PREFACE

In *Human Growth Hormone: Research and Clinical Practice*, we have been fortunate to be able to convince many of the leaders in the field to write about the recent developments in the understanding of basic and clinical research in the field of human growth hormone. During the last few years, there have been major advances in this field, one that has been dramatically enhanced by the discovery of the growth hormone-releasing peptide. This spawned much novel research, and ultimately led to the cloning of the receptor for the growth hormone secretagogues. The understanding of the molecular biology, structure, and function of growth hormone and the growth hormone receptor complex has also set a new standard in the understanding of the structural biology of cell signaling.

Growth hormone secretagogues and GHRH offer new possibilities for the treatment of growth hormone deficiency states. Growth hormone has an important role, not only in stimulating growth, but also in the control of metabolism. With the major recent advance in understanding the molecular mechanisms of the growth hormone axis, it is now possible to identify molecular defects in the axis.

It is our intention that *Human Growth Hormone: Research and Clinical Practice* should serve as an up-to-date summary of the field and should be of benefit both to the basic and clinical researchers as well as clinical endocrinologists who are now beginning to use growth hormone, not only in growth hormone-deficient states in childhood, but in the adult and also for dealing with metabolic derangements associated with catabolic disease.

Roy G. Smith

2

GHRP Historical Perspective
Basic and Clinical

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INTRODUCTION

The growth hormone releasing peptides (GHRPs), in contrast to growth hormone releasing hormone (GHRH), were invented rather than discovered. "Reverse pharmacology," a term recently proposed by Michael Conn, was suggested to designate the developmental GHRP process *(1)*. GHRPs and their mimetics will undoubtedly have a clinical role in the future. Two immediate future objectives of salient importance will be isolation and identification of the putative native hormone for which GHRPs are mimetics and elucidation of its role in the physiological secretion of GH. Moreover, whether this hormone is involved in the pathophysiology of GH deficiency in children and adults is still to be determined.

HISTORICAL BACKGROUND

Between 1976–1980, during the development of GHRPs, a major impetus for the search was an unequivocal belief in the existence of a native (GHRH) in spite of the frustrations of unsuccessful, herculean efforts over a 15-year period (1962–1976). Interestingly, GH releasing activity was demonstrated in more than one partially purified fraction of porcine hypothalamic extracts, suggesting that perhaps more than one GHRH factor existed *(2–5)*.

Listed in Table 1 are the major GHRP milestones in chronological order. The first GHRP (DTrp^2) was developed in 1976 *(4,5)*. The amino-acid sequence is recorded in Table 2. Although DTrp^2 was not potent and was inactive in vivo, it released GH by a direct action on the pituitary. In addition, the GH action was specific in that LH, FSH, TSH, and PRL were not released (Fig. 1). This DTrp^2 pentapeptide, TyrDTrpGlyPheMetNH$_2$, evolved from the natural opiate Met enkephalin pentapeptide, TyrGlyGlyPheMet. Opiates and opiate peptides release GH via a hypothalamic action but not via a direct pituitary action. Furthermore DTrp^2 had no opiate activity.

Table 1
Major GHRP Milestones

1976–77	First GHRPs
1978–80	New types of GHRPs
1980	In vivo active GHRPs
1984	Projection of a new hormone(s)
1989–92	GH release in humans
1992	Increased pulsatile GH secretion, young men
1992	First nonpeptidyl GHRP
1994–95	Increased body-growth velocity, children
1995	Potent new types of GHRP
1996	GHRP receptor cloned

Table 2
Key GHRPs[a]

Active only in vitro[b]		Inactive in vitro[b]
1. TyrDTrpGlyPheMetNH$_2$	(DTrp^2)	1. TyrGly^{2}GlyPheMetNH$_2$
2. TyrAlaDTrpPheMetNH$_2$	(DTrp^3)	2. Trp
3. TyrDTrpDTrpPheNH$_2$	(DTrp^{2,3})	3. Phe
4. TyrDTrpAlaTrpDPheNH$_2$	(DTrp^{2}LTrp^4)	4. Pro
		5. Sar
		6. DVal
		7. DAla
		8. DLeu

[a]Reproduced with permission from ref. *64*.
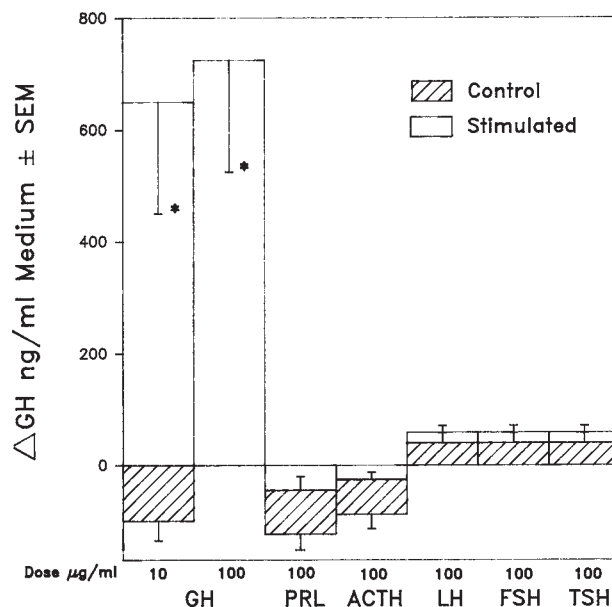[b]Dose: 100 µg/mL in vitro.

Fig. 1. Effect of TryDTrp^{2}GlyPheMetNH$_2$ on GH release in vitro. There was no effect on PRL, ACTH, LH, FSH, or TSH release. Pituitaries of 20-d-old female rats, $n = 6$, *$p = < 0.01$.

Table 3
The In Vitro and In Vivo Specificity of Activity of [His[1]Lys[6]]GHRP in Rats[a]

Peptide dose	GH	TSH	LH	FSH	PRL
μg/mL medium	In vitro (Δmg/mL serum ± SEM)				
—	–203 ± 109	–1372 ± 945	22 ± 11	248 ± 60	
0.03	2165 ± 407	–2308 ± 1230	9 ± 1	210 ± 67	
μg subcutaneously	In vivo (ng/mL serum ± SEM)				
—	6.1 ± 2.4	211 ± 32	0.05 ± 0.04	192 ± 23	4.7 ± 1.8
50	757 ± 40	210 ± 18	0.12 ± 0.04	199 ± 32	2.4 ± 0.2

[a]The in vitro studies (mean of 9 ± SEM) were performed using the pituitary incubate assay and the in vivo studies (mean of 10 ± SEM) using rats treated with 50 μg [His[1]Lys[6]]GHRP daily for 25 d sc at 1500 h. The aforementioned acute study was performed 24 h after the last injection of the peptide. Blood for hormone determinations was collected at +15 min after injection of saline or the peptide. Reproduced with permission from ref. *11*.

Between 1978 and 1980, four different major types of GHRPs were developed, including DTrp[2] (Table 2) *(6–9)*. Despite increased potency, none of these small peptides were active in vitro. Noteworthy was that GH releasing activity was strongly related to the position and stereochemistry of Trp residues. A series of detailed conformational studies by Momany helped to guide the development of the DTrp[2]AlaLTrp[4] sequence of GHRP, which was valuable in the development of the in vitro and in vivo active GHRPs, i.e., GHRP-6, -1, -2 *(10–15)*. From desensitization crossover studies, and from synergistic or additive effects of the GHRPs, evidence strongly indicated that the same receptor and molecular mechanism was activated by structurally different GHRPs. A surprising exception, which suggested finding the possibility of a GHRP receptor subtype, was that in sheep pituitary cell cultures where GHRP-2, but not GHRP-6, raised intracellular cAMP levels; furthermore, a GHRH antagonist inhibited the GHRP-2 GH response *(16)*.

In 1976–77, early results of DTrp[2] were considered indicative that this pentapeptide may be acting via the putative GHRH receptor *(4,9)*. Subsequent studies with GHRP-6 in 1980–81 reinforced the notion. However, following the isolation of a native growth hormone releasing factor and its structural elucidation in 1982, it became apparent that the releasing factor was a natural growth hormone releasing hormone (GHRH) and that GHRP acted via a different receptor. Because GHRPs had characteristics of hypophysiotropic hormones, it was proposed in 1984 that they might mimic another native hormone different from GHRH *(11)*.

Results in Table 3 show that GHRP-6 specifically releases GH in vitro and in vivo *(11)*. The in vivo results were obtained after immature female rats were injected with GHRP-6 or saline once or twice daily subcutaneously (sc) for 25 d. After chronic administration of GHRP-6, the GH response and specificity as well as the increase in body weight gain were maintained (Table 3 and Fig. 2).

