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Sustained Elevation of Pulsatile Growth Hormone (GH) Secretion and Insulin-Like Growth Factor I (IGF-I), IGF-Binding Protein-3 (IGFBP-3), and IGFBP-5 Concentrations during 30-Day Continuous Subcutaneous Infusion of GH-Releasing Peptide-2 in Older Men and Women

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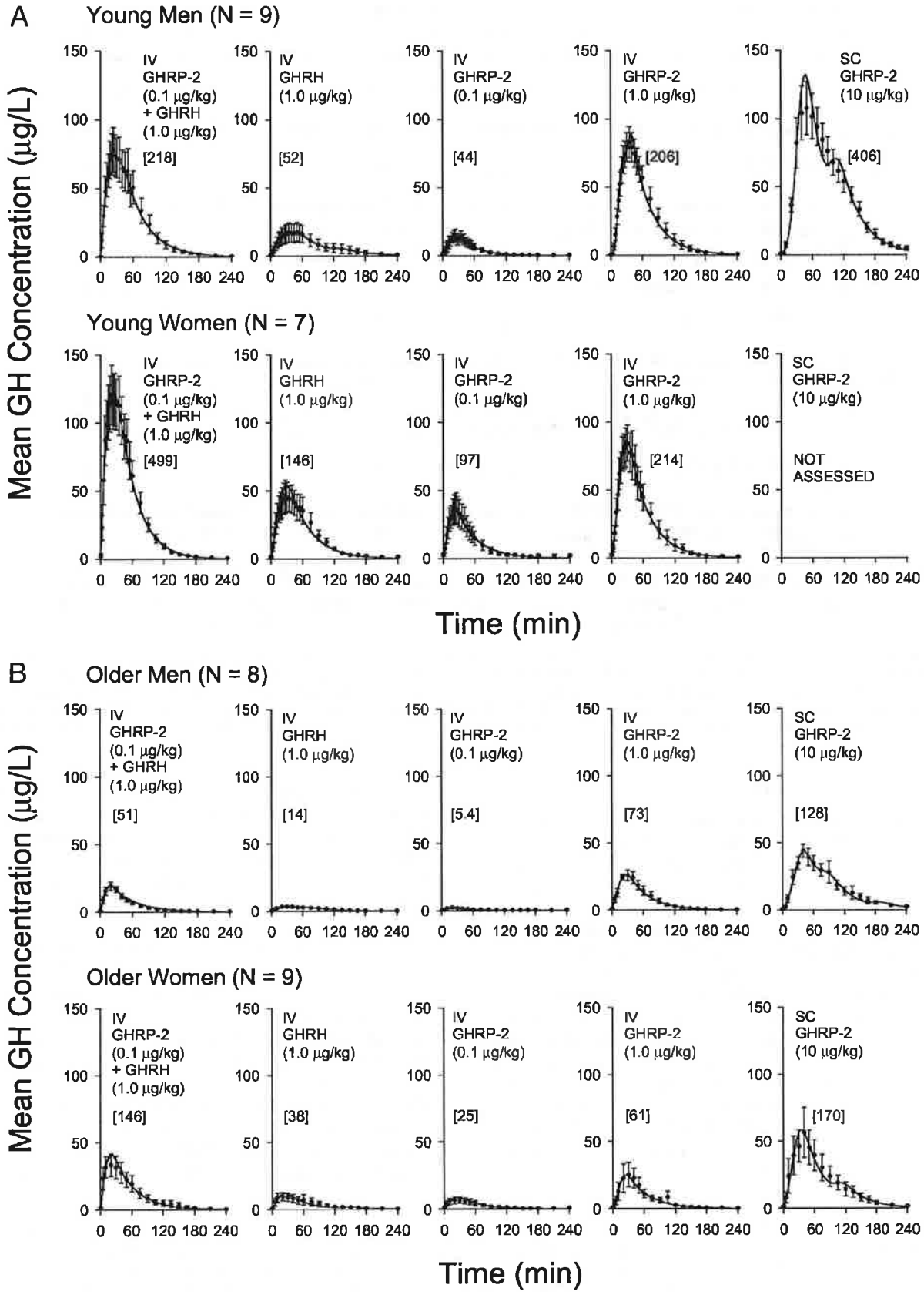
We test the interlinked hypotheses that in healthy older adults: 1) iv injection of GH-releasing peptide-2 (GHRP-2) and GHRH synergizes more in aging women than men; 2) sc infusion of both GHRP-2 (1 μg/kg·h = 1) and GHRH (1, 3, or 10) for 24 h augments GH secretion more than either agonist alone; and 3) continuous sc delivery of GHRP-2 (1) for 30 d stimulates daily GH secretion and IGF-I, IGF-binding protein-3 (IGFBP-3), and IGFBP-5. Acute two-peptide synergy was 3-fold greater in young (n = 16) than older volunteers (n = 17; $P < 0.025$) and was 2.3-fold higher in elderly women than men ($P < 0.025$). The 24-h infusion of GHRP-2 (1) combined with GHRH (3 or 10) in men and with GHRH (10) in women drove GH secretion more than GHRH alone ($P \leq 0.024$). In the entire cohort (n = 11), GHRP-2/GHRH (1/10) stimulated GH secretion more than either GHRP-2 (1; $P = 0.021$) or GHRH (10; $P = 0.012$). The 30-d delivery of GHRP-2 (1; n = 17 subjects): 1) stimulated pulsatile, rhythmic, and entropic GH secretion by more than 3-fold on d 1 and more than 1.8-fold on d 14 and 30 (each $P < 0.001$ *vs.* saline); 2) elevated IGF-I to a stable plateau on d 1, 14, and 30 ($P < 0.025$ *vs.* baseline); and 3) increased IGFBP-3 ($P < 0.01$) and IGFBP-5 ($P < 0.025$) on d 14 and/or 30. Safety screening tests remained normal. In summary, in healthy elderly women and men: 1) acute synergy of GHRP-2 and GHRH is greater in the female; 2) 24-h combined GHRP-2 and GHRH drive is more effective than either agonist alone; and 3) 30-d stimulation with GHRP-2 sustains a physiologically activated somatotropic axis. We conclude that age, gender, stimulus duration, and secretagogue combination determine acute, intermediate, and extended responses of the somatotropic axis in the older adult. (*J Clin Endocrinol Metab* 89: 2290–2300, 2004)

