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# Growth Hormone/Insulin-Like Growth Factor-1 Response to Acute and Chronic Growth Hormone–Releasing Peptide-2, Growth Hormone–Releasing Hormone 1-44$NH_2$ and in Combination in Older Men and Women with Decreased Growth Hormone Secretion

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**To better appreciate the interactions of GHRP-2 and GHRH 1-44$NH_2$ on the release of GH in normal adult men and women with decreased GH secretion and low serum IGF-1 levels, a series of acute and chronic studies have been performed (*n* = 5 men, 5 women). The acute iv bolus GH responses of these subjects to the two peptides alone and together suggest that the decreased GH secretion may be primarily due to a deficiency of the natural endogenous GHRP, ghrelin, rather than a decreased secretion of endogenous GHRH or excess secretion of SRIF. To determine whether the low GH response to GHRH was due to a limited capacity of pituitary to release GH, higher dosages of GHRP-2 alone were administered. At a dose of 1 μg/kg GHRP-2 the GH response was essentially the same as that elicited by 1 μg/kg GHRH + 0.1 μg/kg GHRP-2 while the GH response to 10 μg/kg GHRP-2 sc was about twice as high in both men and women. Although these subjects have a limited pituitary capacity to release GH, which is also an indication of decreased GH secretion in the presence of low serum IGF-1 levels, this alone would not explain the low GH response to GHRH. Furthermore, the finding that a low dose of 0.1 μg/kg GHRP-2 augments the GH response to 1 μg/kg GHRH is strongly against an excess secretion of SRIF. Twenty-four hour profiles of GH secretion during placebo, GHRP-2, and various doses of GHRH alone and together with GHRP-2 were studied. In addition, 1 μg/kg/h GHRP-2 was infused continuously sc to these subjects for 30 d. The normal pulsatile secretion of GH as well as the serum IGF-1 level was increased after 24 h and remained elevated for 30 d. With a deficiency of endogenous GHRH, the GH response of GHRP-2 would be little to none, while in subjects with a deficiency of the natural GHRP, the GH response to GHRH would be more attenuated. Thus, in chronic deficiency the GH response would be expected to depend on the degree of the capacity of the pituitary to release GH as well as the type(s) of hormonal deficiency.**

**Key Words:** GHRP-2; GHRH; continuous infusion; GH pathophysiology; decreased GH and IGF-1; normal elderly

## Introduction

The dimensions of the growth hormone–releasing peptide (GHRP) saga are continuing to progress and in all the right directions but not necessarily ones that most of us readily would have predicted. The GHRP receptor has been cloned and has been identified in all the major anatomic sites that would indicate that this peptide is involved in the physiologic regulation of growth hormone (GH) secretion *(1–3)*.

Isolation and identification of the probable natural GHRP hormone, ghrelin, indeed is the current excitement. This outstanding accomplishment was published by Kojima et al. *(4)* in the December 9, 1999 issue of *Nature*. The chemistry and primary site of origin are novel. The 28 amino acid ghrelin has a covalently linked octanoyl group via an ester linkage to the hydroxyl group of the $Ser^3$ residue, which is an absolute requirement for GH-releasing activity. Although ghrelin has been identified in the ventral lateral part of the arcuate nucleus of the hypothalamus, its major site of origin surprisingly appears to be the stomach. Understanding how the overall GHRP system functions will be an immediate objective. The origin and secretion of ghrelin from the stomach becomes another impetus to reveal the possible physiologic peripheral actions as well as

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**Table 1**
High GH Response of 10 µg/kg sc GHRP-2

| Subject[a] | GHRP-2 10 µg/kg sc Peak GH µg/L | AUC·4 | GHRH 1.0 µg/kg iv Peak GH µg/L | AUC·4 | GHRP-2 + GHRH 1.0 + 0.1 µg/kg iv Peak GH µg/L | AUC·4h |
|---|---|---|---|---|---|---|
| 1 | 358 | 23,173 | 6 | 540 | 308 | 15,031 |
| 2 | 179 | 15,285 | 15 | 1122 | 101 | 9629 |
| 3 | 136 | 12,409 | 10 | 670 | 91 | 6277 |
| 4[b] | 137 | 9767 | 11 | 520 | 172 | 5915 |
| 5 | 109 | 8790 | 5 | 356 | 21 | 723 |
| 6 | 86 | 7079 | 2 | 99 | 5 | 245 |
| 7 | 81 | 6807 | 4 | 330 | 26 | 1298 |
| 8 | 92 | 6379 | 6 | 417 | 43 | 2710 |

[a]Age = 59 ± 4 yr; sex = 7M/1F; IGF-1 = 94 ± 8; BMI = 24 ± 1; 0.1 µg/kg GHRP-2 peak = 12 ± 4/AUC·4 h = 619 ± 217.
[b]Female.

a challenge to reveal how the peripheral secretion might actually regulate the physiologic secretion of GH via a central action on the hypothalamic-pituitary unit.