Between 1981–88, the interrelationship between the actions of GHRP-6, GHRH, and opiates or opiate peptides were studied *(10,17–28)*. Desensitization crossover studies of these three GH-releasing secretagogues revealed the independent action of all three peptides because when the GH response of one secretagogue was desensitized the other one was fully active. When these secretagogues were combined and administered to rats GH was released synergistically, and when all three were administered together, the
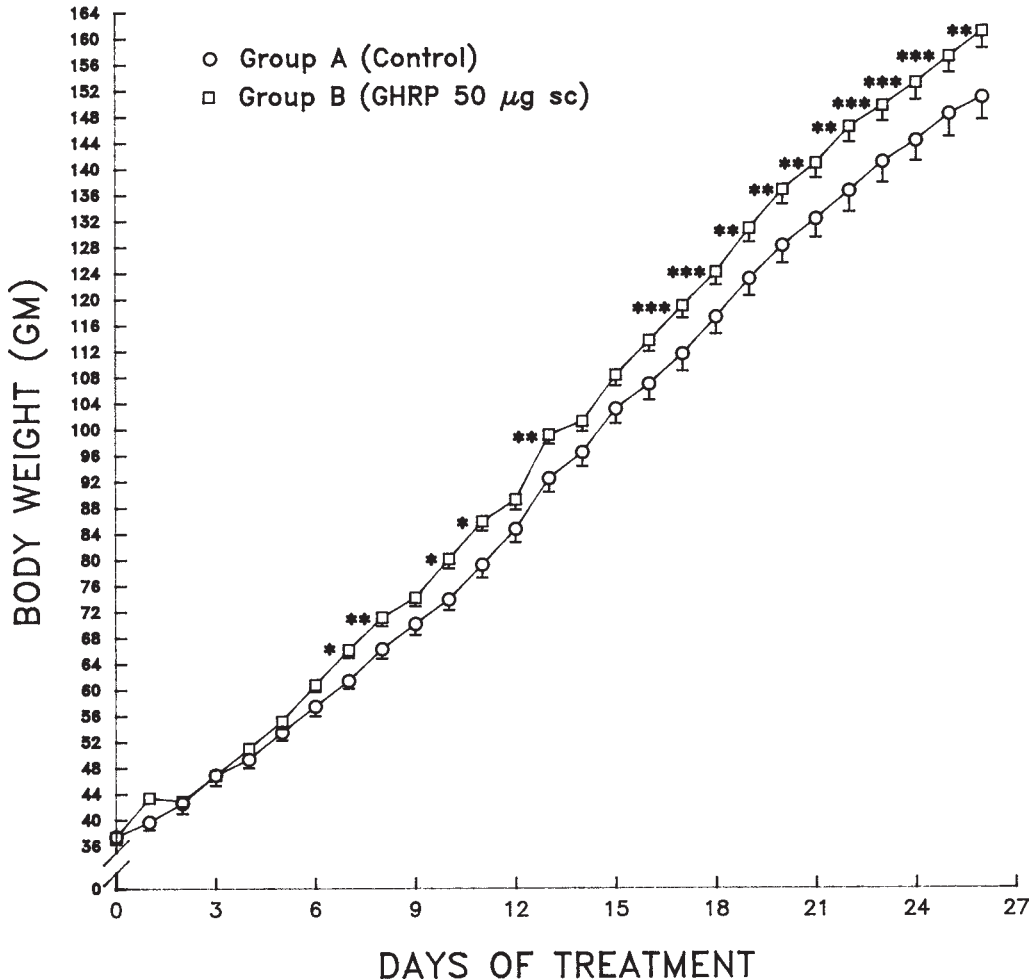
Fig. 2. Chronic treatment of immature female rats with [His^{1}Lys^6]GHRP. Initially immature female rats (16 d of age) were distributed among the mothers so that the BW in groups A and B would be the same, and treatment with saline **(A)** or the peptide **(B)** was started the next day. Saline or the peptide (50 µg) was injected sc daily at 1500 h for 25 d. Mean of 20 ± SEM. P values of treated vs untreated control group (A). *$p < 0.01$, **$p < 0.02$, ***$p < 0.05$. Reproduced with permission from ref. *11*.

synergism was even greater (Fig. 3A). Regardless of the apparent independent action of the three secretagogues, pretreatment with GHRH anti-serum markedly inhibited the GH response of each one of them (Fig. 3B). Complementary studies with somatotropin-release inhibiting factor (SRIF) antiserum pretreatment indicated that it increased the GH response to GHRP-6 and GHRH but not to the benzomorphan opiate 2549 or the opiate

Fig. 3. (A) In vivo studies on the synergistic release of GH in conscious 26-d-old female rats. At zero time, saline, 10 µg GHRP, 10 µg GHRH, 10 µg 2549 opiate, and/or 100 µg dermorphin (DM) were injected iv, and rats were killed at +10 min. Each value represents the mean of 6 ± SEM; P values are given for treated vs saline. *$p < 0.02$, **$p < 0.01$, ***$p < 0.001$. When P values were determined for the various groups vs GHRP, GHRH, plus dermorphin or 2549, the values ranged

A
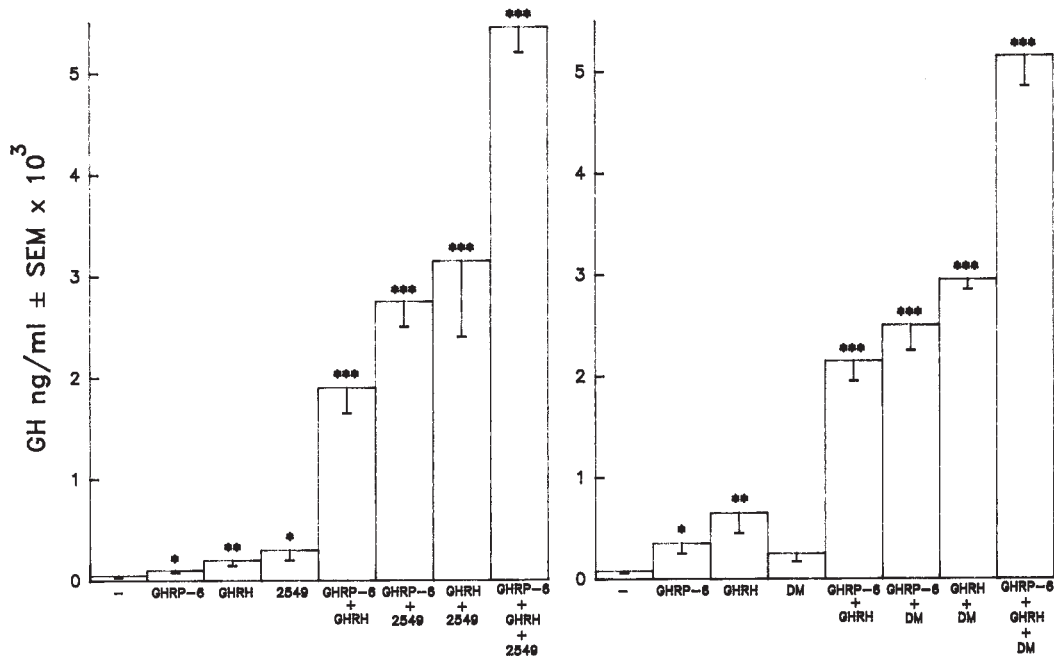
B
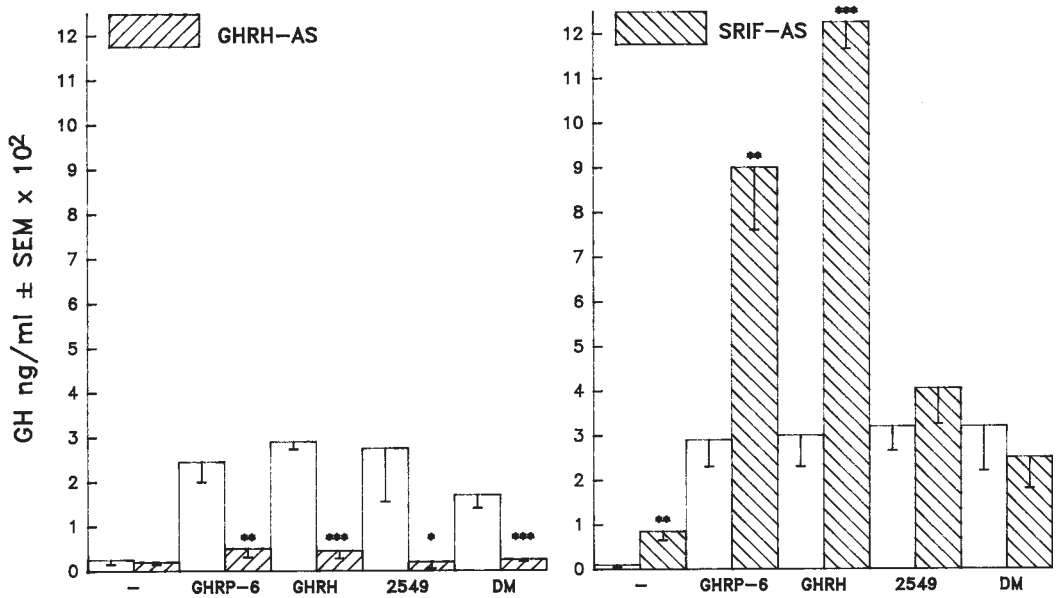
from <0.01–<0.001. **(B)** In vivo GHRH and SRIF antiserum (AS) immunoneutralization studies on the GH responses of GHRP, GHRH 1-43OH, 2549 opiate, and dermorphin (DM) in rats. GHRH AS, SRIF AS, or normal rabbit serum (0.2 mL) was injected iv at −1 h into conscious 26-d-old female rats. At zero time, rats were injected in the tail vein with saline, 10 μg GHRP, 10 μg GHRH, 10 μg 2549 opiate, or 100 μg dermorphin and killed at +10 min. Each value represents the mean of 6 ± SEM. Peptide/2549 vs peptide/2549 plus antiserum: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 4
GHRP Effect on GH Release in the Pituitary (P)
vs Hypothalamus (H) and Hypothalamus Plus Pituitary Incubates In Vitro[a,b]

GHRP dose (ng/mL medium)	Δ GH (ng/mL ± SEM)		
	Pituitary	Hypothalamus + pituitary	Hypothalamus[c]
Control	7874 ± 674	15185 ± 2286	7314 ± 2079
1	9840 ± 1056	15471 ± 1612	5631 ± 805
3	10726 ± 1096	17766 ± 1574	7040 ± 1514
10	19366 ± 1325[d]	32336 ± 3161[d]	12970 ± 3001
30	22630 ± 2148[d]	38661 ± 2180[d]	16031 ± 3694[e]
100	18046 ± 2800[d]	39134 ± 1730[d]	21088 ± 4468[d]

[a]Reproduced with permission from ref. 26.
[b]H and P from 26-d-old female rats. Values are the mean of 9 determinations. Each Δ value was calculated from three consecutive 1 h incubation periods (I_3–I_5) minus basal release of GH during the preincubation period.
[c]H + P – P.
[d]$p < 0.01$ vs control (by Newman-Keuls).
[e]$p < 0.05$ vs control (by Newman-Keuls).

peptide, dermorphin. These studies led to the conclusion that GHRP and the opiate GH responses were dependent on endogenous GHRH and since pretreatment with SRIF antiserum augmented the response of GHRP-6 and GHRH, neither one inhibited the release of SRIF. In contrast, because the SRIF antiserum pretreatment was without an effect on the GH response of the opiates, the opiates did appear to inhibit the release of endogenous SRIF. Thus, each of these three GH secretagogues—GHRP, GHRH, and the opiate peptide—was considered to release GH by a different mechanism and, in addition, the mechanisms or actions were complementary in releasing GH. Importantly, although response to the GHRP is dependent on endogenous GHRH, GHRH apparently plays a permissive role.

