

Endocrine and Metabolic Effects of Long-Term Administration of [Nle²⁷]Growth Hormone-Releasing Hormone-(1-29)-NH₂ in Age-Advanced Men and Women*

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ABSTRACT

Attenuation of the GH and insulin-like growth factor I (IGF-I) axis in aging may be responsible for changes in body composition and metabolism. This relationship has been confirmed by studies of recombinant human GH replacement in aging men and women, but the adverse effects encountered limit its clinical utility. The use of GHRH or its analogs may be an alternative mode for restoring the GH-IGF-I axis in aging individuals. Here we report the endocrine-metabolic changes in response to a GHRH analog in age-advanced men and women.

A single blind, randomized, placebo-controlled trial of 5 months duration was conducted. Ten women and 9 men between the ages of 55–71 yr self-injected placebo (saline) sc nightly for 4 weeks followed by 16 weeks of [Nle²⁷]GHRH-(1-29)-NH₂ at a dose of 10 µg/kg. Subjects underwent 12-h nocturnal (2000–0800 h) frequent blood sampling (10-min intervals) and 24-h urine collection at baseline, after 4 weeks of placebo injections, and after 16 weeks of GHRH analog administration. GH responses to GHRH analog and spontaneous GH pulsatility were assessed. Subjects were also monitored 2, 4, 8, and 12 weeks after commencement of GHRH analog treatment. Blood pressure, body weight, and fasting insulin and glucose levels were recorded at each visit. Serum concentrations of IGF-I, IGF binding protein-1 (IGFBP-1), IGFBP-3, GH-binding protein (GHBP), lipids, and safety laboratory tests (complete blood count and chemistry profile) were measured in fasting samples (0800–0900 h). Body composition was determined by dual energy x-ray absorptiometry scan, and skin thickness was measured at four sites, including the right and left hand and volar forearm, by Harpenden skin calipers. Insulin sensitivity was assessed by a frequently sampled iv glucose tolerance test. Quality of life parameters, including sleep, were evaluated through self-administered questionnaires.

Nightly GHRH analog administration at 2100 h induced, within 10

min, an acute release of GH, which lasted for 2 h. The GH-releasing effect of GHRH analog was sustained during the course of the study. Compared with placebo, GHRH analog induced a significant increase in 12-h integrated nocturnal GH levels in women ($P < 0.01$) and men ($P < 0.05$). This was accompanied, within 2 weeks, by increased serum levels of IGF-I ($P < 0.05$) and IGFBP-3 ($P < 0.001$), but not IGFBP-1, which remained elevated for 12 weeks, returning toward baseline by 16 weeks in both genders. Within 4 weeks, GHBP concentrations were significantly increased ($P < 0.01$) in women, but not in men. Although blood pressure and body weight were unaffected, GHRH analog treatment resulted in a significant increase in skin thickness ($P < 0.05$) in both genders and increased lean body mass in men only ($P < 0.05$), with no other changes in body composition or bone mineral density in either gender. There was a trend for a positive nitrogen balance in both genders, which became significant ($P = 0.03$) when the data were combined. Fasting insulin and glucose levels were unaltered, but a significant increase in insulin sensitivity occurred in men ($P < 0.05$), but not in women. Assessment of quality of life parameters revealed a significant improvement in general well-being ($P < 0.05$) and libido ($P < 0.01$) in men, but not in women, and sleep quality was unaffected in both genders. The only adverse side-effect was transient hyperlipidemia, which resolved by the end of the study.

We conclude that nightly administration of GHRH analog for 4 months in age-advanced men and women activated the somatotrophic axis. Although an increase in skin thickness was found in both genders, increases in lean body mass, insulin sensitivity, general well-being, and libido occurred in men but not in women. These observations suggest that GHRH analog administration induced anabolic effects favoring men more than women. Further studies are needed to define the gender differences observed in response to GHRH analog administration. (*J Clin Endocrinol Metab* 82: 1472–1479, 1997)

THE AGING process is associated with decreases in lean body mass (LBM) and bone mineral density (BMD) and an increase in body fat (1–3). That the reduction in GH and

insulin-like growth factor I (IGF-I) levels with aging contributes to the changes in body composition is supported by their reversal in response to recombinant human GH (rhGH) treatment. Administration of rhGH (0.3 mg/kg, sc), three times a week for 6 months, to a group of healthy men between the ages of 61–81 yr restored the low levels of IGF-I to young adult levels. This was accompanied by increases in LBM, skin thickness, and vertebral bone density and a decrease in adipose tissue mass (4). More recently, Holloway *et al.* (5) reported that in postmenopausal women 6 months of rhGH replacement (0.02 mg/kg) also led to increases in serum levels of IGF-I and IGF-binding protein-3 (IGFBP-3) and in bone turnover markers and to decreases in fat mass. However, in contrast to men, there was no change in LBM (5). In both studies, serious adverse effects were encountered, such as hypertension, impaired glucose tolerance,

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water/salt retention, carpal tunnel syndrome, and elevation of lipoprotein(a) levels, thus constraining the clinical use of rhGH (5–7).