In previous studies, the acute iv bolus GH response to GHRP-2 and GH-releasing hormone (GHRH) alone and together suggested that some older normal men and women with decreased serum insulin-like growth factor-1 (IGF-1) levels and decreased GH secretion have a deficiency of the natural GHRP hormone rather than decreased secretion of GHRH or excess secretion of SRIF *(5)*. To better appreciate the interactions of GHRP-2 and GHRH on the release of GH in normal older men and women with low serum IGF-1 levels and decreased GH secretion, we conducted a series of acute and chronic studies.

## Results

In the first series of studies recorded in Table 1, 10 µg/kg sc GHRP-2 was administered to 45 normal older men and women with low serum IGF-1 levels (<125 µg/L) in order to assess the pituitary GH stores by maximal stimulation with high-dose GHRP-2. Low, moderate, and high GH responses were categorized according to the GH area under the curve (AUC)·4 h, i.e., <1300, 1300–5000, and >5000, respectively. Most of the GH responses were in the moderate group (data not recorded). Results of eight subjects with high GH responses to the 10 µg/kg sc GHRP-2 dose are shown in Table 1. Table 1 also gives the GH responses of these eight subjects to acute 1 µg/kg iv bolus GHRH, which were relatively low. Acute iv administration of 0.1 µg/kg of GHRP-2 + 1 µg/kg of GHRH markedly enhanced the GH response of GHRH in five of eight subjects. As recorded, the mean GH response to 0.1 µg/kg of GHRP-2 alone was 12 ± 4 µg/L.

Subjects 1 and 4 of Table 1 were further studied by 24-h and/or 30-d continuous infusion of the peptides. Figure 1

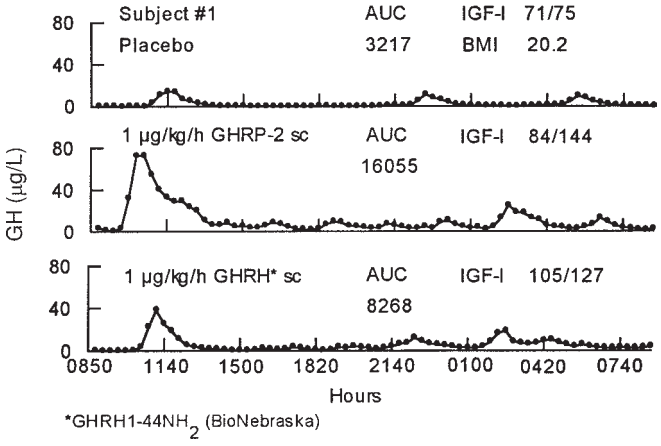


**Fig. 1.** GHRP-2 continuous infusion for 24 h in a normal 61-yr-old man.

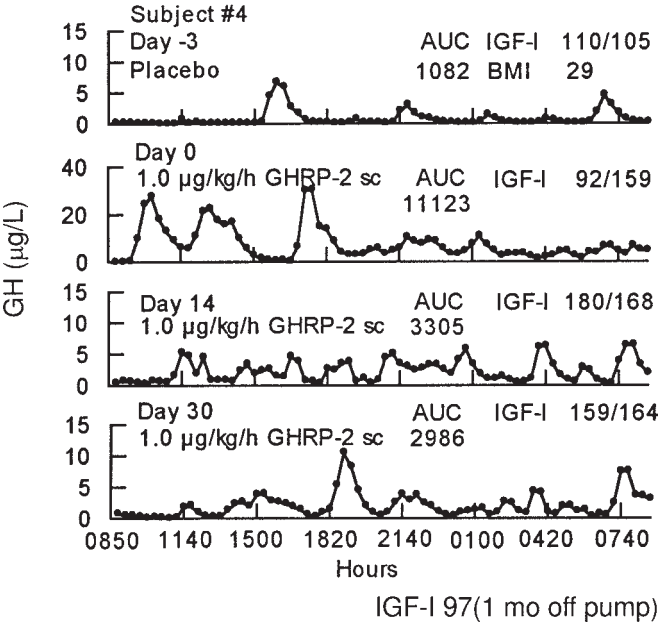


**Fig. 2.** GHRP-2 continuous infusion for 30 d in a normal 49-yr-old woman.

gives the 24-h results of subject 1. They were surprising because the 24-h GH secretion during placebo was not decreased (GH AUC-3217) and the serum IGF-1 level was very low (70–75 µg/L). Particularly notable, however, was that only three spontaneous GH peaks were observed over the 24-h period, possibly indicating the importance of the frequency in addition to the magnitude of the GH pulses in maintaining the normal level of serum IGF-1. Figure 1 also shows the results of the 24-h infusion of 1 µg/kg·h GHRP-2 or GHRH. Both peptides markedly increased the frequency and pulsatile secretion of GH over the 24-h period and raised the serum IGF-1 level.