In addition to a direct action on the pituitary gland, a direct hypothalamic action of GHRP has been demonstrated (Table 4) (26). In three different in vitro assay systems, pituitary incubate, dispersed pituitary monolayer cell culture, and perifusion of pituitary cells, the GH response to GHRP+GHRH was essentially additive or only slightly synergistic (≈30%), and thus the direct pituitary action of the two peptides was insufficient to account for the magnitude of the synergism induced by GHRP+GHRH (26). Even when the in vitro GHRP+GHRH results in the pituitary cell culture and incubate assay were obtained under different experimental conditions and the time of the GH response was varied the effect on GH release was essentially additive. Other investigators have found synergism in vitro, but this has been the exception (29).

The in vivo synergistic release of GH induced by GHRP+GHRH has been a hallmark of the GHRP effect on GH release in that it occurs in multiple animal species and in humans of all ages and both sexes. The exact mechanism(s) involved has not been elucidated. The fact that synergism has been such a consistent finding, even at very low dosages (≈2 µg) in humans, has led us to believe that understanding how this occurs will substantially aid in elucidation of the action of GHRP especially on the hypothalamus. Examples of the synergistic GH response induced by GHRP-6+GHRH in male and female rats, rhesus monkeys, and cows are recorded in Table 5.

Table 5
Synergistic GH Response[a]

Immature rat

Peptide	Dose μg/kg	GH ng/mL ± SEM (n = 6)	
		Male	Female
Control	—	3 ± 1.7	10 ± 2
GHRP-6	200	289 ± 69	248 ± 31
GHRH-44	200	102 ± 19	173 ± 28
GHRP-6+GHRH-44	200 + 200	1063 ± 343	1184 ± 145

Monkey

Peptide	Dose μg/kg	GH ng/mL ± SEM (n = 6)	
		Male	Female
Control	—	2 ± 1.6	6 ± 5
GHRP-6	5	1 ± 0.6	1 ± 0.5
GHRH	5	6 ± 2	2 ± 1
GHRP-6+GHRH	5 + 5	21 ± 8	26 ± 9

Nonlactating Holstein Cow

Peptide	Dose μg/kg	GH ng/mL ± SEM (n = 4)
Control	—	0.17 ± 0.19
Ala[1]GHRP-6	3	8.6 ± 2.5
GHRH	3	5.7 ± 0.58
Ala[1]GHRP-6+GHRH	3 + 3	88.0 ± 19.0

[a]Blood collected +10 (26-d-old rat), +20 (rhesus monkey), +15 (cow) min after iv peptide.

A series of GHRP antagonists were synthesized between 1980–83. The in vitro results of HisDTrpDLysTrpDPheLysNH$_2$, a GHRP antagonist, and the GHRH antagonist, DArg^2,Ala^{8,9,15}-GHRH, developed by Coy et al. are recorded in (Table 6) *(26)*. The GHRP antagonist inhibits the GH response to GHRP but not GHRH and the GHRH antagonist inhibits GHRH but not GHRP. In 1991, certain substance P antagonists were found to inhibit the GH response to GHRP *(30)* as well as labeled GHRP in the pituitary GHRP RRA *(31)*.

Clark and Robinson *(32)* continuously infused GHRP-6 to freely moving conscious rats over 8 h and a pulse of GHRH was administered each hour. GHRH pulses inconsistently released GH in saline treated control rats, whereas in the GHRP-6 treated rats, GH was consistently released by the pulses of GHRH. These results were interpreted to reflect a hypothalamic action of GHRP. Since exogenous GHRH was administered in this study, it is obvious that the hypothalamic action of GHRP is not due to the endogenous release of GHRH. Other results in support of a GHRP hypothalamic action include demonstration of high affinity binding studies in membranes of both the hypothalamus and pituitary by Codd et al. *(33)* and Sethumadhavan et al. *(31)* as well as those of Dickson et al. *(34)*, who showed that by both iv and icv routes, GHRP-6 increased c-*Fos* production in select neurons of the arcuate nucleus of the hypothalamus *(35,36)*, Guillaume et al. showed that GHRH was increased in hypophyseal portal blood of sheep following treatment with hexarelin *(37)*. Also, results of Mallo et al. *(38)* demonstrated a GHRP hypothalamic site of action following hypophyseal stalk section and pituitary transplan-

Table 6
In Vitro Effects of GHRH and GHRP Competitive Antagonists
on the GH Responses of GHRP and GHRH

Peptide	Dose ng/mL	$DArg^2Ala^{8,9,15}GHRH$ Dose μg/mL	GH (ng/mL ± SEM)[a]
Control	—	—	179 ± 4
GHRP	10	—	930 ± 25
GHRP	10	1.0	856 ± 27
GHRP	10	3.0	933 ± 27
GHRP	10	10.0	920 ± 22
GHRH	10	—	1197 ± 17
GHRH	10	1.0	454 ± 23[b]
GHRH	10	3.0	309 ± 15[b]
GHRH	10	10.0	253 ± 15[b]
Peptide	Dose ng/mL	$DLys^3$-GHRP Dose μg/mL	GH (ng/mL ± SEM)[a]
Control	—	—	172 ± 15
GHRP	10	—	1020 ± 11
GHRP	10	1.0	626 ± 9[b]
GHRP	10	3.0	555 ± 36[c]
GHRP	10	10.0	357 ± 45[b]
GHRH	10	—	1197 ± 17
GHRH	10	1.0	1242 ± 18
GHRH	10	3.0	1206 ± 25
GHRH	10	10.0	1080 ± 52

[a]$n = 3$.
[b]$p = < 0.001$.
[c]$p < 0.01$ vs peptide alone.

tation. The recent important studies by Dickson et al. *(36)* demonstrate that GHRP-6 acts directly on the hypothalamus in vitro. Recently reported in vitro studies of Korbonits et al. failed to demonstrate that GHRP increased or decreased GHRH or SRIF release from the hypothalamus *(39)*. Furthermore, peptidomimetics of the GHRPs did not induce a reproducible rise of GHRH and/or fall in SRIF hypophyseal portal blood in vivo *(40)*.

Mechanism of action studies by Cheng in 1989 *(29)* demonstrated that GHRP-6 did not activate the adenyl cyclase cAMP pathway, but together with GHRH, synergistically raised intracellular cAMP levels by acting through the protein kinase C pathway. In 1983, we also reported that neither $DTrp^2$ nor $DTrp^3$ in vitro raised pituitary cAMP or cGMP levels *(9)*. Later results of Adams et al. *(41)* and Mau et al. *(42)* demonstrated that although GHRH stimulated the cAMP pathway GHRP-6 stimulated the phospholipase-C IP_3 (inositol triphosphate) pathway. In vitro results have supported the role of GHRP as a functional SRIF antagonist at the molecular level in that the peripheral membrane of the somatotroph is depolarized by GHRP by blocking the K^+ channels and inhibiting hyperpolarization by SRIF *(43,44)*. Intracellular Ca^{2+} is raised via voltage-activated L-type channels and by release from intracellular stores *(44,45)*. Recently, details of these studies were discussed by Smith et al. *(40)* and Chen *(46)*.

In 1989, our group, together with Michael Thorner's and as Ilson et al. at Smith-Kline Beecham, found that GHRP-6 very effectively released GH in normal young men *(47,48)*.

There was a small concomitant transient rise of serum PRL and cortisol, both of which were still within the normal range. Similar to that found in animal models, i.e., rats, monkeys, and cows, the combined administration of GHRP-6 and GHRH on GH release was synergistic in humans. These results underscore that, in humans also, GHRP and GHRH act differently. Another important property of the GHRPs was revealed when Huhn and Thorner et al. *(49)* and Jaffe and Barkan et al. *(50)* independently demonstrated that continuous iv infusion of GHRP-6 administered for 24–36 h to normal young men increased the amplitude of the spontaneous pulsatile secretion of GH. Because the GH response to GHRP-6 was readily desensitized after repeated administration to rats *(21)*, as well as by continuous administration during perifusion of dispersed rat pituitary cells *(18)*, these results in humans were surprising. However, the results of Clark and Robinson in conscious rats suggested that continuous infusion of GHRP-6 to humans might increase the amplitude of the spontaneous GH pulsatility and that this could occur despite desensitization of the GH response *(32)*.

Between 1991–1997, a series of detailed and noteworthy studies were performed with the very potent GHRP-6-like hexapeptide hexarelin, HisD2MeTrpAlaTrpDPheLysNH$_2$ that had been developed by Dengheni et al.: The effects of hexarelin essentially paralleled those of the other GHRPs *(51,52)*. Also, during this time, Walker and Bercu *(53)* reported the results of chronic administration of GHRP-6 to rats. They investigated the corrected effects of GHRP-6+GHRH co-administration, relationships to endogenous GHRH, TRH and GnRH secretion as well as secretion of PRL, body weight gain, and effect on serum lipids and hepatic mRNA levels for low-density lipoproteins (LDLs).

In 1992, a seminal accomplishment and a major GHRP milestone was the development of a substituted benzolactam peptidomimetic L-692,429 by Merck and Co. *(54)*. This was a special achievement because a peptidomimetic agonist was developed from a peptide agonist. In contrast, the development of a peptidomimetic antagonist from a peptide agonist is not such an unusual event. Undoubtedly this peptidomimetic will catalyze efforts to develop other peptidomimetic agonists that mimic the actions of small peptide hormones. A point of note has been the finding that the peptides and peptidomimetics act on the same receptor and activate GH release by the same intracellular signal transduction pathway *(55)*. An important improvement of the benzolactam GH secretagogue was reported by the Merck group in 1995. This spiroindoline derivative [MK-0677 (L-693,191)] is more potent, has higher oral bioavailability ($\approx$60%) and increases pulsatile GH secretion with an associated increase of serum IGF-I levels during chronic oral administration to normal younger and older subjects *(56)*.

Also, in 1995, highly potent GHRPs were developed by the Genentech *(57,58)* and Novo Nordisk groups *(59,60)*. These GHRPs were developed primarily from the DTrp^{2,3} type of GHRP with an aromatic core in the center of the molecule and special functional groups at each end. The Genentech group has reported potent GHRPs that are low in molecular weight ranging from 496 to 508. Gradually, small partial peptide GHRPs are being developed with more substitutions of the amino acids by organic chemical nonpeptide groups. Besides the four or more major types of GHRPs, there are now three major chemical classes of GHRPs, i.e., peptide, partial peptide, and peptidomimetics. Regardless of the broad range of the GHRP SARs, all of them appear to act on the same receptor and by the same molecular mechanism(s). What is different among these GH secretagogues is the pharmacokinetics. In principle, the pharmacokinetics do not alter the

action on GH release, but MK-0677, with a more prolonged serum half-life, appears advantageous in terms of increasing pulsatile GH secretion and serum IGF-I levels after oral administration. These same results have been observed with continuous infusion of GHRP-6 and GHRP-2 *(49,50,61,62)*.