An alternative mode for restoring the GH-IGF-I axis in aging would be through the use of GHRH. The potential advantage of this approach is that the quantity of GH release induced by exogenous GHRH would be modulated according to the ambient IGF-I levels, thereby circumventing excessive GH secretion (see review in Ref. 3). Corpas *et al.* (8), using a synthetic analog of GHRH-(1–29) at a dose of 1 mg administered sc twice daily to 10 old men (68 ± 6.2 yr) for 14 days, reported activation of the GH-IGF-I axis. In an extended study by the same group, a single nightly sc injection of GHRH (2 mg) for 6 weeks in 11 healthy elderly men increased all parameters of GH secretion, but serum levels of IGF-I, IGFBP-3, and GH-binding protein (GHBP) and body composition were unaltered (9). To our knowledge, assessments of endocrine-metabolic responses to longer duration GHRH analog treatment involving both older men and women have not been investigated. We report here the efficacy and safety of the administration of a GHRH analog, [Nle²⁷]GHRH-(1–29)NH₂, for a period of 16 weeks in activating the somatotrophic axis and its downstream metabolic effects in age-advanced men and women.

Subjects and Methods

Subjects

Nineteen (10 women and 9 men) of 20 volunteers completed the study. One subject dropped out due to personal reasons. Subjects were healthy nonsmokers, taking no medications other than daily continuous hormone replacement therapy (HRT; premarin, 0.625 mg; provera, 2.5 mg; 8 of 10 women). Medical illness was excluded by history, physical examination, complete blood count, and chemistry profile. The clinical characteristics and baseline values of somatotrophic axis parameters for the study subjects are given in Table 1. Men had a mean age of 66.9 yr

(range, 61–71) and body mass index of 24.5 kg/m² (range, 20–28), and women had a mean age of 64.6 yr (range, 55–70) and body mass index of 24.3 kg/m² (range, 17–31). The protocol was approved by the committee on investigations involving human subjects of the University of California-San Diego. All subjects gave oral and written informed consent.

Study design and procedures

The study design was a single blind, placebo-controlled trial of 20 weeks duration. At 2100 h, subjects self-injected sc into their thighs placebo (saline) for the first 4 weeks, followed by 16 weeks of [Nle²⁷]GHRH-(1–29)NH₂ at a dose of 10 µg/kg BW. The GHRH analog (provided by Dr. J. Rivier, The Salk Institute, La Jolla, CA) has been shown to have full biological activity (10). A solution of GHRH analog in 5-mL vials was provided to the subjects monthly at a concentration of 2 mg/mL. Vials were stored frozen until dispensed to the subjects, then kept at 4°C for 10 days at home. High performance liquid chromatographic analysis showed that the peptide was stable at 4°C for at least 2 weeks. All subjects were instructed to continue their current and usual dietary and exercise regimens. Seven-day diet records were analyzed by the Clinical Research Center (CRC) dietitian using the Nutritionist III (version 7) computer program (N-Squared Computing, Salem, OR). Out-patient visits to the CRC were scheduled 2, 4, 8, and 12 weeks after commencement of GHRH analog administration. During each visit, fasting blood samples were obtained between 0800–0900 h for hormone measurements and safety laboratory tests (complete blood count and chemistry profile). Each subject was interviewed regarding potential side-effects, and the vials of GHRH analog used were checked for compliance.

Subjects were admitted to the CRC at baseline, after 4 weeks of placebo treatment, and at the end of GHRH analog treatment at 0700 h after an overnight fast. Meals were served at 0800, 1200, and 1700 h, and subjects slept from 2200–0700 h. They were instructed on the sc injection technique during the initial CRC admission. At 1800 h, an iv line was inserted, and blood samples were obtained at 10-min intervals for 12 h beginning at 2000 h for GH determinations. At 2100 h on each admission, the correctness of sc administration of placebo (saline) or GHRH analog was checked. Twenty-four-hour urine collections were made for determination of creatinine and nitrogen. Total calories and the percentages of carbohydrates, fat, and protein consumed by the subjects in the 24-h period before admission to the CRC were determined by the dietitian.

TABLE 1. Clinical and baseline parameters of the somatotrophic axis in 9 men and 10 women who participated in placebo and GHRH analog treatments

Subject (sex)	Age (yr)	BMI (kg/m ²)	GH (AUC ^a 2000–0300 h)	IGF-I (ng/ mL)	IGFBP-3 (mg/L)	IGFBP-1 (µg/L)	GHBP (pmol/L)
1 (M)	62	26.6	761	136	2.3	2.3	235
2 (M)	69	22.1	1077	237	2.9	9.1	314
3 (M)	71	24.9	n/a	154	2.3	11	228
4 (M)	69	25.6	886	134	2.4	5.9	325
5 (M)	66	24.1	946	127	2.3	5.7	625
6 (M)	61	22.1	893	190	3.5	11.2	978
7 (M)	65	20.1	1511	211	3.5	14.5	112
8 (M)	68	26.1	1170	270	3.5	5.4	432
9 (M)	71	28.7	1448	140	2.2	5.1	211
Mean ± SE	66.8 ± 1.2	24.5 ± 0.9	1086 ± 96	178 ± 17	2.8 ± 0.2	7.8 ± 1.3	384 ± 89
10 (F)	70	25.1	2219	170	4	19.4	949
11 (F)	63	29.8	975	159	4.8	6.1	1006
12 (F)	61	25.5	1658	94	2.5	9.3	n/a
13 (F)	63	21.8	2153	133	2.9	18.4	499
14 (F)	70	21.9	1116	67	2	51.2	368
15 (F)	66	21.8	1706	140	2.9	22.4	387
16 (F)	55	27.8	2420	121	2.8	8.7	389
17 (F)	59	21.2	1986	188	3.1	5.5	155
18 (F)	67	16.8	4109	93	2.4	16.3	157
19 (F)	72	31.3	1274	124	3	7.1	779
Mean ± SE	64.6 ± 1.7	24.3 ± 1.4	1960 ± 283 ^b	129 ± 12 ^c	3.0 ± 0.26	16.4 ± 4.3	521 ± 106

