

## Comment

# The Decrease in Growth Hormone (GH) Response after Repeated Stimulation with GH-Releasing Hormone Is Partly Caused by an Elevation of Somatostatin Tonus\*

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### ABSTRACT

Repeated injection of GHRH leads to a decrease in the GH response in normal subjects. Arginine (Arg) stimulates GH secretion by suppression of hypothalamic somatostatin. To confirm these findings, eight normal men were examined in a series of five settings: test 1 (GHRH/GHRH-TRH), 100 µg GHRH injected iv, followed by 100 µg GHRH, iv, after 120 min and 200 µg TRH, iv, after 150 min; test 2 (GHRH/Arg-TRH), like test 1, but instead of the second GHRH injection, a 30 g Arg infusion over 30 min; test 3 (GHRH/GHRH-Arg-TRH), like test 1, but additionally a 30 g Arg infusion after 120 min; test 4 (GHRH-Arg-TRH), iv GHRH and Arg infusion initially, followed by iv TRH after 30 min; and test 5 (TRH), 200 µg TRH, iv, at 0 min.

For statistical evaluation, the area under the GH curve (AUC) from 0–120 min was compared with the AUC from 120–240 min. The GH response to the second administration of GHRH was significantly lower ( $P < 0.02$ ) than the first increase [AUC,  $0.5 \pm 0.1$  min·mg/L (mean  $\pm$  SE) vs.  $1.2 \pm 0.3$ ]. No significant differences were found

between the GH responses to either GHRH or Arg alone (AUC,  $0.9 \pm 0.2$  min·mg/L vs.  $0.9 \pm 0.2$ ). A larger GH increase ( $P < 0.02$ ) was seen after GHRH-Arg compared to GHRH alone (AUC,  $1.9 \pm 0.4$  min·mg/L vs.  $1.2 \pm 0.3$ ). The GH response ( $P < 0.02$ ) to GHRH-Arg stimulation was lower after previous GHRH injection than after GHRH-Arg stimulation alone (AUC,  $1.9 \pm 0.4$  min·mg/L vs.  $3.5 \pm 0.9$ ). There was a statistically significant difference between the TRH-stimulated TSH response in test 4 compared to that in test 5. We could show that decreasing GH responses to repeated GHRH can be avoided by a combined stimulation with GHRH/Arg. These findings suggest that the decreased GH response to a second GHRH bolus may be partly due to an elevated hypothalamic somatostatin secretion, which can be suppressed by Arg. The lower GH response to GHRH-Arg stimulation after a previous GHRH bolus suggests, furthermore, that the readily available GH pool in the human pituitary may be limited. (*J Clin Endocrinol Metab* 81: 1994–1998, 1996)

GH SECRETION is controlled by the hypothalamus via GHRH and its inhibitory opponent, somatostatin (1). Acute injection of GHRH stimulates GH secretion in both normal subjects (2, 3) and acromegalic patients (4, 5). Continuous GHRH infusion fails to sustain elevated GH levels, and pulsatile stimulation with GHRH also leads to decreasing GH response in normal subjects (6). It was suggested that two different mechanisms may be responsible for these findings; possibly, there is a limitation of GH release from the pituitary on short term stimulation with GHRH due to partial depletion of intracellular GH stores (7), or the stimulation of GH secretion by GHRH may be antagonized by somatostatin secretion from the hypothalamus. As the half-life of somatostatin is extremely short (8), and somatostatin is also produced in the

pancreas and gut, somatostatin levels in peripheral blood do not reflect any fluctuations of its concentration in the portal circulation. Arginine (Arg) stimulates GH secretion by suppressing hypothalamic somatostatin (9), allowing indirect conclusions about the hypothalamic somatostatin tonus.

The following study on Arg application was designed to determine whether decreases in GH release after repeated stimulation by GHRH are due to exhaustion of the readily releasable GH pool in human pituitary or whether the GH secretion is specifically suppressed by increasing portal levels of somatostatin.