GH-RELEASING PEPTIDES (GHRP) were synthesized more than 2 decades ago as derivatives of met-enkephalin that stimulate GH secretion directly *in vitro* and more markedly *in vivo* (1, 2). The cognate G protein-coupled receptor and natural GHRP, ghrelin, were cloned in 1996 and 1999, respectively (3, 4). Clinical studies using peptidyl agonists (*e.g.* GHRP-6, GHRP-1, hexarelin, and GHRP-2), nonpeptidyl mimetics of GHRP, and native ghrelin establish that joint hypothalamo-pituitary actions mediate maximal stimulation of GH release by this class of secretagogues (5–9). From a mechanistic vantage, GHRP synergizes acutely with GHRH in humans and experimental animals (5, 9–13), releases hypothalamic GHRH into portal blood in the sheep (14, 15), and opposes certain central inhibitory effects of somatostatin in rodents (16–18). A functional role for the murine GHRP receptor-effector pathway was inferred recently by partial molecular silencing of the central nervous system ghrelin receptor; this intervention reduced GH and IGF-I concentrations and GH pulse height in the adult female, but not the male, animal (19).

Acute administration of a high dose of GHRP downregulates homologous responsiveness of the somatotropic axis in all mammalian species studied (20–24). However, limited indirect data suggest that continuous delivery of or repeated exposure to a low dose of GHRP can maintain elevated GH secretion and IGF-I concentrations for 12 h to 4 d (25–31). We hypothesized that constant exposure to a submaximal concentration of GHRP may mimic the physiological pattern of relatively stable systemic ghrelin concentrations (32, 33).