^a Integrated area under the curve, units (micrograms per L/min). n/a, Not available.

^b $P < 0.01$ vs. men.

^c $P < 0.05$ vs. men.

At 0800 h on day 2 of the CRC admission, insulin sensitivity was assessed by the modified frequently sampled iv glucose tolerance test (11): iv lines were established in each arm for administration of 300 mg/kg dextrose as an iv bolus over 1 min in one line, followed 20 min later by an iv bolus of regular insulin (0.03 U/kg) in the opposite iv line. Blood samples were obtained (at 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90, and 180 min) for determination of plasma glucose and serum insulin concentrations. Insulin sensitivity was analyzed using the MINMOD computer program (12).

Assays

Plasma glucose concentrations were determined by the glucose oxidase method (Yellow Springs Instrument Co., Yellow Springs, OH) with an intraassay coefficient of variation (CV) less than 2% and an interassay CV of 3%. Serum insulin levels were analyzed by a double antibody RIA with a sensitivity of 15 pmol/L and intra- and interassay CVs of 7% and 9%, respectively. Serum GH concentrations were determined using a RIA with an interassay CV of 6% at 1.4 and 6.0 $\mu\text{g/L}$, intraassay CVs of

TABLE 2. Serum lipids (milligrams per dL) in response to placebo and GHRH analog treatments

	Placebo	Weeks of treatment			
		4	8	12	16
Cholesterol					
Men	209.9 $\pm$ 11	233.7 $\pm$ 9.2 ^a	214.7 $\pm$ 11.6	220.9 $\pm$ 13.2	198.7 $\pm$ 12.2
Women	197.3 $\pm$ 11.9	236.0 $\pm$ 10.8 ^b	220.9 $\pm$ 15.0 ^a	213.5 $\pm$ 11.1	195.2 $\pm$ 7.2
HDL					
Men	39.0 $\pm$ 2.8	44.4 $\pm$ 3.8 ^a	42.2 $\pm$ 3.0	42.6 $\pm$ 3.5	37.7 $\pm$ 1.7
Women	47.2 $\pm$ 3.3	57.7 $\pm$ 3.2 ^b	55.0 $\pm$ 2.9 ^c	53.0 $\pm$ 2.7	48.0 $\pm$ 2.6
LDL					
Men	134.4 $\pm$ 13.8	164.6 $\pm$ 9.7 ^a	154.9 $\pm$ 12.3	160.4 $\pm$ 13.3 ^a	136.2 $\pm$ 13.1
Women	127.2 $\pm$ 8.7	154.1 $\pm$ 12.2 ^b	149.6 $\pm$ 11.4 ^c	140.7 $\pm$ 10.5	124.7 $\pm$ 8.1
TG					
Men	174.1 $\pm$ 51.2	220.9 $\pm$ 76.3	149.0 $\pm$ 40.1	152.7 $\pm$ 46.0	152.9 $\pm$ 28.4
Women	137.3 $\pm$ 21.3	146.1 $\pm$ 18	133.2 $\pm$ 15.7	118.9 $\pm$ 14.3	137.4 $\pm$ 16.5
Lp(a)					
Men	26.4 $\pm$ 8.2	30.6 $\pm$ 9.4	24.6 $\pm$ 8.3	28.0 $\pm$ 9.4	31.0 $\pm$ 8.9
Women	16.9 $\pm$ 8.2	20.3 $\pm$ 8.6	16.9 $\pm$ 6.8	13.6 $\pm$ 5.0	13.2 $\pm$ 4.8
Apo A1					
Men	139.0 $\pm$ 5.6	163.7 $\pm$ 10.4 ^c	152.3 $\pm$ 7.9	152.7 $\pm$ 10	142.3 $\pm$ 8.8
Women	174.0 $\pm$ 10.6	195.2 $\pm$ 7.7 ^a	186.9 $\pm$ 7.4	180.9 $\pm$ 8.0	154.7 $\pm$ 7.3 ^a
Apo B					
Men	123.1 $\pm$ 7.8	145.0 $\pm$ 8.9 ^a	126.3 $\pm$ 8.1	129.0 $\pm$ 9.6	116.7 $\pm$ 8.6
Women	103.5 $\pm$ 6.0	126.7 $\pm$ 7.7 ^b	117.7 $\pm$ 8.1 ^a	111.0 $\pm$ 8.3	95.8 $\pm$ 6.5