### Materials and Methods

We studied eight healthy men (mean age, 26.3 yr; range, 19–33), all of normal height, within 15% of the ideal body weight, and taking no medication known to interfere with GH secretion. The volunteers agreed to participate in the study and gave informed consent in written form. The men were studied in a randomized fashion in a series of five different settings separated by at least 7 days (Fig. 1). All tests were performed in the morning between 0830 and 0900 h after an overnight fast. An antecubital venous catheter was placed and kept patent by slow saline infusion. The volunteers remained recumbent throughout the test. The experiments satisfied the ethical precepts of the responsible committee on human experimentation and of the Helsinki Declaration.

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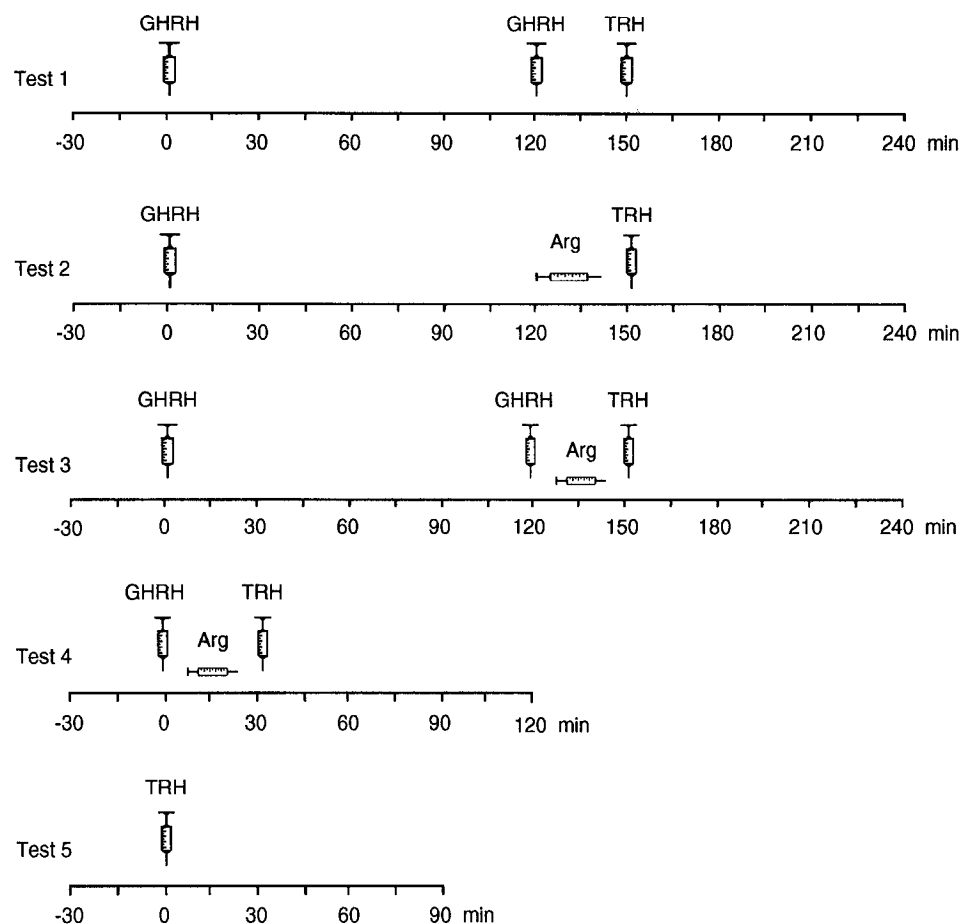


FIG. 1. Procedures of tests 1, 2, 3, 4, and 5. All injections were given iv (GHRH, 100  $\mu$ g; TRH, 200  $\mu$ g; Arg, 30 g as an infusion over 30 min).

#### Test 1 (GHRH/GHRH-TRH)

After a stabilization period of 30 min, the volunteers were given an iv bolus of 100  $\mu$ g GHRH-(1-44) (Somatobiss, Bissendorf Biochemicals, Bissendorf bei Hannover, Germany) over 30 s. After 120 min, this procedure was repeated. After 150 min, 200  $\mu$ g TRH (Relefact TRH 200, Hoechst, Bad Soden, Germany) were injected iv. Blood samples were drawn at intervals of 15 or 30 min from the beginning of the stabilization period up to 240 min after starting the test.