In 1996, another seminal milestone was accomplished by the Merck group by cloning the MK-0677 receptor and characterizing it as the GHRP receptor *(63)*. This is a new seven transmembrane domain G-protein coupled receptor. Anatomically it has been localized in the hypothalamic arcuate nucleus and the infundibulum as well as in the pituitary on the somatotrophs. All of the various types and classes of GHRPs specifically bind to the transfected cloned receptor with high affinity. Genomic analysis of the receptor supports the presence of a single highly conserved gene in human, chimpanzee, swine, bovine, rat, and mouse genomic DNA.

The SARs of GHRP strongly support that the putative native GHRP-like hormone is a peptide. Because of the substitution of unnatural D amino acid stereoisomers in the GHRPs, it is probable that the amino acid sequence of the putative native GHRP-like hormone will not closely simulate the sequence(s) of the current peptide GHRPs. In regard to how GHRP releases GH, it is well established that it acts on both the hypothalamus and pituitary *(27)*. What is still unanswered is the relative importance of the action of GHRP at these two anatomical sites as well as the type of action(s) GHRP has on the hypothalamus, i.e., increased GHRH and/or decreased SRIF release or even the seemingly likely possibility of increased release of a yet unidentified factor. It has been postulated that the hypothalamic action of GHRP involves the release of U-factor (unknown factor) which in part mediates its effect on GH release *(27)*. The latter has been proposed because of an inability to explain the action of low dose GHRP via an effect on GHRH or SRIF release or as a functional SRIF-antagonist. Sequential events of the GHRP story also were outlined in 1996 *(64)*.

What has become gradually more apparent is that the type of action(s) induced by GHRP is probably dose dependent. High dosages are considered to reflect a pharmacological action and low dosages presumably a physiological action of a putative endogenous GHRP-like hormone. Conceptual models of the role of the putative GHRP system in the physiological regulation of GH secretion can be categorized in terms of three different types, hypothalamic, pituitary, and hypothalamic-pituitary *(27)*. Because GHRP acts on both the hypothalamus and pituitary, the hypothalamic-pituitary model is the most logical choice, but this model is particularly difficult to envision without knowing more about the hypothalamic action of GHRP and to what degree the quality and quantity of this effect is dosage-dependent.

Unusual and unexpected effects of GHRP in humans have been exemplified by the not infrequent unique actions of this new class of GH secretagogues. Figure 4 shows that each of the three GHRPs, GHRP-6, -1 and -2, increasingly released more GH in normal young men than GHRH when 1 µg/kg of the peptides was administered by iv bolus injection. Data recorded in Fig. 5 demonstrate another important aspect of these three initial GHRPs in that even though they are peptides they very effectively release GH after oral administration in normal young men. Figure 6 shows the high reproducibility and marked effect on GH release of four different oral formulations of GHRP-2 including small tablets at a dosage of 10 mg in normal young men. The low and consistent serum concentration of GHRP-2 after oral administration supports the consistency of the GH effect as well as the high potency of the peptide.
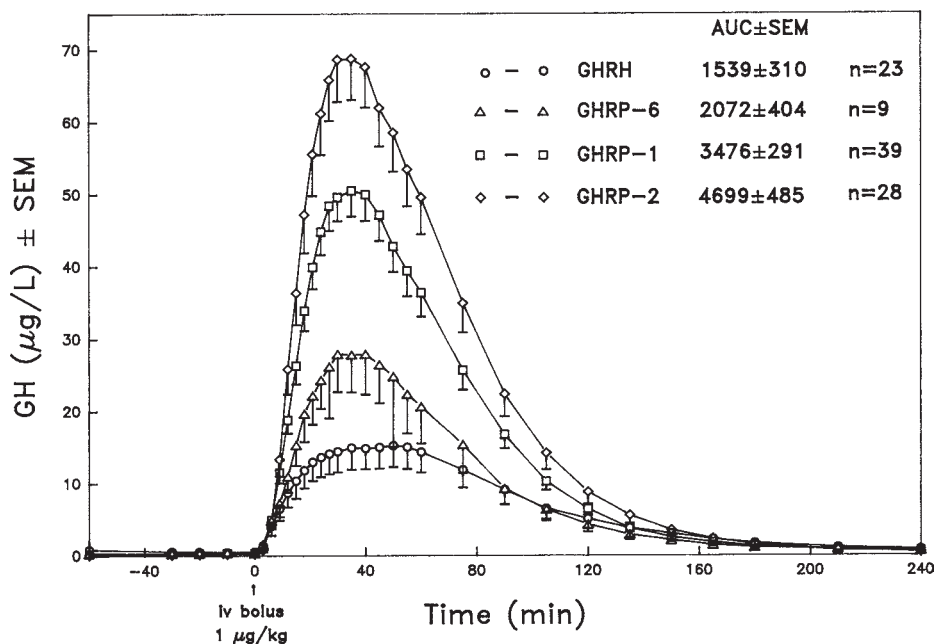
Fig. 4. Comparative mean responses to 1.0 µg/kg GHRH(1-44)NH2, GHRP-6, GHRP-1 and GHRP-2 in normal young men. Reproduced with permission from ref. *64*.

The results of a continuous infusion of GHRP-6 for 36 h to normal young men are recorded in Fig. 7 and demonstrate that the GH response to GHRP is both sensitized and desensitized *(50)*. The amplitude but not the frequency of the spontaneous GH pulses was increased during the infusion. Near the end of the infusion, the GH response to iv bolus GHRH was increased while that of iv bolus GHRP-6 was almost completely inhibited.

As recorded in Fig. 8, another dimension of the action of GHRP was observed when the effect of a very small amount of GHRP-2 ($\approx$2 µg or 0.03 µg/kg) was administered to normal young men *(65)*. GHRP-2 alone in this small dosage was without an effect on GH release but when given together with 1 µg/kg GHRH, GH was synergistically released. When this study was performed in normal young women, essentially the same results were obtained. A number of interpretations and implications appear to evolve from this study. Because this was usually a subthreshold GH releasing dosage of GHRP-2, and because the in vitro effects of combined GHRP-2 and GHRH on GH release are usually additive or only marginally synergistic, the synergistic release of GH induced by low-dose GHRP-2+GHRH is unlikely mediated by the action of this small dose of GHRP-2 directly on the pituitary. Thus, the synergism is probably mediated via a hypothalamic action of GHRP. Because of the large amount of GHRH administered, the GHRP-2 hypothalamic action obviously is not mediated via an increased release of endogenous GHRH. In addition, the GHRP-2 hypothalamic action probably involves an action outside the blood brain barrier on the median eminence rather than on the arcuate nucleus because evidence indicates the blood brain barrier limits the access of GHRP *(35,66)* and a 2 µg dose of GHRP-2 is very low. Also, in a number of studies in which SRIF release was inhibited by different agents, i.e., pentobarbital, SRIF antiserum, opiates, pyridostigmine, the GH response to GHRP was increased, thus indicating GHRP does not
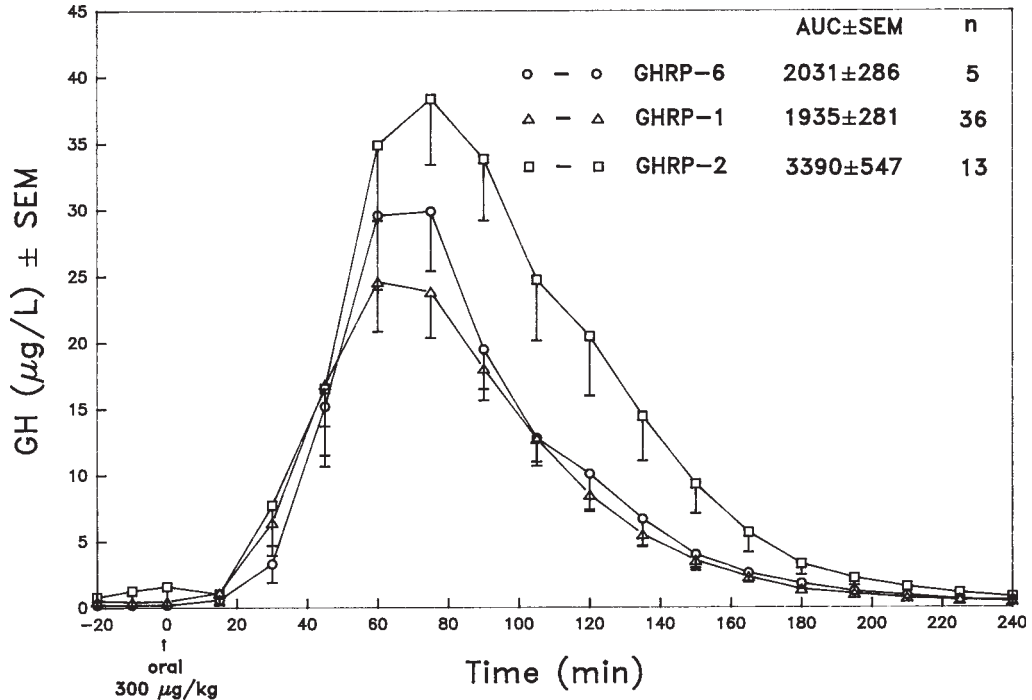
Fig. 5. Comparative mean GH responses to 300 µg/kg oral GHRP-6, GHRP-1, and GHRP-2 in normal young men.
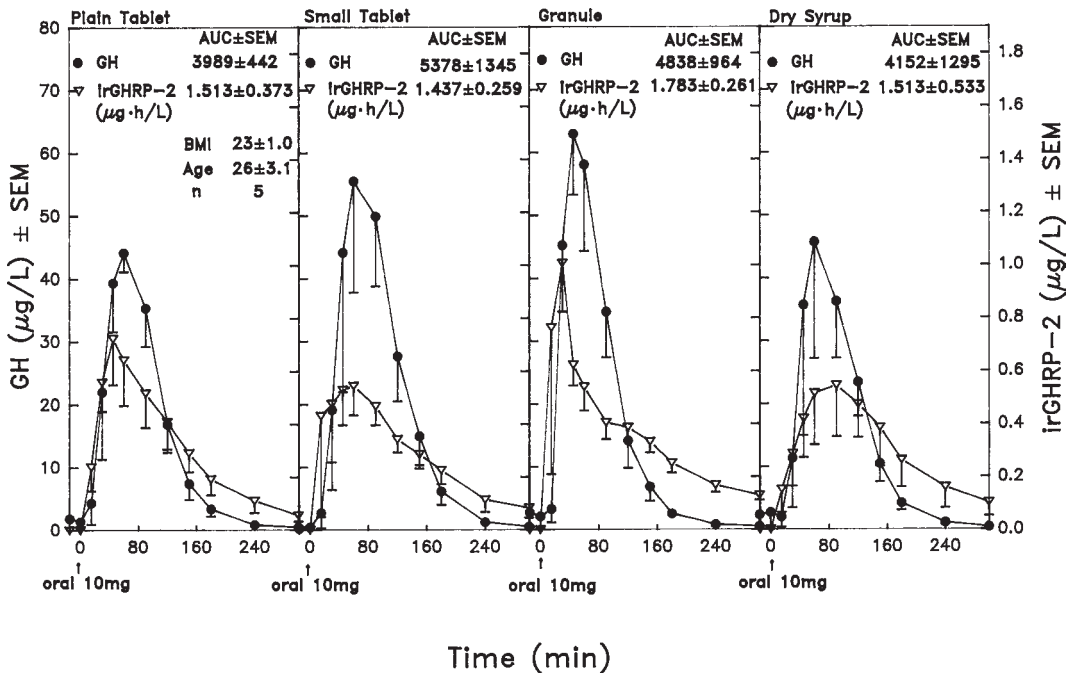