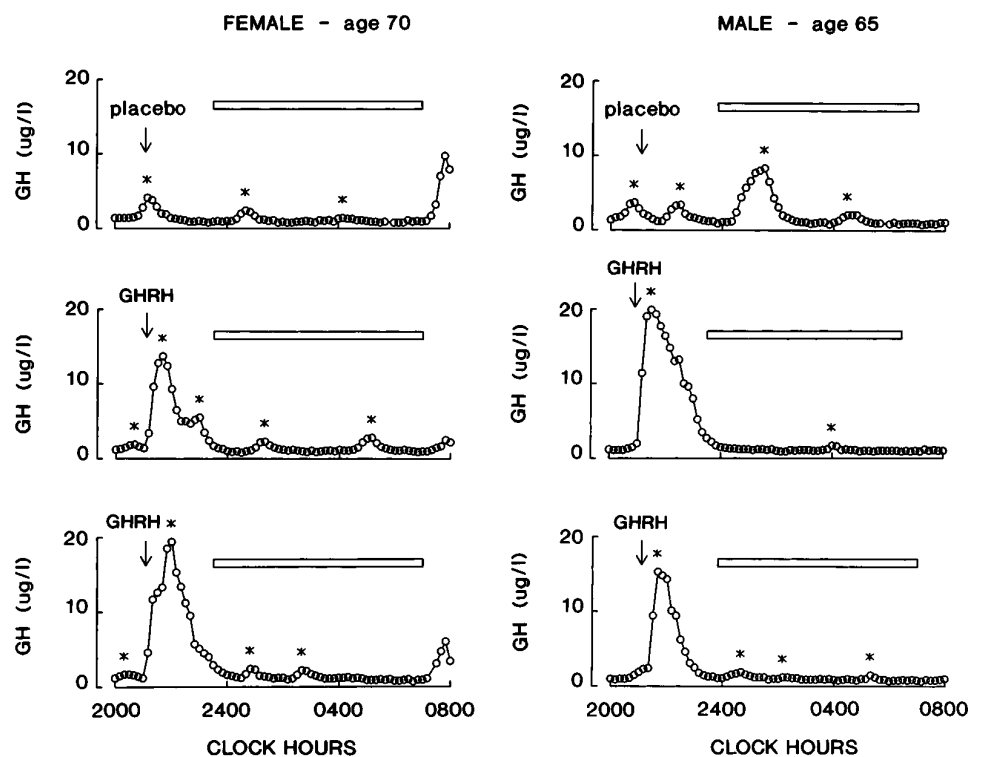
Values are the mean $\pm$ SE. Apo A1, Apolipoprotein A1; Apo B, apolipoprotein B; TG, triglyceride; Lp(a), lipoprotein(a).

^a $P < 0.05$ vs. placebo.

^b $P < 0.005$ vs. placebo.

^c $P < 0.01$ vs. placebo.

FIG. 1. Representative 12-h GH profiles with responses to sc injection at 2100 h of placebo (top panel) and GHRH analog at 1 (middle panel) and 4 months (bottom panel). Bars indicate sleep. *, GH pulses.



8% at 1.0 $\mu\text{g/L}$ and 2.5% at 4.2 $\mu\text{g/L}$, and a sensitivity of 0.9 $\mu\text{g/L}$. IGFBP-1 was measured by time-resolved immunofluorometric assay (sensitivity, 0.06 $\mu\text{g/L}$; intra- and interassay CVs, 4% and 10%, respectively) (13). IGF-I levels were measured after acid-ethanol extraction using the Corning Nichols Institute RIA kit; an intraassay CV of 6%. IGFBP-3 was measured using a RIA kit (Corning Nichols Institute) with a sensitivity of 0.1 mg/L and an intraassay CV of 3%. Total functional GHBP concentrations were measured by a ligand-mediated immunofunctional assay with a sensitivity of 8 pmol/L and an intraassay CV of 6.4% (14). Serum lipids were measured in a commercial laboratory.

Metabolic parameters

Body composition and total BMD were determined by dual energy x-ray absorptiometry scan. Skin thickness was measured at four sites (the right and left hand and the right and left volar forearm) using Harpenden calipers (15). Nitrogen balance was determined from the amount of protein consumed during 24 h, and urinary urea nitrogen was measured in 24-h urine corrected by creatinine values. Nitrogen balance was calculated according to the following formula: daily protein intake (grams)/6.25 - [24-h urinary urea nitrogen (grams) + 2.5] (2). Urinary pyridinoline, a biochemical marker of bone resorption, was measured by RIA (Pyrilinks™, Metra Biosystems Inc., Palo Alto, CA).

Quality of life

Subjects completed three questionnaires to assess the quality of life at baseline, after placebo treatment, and after 16 weeks of GHRH analog treatment. These included a general well-being questionnaire, comprising six subscales of anxiety, depression, well-being, self-control, health, and vitality (16); the Pittsburgh sleep quality index, measuring sleep satisfaction (17); and a visual analog scale for libido.

Statistical analysis

GH pulsatility was analyzed by the Cluster pulse detection algorithm, with a cluster configuration of 2×2 and t statistics of 2.5×2.5 (18). The integrated area under the curve (AUC) was determined by the trapezoid method. Outcome variables were compared by two-factor ANOVA with repeated measures. *Post-hoc* testing compared responses to the GHRH analog and placebo by Fischer's exact test. Linear or multiple regression analyses were performed as indicated. The metabolic data were analyzed by paired Student's t test (two-tailed). Data are presented as the mean $\pm$ SE, and $P < 0.05$ was considered significant.