GH secretion and IGF-I concentrations decline significantly in healthy aging individuals. Postulated mechanisms include impaired stimulation by endogenous peptidyl secretagogues, such as GHRH and possibly ghrelin, and accentuated inhibition by hypothalamic somatostatin (34–36). We hypothesized that if relative hyposomatotropism in elderly adults arises at least in part from reduced peptidyl drive, then combined stimulation with GHRP-2 and GHRH acutely

Abbreviations: ApEn, Approximate entropy; BMI, body mass index; GHRP, GH-releasing peptide; PRL, prolactin.


A
Young Men (N = 9)
Young Women (N = 7)
Mean GH Concentration (μg/L)
IV GHRP-2 (0.1 μg/kg) + GHRH (1.0 μg/kg)
IV GHRH (1.0 μg/kg)
IV GHRP-2 (0.1 μg/kg)
IV GHRP-2 (1.0 μg/kg)
SC GHRP-2 (10 μg/kg)
[218]
[52]
[44]
[206]
[406]
[499]
[146]
[97]
[214]
NOT ASSESSED
Time (min)
B
Older Men (N = 8)
Older Women (N = 9)
[51]
[14]
[5.4]
[73]
[128]
[146]
[38]
[25]
[61]
[170]

and any single dose of GHRH, albeit not GHRP-2 ($P < 0.001$; Table 1). In men, combined stimulation with GHRP-2/GHRH (1/3) was more effectual than any one of GHRH (1; $P = 0.013$), GHRH (3; $P = 0.019$), or GHRH (10; $P = 0.024$), but not GHRP-2 alone ($P > 0.15$). Combined administration of GHRP-2/GHRH (1/10) was more effectual than any dose of GHRH (1, 3, and 10 μg/kg·h; $P < 0.005$), but not GHRP-2 ($P = 0.067$). In women, GHRP-2/GHRH (1/10) was more effective than GHRH (10 μg/kg·h; $P = 0.016$), but not GHRP-2 alone ($P = 0.096$). In the combined group of subjects (n = 11), combined GHRP-2/GHRH (1/10) was more effectual in increasing daily GH secretion than either GHRP-2 ($P = 0.021$) or GHRH (10; $P = 0.012$) alone.

Postinfusion mean IGF-I concentrations rose significantly and equivalently among the three GHRH doses from less than 100 to more than 150 μg/liter ($P < 10^{-5}$). GHRH (10 μg/kg·h) was as effective as GHRP-2 alone. Concomitant stimulation with GHRP-2 and GHRH (3 or 10 μg/kg·h) augmented IGF-I concentrations to more than 165 and more than 200 μg/liter, which exceeded values for any single dose of GHRH or GHRP-2 ($P < 0.05$ and $P < 0.01$).

Comparisons of deconvolution-calculated basal (nonpulsatile) GH secretion revealed that in the combined group (n = 11), only concurrent infusion of GHRP-2 and GHRH (3 or 10 μg/kg·h) elevated this measure ($P = 0.0036$ and $P = 0.0015$, respectively). The percent basal (of total) GH secretion remained less than 10% in each stimulation protocol.