#### Test 2 (GHRH/Arg-TRH)

One hundred micrograms of GHRH-(1-44) were given iv at 0 min. Thirty grams of Arg were administered in 500 mL saline from 120-150 min, followed by an injection of 200  $\mu$ g TRH, iv. Blood samples were drawn as described in test 1.

#### Test 3 (GHRH/GHRH-Arg-TRH)

One hundred micrograms of GHRH-(1-44) were injected iv at 0 min. The volunteers received a second 100- $\mu$ g GHRH-(1-44) injection after 120 min, immediately before starting the 30 g Arg infusion (over 30 min). Two hundred micrograms of TRH were given iv after 150 min. Blood samples were drawn as described in test 1.

#### Test 4 (GHRH-Arg-TRH)

One hundred micrograms of GHRH-(1-44) were administered at 0 min, immediately followed by an infusion of 30 g Arg over 30 min and 200  $\mu$ g TRH, iv, thereafter. Blood samples were drawn 30 min before until 120 min after starting the test, at 15- and 30-min intervals.

#### Test 5 (TRH)

Two hundred micrograms of TRH were injected iv at 0 min. Blood samples were drawn from 30 min before to 90 min after starting the test, at 15- and 30-min intervals.

#### Assays

GH was measured by a commercially available IRMA [immunoradiometric assay, Medgenix (Medgenix Diagnostics, Ratingen, Germany); intraassay coefficients of variation (CVs), 4.5% and 3.9% for low and high concentrations, respectively; interassay CVs, 8.3% and 5.9%). TSH was measured as previously described (10). The intraassay CVs were 5.9% and 7.7% for low and high concentrations, respectively; the interassay CV was 13%. Serum samples for GH and TSH determinations were stored at  $-20^{\circ}\text{C}$  until the time of assay.

#### Statistical analysis

All values are given as the mean  $\pm$  SE. For statistical evaluation, baseline GH (0 or 120 min) and maximum GH (45 or 165 min) were compared by Student's *t* test, and significance was corrected using the Bonferroni rule. The area under the GH curve (AUC) from 0-120 min was compared to the AUC from 120-240 min by ANOVA. The TSH AUC was evaluated up to 90 min after TRH injection using ANOVA. If ANOVA proved to be significant, further testing with paired Wilcoxon's rank test was performed. The results were considered significant at a level of  $P \leq 0.05$ .

#### Results

No serious side-effects were recorded after GHRH-(1-44) or TRH iv bolus injection or during any of the Arg infusions.

Minor complaints were transient facial flushing soon after GHRH bolus administration and an urge to urinate immediately after TRH injection, which subsided within 3–5 min.

### Test 1

After the first GHRH injection, the mean GH concentration of eight normal men increased significantly from  $0.3 \pm 0.0 \mu\text{g/L}$  (mean  $\pm$  SE) at 0 min to a maximum of  $14.4 \pm 3.9 \mu\text{g/L}$  at 45 min (Fig. 2). Compared to this, the mean GH increase after the second GHRH injection at 120 min was significantly lower (from  $4.5 \pm 1.6 \mu\text{g/L}$  to  $5.6 \pm 1.6$  after 165 min). The AUC of the GH response was also significantly lower ( $P < 0.02$ ) after the second stimulation ( $0.5 \pm 0.1 \text{ min}\cdot\text{mg/L}$  vs.  $1.2 \pm 0.3$  after the first GHRH stimulation).

### Test 2

After Arg-TRH administration, the mean GH concentration increased significantly from  $2.9 \pm 0.8 \mu\text{g/L}$  at 120 min to a maximum of  $13.6 \pm 3.9 \mu\text{g/L}$  (Fig. 3) after 165 min. There were no significant differences between the GH response to GHRH injected at 0 min (AUC,  $0.9 \pm 0.2 \text{ min}\cdot\text{mg/L}$ ) and that to Arg given 120 min after GHRH (AUC,  $0.9 \pm 0.2 \text{ min}\cdot\text{mg/L}$ ), as shown in Fig. 3.