Results

General observations

As the values of all parameters examined were not significantly different between baseline and placebo, the effects of GHRH analog administration were determined by comparing postplacebo and post-GHRH analog values. Subjects did not report adverse side-effects, and their physical examinations at the end of the study remained unchanged. There was no reaction at the injection sites (thighs), and there was full compliance with self-administration of placebo or GHRH analog. Treatment with GHRH analog did not induce detectable antibody titer against GHRH analog when the sera

were tested at dilutions of 1:5 and 1:50. Complete blood count, chemistry profile, blood pressure, and body weight were unaffected. Relative to placebo, there was a significant transient increase in serum cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), apolipoprotein A1 (Apo A1), and Apo B after 4 weeks of GHRH analog treatment in both genders. All but Apo A1 were reversed to baseline levels by 16 weeks of GHRH analog treatment. There was a 12% decrease in Apo A1 levels at 16 weeks in women only ($P < 0.05$; Table 2).

Somatotropic axis

GHRH analog administration, but not placebo, stimulated within 10 min an acute increase in GH levels in men ($18.5 \pm 5 \mu\text{g/L}$) and women ($20.5 \pm 4.3 \mu\text{g/L}$), and levels returned to baseline in 2 h (2100–2300 h) in both genders. Other than the episode of GH release induced by GHRH analog, the profile of GH pulsatility remained intact during the 12-h study, with no changes in pulse frequency or amplitude (Fig. 1 and Table 3). Relative to placebo, GHRH analog treatment in men significantly ($P < 0.05$) increased the integrated GH response in the 2 h (2100–2300 h) after injection and for the 12-h nocturnal (2000–0800 h) period at 4 and 16 weeks (Fig. 2 and Table 4). In women, a similar incremental change was seen, but the acute GH response to GHRH analog was further

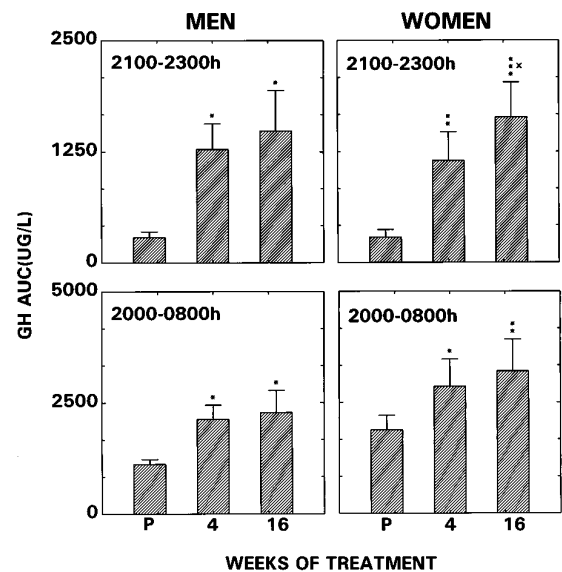


FIG. 2. GH AUC in acute response (2100–2300 h; top) and integrated 12-h values (2000–0800 h) in response to placebo (p) and GHRH analog treatments in men and women. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (vs. placebo). X, $P < 0.01$ (vs. 4 weeks in women).

TABLE 3. Twelve-hour nocturnal (2000–0800 h) GH pulsatile parameters in response to placebo and GHRH analog in age-advanced men and women

Treatment	Women			Men		
	No. of peaks (12 h)	Frequency (min)	Amplitude ($\mu\text{g/L}$)	No. of peaks (12 h)	Frequency (min)	Amplitude ($\mu\text{g/L}$)
Placebo (4 weeks)	4.7 $\pm$ 0.9	222 $\pm$ 64	4.4 $\pm$ 1.2	4.7 $\pm$ 0.3	147 $\pm$ 17	1.8 $\pm$ 0.4
GHRH (4 weeks)	5.3 $\pm$ 0.4	142 $\pm$ 11	6.3 $\pm$ 2.0	3.3 $\pm$ 0.6	248 $\pm$ 39	7.3 $\pm$ 2.2
GHRH (16 weeks)	4.6 $\pm$ 0.7	194 $\pm$ 33	6.7 $\pm$ 1.3	3.5 $\pm$ 0.7	288 $\pm$ 92	12.5 $\pm$ 7.4

Values are the mean $\pm$ SE.

TABLE 4. Integrated GH responses during the first 2 h after GHRH analog administration and during the 12-h nocturnal period in age-advanced men and women

	Men		Women	
	2 h (2100–2300 h)	12 h (2000–0800 h)	2 h (2100–2300 h)	12 h (2000–0800 h)
Placebo (4 weeks)	177 ± 28	1115 ± 106	293 ± 75	1873 ± 350
GHRH (4 weeks)	1277 ± 289 ^a	2126 ± 319 ^a	1083 ± 289 ^b	2850 ± 616 ^a
GHRH (16 weeks)	1484 ± 450 ^a	2276 ± 496 ^a	1567 ± 338 ^{c,d}	3162 ± 707 ^a

Values are the mean ± SE.

^a $P < 0.05$ vs. placebo.