Fig. 6. GH and GHRP-2 concentration time-profiles after different formulations of 10 mg GHRP-2 orally on different occasions to the same five normal young men. Values are the mean ± SEM.
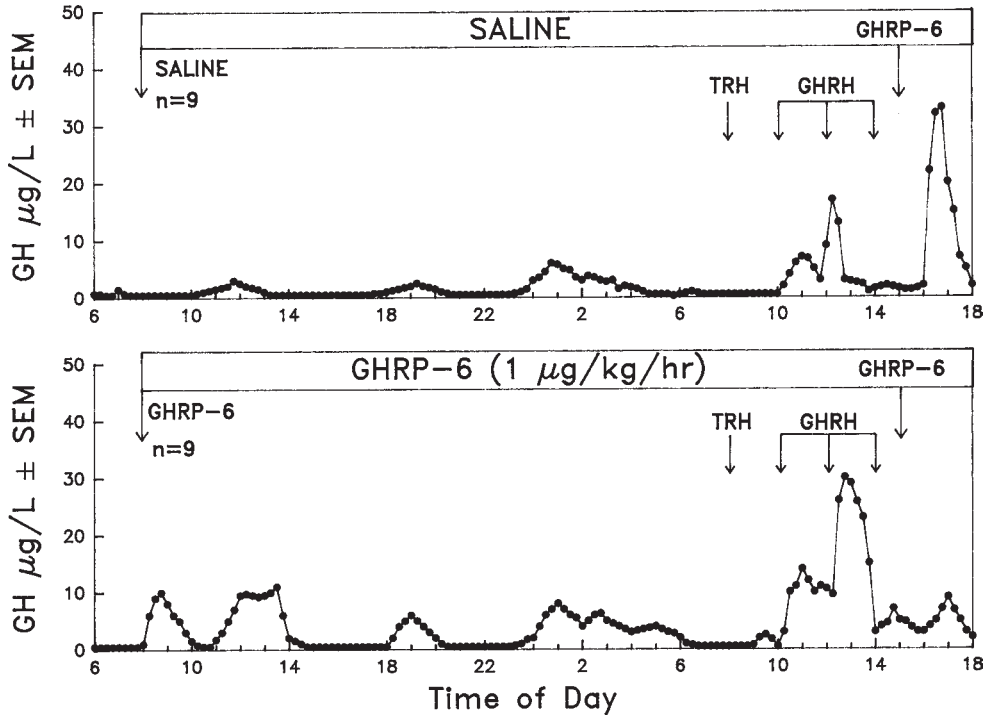
Fig. 7. Effect of continuous 36-h infusion of saline or GHRP-6 in normal young men. Reproduced with permission from ref. *50*.
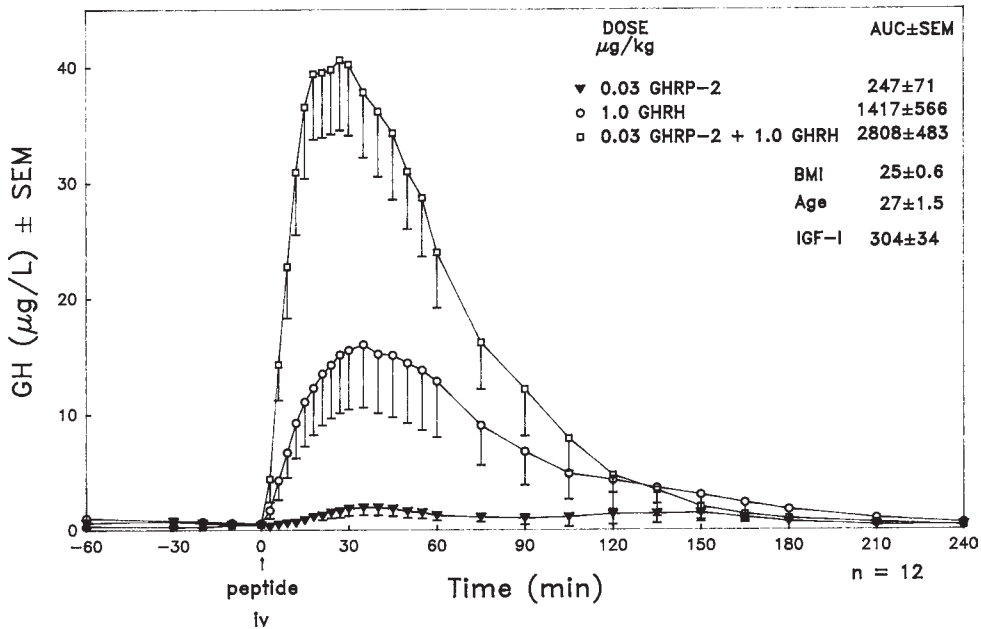

Fig. 8. Effect of a very low dosage of GHRP-2 (0.03 µg/kg) combined with a high dosage of GHRH (1.0 µg/kg) on the synergistic release of GH in normal young men. Reproduced with permission from ref. *81*.

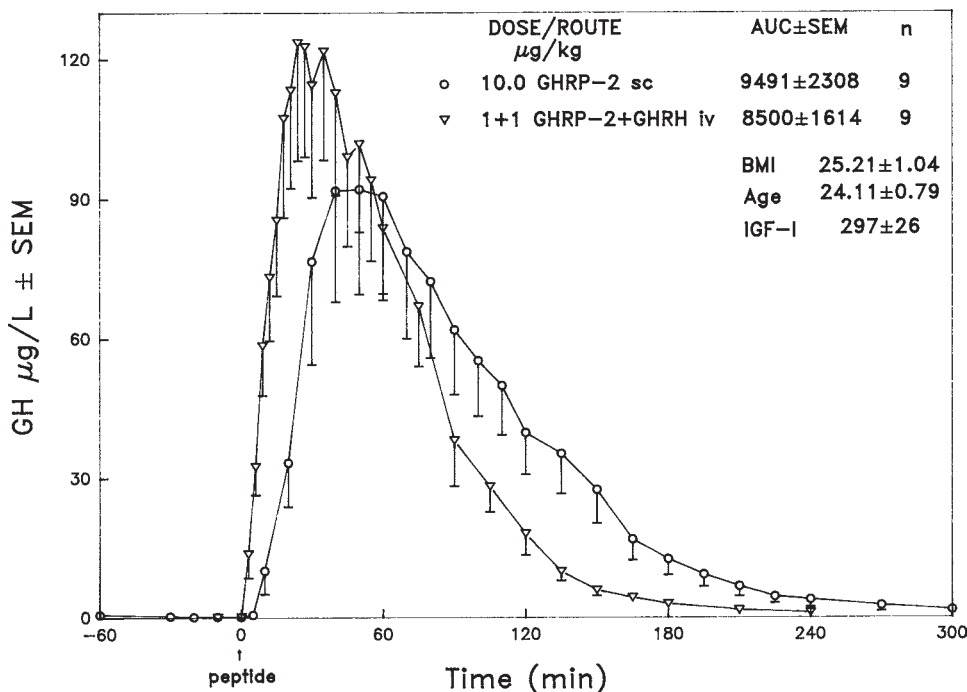**Fig. 9.** Comparative effects on the GH release to 10 µg/kg sc high dose GHRP-2 vs 1+1 µg/kg iv bolus GHRP-2+GHRH in the same nine normal young men. Reproduced with permission from ref. *81*.

release GH by inhibition of SRIF release or by attenuation of the SRIF inhibitory action on the pituitary. Although GHRP can be categorized as a functional SRIF antagonist at the pituitary and possibly the hypothalamic level *(66,67)*, such a small dose of GHRP-2 would be unlikely to attenuate the pituitary or hypothalamic action of SRIF. A seemingly convoluted issue is to what degree is it possible to relate the hypothalamic action(s) of exogenous low dose GHRP-2 and a putative endogenous GHRP-like hormone. Because it has been impossible to explain the synergistic release of GH by a very low dosage of GHRP-2 via a hypothalamic action on the release of GHRH or SRIF, it has been hypothesized that a third factor designated U-factor mediates this synergism. U-factor is envisioned to be released from the hypothalamus via the action of GHRP. In concert with GHRH and sometimes with GHRP when higher dosages of GHRP are administered, U-factor acts on the pituitary to synergistically release GH, seemingly by a complementary intracellular signal transduction action and in part by possibly attenuating the pituitary inhibition of SRIF on GH release. A seemingly general valuable point is that GHRP studies alone and in combination with GHRH in humans can reveal new dimensions about the secretion of GH, as well as add new insight into the actions of GHRP.