### Test 3

After another GHRH injection, followed by Arg-TRH administration, the mean GH level increased significantly from  $6.3 \pm 3.0 \mu\text{g/L}$  at 120 min to a maximum of  $30.3 \pm 6.8 \mu\text{g/L}$  (Fig. 4) at 165 min. The AUC of the GH increase seen after the first GHRH injection at 0 min was  $1.2 \pm 0.3 \text{ min}\cdot\text{mg/L}$ . Compared to this, the GH increase after combined GHRH-Arg administration (AUC,  $1.9 \pm 0.4 \text{ min}\cdot\text{mg/L}$ ) was significantly larger ( $P < 0.02$ ), as shown in Fig. 4.

### Test 4

After iv GHRH followed by immediate Arg infusion and TRH administration after 30 min, the mean GH concentration increased significantly from  $0.3 \pm 0.0 \mu\text{g/L}$  at 0 min to a

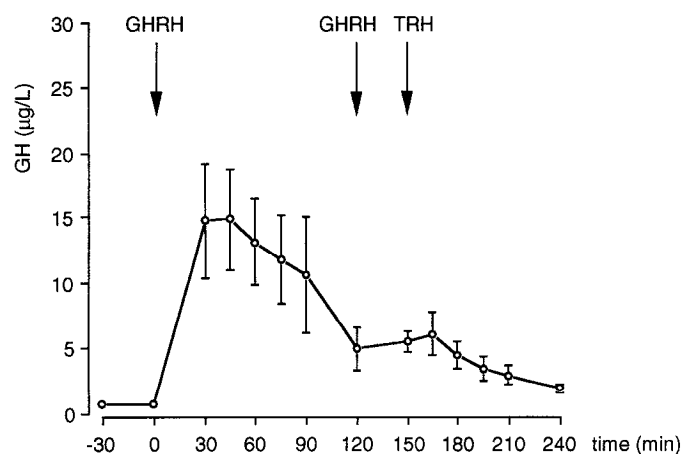


FIG. 2. Test 1 (GHRH/GHRH-TRH): mean GH levels before and after iv stimulation. Arrows indicate the times of GHRH (0 and 120 min) and TRH (150 min) injections. The AUC of the GH response after the second GHRH injection was significantly lower than the AUC of the first GH increase ( $P < 0.02$ ).

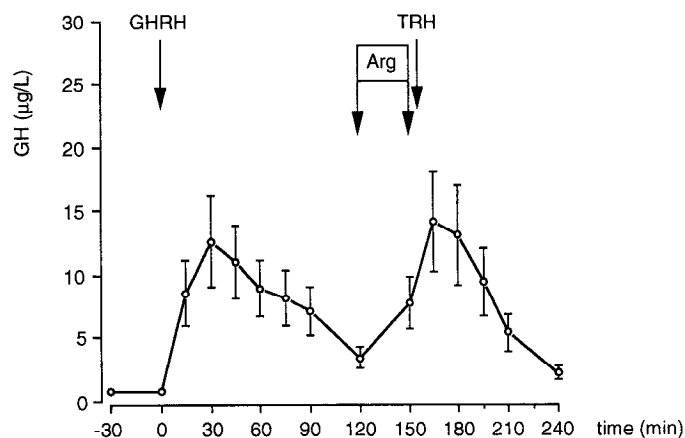


FIG. 3. Test 2 (GHRH/Arg-TRH): mean GH levels before and after iv stimulation. Arrows indicate the times of GHRH (0 min) and TRH (150 min) injections. Arg infusion was given after 120 min over 30 min. There were no significant differences in GH increase after GHRH injected at 0 min and that after Arg infusion at 120 min.

maximum of  $46.5 \pm 11.8 \mu\text{g/L}$  (Fig. 5) at 60 min. The GH response ( $P < 0.02$ ) to combined GHRH-Arg-TRH stimulation was significantly lower after previous GHRH injection (AUC,  $1.9 \pm 0.4 \text{ min}\cdot\text{mg/L}$ ) than after GHRH-Arg-TRH stimulation alone (AUC,  $3.5 \pm 0.9 \text{ min}\cdot\text{mg/L}$ ), as shown in Figs. 4 and 5.

Summing up the AUCs of tests 1 and 2 between 120–240 min gives a result that is still significantly lower ( $P < 0.05$ ) than the AUC of combined GHRH-Arg-TRH stimulation after previous GHRH injection (test 3), as shown in Figs. 2–4.

