 – 23.6</td>
<td>9.8 – 37.4</td>
<td>30.8 – 22</td>
<td>20.2 – 14.4</td>
<td>7.4 – 52.6</td>
</tr>
</tbody>
</table>

* Values are medians and ranges; p = 0.051 vs II (0.2 µg/kg), II vs III (saline, 0.2 µg/kg, 0.6 µg/kg and 2 µg/kg); p = 0.0005, I vs II (saline, 0.4 µg/kg, 0.6 µg/kg and 2 µg/kg), I vs III (saline, 0.2 µg/kg, 0.4 µg/kg, 0.6 µg/kg, and 2 µg/kg) and II vs III (0.4 µg/kg).

IGHD = isolated growth hormone deficiency; MPHD = multiple pituitary hormone deficiency.

Statistical analysis

The GH releases in response to continuous infusion of escalating GHRH(1–29) and to the iv bolus were analysed for the five time periods corresponding to different stimulation modalities: −30 to 0 min (saline), 15–60 min (0.2 µg/kg·h−1), 75–120 min (0.4 µg/kg·h−1), 135–180 min (0.6 µg/kg·h−1) and 185–210 min (2 µg/kg). The Kruskal–Wallis test was used to compare the GH response between the three groups. The Mann–Whitney test was used to compare GH and IGF-I levels during the infusion periods. The area under the curve (AUC) was calculated by trapezoidal integration. Statistical analyses were performed using the Statistica modeling package (Statsoft Inc, USA). Multiple regression analysis was employed to assess whether patients' characteristics, such as sex, age at the time of diagnosis, age at the time of study, period of GH treatment and BMI, had influenced the GH response to GHRH(1–29) infusion. Results are expressed as the median and mean±SEM, and statistical significance was assumed when p < 0.05.

Results

Facial flushing in a few patients during the first 10 min after GHRH(1–29) infusion was the only side effect. Blood pressure, pulse rate and temperature did not vary significantly throughout the entire infusion.

Saline infusions

During 30 min of saline infusion, spontaneous GH
The infusion of GHRH(1–29) resulted in a GH response of >20 mU/L in three patients of group III at 15 min, indicating that 0.2 µg·kg⁻¹·h⁻¹ is able to stimulate the anterior pituitary. Furthermore, 0.4 µg·kg⁻¹·h⁻¹ was enough to obtain the maximum GH response from patients with a morphologically normal pituitary gland (group III). In contrast, 0.6 µg·kg⁻¹·h⁻¹ was necessary to obtain a regular and sustained GH response from those with small anterior pituitary/empty sella (group II). Moreover, in group II there was no tendency for the pulse frequency to decrease as it did in group III (Table 2 and Fig. 1). The GH range and median were significantly lower in the group I patients, with minimal changes in the MPHGD patients (group IIb) compared to those with IGHD (group Ia) (Table 2). One patient of group Ia showed a peak GH response of 34.6 mU/L at the dose of 0.6 µg·kg⁻¹·h⁻¹ of GHRH infusion, which was much higher than that observed at the time of presentation (Fig. 2).

Responses to GHRH (1–29) bolus injection, when they occurred, were observed 5–10 min after the injection. All group II patients showed a GH response of >20 mU/L. The GH response in the two patients from group III with spontaneous GH secretion of >20 mU/L during saline infusion did not differ from that in the remaining six patients.

Significant differences in the GH response to GHRH infusion were present at the different time periods by the Kruskal–Wallis test. The significances were p = 0.03, p = 0.02, p = 0.001, p = 0.0002 and p = 0.0001 at the five different time periods of −30 to 0 min, 15–60 min, 75–120 min, 135–180 min and 185–210 min, respectively. The significant differences by the Mann–Whitney test between the groups are reported in Table 2. The mean AUC of GH was significantly lower in group I (154.2 ± 27.4 mU/L) than in groups II (839.7 ± 65.3 mU/L, p < 0.0001)

Fig. 1. Mean serum GH concentrations in 29 patients with GH deficiency during the 4h study period (08.30–12.30 h). The concentrations were measured during 0.9% saline infusion at 08.30–9.00 h as well as after infusion of GHRH (1–29): 0.2 µg/kg at 09.00–10.00 h, 0.4 µg/kg at 10.00–11.00 h, 0.6 µg/kg at 11.00–12.00 h and 2 µg/kg at 12.00–12.30 h. Three different patterns of GH response in the patients with multiple MRI pituitary abnormalities (group I), small anterior pituitary/empty sella (group II) and normal pituitary gland (group III) are shown.