Another dimension of the action of GHRP-2 on GH release was revealed when a large dose of 10 µg/kg was administered sc to normal young men (Fig. 9) *(65)*. Because this large dose of GHRP-2 alone released the same amount of GH as that induced by iv bolus 1+1 µg/kg GHRP-2+GHRH, GHRP-2 in this high dosage was considered to release endogenous GHRH from the hypothalamus and, in this way, release a large amount of GH possibly via the synergistic action of GHRP + endogenous GHRH.
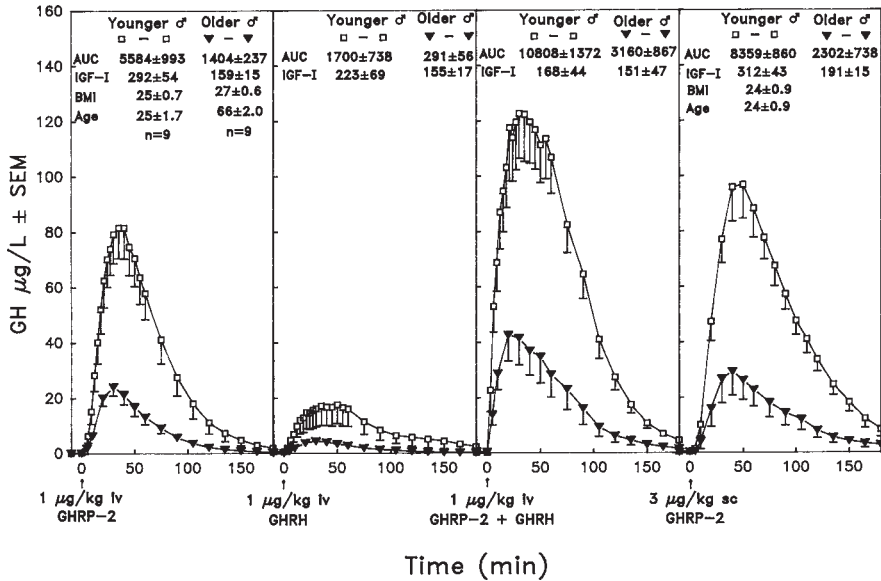
Fig. 10. Comparative effects on the GH response to GHRP-2, GHRH, and GHRP-2+GHRH in the same normal younger and the same normal older men. Three µg/kg sc GHRP-2 (last panel) was administered to these subjects, with the exception that there were only 5 of 7 younger female subjects.
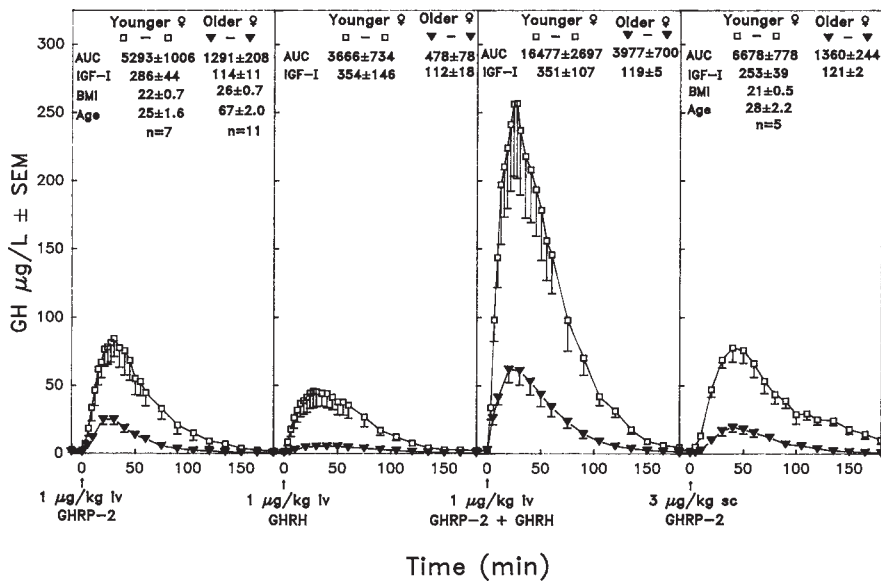

Fig. 11. Comparative effects on the GH response to GHRP-2, GHRH, and GHRP-2+GHRH in the same normal younger and the same normal older women. Three µg/kg sc GHRP-2 (last panel) was administered to these subjects, with the exception that there were only 5 of 7 younger female subjects.

The GH response to GHRP-2 and GHRH as well as the marked synergistic release of GH induced by these combined peptides was greater in younger than in older men and women (Figs. 10 and 11) *(68)*. GHRP-2 consistently released more GH than maximal
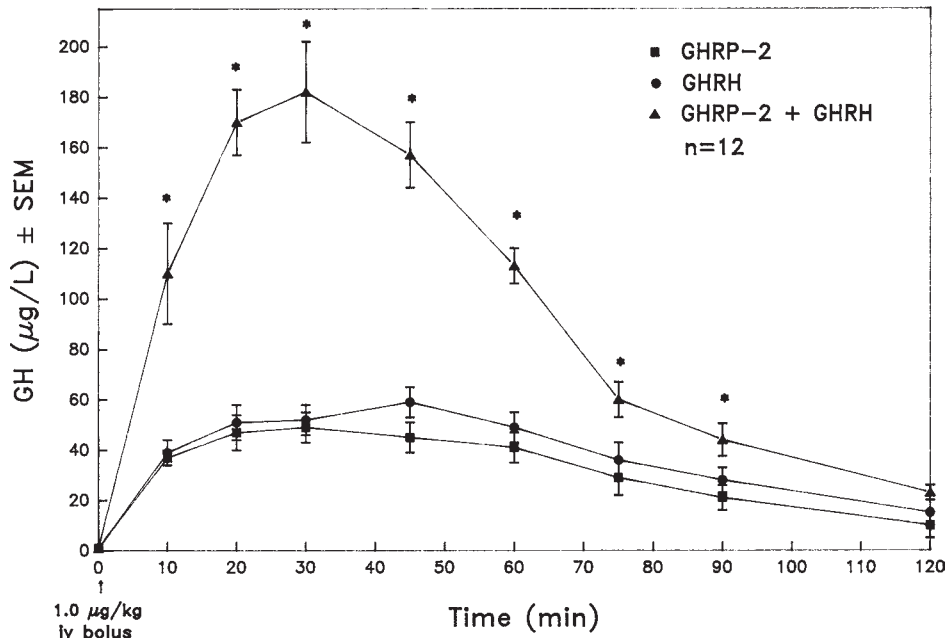
Fig. 12. GH responses over time with administration of iv GHRH (1 µg/kg), GHRP-2 (1 µg/kg), and GHRH+GHRP-2 (each at a dose of 1 µg/kg) in children with GH insufficiency. Mean ± SEM. Reproduced with permission from ref. *69*.

dosages of GHRH even in the older subjects, indicating the pituitary capacity to release GH is not the reason the GHRH GH response is lower in older subjects.

GHRPs are very effective in children. When Pihoker et al. *(69)* acutely administered GHRP-2+GHRH to short-statured children with various degrees of GH deficiency, GH was synergistically released (Fig. 12). In these children, GHRP-2 alone very effectively released GH. Three separate chronic studies by Laron et al. *(70,71)*, Pihoker et al. *(69,72)* and Mericq et al. *(73)* have been performed with hexarelin or GHRP-2 administered intranasally or subcutaneously to short-statured children with partial GH deficiency. In each study, the height velocity was increased by 2.5–3 cm/yr. The results recorded in Fig. 13, obtained by Pihoker et al., indicate that after 6 mo of intranasal GHRP-2 administration 2–3 times/d, the GH response was not desensitized and tended to be increased or up-regulated.

Since 1993, Casanueva and Dieguez et al. *(74)* have performed a series of important studies with GHRP-6 in patients with obesity, Cushings syndrome, and hypothalamic-pituitary disconnections. In obesity, GHRP released a remarkable amount of GH, especially when GHRP-6+GHRH was administered. The GH response to GHRP-6 alone and together with GHRH was markedly decreased in Cushing's syndrome. In patients with a hypothalamic-pituitary disconnection, GHRH released a normal amount of GH and GHRP-6 a lesser amount. These results indicate new dimensions in the secretion of GH and eventually understanding them in more detail will reveal new insight into the physiological and pathophysiological secretion of GH in humans. Also, other studies reveal that GHRP releases GH from pituitary tumors of acromegalic patients in vitro and in vivo *(28,41,75,76)*.
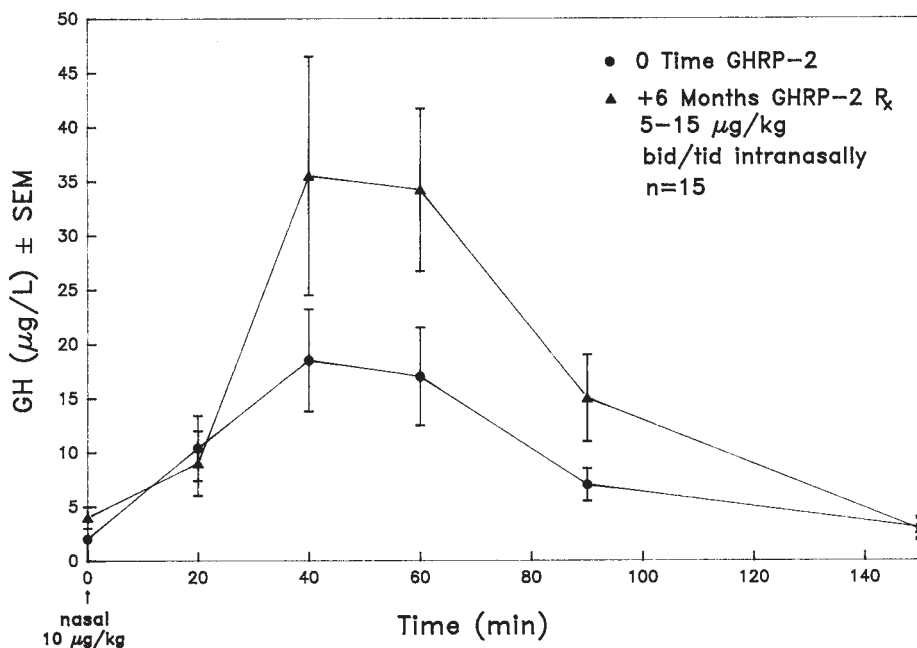
Fig. 13. GH responses to an acute intranasal dose of GHRP-2, 1 µg/kg on initial testing and 6 mo after daily GHRP-2 administration in children with GH insufficiency. Initial testing, $n = 12$, + 6 mo, $n = 9$. Mean ± SEM. Reproduced with permission from ref. *72.*

Results of the GH responses to GHRP-2 or GHRP-2+GHRH during chronic administration to normal older subjects every other day for 60 d are recorded in Figs. 14 and 15 *(77)*. The acute GH response to GHRP-2, GHRH, or GHRP-2+GHRH was not differentially influenced by GHRP-2 alone or GHRP-2+GHRH administered chronically. Neither GHRP-2 nor GHRP-2+GHRH desensitized or up-regulated the acute GH response to GHRP-2 or GHRH. In these studies, serum IGF-I levels remained unchanged during chronic administration of GHRP-2 as well as GHRP-2+GHRH.