The impact of constant sc infusion of GHRP-2 (1.0 μg/kg·h) on GH secretion was assessed by multiparameter deconvolution analysis, the ApEn statistic, and cosine regression. Analyses were performed at baseline (saline infusion, d −3) and on d 1, 14, and 30 of continuous GHRP-2 stimulation. Principal findings included 1) no significant alteration in the slow component half-life of GH or GH secretory burst frequency, interpulse interval, or calculated secretory burst half-duration; 2) augmentation of total (basal plus pulsatile) 24-h GH secretion (micrograms per liter per day) by 3- to 5-fold control on d 1 ($P < 10^{-4}$) and by 1.8- to 2.1-fold on d 14 and 30 ($P < 0.01$); 3) 1.8- to 2.5-fold greater total daily GH secretion in women than men at baseline (control) and each time point during GHRP-2 stimulation ($P < 0.01$ each), except d 1 (Fig. 3); 4) elevation of IGF-I concentrations on d 1 to a plateau value of 180 ± 15 μg/liter, which was maintained thereafter on d 14 and 30 ($P < 0.01$) with no difference by sex; 5) time-dependent and gender-distinguishable stimulation of pulsatile and basal GH secretion ($P < 10^{-4}$ for both measures) and the cosine mesor (regressed mean of the 24-h rhythm in GH concentrations; $P < 0.001$; Fig. 4); 6) elevation of GH ApEn (a feedback-sensitive regularity statistic; $P < 10^{-4}$; $P = 0.0019$ in men and $P = 0.0049$ in women) on d 1, 14, and 30; and 7) 2- to 3-fold increased amplitude of 24-h rhythmic GH release (50% of the arithmetic difference between the maximum and nadir values; $P < 10^{-5}$) with no change in the daily acrophase of 2340 h (clock time of the maximum).

The effects of GHRP-2 were maximal on d 1 in both men and women with respect to pulsatile and total daily GH secretion, the mass of GH released/burst, and the 24-h rhythmic cosine amplitude and mesor ($P < 0.01$ for each). For

TABLE 1. Total (basal plus pulsatile) GH secretion rates in elderly men and women during single or combined sc peptide infusions for 24 h

Intervention (dose, μg/kg·h)	Men (n = 5)	Women (n = 6)
Placebo (0)	19 ± 7[a] (13)	28 ± 5[a] (28)
GHRP-2 (1)	121 ± 32[b,c] (117)	177 ± 53[b,c] (145)
GHRH (1)	86 ± 29[b] (85)	97 ± 17[b] (102)
GHRH (3)	72 ± 14[b] (66)	165 ± 60[b] (140)
GHRH (10)	75 ± 16[b] (59)	195 ± 77[b] (104)
GHRP-2/GHRH (1/1)	185 ± 32[c] (172)	275 ± 61[b] (255)
GHRP-2/GHRH (1/3)	238 ± 28[c,d] (239)	452 ± 106[c] (325)
GHRP-2/GHRH (1/10)	255 ± 55[c,e] (176)	501 ± 147[e,f] (339)
Within-gender contrast (*P*)	$<10^{-5}$	0.0011

Data are the mean ± SEM. *P* values give the overall interventional effect. Unique (unshared) alphabetic superscripts within-gender (column) and between-gender differ as: *a vs. b*, $P < 0.01$ and *b vs. c*, $P < 0.001$. *bc* does not differ from either *b* or *c*.

[d] GHRP-2/GHRH (1/3) *vs.* each of GHRH 1, 3, and 10: $P = 0.013$, $P = 0.019$, and $P = 0.024$, respectively.

[e] GHRP-2/GHRH (1/10) *vs.* each of GHRH 1, 3, and 10: $P < 0.005$; and *vs.* GHRP-2: $P = 0.067$.

[f] GHRP-2/GHRH (1/10) *vs.* GHRH (10): $P = 0.016$; and *vs.* GHRP-2: $P = 0.096$.