Figures 2 and 3 present the results of subject 4 during the 24-h infusion of placebo, GHRP-2, GHRH and GHRP-2 + GHRH. The placebo 24-h GH AUC value of ~1000 is in the

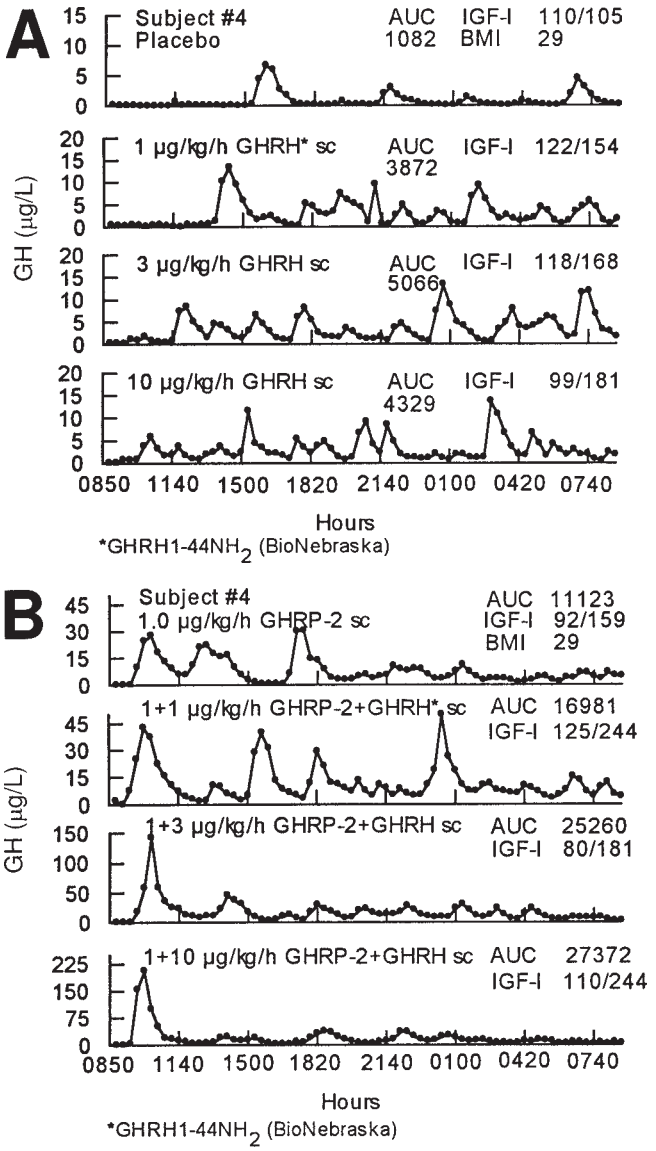


**Fig. 3**. GHRP-2 continuous infusion for 24 h in a normal 49-yr-old woman. A = Peptide alone, B = Peptide combination.

low normal range; however, only two spontaneous GH pulses appear normal in magnitude. The 24-h infusion of both GHRP-2 and GHRH alone increased the frequency and magnitude of the GH pulses as well as the IGF-1 level. When the two peptides were infused together, GH was additively or synergistically released and serum IGF-1 levels increased further. As previously suggested, the possibility is being considered that subjects 1 and 4 are in an early stage of a deficiency of the natural GHRP hormone.

In the second series of studies, recorded in Figs. 4–7 and Tables 2–5, 10 normal older men and women with low serum IGF-1 levels (five men and five women) received acute iv bolus administration of GHRP-2 and GHRH alone and together, 24-h infusion of GHRP-2 and GHRH alone and together, and chronic infusion of GHRP-2 for 30 d. By this approach, the acute and chronic results could be compared and evaluated in the same subject. The mean age, body mass index (BMI), and IGF-1 levels were 65 ± 3, 24.4 ± 1, and 95.4 ± 12 (men) and 67.4 ± 5.2, 26.8 ± 1.8, and 87.4 ± 9.5 (women), respectively.

The results in Fig. 4 demonstrate that after acute iv bolus administration in men, the GH response to 1 μg/kg of GHRH and 0.1 μg/kg of GHRP-2 alone were low whereas 0.1 μg/kg of GHRP-2 + 1 μg/kg of GHRH synergistically released GH. The acute GH response to 1 μg/kg of GHRP-2 was essentially the same as that of the combined peptides. A moderate GH response was induced by 10 μg/kg of GHRP-2 subcutaneously, indicating that pituitary GH stores were decreased but not to the degree that would account for the low GH-releasing action of the peptides at the dosages of 0.1 and 1 μg/kg alone and together. The acute GH responses of the five women (Fig. 5) were essentially the same as those of the men but the variation was much greater.

The 24-h continuous sc infusion results of the GH AUC as well as the IGF-1 levels of the combined 10 subjects (5 men and 5 women) are given in Tables 2 and 3. Infusion of both peptides alone increased the GH release and IGF-1 level. As shown in Table 2, 1 μg/kg·h of GHRP-2 released more GH than GHRH at the dosages of 1, 3, and 10 μg/kg·h. The GH release was essentially the same by all three dosages of GHRH. Serum IGF-1 levels were increased by all dosages of the peptides and were not significantly different among the four peptide groups. When GHRP-2 and GHRH were administered together (Table 3), GH was increased additively or synergistically and IGF-1 levels were higher than when the peptides were administered alone. GH release was additively increased by combined 1 + 1 μg/kg·h of GHRP-2+GHRH and synergistically increased by 1 + 3 and 1 + 10 μg/kg·h. GH release induced by the 1 + 3 and 1 + 10 μg/kg·h dosages was the same. Although the combined peptides increased serum IGF-1 more than GHRP-2 or GHRH alone, all three dose combinations of the peptides induced essentially the same degree of rise in the serum IGF-1 level.