Fig. 2. Serum GH concentrations in 12.8-year-old boy with isolated growth hormone deficiency (IGHD) and multiple MRI pituitary abnormalities after bolus GHRH at the time of first evaluation and during the 4h study period (08.30–12.30 h). The concentrations were measured as stated in the legend to Fig. 1. The GH response during the infusion is higher than that obtained after a 2 µg/kg bolus of GHRH (1–29) given at the time of presentation.

Growth hormone-releasing hormone infusions

Progressive increase in GH secretion during the 3 h of infusion was documented. The magnitude and the time of GH response, however, differed widely between the groups. The maximum GH response was delayed and very low in the group I patients, delayed and persistently high in group II and early high, but rapidly declining in group III (Table 2 and Fig. 1).

Fig. 3. Mean plasma IGF-I concentrations in 29 patients with GH deficiency during the 4h study period (08.30–12.30 h). The concentrations were measured as stated in the legend to Fig. 1. Mean basal (time 0) concentration is statistically lower in groups I and II than in group III patients, p < 0.01 for group I vs group II and p < 0.001 for group I vs group III and group II vs group III. No increase in IGF-I level was documented during the infusion.
and III (624.7 ± 61.8 mU/l, p < 0.0001). There was a slight difference between groups Ia and Ib (84.7 ± 26.4 vs 41.6 ± 3.8 mU/l, p < 0.05).

Multiple regression indicated that facial flushing, sex, age at the time of diagnosis, age and BMI at the time of the study and duration of GH treatment were not significant variables to the GH response.

**IGF-1 levels**

The mean basal (time 0) IGF-1 levels were 0.22 ± 0.06 (median 0.08 U/ml) in group Ia and 0.24±0.09 U/ml (median 0.11 U/ml) in group Ib. In group I as a whole they were 0.23 ± 0.05 U/ml (median 0.11 U/ml), which is significantly lower than in groups II (0.39 ± 0.13 U/ml, p < 0.01, median 0.29 U/ml) and III (1.54 ± 0.46 U/ml, p < 0.001; median 1.35 U/ml) (Figures 4 and 5). The mean IGF-1 levels did not vary significantly during the GHRH(1–29) infusion.

**Discussion**

The GH response to a 3 h infusion of escalating doses of GHRH(1–29) in patients with GH deficiency and multiple pituitary MRI abnormalities is qualitatively and quantitatively different from that in patients with small pituitary/empty sella or with apparently normal anterior pituitary. It is difficult to be certain whether the progressive rise of GH was due to the dose effect of the GHRH(1–29), to the duration of the stimulus or to a synergistic effect. The different GH patterns obtained with identical stimulus of the same duration may be a manifestation of different etiologies expressed by different pituitary features.

In particular, GH response after GHRH(1–29) infusion was delayed and extremely low only in patients with multiple pituitary MRI abnormalities (anterior pituitary hypoplasia, unidentified pituitary stalk and ectopic posterior pituitary), regardless of whether they had IGHD or MPHD. This is also in agreement with recent observations after bolus GHRH in MPHD patients with the same hypothalamic–pituitary abnormalities (20, 21), except for one case (20). Although the precise locus of the GH defect in such patients was not defined, delayed TSH response to TRH and the high PRL level point to involvement of the hypothalamus. They appear, however, to have mixed severe congenital/ perinatal hypothalamic–pituitary, i.e. GHRH/GH damage, the GH response depending on the residual pituitary GH cells as suggested by the GH response of 34.6 mU/l in the patient of group I with IGHD. Moreover, the greater increase in GH response during GHRH infusion than that in response to the bolus GHRH performed at the time of presentation is interesting. This might be due to the variability of GH response to GHRH or to the longer GHRH infusion. It also indicates that a long-term survival of GH pituitary cells is possible even in the absence of pituitary stalk. The absence of GH response to GHRH has been reported at birth in a patient with the same pituitary abnormalities (22), and Borges and co-workers (7) reported that 5 days of pulsatile priming with GHRH (1–40) did not increase the GH response to more than 5 µg/l in MPHD patients. We suppose that these last had the same hypothalamic–pituitary abnormalities as described in our cases. In the presence of the same multiple pituitary MRI abnormalities, the patients with MPHD have a GH response to GHRH(1–29) that is not only low, but is significantly lower than in patients with IGHD. This could be related to the degree of endogenous GH deficiency but we believe that the greater the pituitary deficits in children (evidently due to the other pituitary hormones), the more severe is the GH response, as has been reported in adults with pituitary disease (23).