^b $P < 0.01$ vs. placebo.

^c $P < 0.005$ vs. placebo.

^d $P < 0.05$, 16 weeks vs. 4 weeks.

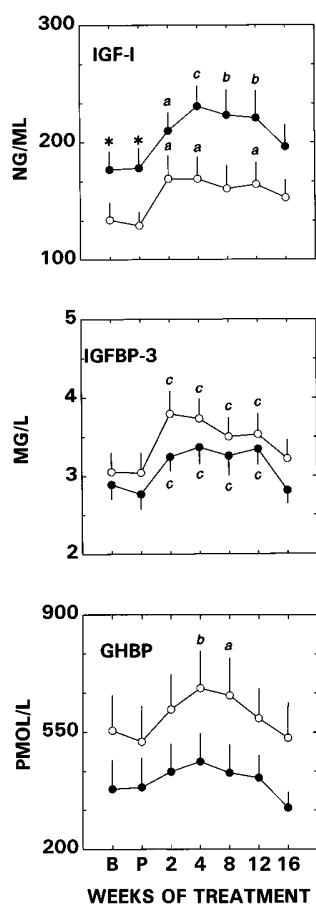


FIG. 3. Serum levels of IGF-I, IGFBP-3, and GHBP in men (solid circles) and women (open circles) at baseline (B), after placebo (P), and during GHRH analog treatment. a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$ (vs. placebo). *, $P < 0.05$ (comparing IGF-I levels between men and women).

augmented ($P < 0.01$) at 16 weeks from that seen at 4 weeks (Fig. 2 and Table 4).

Activation of GH secretion by GHRH analog led to increases in IGF-I, IGFBP-3, and GHBP levels (Fig. 3), without affecting IGFBP-1 levels (Table 5). Serum IGF-I levels were higher in men than in women at baseline (Table 1) and after placebo treatment ($P < 0.05$; Fig. 3). Within 2 weeks of GHRH analog treatment, serum IGF-I levels rose significantly ($P < 0.05$) to a maximum of 256 ± 20 ng/mL, in men which was significantly higher ($P < 0.05$) than the maximal level of

197.2 ± 18 ng/mL in women (Fig. 3). IGF-I levels remained elevated at 12 weeks for both genders and declined toward baseline by 16 weeks. The relative increases in GH AUC and IGF-I levels in response to GHRH analog were similar between men (GH, $107 \pm 43\%$; IGF-I, $28 \pm 6\%$) and women (GH, $70 \pm 21\%$; IGF-I, $27 \pm 8\%$; Fig. 4). There was no correlation between the increments in GH and IGF-I levels in men ($r = 0.09$; $P = 0.85$) or women ($r = 0.18$; $P = 0.63$). Two men and one woman did not show an increase in GH AUC (2000–0800 h), although for one of these men and for the woman a rise in IGF-I levels occurred (Fig. 4). One woman did not show an increased IGF-I level (mean of months 1–3) despite a normal GH response; however, for this subject an increase in IGF-I levels did occur at month 4. There was a highly significant rise ($P < 0.001$) in serum IGFBP-3 levels, which followed a similar time course as the increase in IGF-I levels in both men and women (Fig. 3). A positive correlation was found between baseline IGFBP-3 and IGF-I levels in women ($r = 0.72$; $P = 0.01$) and men ($r = 0.79$; $P = 0.03$). In women, but not men, GHBP levels rose significantly ($P < 0.01$) at 4 and 8 weeks and declined to baseline levels by 12 weeks (Fig. 3).

Metabolic effects

No significant changes occurred in total calories and dietary composition consumed or in exercise regimens during the course of the study. Body weight and blood pressure were unaffected by GHRH analog administration (Table 5). As shown in Table 5, there were no significant changes in the body fat mass in either gender. However, in six of the eight women who were receiving HRT, a small increase in body fat mass was found (mean change, 1872 ± 463 g, $P = 0.002$). In men, but not women, a gain of 1.26 ± 0.52 kg ($P < 0.05$) in LBM was found. Skin thickness increased significantly in both men ($P < 0.05$) and women ($P < 0.01$) after 16 weeks of GHRH analog treatment. There was a trend for an increase in nitrogen retention, which attained statistical significance ($P = 0.03$) when the data for both genders were combined. Serum testosterone levels in men were unaltered, with a mean of 10.3 ± 0.76 nmol/L during placebo treatment and 11.4 ± 1.2 nmol/L after GHRH analog administration. No significant changes in BMD or urinary pyridinoline were found. Although fasting insulin and glucose concentrations were unaltered, GHRH analog treatment induced a significant increase ($P < 0.01$) in insulin sensitivity in men, but not women (Table 5).