The results of the 30-d sc continuous infusion of 1 μg/kg·h GHRP-2 to the same 10 subjects are given in Table 4. The GH release was increased over the entire 30 d. Compared to the effects of GHRP-2 on d 0, the GH release was less on d 14 and d 30; however, the IGF-1 remained elevated at essentially the same level over the entire 30-d period. Compared with the placebo day, the pulsatile secretion of GH increased during the entire 30-d period (data not recorded).

The results recorded in Table 5 demonstrate that the 24-h mean serum cortisol levels did not change during the 30-d infusion of GHRP-2 or the 24-h infusion of GHRH and GHRP-2+GHRH. Also, as seen in Figs. 6 and 7, there was no change in the normal 24-h circadian rhythm of cortisol secretion in the man (Fig. 6) or woman (Fig. 7) during the

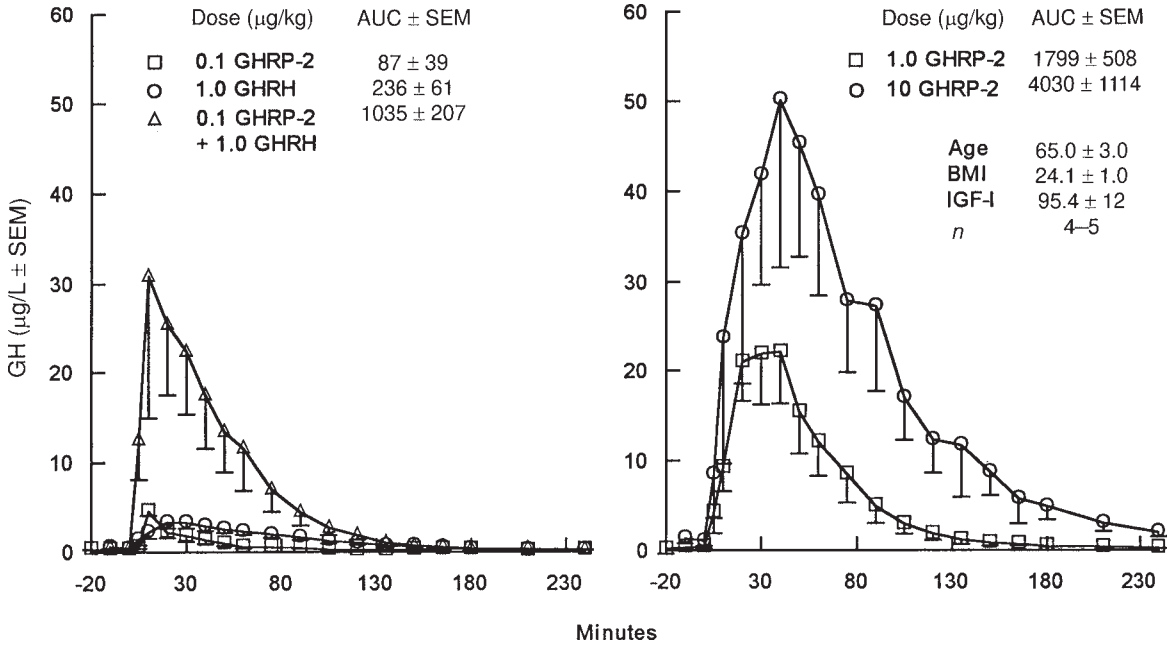


**Fig. 4.** Possible natural GHRP hormone deficiency in normal older men ($n$ = 5). Values are mean ± SEM.

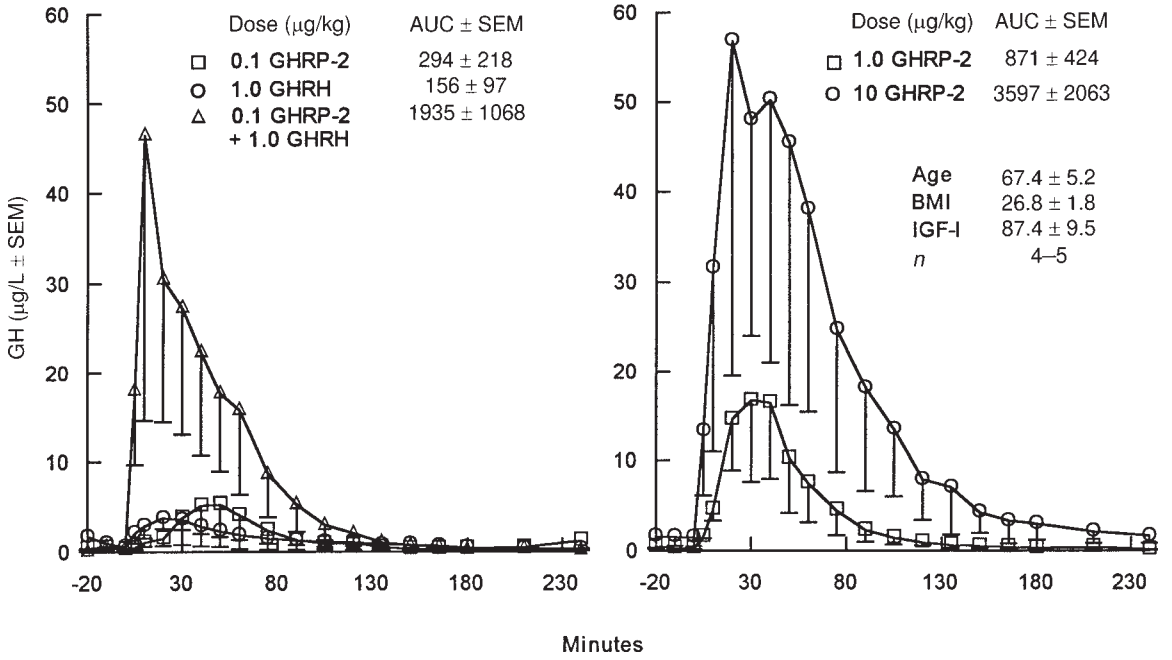


**Fig. 5.** Possible natural GHRP hormone deficiency in normal older women ($n$ = 5). Values are mean ± SEM.

30-d continuous sc infusion of GHRP-2. The cortisol 24-h circadian rhythm was measured in all 10 subjects and found to be unchanged (data not recorded).

## Discussion

We hypothesized that normal older men and women with low serum IGF-1 levels would have low GH responses even to the acute sc maximal stimulus of 10 μg/kg of GHRP-2. In the group of 45 older men and women, it was determined that 37 subjects did have decreased GH responses, most of which were in the moderately decreased category. However, the GH responses of eight subjects (Table 1) were unexpectedly very high and are envisioned to represent a possible early stage of deficiency of the natural GHRP hormone.