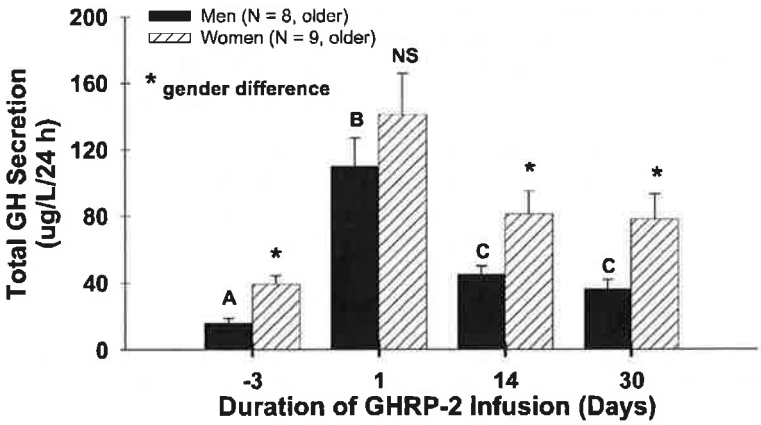


FIG. 3. Total (pulsatile plus basal) daily GH secretion rates determined in 17 healthy older adults at baseline (d −3) and on d 1, 14, and 30 of continuous sc infusion of GHRP-2 (1 μg/kg·h beginning on d 1). *Post hoc* contrasts in means are marked by unshared (unique) alphabetic superscripts. *Asterisks* denote significant differences by gender; women > men, $P < 0.025$; NS, $P > 0.05$ (n = 8 men; n = 9 women).

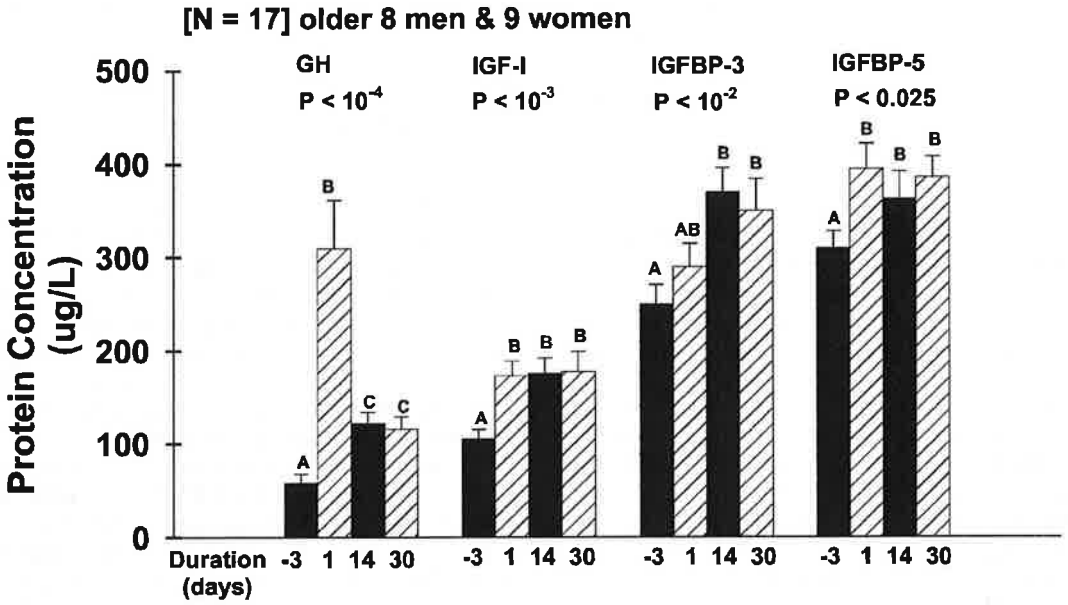


FIG. 5. Time-dependent stimulation of somatotropic hormones by continuous sc infusion of GHRP-2 on d 1, 14, and 30 compared with baseline d −3 (saline) in healthy elderly adults (n = 17). Measures include (*left* to *right*) fasting morning concentrations of GH, IGF-I, IGFBP-3, and IGFBP-5. See Fig. 3 for format of data presentation.