TABLE 5. Metabolic responses to GHRH analog and placebo treatments in age-advanced men and women

Parameters	Men		Women	
	Placebo	GHRH	Placebo	GHRH
Blood pressure				
Systolic (mm Hg)	132 ± 3	132 ± 2	127 ± 4	121 ± 4
Diastolic (mm Hg)	73 ± 2	74 ± 2	78 ± 5	68 ± 3
Anabolic parameters				
BW (kg)	74.27 ± 3.24	75.70 ± 3.69	61.97 ± 4.09	62.81 ± 4.21
Lean mass (kg) ^a	54.74 ± 2.36	56.00 ± 2.55 ^b	41.23 ± 1.49	41.25 ± 1.47
Fat mass (kg)	16.97 ± 1.25	17.13 ± 1.36	18.80 ± 2.76	19.33 ± 1.36
% Body fat	22.8 ± 1.2	22.5 ± 1.2	29.0 ± 2.4	29.5 ± 2.7
BMD (g/m ²) ^a	1.1056 ± 0.043	1.101 ± 0.038	0.9648 ± 0.037	0.9612 ± 0.038
Pyridinoline (nmol/mmol creatinine)	50.4 ± 5.8	60.0 ± 11.2	43.4 ± 9.9	58.6 ± 6.5
Skin thickness (mm) ^c	9.2 ± 0.5	10.5 ± 0.5 ^b	8.7 ± 0.5	9.8 ± 0.6 ^d
Nitrogen balance (g/day) ^e	3.75 ± 0.90	6.50 ± 1.50	6.37 ± 1.43	9.14 ± 1.39
Glucose metabolism				
Fasting glucose (mmol/L)	5.07 ± 0.19 ^f	5.11 ± 0.10	4.43 ± 0.15	4.68 ± 0.16
Fasting insulin (pmol/L)	32 ± 9	36 ± 14	29 ± 5	22 ± 3
Insulin sensitivity ^f	1.86 ± 0.50	2.90 ± 0.68 ^b	2.92 ± 0.43	3.36 ± 0.65
IGFBP-1 (μg/L)	7.8 ± 1.3	6.2 ± 0.9	16.4 ± 4.3	15.2 ± 4.4

Values are the mean ± SE.

^a Body composition/bone density by DEXA.

^b $P < 0.05$ vs. placebo.

^c Sum of four sites.

^d $P < 0.01$ vs. placebo.

^e Combined nitrogen balance (placebo 5.1 ± 0.89 ; GHRH, 7.89 ± 1.0 ; $P < 0.01$).

^f $P < 0.05$ vs. corresponding value in women.

^g Determined by modified FSIGT ($\times 10^{-4}$ min/ μ U · mL).

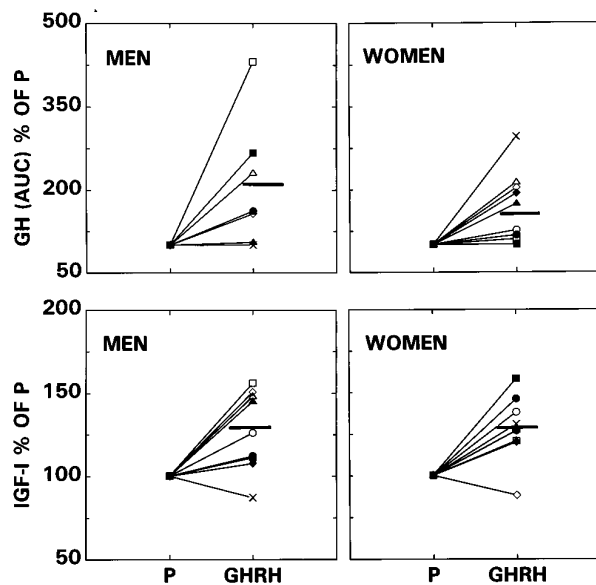


FIG. 4. Percent changes in integrated 12-h nocturnal (2000–0800 h) GH values and mean IGF-I levels (months 1–3) in response to 4 months of GHRH analog treatment in each individual age-advanced man and woman. Horizontal lines represent mean values.

Quality of life

Men, but not women, reported a significant improvement in general well-being ($P < 0.05$) and libido ($P < 0.01$). There were no changes in sleep quality in either gender (Fig. 5).

Discussion

Treatment with a single nightly sc injection of the GHRH analog (10 μg/kg) for 16 weeks significantly increased 12-h nocturnal integrated (AUC) GH release in age-advanced men

and women. This effect was largely due to GH peaks induced by the GHRH analog injection without alteration of spontaneous GH pulsatility. With relatively long term treatment, desensitization to the GH-releasing effect of the GHRH analog did not occur. In women, but not in men, a priming effect of GHRH analog was observed, with a greater ($P < 0.05$) GH release at 16 weeks than at 4 weeks. The enhancement of GH secretion reported here is consistent with observations during a shorter duration of GHRH analog administration (8, 9, 19).

Downstream effects of the enhanced GH secretion occurred, as both IGF-I and IGFBP-3 levels were significantly elevated. The maximal percent increment in serum IGF-I levels after treatment with the analog was similar in men ($28 \pm 6\%$) and women ($27 \pm 8\%$). Serum levels of IGFBP-3, the major circulating binding protein for IGF-I, rose within 2 weeks of GHRH analog treatment and remained elevated for 12 weeks in both genders. The parallel rise of serum levels of IGF-I and IGFBP-3 is consistent with their GH dependence (20). The decline of IGF-I and IGFBP-3 toward baseline levels at 16 weeks in the face of elevated GH levels is puzzling. As IGFBP-1 is primarily regulated by insulin (21), and insulin levels remained unaltered, the finding of unchanged IGFBP-1 levels in response to GHRH analog treatment is expected. Plasma levels of GHBP decline after age 60 yr, and this decrease has been postulated to be a contributing factor to the age-related GH resistance in target tissue (22). Although there were no gender-related differences in baseline GHBP levels, a significant increase in GHBP levels, which lasted for 8 weeks, occurred in women, but not in men. The mechanism subserving this transient rise in GHBP levels selectively in women is unclear and may be related to a modulating effect of estrogen on the GHBP response to GHRH analog treatment. This possibility is consistent with