In test 4, the GHRH/Arg/TRH-stimulated TSH secretion (AUC,  $537 \pm 181 \text{ min}\cdot\text{mU/L}$ ) was significantly higher ( $P \leq 0.02$ ) than that after TRH alone (test 5,  $468 \pm 151 \text{ min}\cdot\text{mU/L}$ ). In tests 1, 2, and 3 (AUC in test 1,  $565 \pm 228 \text{ min}\cdot\text{mU/L}$ ; test 2,  $537 \pm 192$ ; test 3,  $490 \pm 143$ ), there was no significant

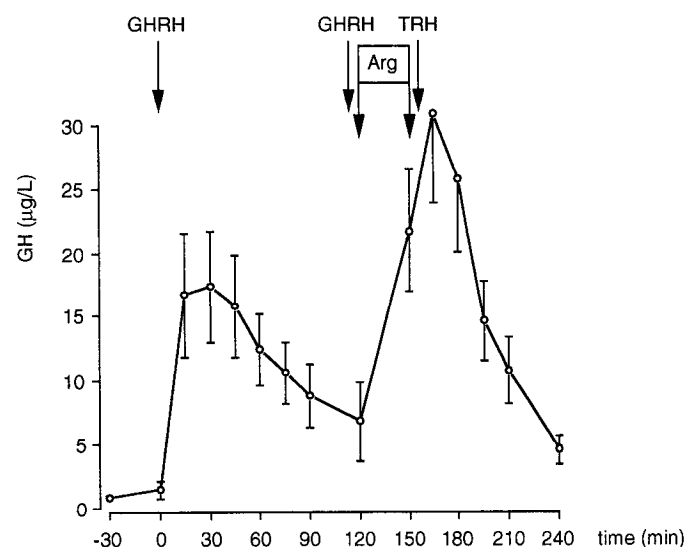


FIG. 4. Test 3 (GHRH/GHRH-Arg-TRH): mean GH levels before and after iv stimulation. Arrows indicate the times of GHRH (0 and 120 min) and TRH (150 min) injections. Arg infusion was given after 120 min over 30 min. Compared to AUC after iv GHRH injection at 0 min, GH release after combined GHRH/Arg/TRH stimulation was significantly greater ( $P < 0.02$ ).

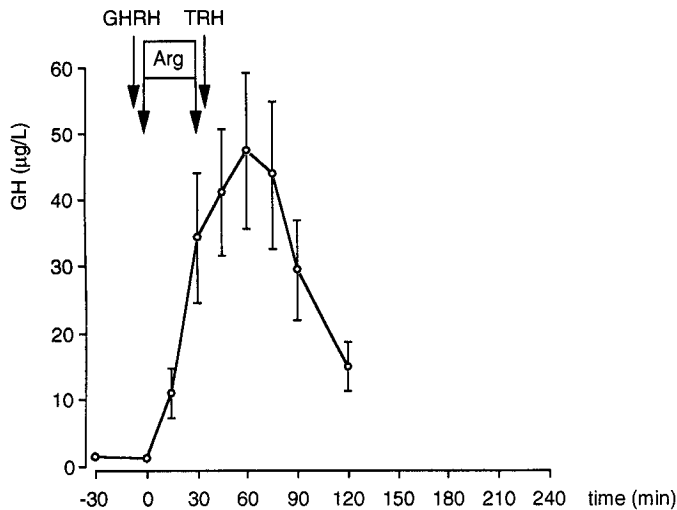


FIG. 5. Test 4 (GHRH-Arg-TRH): mean GH levels before and after iv stimulation. Arrows indicate the times of GHRH (0 min) and TRH (30 min) injections. Arg infusion was given over 30 min, immediately after GHRH stimulation. Mean GH concentrations increased significantly, reaching a maximum at 60 min.

difference in stimulated TSH secretion compared to that after TRH alone (test 5).