TABLE 2. Selected hormone concentrations during continuous infusion of GHRP-2 for 30 d

	d	IGF-II (μg/liter)	IGFBP-4 (μg/liter)	Cortisol (μg/dl)	Prolactin (μg/liter)	Leptin (μg/liter)	Adiponectin (μg/liter)
Men							
Placebo	−3	347 ± 27	548 ± 32	10.3 ± 1.0	12.3 ± 0.6	6.4 ± 1.6	12.5 ± 2.2
GHRP-2	1	334 ± 16	512 ± 25	11.3 ± 1.4	13.9 ± 1.1	7.0 ± 1.7	11.6 ± 1.8
GHRP-2	14	413 ± 53	524 ± 44			7.5 ± 1.9	13.5 ± 2.0
GHRP-2	30	354 ± 40	519 ± 47	11.4 ± 1.3	14.9 ± 0.8[a]	6.7 ± 1.5	12.6 ± 2.0
Women							
Placebo	−3	338 ± 17	543 ± 37	13.5 ± 2.1	11.5 ± 1.1	20.2 ± 4.2	17.0 ± 2.2
GHRP-2	1	379 ± 20	597 ± 24	15.1 ± 2.2	16.6 ± 1.7[a]	21.0 ± 3.8	15.6 ± 1.6
GHRP-2	14	355 ± 19	560 ± 24			23.1 ± 3.3	19.5 ± 1.9
GHRP-2	30	373 ± 19	513 ± 29	14.3 ± 1.3	16.6 ± 1.2[b]	22.2 ± 3.8	17.4 ± 2.2

Men, n = 8; women, n = 9.
[a] $P < 0.05$ *vs.* placebo.
[b] $P < 0.025$ *vs.* placebo.

apparent resistance to down-regulation of secretagogue effects under uninterrupted and submaximal feedforward is not known. Laboratory data indicate that prolonged constant sc delivery of GHRP induces expression of the pituitary GH gene in the GHRH-depleted infantile rat (65). In addition, intragastric administration of a high dose of a nonpeptidyl mimetic of GHRP down-regulates GH and IGF-I production within 24 h, whereas daily stimulation with a minimally effective dose of the same agonist progressively increases pulsatile and entropic modes of GH secretion and IGF-I concentrations (26). In short-term clinical studies in postmenopausal women, continuous iv infusion of GHRP-2 separately or combined with GHRH for 24 h amplifies pulsatile and total GH secretion by 30- to 120-fold and elevates fasting IGF-I concentrations (27–29). The present outcomes extend such inferences in older men and women by demonstrating that unvarying sc infusion of GHRP-2 for 1 month augments pulsatile, total, 24-h rhythmic, and entropic measures of GH secretion and IGF-I, IGFBP-3, and IGFBP-5 concentrations. From a qualitative viewpoint, the foregoing specific ensemble of neuroendocrine and systemic responses is identical to that observed at the time of maximal linear growth in healthy pubertal girls and boys (66). From a quantitative vantage, continuous GHRP-2 administration for 2 and 4 wk increased the 24-h GH secretion rate in elderly adults to approximately 0.5 mg (assuming a 7% distribution volume). The latter value is approximately 50% of that attained in adolescents at peak growth velocity (66–68).

In earlier pilot studies we administered GHRP-2 and/or GHRH by bolus injection in doses ranging from 0.1–3 μg/kg, sc, and at frequencies varying from once every other day to twice daily for 1–2 wk in elderly volunteers (30, 33, 69–71). In these short-term protocols, a high dose of GHRP-2 blunted the subsequent effect of the same peptide as well as that of GHRH. On the other hand, twice (but not single) daily iv pulses of GHRH for 7 d amplified the effects of later bolus injection of either GHRP-2 or GHRH. Once daily iv pulses of GHRP-2 and GHRH together for 1 wk also maintained the

menopausal women than in men of comparable age; 2) short-term (24-h) constant combined sc infusion of GHRP-2 and GHRH stimulates GH secretion and elevates IGF-I concentrations more than either peptide alone; and 3) prolonged (1-month) continuous sc delivery of GHRP-2 drives pulsatile, total, 24-h rhythmic, and entropic (feedback-adaptive) GH secretion and increases IGF-I, IGFBP-3, and IGFBP-5 concentrations. Further investigations will be required to elucidate the mechanisms that transduce the evidently sustained hypothalamo-pituitary responsiveness to unabated feedforward by GHRP.

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