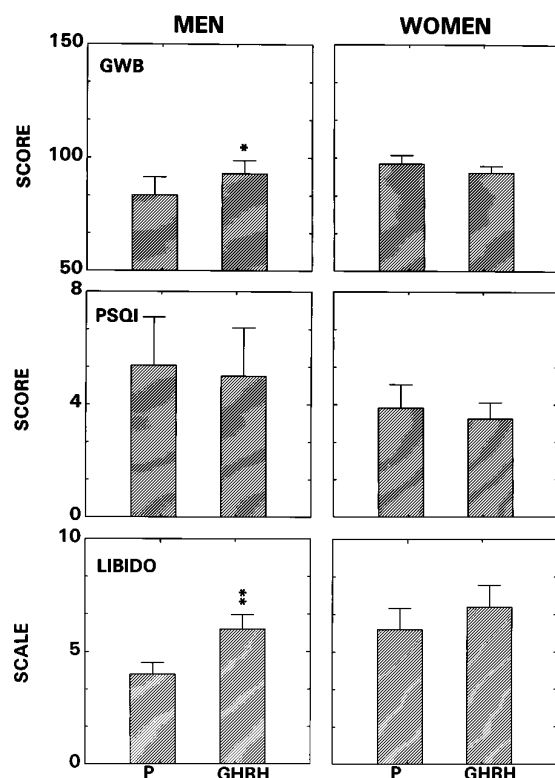


FIG. 5. General well being (GWB), Pittsburgh sleep quality index (PSQI), and libido scales in age-advanced men and women after 1 month of placebo (P) and 4 months of GHRH analog treatment. *, $P < 0.05$; **, $P < 0.01$ (vs. P).

the study by Kelly *et al.* (23) showing that oral estrogen replacement through a first pass hepatic effect enhances GHBP production.

There were similarities and differences in the metabolic responses to the administration of GHRH analog *vs.* rhGH. In our study, men had a 1-kg gain in LBM after 4 months of GHRH analog treatment, which was comparable to that after 6 months of rhGH replacement in men (4). In contrast to rhGH, there was no decrease in fat mass in men in our study, and the majority of women receiving HRT actually had a small gain in their fat mass, a finding similar to that reported by Holloway *et al.* in postmenopausal women receiving rhGH (5). In this connection, O'Sullivan *et al.* (24) recently reported that oral, but not transdermal, estrogen replacement in postmenopausal women resulted in an increase in fat mass and a decrease in LBM. It should be noted that determination of body composition by dual energy x-ray absorptiometry has its limitations because of the inability to quantitate visceral fat. GHRH analog treatment resulted in an increased insulin sensitivity in men, but not in women, an effect that may be mediated by the elevation of IGF-I levels, which can enhance muscle insulin sensitivity (25). If so, the lack of a similar effect in women may also be related to a modulatory effect of oral estrogen. Nonetheless, the improved insulin sensitivity in men provides an advantage over rhGH replacement, which has the opposite effect. GHRH analog treatment did not affect BMD or indexes of bone turnover, similar to the negative findings of Holloway *et al.* with 6 months of rhGH replacement in postmenopausal women (5),

but in contrast to the trophic effects of rhGH on BMD in men (4). This lack of effect of GHRH analog on bone turnover is probably due to the degree and shorter duration of GH-IGF-I activation. Collectively, these findings suggest that the sex steroid milieu and the route of HRT administration may be confounding factors in determining the metabolic response to both rhGH and GHRH analog treatments, a proposition remaining to be determined.

Given the limitations associated with self-administered questionnaires, a significant enhancement of a sense of general well-being and libido was found in men, but not in women. The improved libido was unrelated to changes in serum androgens, as serum testosterone, dihydrotestosterone, and sex hormone-binding globulin (data not shown) were unaltered. Although iv pulses of GHRH administration at 4-h intervals have been shown to promote slow wave sleep, as determined by electroencephalogram monitoring (26, 27), an improvement in sleep quality was not found in this study. Blackman *et al.* (9), using a single sc dose of GHRH analog in older men, also did not detect changes in sleep quality. This discrepancy may have been due to differences in the route and frequency of GHRH administration as well as in the methods of assessing sleep quality.

In conclusion, administration of GHRH analog for 4 months is a safe and effective means to activate the somatotrophic axis in age-advanced men and women. No adverse effects were encountered. The only significant side-effect of GHRH analog treatment was transient hyperlipidemia. The increases in skin thickness and nitrogen retention suggest a shift toward anabolic metabolism in both genders, but differences in metabolic responses to GHRH treatment were found, with men displaying an increase in LBM and insulin sensitivity, and enhancement of general well-being and libido not seen in women. Further studies are warranted with a longer term of GHRH analog treatment and assessment of the impact of oral estrogen in blunting the beneficial effects of GHRH analog on body composition in women.

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