In these eight subjects, the marked discrepancy between the GH responses to maximal dosages of GHRH and GHRP-2 appears to be the result of an impaired pituitary

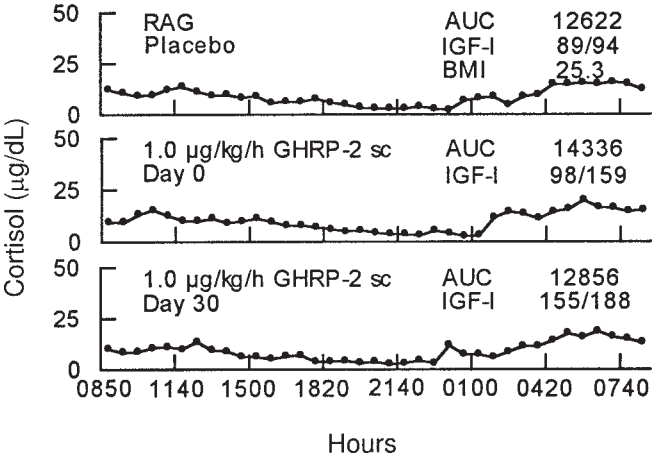


**Fig. 6.** Cortisol levels after GHRP-2 continuous infusion for 30 d in a 67-yr-old man.

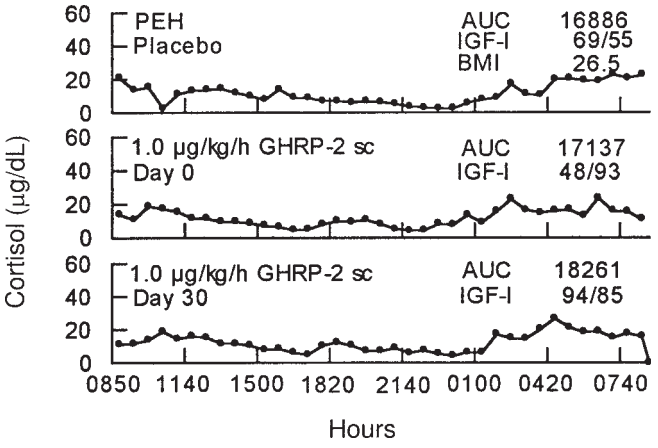


**Fig. 7.** Cortisol levels after GHRP-2 continuous infusion for 30 d in a 76-yr-old woman.

GH-releasing mechanism that greatly attenuates the action of GHRH on GH release. What is so dramatic is the reversal of this impaired pituitary GH-releasing action of GHRH by the low 0.1 µg/kg dose of GHRP-2 in five of the eight subjects, which suggests that a deficiency of the natural GHRP hormone may be the missing link responsible for the impaired GH response. GHRH secretion is speculated to be normal or even possibly increased in these subjects and thus continues to effectively stimulate GH synthesis but ineffectively release GH. Presumably the concomitant increased synthesis and decreased release of GH results in large pituitary stores of GH that, depending on the amount of GH stored, can be readily released by restoring the natural GHRP hormone or by administering an unnatural GHRP such as GHRP-2. The greater the degree and duration the GHRH secretion continues to be normal or increased and the more the endogenous GHRP hormone secretion is decreased, the greater the pituitary GH stores will be increased and the GH release decreased.