### Discussion

The present report confirms that the mean GH response to a second GHRH injection is significantly lower than the first GH increase. These findings are in agreement with data presented by Losa *et al.* (6), who showed that intermittent stimulation of GH secretion with 50 µg GHRH-(1-44) within 2 h as well as continuous stimulation by GHRH infusion over 2 h result in a decreased GH response in normal subjects. Shibasaki *et al.* (11) and Page *et al.* (12) described similar changes in plasma GH levels in normal men pretreated with 200 µg GHRH-(1-44). To clarify the mechanism of persistent GHRH-induced GH hypersecretion, additional studies during 24-h GHRH infusion were performed (13, 14). Continuous sc infusions of GHRH increased GH peak frequency during both the day and the night, but mean GH levels and AUC increased only during the daytime hours (14). It was speculated that the decrease in nocturnal GH peak amplitude and the decreased GH response after short term repeated stimulation with GHRH may be due to either a depletion of the readily available GH pool or, alternatively, to hypothalamic somatostatin antagonizing GHRH-stimulated GH secretion (6, 14). Vance *et al.* (15) found an augmentation of naturally occurring GH pulses during continuous infusion of GHRH and concluded that these GH pulses are the result of GHRH secretion associated with a diminution or withdrawal of somatostatin secretion. Ross *et al.* (16) showed that GH may regulate its own secretion, probably at the hypothalamic or pituitary level, via changes in hypothalamic somatostatin secretion. The results observed by Shibasaki *et al.* (11) and Ghigo *et al.* (17) also suggested that a desensitization of GHRH receptors in somatotrophs and/or enhanced somatostatin release induced by an increase in GH levels after a previous GHRH injection may be involved in the mechanism by which the prior GHRH injection suppresses the GH re-

sponse to the second GHRH bolus. Arg is known to stimulate GH secretion by suppression of hypothalamic somatostatin (9). We demonstrated that Arg administered together with GHRH led to higher serum GH levels than a single dose of GHRH or Arg alone and concluded that the stimulatory effect of Arg was mediated by suppression of endogenous hypothalamic somatostatin secretion (9). Further support of this hypothesis was given by Ghigo *et al.* in 1990 (18), who showed that both Arg and pyridostigmine induce a clear GH increase in children with familial short stature by the same mechanism, namely suppression of somatostatin release. These data in normal adults were confirmed in our study. In contrast, Magnan *et al.* (19) demonstrated a direct effect of neostigmine on hypothalamic GHRH release in rams and showed a synergistic effect of GHRH and neostigmine on GH secretion. These differences from human results are probably due to species differences and may be explained by the use of a nonmaximal GHRH stimulation dose in rams. Corresponding to the reports by Page *et al.* (12) and Ghigo *et al.* (17), we showed that after prior stimulation with GHRH, the second GH response (120 min) is not blunted when performed with Arg. Our findings also demonstrate that decreasing GH responses to a short term repeated GHRH bolus can be partially avoided by a combined stimulation with GHRH-Arg. The GH response to combined administration of GHRH-Arg after previous GHRH stimulation is even higher than the sum of the individual GH responses to GHRH only and Arg only, showing that Arg administration potentiates GH increase even after prior GHRH exposure. Without prior GHRH administration, this potentiation was significantly higher, which shows that suppression of hypothalamic somatostatin tonus by Arg could only partially restore the diminished GH response after prior GHRH stimulation or was not complete. This indicates that the decreased GH response after repeated GHRH administration may be partially induced by elevated somatostatin tonus and partially by exhaustion of the readily releasable GH pool.

Confirming our observations (9), Merola *et al.* (20) demonstrated that Arg infusion potentiates the TSH responsiveness to TRH in healthy subjects by antagonizing the inhibitory effect of somatostatin on TSH secretion (21). Although the TSH increase was significantly higher after stimulation in test 4 than after TRH alone, there was no significant difference between tests 2 and 3, probably due to the small number of subjects and to interindividual fluctuations of the TSH response. Additionally, a blunting influence of long term GHRH stimulation on TRH-stimulated TSH (tests 1, 2, and 3) cannot be excluded, masking the effect of enhanced somatostatin tonus on stimulated TSH secretion.

In conclusion, we propose that a decreased GH response to repeated GHRH injection may be due to an elevated hypothalamic somatostatin secretion, which can be suppressed by Arg, and to a limitation of the ready available GH pool in the human pituitary.

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