In the patients with IGHD and small anterior pituitary/empty sella, the GH response was delayed and persistently high, suggesting primary hypothalamic dysfunction due to endogenous GHRH deficiency. It is worth noting that, in contrast to group I patients and in the presence of similar pituitary volumes, the short-term stimulation was able to increase the GH response to very high levels. In this case a persistent lack of endogenous GHRH in these patients seems not to affect the intracerebral content of GH within the somatotropes. In newborn rats, deprivation of endogenous GHRH with anti-GHRH antibodies permanently impairs growth rate, GH synthesis and pituitary morphology, but not GH concentration (24). The concomitant GH treatment seems to counteract some of these effects, improving the ability of the gland to respond to an acute GHRH challenge by increasing the cellular activity (25).

In the patients with normal pituitary morphology, the pattern of GH response to GHRH (1–29) was similar to that described previously in normal subjects (1–5). One wonders whether these patients are hypothalamic GHRH-dependent GH–deficient; if so, it remains questionable why the pituitary gland is still of the normal size and quickly responsive (26). Evidence of normal spontaneous GH secretion, probably stress-related, in two patients suggests that they were transient or false-positive GH-deficient (27). The presence of spontaneous GH secretion indicates that the negative feedback of GH treatment on endogenous GH secretion (28, 29) has been overcome after a 1 month GH washout. Physiological GH secretion and GH response to provocative stimulation tests recover after 48 h both in treated idiopathic short-stature and in normal subjects (30, 31).

Growth hormone response to GHRH(1–29) bolus differed among the three groups of patients. The low response in patients with multiple MRI abnormalities may be due to complete pituitary cell depletion in the presence of a dysgenetic pituitary gland. On the contrary, the high GH response in the patients with a small anterior pituitary gland/empty sella could be related to the GH secretory status at the time of GHRH
The administration of GHRH(1–29) bolus while GH secretion was in progress probably caused the high GH response in our patients, as reported in man (31, 32) and in animals (33). The GH response to bolus GHRH(1–29) in the patients with a normal pituitary morphology might be due to a decrease of GH level in progress at the time of bolus injection. A partial desensitization of the somatotropes or a depletion of readily-releasable pools probably occurred.

Basal IGF-I level was always less than 0.4 U/ml in the patients with multiple pituitary MRI abnormalities and less than 0.2 U/ml in those with MPHD. It was between 0.4 and 0.8 U/ml in those with small anterior pituitary/empty sella, and variable in those with normal pituitary morphology, indicating a less severe GH deficiency, if any. Stable IGF-I levels during the study are in keeping with previous reports of 6–8 h latency for increase after GH administration (34), which excludes a possible negative feedback at the level of the pituitary during GHRH infusion.

Our data are not fully comparable with those of previous studies in terms of the selection of patients (number, age, MRI findings), the doses of GHRH and the time and duration of GHRH infusion. The present study demonstrates that in the presence of a different pituitary morphology 3 h of continuous infusion of escalating doses of GHRH(1–29) provides greater insight into the pathophysiological mechanisms of GH secretion, strengthening the case for use of GHRH infusion in the differential diagnosis of GH deficiency. In particular, the impaired GH response to GHRH was strikingly indicative of multiple pituitary abnormalities (pituitary hypoplasia, ectopic posterior pituitary and stalk agenesis), unlike the results in our patients with small pituitary/empty sella who have a high, even though delayed, GH response. Long-term priming to exclude down-regulation of GHRH receptors or decreased GH gene expression may provide meaningful information in patients with multiple MRI pituitary abnormalities, because they probably do not benefit from GHRH or GH-releasing peptide GHRH-mediated treatment.

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