Whether a deficiency in the natural GHRP hormone begets a deficiency in GHRH is unknown. Most of the older men and women we have studied with low serum IGF-1 levels also have had decreased rather than high GH responses to 10 µg/kg of sc GHRP-2. Since indirect evidence indicates that the pituitary stores of GH are decreased in the majority of these subjects, our current working hypothesis is that most of the time deficiencies in both the natural GHRP and GHRH coexist because GHRH alone presumably is the regulator of GH synthesis. So far we have found little evidence for a selective deficiency in GHRH alone that would result in a low GH response to GHRP-2 and, depending on the degree and duration of the GHRH deficiency as well as the pituitary GH stores, a moderately low, low, or absent GH response to GHRH.

It can be seen from the GH-releasing results of the 10 subjects presented in Figs. 1 and 2 that the continuous 24-h infusion of GHRH alone effectively increases the pulsatile secretion of GH and raises serum IGF-1 levels. GHRH also induced pulsatile secretion in all 10 subjects recorded in Table 2 over the entire 24 h. Because of the postulated deficiency in the natural GHRP hormone, the GH release mechanism is envisioned to be inefficient and relatively ineffective as well as nonphysiologic, as indicated by the abnormal spontaneous pulsatile secretion of GH during administration of the placebo and the low GH response to acute iv bolus 1 µg/kg GHRH. A seemingly relevant difference between the acute and chronic studies is the administration of GHRH acutely by iv bolus vs its prolonged continuous infusion. Since the GHRH infusion compared to acute iv bolus GHRH much more effectively released GH, the magnitude and pattern of secretion of GHRH may be essentially normal but yet its pituitary GH-releasing action may be inappropriate to maintain normal pulsatile GH secretion because of a deficiency in the natural GHRP hormone. Alternatively, it may be possible that these subjects secrete GHRH but not in an optimal physiologic pattern to support normal pulsatile GH secretion. However, with normal secretion of GHRH, decreased pituitary GH stores would not be expected. A possible exception to this explanation would be if normal secretion of GHRH were unable to maintain normal pituitary synthesis of GH in the presence of a chronic deficiency in the natural GHRP hormone. Some studies in rats have indicated that GHRP increases pituitary GH mRNA levels *(6)*. In addition, GHRP augments the action of GHRH on pituitary cyclic adenosine monophosphate levels in rats, which, in turn, may result in the increased synthesis of pituitary GH *(7)*. Obviously, in the future, a number of different ideas and possibilities will be considered and investigated to explain the results of pituitary GH synthesis.

Provided that the natural GHRP hormone is not found to stimulate pituitary GH synthesis to a clinically meaningful degree, collectively the acute GH results of the 10 subjects

**Table 2**
24 h Continuous sc Infusion in Five Men and Five Women ($n$ = 10)[a]

| | Dose µg/kg·h | AUC·24 h GH µg/L ± SEM | | | IGF-1 µg/L ± SEM | | |
|---|---|---|---|---|---|---|---|
| | | | *p* | *p* | | *p* | *p* |
| Placebo | — | 740 ± 109 | — | 0.003 | 97 ± 10 | — | 0.001 |
| GHRP-2 | 1 | 4668 ± 904 | 0.003 | — | 155 ± 9 | <0.001 | — |
| GHRH | 1 | 2103 ± 434 | 0.023 | 0.032 | 136 ± 14 | 0.040 | 0.182 |
| GHRH | 3 | 3081 ± 423 | <0.001 | 0.080 | 139 ± 15 | <0.001 | 0.080 |
| GHRH | 10 | 2815 ± 300 | <0.001 | 0.083 | 173 ± 16 | <0.001 | 0.544 |

[a]Age 67 ± 2.9yr; BMI 26 ± 1.2

**Table 3**
24 h Continuous sc Infusion in Five Men and Five Women ($n$ = 10)[a]

| | Dose µg/kg·h | AUC·24 h GH µg/L ± SEM | | | IGF-1 µg/L ± SEM | | |
|---|---|---|---|---|---|---|---|
| | | | *p* | *p* | | *p* | *p* |
| Placebo | — | 740 ± 109 | — | 0.003 | 97 ± 10 | — | <0.001 |
| GHRP-2 | 1 | 4668 ± 904 | 0.003 | — | 155 ± 9 | <0.001 | — |
| GHRP-2 ± GHRH | 1 + 1 | 7493 ± 1275 | 0.012 | 0.012 | 181 ± 12 | <0.001 | 0.031 |
| GHRP-2 ± GHRH | 1 + 3 | 10,868 ± 1778 | 0.001 | 0.002 | 192 ± 10 | 0.001 | 0.001 |
| GHRP-2 ± GHRH | 1 + 10 | 11,146 ± 2066 | 0.001 | 0.003 | 211 ± 10 | <0.001 | <0.001 |

[a]Age 67 ± 2.9; BMI- 26 ± 1.2; $p$ = <0.001 (GH AUC of GHRH alone vs all combined peptides); $p$ = 0.015, 0.008, 0.002 low dose to high dose, respectively (IGF-1 of GHRH alone vs combined peptides).