Although demonstration of a decreased GH response to 1 µg/kg iv bolus GHRH is essential for understanding the pathophysiology and for making the diagnosis of pathological secretion of GH in older men and women, the GHRH response alone is considered insufficient for these two purposes. Almost all normal elderly men and women have considerably lower GH responses to 1 µg/kg GHRH than normal younger men and women and therefore, utilization of this criteria alone would tend to include all normal elderly subjects. The results of age-dependency of GH release are recorded in Figs. 10 and 11 for GHRP-2 with and without GHRH *(68)* and Fig. 16 for GHRP-1 with and without GHRH after iv bolus administration of the peptides *(78)*. Furthermore, the GHRH approach alone would not distinguish a low response due to excess secretion or action of SRIF.

Particularly needed is a new approach based on a better understanding of the pathophysiology in order to distinguish the decreased secretion of GH associated with aging *per se* from a pathological decreased secretion of GH due to a possible specific hormonal deficiency. As unlikely and illogical as this may seem at first, the putative GHRP-like hormone and GHRP-2 appear intimately and perhaps fundamentally related to the pathophysiology and to the diagnosis of the pathological decreased GH secretion in the elderly.
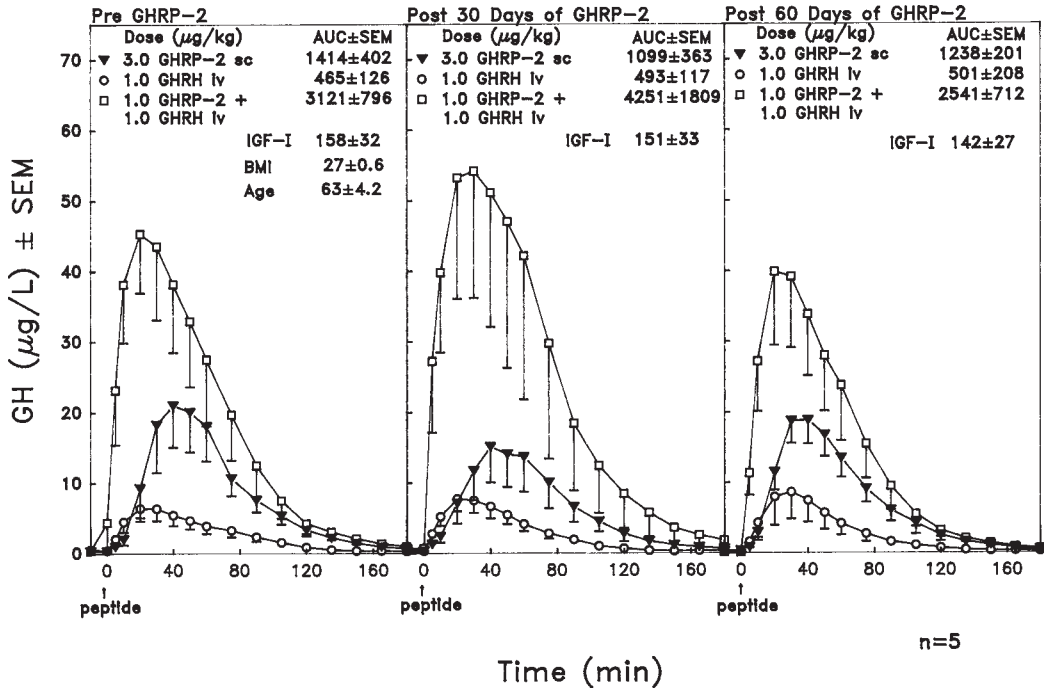
Fig. 14. Effect of 3 µg/kg GHRP-2 sc every other day in the AM for 60 d in normal older adults. The GH responses to iv bolus GHRP-2, GHRH, and GHRP-2+GHRH were the same before treatment, at +30 d, and at +60 d. The IGF-I mean levels did not change.
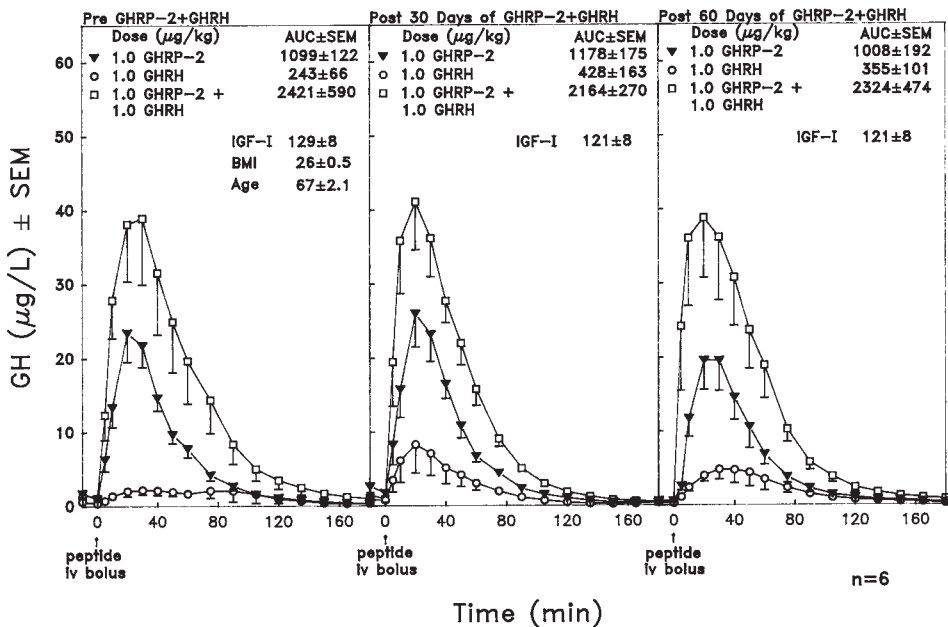

Fig. 15. Effect of 1 µg/kg GHRP-2+GHRH every other day in the AM for 60 d in normal older adults. The GH responses to iv bolus GHRP-2, GHRH, and GHRP-2+GHRH were the same before treatment, at +30 d, and at +60 d. The IGF-I mean levels before and after treatment were unchanged.
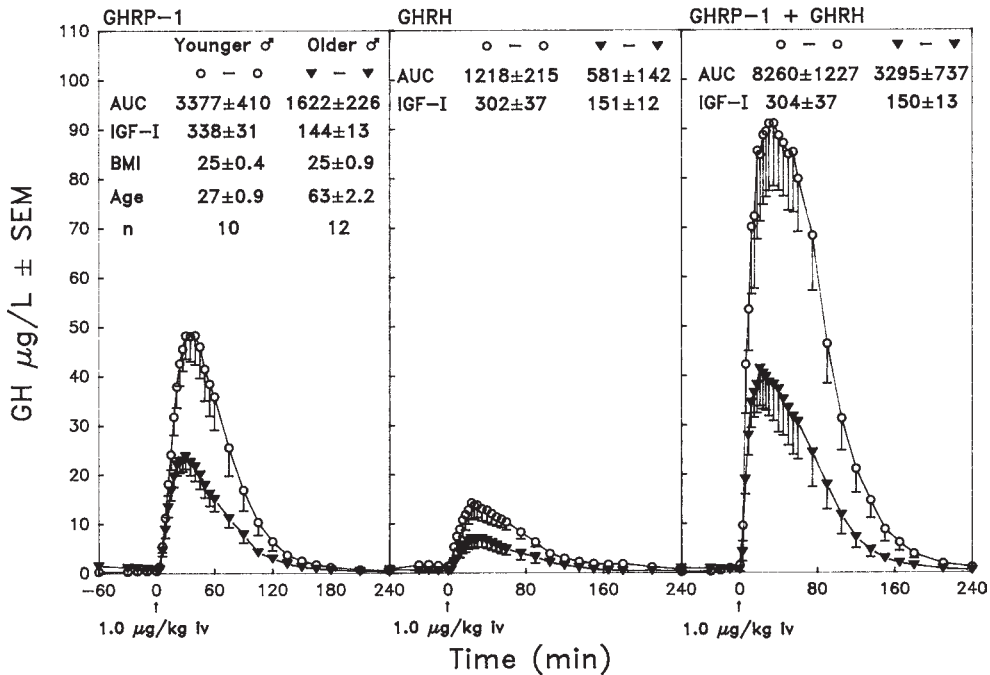
Fig. 16. Comparative mean GH responses to GHRP-1, GHRH and GHRP-1+GHRH in normal younger and older men. Values are the mean ± SEM. Reproduced with permission from ref. *27*.

Bercu and Walker *(79)* have performed a series of studies in animals and humans in order to understand the pathophysiology of decreased GH secretion that occurs during aging and also to develop an approach to diagnose this endocrine abnormality(s).

In order to understand this pathophysiology and to develop a way to distinguish the decreased GH secretion due to normal aging from that due to a pathological abnormality in older men and women, our approach has been to assess and establish the clinical value of a dual linked index of GH release designated a quantitative GH release index and a qualitative GH release index. Our hypothesis is that the pathophysiology of the pathological decreased GH secretion in older men and women is due to a deficiency of the putative hypothalamic GHRP-like hormone rather than a primary deficiency of GHRH or an excess of SRIF. The basic finding that has led to this hypothesis is that the pituitary action of 1 µg/kg GHRH on GH release is quantitatively impaired and that this impairment is reversed by iv bolus 0.1 µg/kg GHRP-2 + 1 µg/kg GHRH *(80,81)*. An example of these GH responses in a normal older woman is recorded in Table 7. Also in Fig. 17 is recorded the results of an acute GH response of this same older woman before and during twice daily 0.1 µg/kg sc GHRP-2 chronically for 30 d. Noteworthy is that 0.1 µg/kg GHRP-2 consistently and dramatically reversed the markedly impaired GH responses of 1 µg/kg GHRH on d 0, 15, and 30. This supports that the impaired action of GHRH on GH release is basically and primarily a hypothalamic rather than a pituitary pathological abnormality.