given in Figs. 4 and 5 have been interpreted to reflect that a deficiency in the endogenous natural GHRP hormone and endogenous GHRH probably coexist. Reversal of the low GH response by administration of low-dose GHRP-2 with high-dose GHRH has not been explainable by a selective deficiency in endogenous GHRH secretion or excess SRIF secretion *(8)*. Even if GHRH were secreted in substantial amounts in these subjects, the impaired GH-releasing activity of high-dose exogenous GHRH indicates that it would be ineffective in releasing GH. Also, the GH response to 1 µg/kg of GHRP-2 alone supports that endogenous GHRH is being secreted because without the presence of endogenous GHRH, the GH response to GHRP is very low in humans and animals. In our studies, it is apparent that the pituitary GH stores are not the limiting factor determining the low GH response to acute iv bolus GHRH. Presumably the GH stores are being maintained mainly by the secretion of endogenous GHRH or possibly by GHRH together with the natural GHRP hormone. As previously hypothesized, low-dose GHRP, which is considered to reflect the physiologic rather than pharmacologic action of the natural GHRP hormone, requires endogenous GHRH permissively rather than as the mediator of the induced GH release. In part, the low-dose GHRP GH-releasing action is envisioned to be mediated by hypothalamic U-factor (unknown factor) as well as a direct GHRP GH-releasing action on the somatotrophs. U-factor is considered to augment the pituitary GH-releasing action of GHRH *(8)*. By contrast, high pharmacologic doses of GHRP presumably do release endogenous GHRH and, in part, this is the reason it so dramatically releases GH in vivo *(5)*.

In ambulatory normal men ($n$ = 5) and women ($n$ = 5) with decreased serum IGF-1 levels and 24-h GH secretion, 30-d continuous sc infusion of 1 µg/kg of GHRP-2 increased GH secretion and serum IGF-1 levels over the entire infusion period (Table 4). Even though GH secretion decreased on d 14 and 30, it was pulsatile and increased above the values on the placebo day. Furthermore, serum IGF-1 levels remained elevated by twofold or greater over the placebo day vs d 0, 14, and 30. One month after stopping the infusion, the serum IGF-1 decreased to the baseline level.

Note that on d 14 and 30 of the GHRP-2 infusion, the 24-h secretion was sometimes relatively low, being only slightly higher than that of the placebo day whereas the IGF-1 level remained substantially elevated at the same level after infusing GHRP-2 for 24 h on d 0. This equilibrium state is considered to be due not only to the negative feedback effect of IGF-1 on GH secretion but also the par-

**Table 4**
30 Day Continuous sc Infusion in Five Men and Five Women ($n = 10$)[a]

| | Dose µg/kg·h | | AUC·24 h GH µg/L ± SEM | | | IGF-1 µg/L ± SEM | | |
|---|---|---|---|---|---|---|---|---|
| | | | | *p* | *p* | | *p* | *p* |
| Placebo | — | Day–3 | 740 ± 109 | — | 0.003 | 97 ± 10 | — | <0.001 |
| GHRP-2 | 1 | Day 0 | 4668 ± 904 | 0.003 | — | 155 ± 9 | <0.001 | — |
| GHRP-2 | 1 | Day 14 | 1712 ± 235 | <0.001 | 0.005 | 161 ± 14 | <0.001 | 0.479 |
| GHRP-2 | 1 | Day 30 | 1719 ± 230 | <0.001 | 0.006 | 148 ± 11 | <0.001 | 0.427 |

[a]Age 67 ± 2.9 yr; BMI-26 ± 1.2; IGF-1 = 87 ± 15 (1 mon off pump).

**Table 5**
Cortisol Levels during GHRP-2 continues Infusion in Five Men and Five Women ($n = 10$)[a]

| Group condition | Peptide | | Dose µg/kg·h | Cortisol µg/dL ± SEM |
|---|---|---|---|---|
| 30-d Infusion | Placebo | Day–3 | — | 10.8 ± 1.5 |
| in Men | GHRP-2 | Day 0 | 1 | 12.0 ± 2.3 |
| | | Day 30 | 1 | 11.3 ± 1.9 |
| 30-d Infusion | Placebo | Day–3 | — | 16.6 ± 2.8 |
| in Women | GHRP-2 | Day 0 | 1 | 16.9 ± 3.3. |
| | | Day 30 | 1 | 16.3 ± 1.9 |
| 24-h Infusion | GHRH(G) | | 3 | 11.0 ± 2.0 |
| in Men | GHRP-2 + G | | 1+ 3 | 10.4 ± 3.0 |
| 24-h Infusion | GHRH (G) | | 3 | 14.9 ± 2.9 |
| in Women | GHRP-2 ± G | | 1 + 3 | 14.2 ± 2.0 |

[a]Cortisol was determined every 40 min over 24 h (n = 36). *p* value >0.05.

tial desensitization of the GH-releasing action of GHRP. It appears that elevated IGF-1 levels once induced by GHRP-2 can be maintained with low levels of pulsatile GH secretion. In support of this conclusion is the return to the baseline of the IGF-1 levels 1 mo after stopping the GHRP-2 infusion. Recently studies have been reported that exogenous IGF-1 more effectively inhibits the GH response of GHRP (hexarelin) than GHRH *(9)*.

When GH was measured during the day vs night (i.e., 9:00 AM to 9:00 PM vs 9:00 PM to 9:00 AM, equal amounts of GH were secreted on d 0 and slightly more during the night on d 14, and by d 30 the night/day GH ratio was significantly greater (data not shown). Thus, prolonged continuous GHRP-2 infusion supports the normal circadian secretion of GH. When the acute iv bolus GH response to GHRH was determined before and during the 30-d GHRP-2 infusion, the acute GHRH GH response remained the same or was increased (data not shown). Previously, the acute iv bolus GHRP GH response had been demonstrated to be decreased in young men during a 36-h continuous infusion of GHRP whereas the GH response to acute iv bolus GHRH was increased *(10)*.

In the 10 older men and women with decreased IGF-1 levels (mean 93 ± 8), the effects on 24-h GH secretion and serum IGF-1 levels also were determined during continuous sc infusion of 1, 3, and 10 µg/kg·h of GHRH alone and with 1 µg/kg·h of GHRP-2 (Tables 2 and 3). At these dosages, the combined peptides additively released GH by 1 + 1 µg/kg·h and synergistically by 1 + 3 and 1 + 10 µg/kg·h of GHRP-2+GHRH. Depending on the dose and/or peptide or peptide combination as well as the duration of administration, there was a variable effect on the magnitude of the 24-h GH secretion and serum IGF-1 level.

The final results recorded in Table 5 show that the continuous 24-h infusion of GHRP-2 and GHRH alone and together did not increase the mean 24-h serum cortisol levels. Furthermore, the results in Figs. 6 and 7 show that during the continuous 30-d sc infusion of 1 µg/(kg·h) of GHRP-2, the normal 24-h circadian rhythm of serum cortisol was maintained.

Our conclusions and projections are based on the premise that in subjects with a deficiency in endogenous GHRH, the GH response to GHRP-2, but not GHRH, would be little to none and more impaired than the GH response to GHRH, whereas in subjects with a deficiency in the natural GHRP hormone, the GH response to GHRH compared to GHRP-2 would be more attenuated. Although this would reflect the GH response during acute or early deficiency in these

hormones, in chronic deficiency the GH response would be expected to depend on the degree of the pituitary's capacity to release GH as well as the type of hormonal deficiency, i.e., endogenous natural GHRP vs GHRH or both of these hormones.

Results in normal older men and women with decreased serum IGF-1 levels and GH secretion have been obtained that support the possibility that continuous administration of GHRP-2 and GHRH alone and together may be uniquely utilized to "tailor" the physiologic secretion of GH and increase the serum IGF-1 level.

## Materials and Methods

All protocols were approved by the Committee on Use of Human Subjects at Tulane University Medical School, and each subject enrolled into the study gave written informed consent.

GHRP-2 and GHRH 1-44$NH_2$ were administered alone and together to normal older men and women with low serum IGF-1 levels (<125 μg/L). Acute studies were performed by iv bolus and chronic studies by continuous sc infusion of the peptides for 24 h or 30 d via an ambulatory pump. The acute iv bolus dosages were 0.1 and 1 μg/kg of GHRP-2 and 1.0 μg/kg of GHRH as well as 0.1 μg/kg of GHRP-2 + 1 μg/kg GHRH. In addition to these four acute iv studies, 10 μg/kg of GHRP-2 was administered as a single acute sc bolus. The dosages of the sc infusion studies consisted of 1 μg/kg·h of GHRP-2 for 24 h or 30 d, while GHRH was administered at 1, 3, and 10 μg/kg·h for 24 h alone and together with 1 μg/kg·h GHRP-2. In the acute studies, blood was collected every 10–30 min over 4 h, and for the chronic infusion studies blood was collected every 20 min. IGF-1 was measured before and after the administration of peptide. Serum cortisol was measured over the 24-h infusion period at 40-min intervals. For the 30-d GHRP-2 infusion studies, GH was measured every 20 min over 24 h on day –3 during placebo and on d 0, 14, and 30 during the administration of peptide.

Serum GH concentrations were measured in duplicate by the GH chemiluminescence assay (Nichols Institute Diagnostics, San Juan Capistrano, CA; sensitivity of 0.005 μg/L). The median inter- and intraassay coefficients of variation were 7.4 and 6.7%, respectively. Serum IGF-1 (Nichols Institute Diagnostics) and cortisol (ICN, Costa Mesa, CA) concentrations were measured in duplicate by radioimmunoassay. The AUCs were calculated by the trapezoidal rule.

### *Statistical Analyses*

Statistical analyses were performed with SigmaStat for Science (SPSS, Chicago, IL). Data were analyzed by repeated measure analysis of variance (ANOVA) using a general linear model and multiple pairwise comparisons. GH peak, AUC, and IGF-1 levels within and between analyses were measured by within and between subject two-way ANOVA. Mean AUC was determined by the trapezoidal rule. Data are recorded as the mean ± SEM.

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