Whether the impaired GHRH GH release is reversed by a low dose of GHRP-2 can be qualitatively decided in an all or none way as being positive or negative. A qualitative positive index would be when the ratio of the peak GH release of the combined peptides (0.1+1 µg/kg) is at least threefold greater than that of the arithmetic sum of the individual

Table 7
Effect of Chronic GHRP-2

	Dose μg/kg iv bolus[a]	Peak GH μg/L	AUC GH μg/L × 4 h
GHRH	1.0	2.7	183
GHRP-2	0.1[a]	0.7	221
GHRH+GHRP-2	1.0 + 0.1	44.1	2436
GHRP-2	1.0	47.6	2540

[a]sc.
GF-I, 85 μg/L.
BMI, 21.4.
GHRP-2, 0.1 μg/kg sc administered to a 66-yr-old female 2×/d for 30 d.

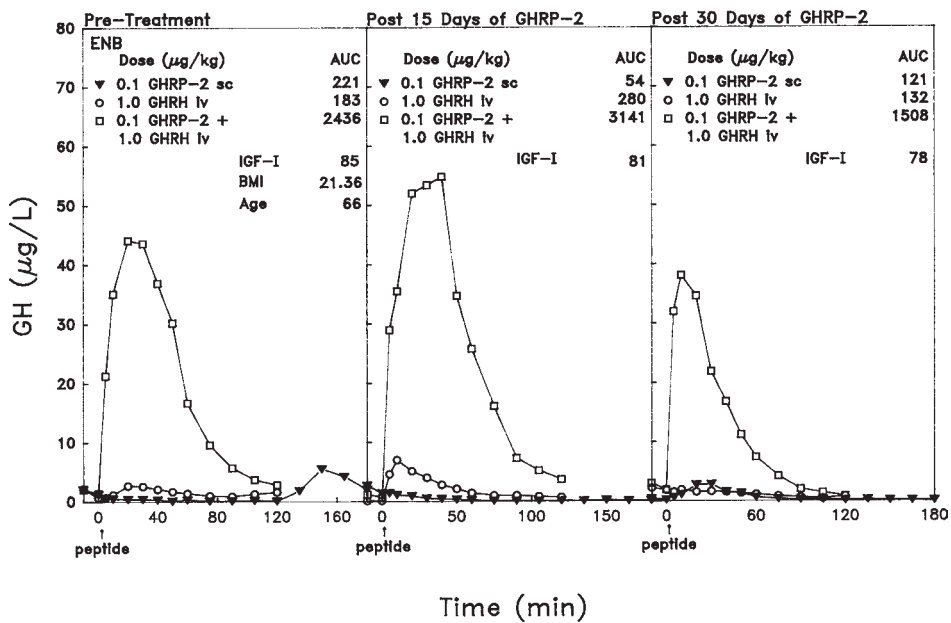**Fig. 17.** Effect of 0.1 μg/kg GHRP-2 administered sc 2×/d for 30 d in a 66-yr-old female. Subject tested before treatment, at +15 d, and at +30 d after treatment. There was no change in the IGF-I levels.

peptides. The GH response to 1 μg/kg GHRP-2 is considered to impart more insight into the pathophysiology and more insight into the pituitary capacity to release GH. Without endogenous GHRH secretion GHRP-2 does not release GH and thus the GH release induced by GHRP-2 indicates the secretion of endogenous GHRH. Although still impaired in comparison to younger adults, 1 μg/kg GHRP-2 releases considerably more GH than 1 μg/kg GHRH in older normal adults and thus, this indicates more about the capacity of the pituitary to release GH. However, because 1+1 μg/kg GHRP-2+GHRH or 10 μg/kg sc GHRP-2 releases much larger amounts of GH than 1 μg/kg GHRP-2 alone in normal young men, eventually one of these two approaches may be considered more optimal to assess the maximal capacity of the pituitary to release GH.

In Table 8 are the results of the acute iv bolus GH responses to 1 μg/kg GHRP-1, GHRH and the combined peptides in normal older men and women *(78)*. The results of these 19

Table 8
Comparison of GH Responses (Peak GH and AUC × 4 H) in the Elderly to GHRP-1, GHRH(1-44)NH$_2$, and GHRP-1+GHRH[a,b]

Age (yr)	BMI[c]	IGF-I µg/L	GHRP-1 µg/L ± SEM		GHRH-1 µg/L ± SEM		GHRP-1+GHRH µg/L ± SEM	
			Peak GH	AUC × 4 h	Peak GH	AUC × 4 h	Peak GH	AUC × 4 h
No synergism 2♀, 5♂								
64.3 ± 1.9	27.9 ± 1.4	136.3 ± 13.3	18.7 ± 2.6	1067.0 ± 166.0	2.5 ± 0.7	201.0 ± 44.0	23.6 ± 2.4	1349.0 ± 182.0
Synergism 5♀, 1♂								
65.5 ± 2.9	27.9 ± 2.0	104.6 ± 12.0	13.6 ± 1.8	746.0 ± 105.0	3.9 ± 0.9	373.0 ± 65.0	40.0 ± 5.4	2539.0 ± 424.0
High synergism 3♀, 3♂								
60.5 ± 2.3	23.6 ± 1.1	150.6 ± 22.8	24.8 ± 4.8	1522.0 ± 376.0	13.6 ± 2.5	980.0 ± 199.0	79.6 ± 9.3	6367.0 ± 845.0

[a]Reproduced with permission from ref. *78*.
[b]Mean ± SEM.
[c]Body Mass Index.

subjects are grouped according to whether the synergistic GH response of the combined peptides was absent, normal or high. In all three groups, the peak GH responses to GHRP-1 were nearly the same (18.7 ± 2.6, 13.6 ± 1.8, 24.8 ± 4.8), but for GHRH were different in the third group (2.5 ± 0.7, 3.9 ± 0.9, 13.6 ± 2.5). The results of the mean GH AUC paralleled the peak GH responses. Apparent is that the GH response to 1 µg/kg GHRH was markedly impaired in the first two groups and in the second group, 1 µg/kg GHRP-1 reversed the impaired GH response to 1 µg/kg GHRH by synergistically releasing GH. It is assumed that synergism in the first group was not induced because of the limited capacity of the pituitary to release GH. From our later GHRP-2 studies, it could be postulated that if a lower 0.1 µg/kg GHRP-1 dose had been administered in combination with the maximal 1 µg/kg dose of GHRH, a synergistic release of GH would have been elicited in all three groups. What is seemingly so fundamentally important is that the action of GHRH on the pituitary is markedly impaired and this impairment can be uniquely reversed by administering low dose GHRP + high dose GHRH.

Presumably the variable capacity of the pituitary to release GH will depend on the duration and severity of the putative GHRP-like hormone deficiency as well as the amount of endogenous GHRH and SRIF being secreted. The high sensitivity of the GHRP-2 effect on the reversal of the impaired GH releasing action of GHRH is against a primary decreased function of the somatotroph *per se* or a primary excess secretion or action of SRIF as the immediate cause of the pathological decreased GH secretion in older men and women. Envisioned is that when endogenous GHRH is secreted in greater amounts or SRIF is secreted in smaller amounts, 0.1 µg/kg GHRP-2 will be more effective in enhancing the GHRH GH response and thus the effect of low dose GHRP-2 will be an indicator of endogenous GHRH secretion and also SRIF secretion.

To what degree the pituitary capacity to release GH will parallel and determine the type and efficacy of the neuroendocrine therapeutic approach will require special evaluation. The secretory status of endogenous GHRH, SRIF, and the putative GHRP-like hormone as well as the pituitary somatotrophs, alone and collectively, probably will significantly dictate the type and design of neuroendocrine therapeutic approach.

At present, if the quantitative GH release index is abnormally low, i.e., the GHRH peak GH response is <6 µg/L to 1 µg/kg iv bolus GHRH and the qualitative GH release index is threefold or greater, i.e., synergistic release of GH is induced by 0.1+1 µg/kg iv bolus GHRP-2+GHRH, the subject's decreased GH secretion would be considered to be pathological possibly due to a deficiency of the hypothalamic GHRP-like hormone.

Major results which have evolved so far from our studies on the pathophysiology of pathological GH deficiency in elderly men and women are the following:

1. Impaired pituitary GH response to a maximal 1 µg/kg dosage of GHRH;
2. Increased GH release to 0.1 µg/kg low dose GHRP-2 + high dose GHRH;
3. Relatively high GH response to 1 µg/kg GHRP-2.

Major conclusions about elderly subjects are:

1. GH release induced by 1 µg/kg GHRP-2 alone indicates endogenous GHRH is being secreted because without endogenous GHRH secretion GHRP-2 does not release GH;
2. GHRH pituitary action on GH release is impaired, which is necessary but not a specific indicator of the pathological decreased GH secretion;
3. Impaired pituitary action of GHRH is mainly due to a secondary hypothalamic abnormality rather than a primary pituitary abnormality, because a maximal GHRH dose releases a subnormal amount of GH;

4. Low-dose GHRP-2 reverses the high-dose, GHRH-impaired pituitary GH response, indicating this occurs via a hypothalamic action of GHRP-2 rather than a pituitary action, which does not involve release of endogenous GHRH;
5. High sensitivity of GHRP-2 in reversing the impaired pituitary action of GHRH is against increased release or action of SRIF as the reason GH secretion is decreased;
6. Reversal of the GHRH-impaired pituitary action by GHRP-2 is considered to be mediated via the hypothalamic action of GHRP-2 to release U-factor (unknown factor) rather than to release GHRH or to inhibit SRIF;
7. Low-dose GHRP-2 has such a unique effect on the GHRH pituitary action that the pathological decreased secretion of GH in some older men and women may result from a deficiency of the putative hypothalamic GHRP-like hormone.

In conclusion, it is postulated that a putative GHRP-like system probably does exist and is involved in the physiological regulation of GH secretion. In addition, because of the unique actions of GHRP on GH release, it is likely to be valuable clinically both diagnostically and therapeutically.

ACKNOWLEDGMENT

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