Unequal impact of age, percentage body fat, and serum testosterone concentrations on the somatotrophic, IGF-I, and IGF-binding protein responses to a three-day intravenous growth hormone-releasing hormone pulsatile infusion in men

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Abstract

We here investigate the potential rescue of the relative hyposomatotropism of aging and obesity by 3-day pulsatile GHRH infusions (i.v. bolus $0.33 \mu\text{g/kg}$ every 90 min) in 19 healthy men of varying ages (18 to 66 years) and body compositions (12 to 37% total body fat). Baseline (control) and GHRH-driven pulsatile GH secretion (in randomly ordered sessions) were quantitated by deconvolution analysis of 24-h (10-min sampling) serum GH concentration profiles measured in an ultrasensitive (threshold $0.005 \mu\text{g/l}$) chemiluminescence assay. GHRH infusion significantly increased the mean (24-h) serum GH concentration (0.3 ± 0.1 basal vs $2.4 \pm 0.4 \mu\text{g/l}$ treatment; $P = 0.0001$), total daily pulsatile GH production rate (21 ± 9.5 vs $97 \pm 17 \mu\text{g/l/day}$; $P = 0.01$), GH secretory burst frequency (11 ± 0.5 vs 17 ± 0.3 events/day; $P < 0.01$), and mass of GH released per burst (1.1 ± 0.4 vs $5.9 \pm 1 \mu\text{g/l}$; $P < 0.01$), as well as serum IGF-I (261 ± 33 vs $436 \pm 37 \mu\text{g/l}$; $P = 0.005$), insulin (45 ± 13 vs $79 \pm 17 \text{mU/l}$; $P = 0.0002$), and IGF binding protein (IGFBP)-3 (3320 ± 107 vs $4320 \pm 114 \mu\text{g/l}$; $P = 0.001$) concentrations, while decreasing IGFBP-1 levels (16 ± 1.2 vs $14 \pm 0.09 \mu\text{g/l}$; $P = 0.02$). Serum total testosterone and estradiol concentrations did not change. GHRH treatment also reduced the half-duration of GH secretory bursts, and increased the GH half-life. GHRH-stimulated 24-h serum GH concentrations and the mass of GH secreted per burst were correlated negatively with age ($R[\text{value}]:P[\text{value}] = -0.67:0.002$ and $-0.58:0.009$ respectively), and percentage body fat ($R:P = -0.80:0.0001$ and $-0.65:0.005$ respectively), but positively with serum testosterone concentrations ($R:P = +0.55:0.016$ and $+0.53:0.019$ respectively). GHRH-stimulated plasma IGF-I increments correlated negatively with age and body mass index, and positively with serum testosterone, but not with percentage body fat. Cosinor analysis disclosed persistent nyctohemeral rhythmicity of GH secretory burst mass (with significantly increased 24-h amplitude and mesor values) but unchanged acrophase during fixed pulsatile GHRH infusions, which suggests that both GHRH- and non-GHRH-dependent mechanisms can modulate the magnitude (but only non-GHRH mechanisms can modulate the timing) of somatotrope secretory activity differentially over a 24-h period.

In summary, diminished GHRH action and/or non-GHRH-dependent mechanisms (e.g. somatostatin excess, putative endogenous growth hormone-releasing peptide deficiency etc.) probably underlie the hyposomatotropism of aging, (relative) obesity, and/or hypoandrogenemia. Preserved or increased tissue IGF-I responses to GHRH-stimulated GH secretion (albeit absolutely reduced, suggesting GHRH insensitivity in obesity) may distinguish the pathophysiology of adiposity-associated hyposomatotropism from that of healthy aging.

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Introduction

Increasing age and body fat singly and jointly suppress pulsatile growth hormone (GH) secretion (1–9). However,

the pathophysiological disruption of GH secretion in these hyposomatotropic states has been difficult to quantitate, since 22–97% of daytime (awake, nonfasting) and fed serum GH concentrations fall

below the limits of detectability of most RIA and IRMA techniques (5, 7, 8, 10). More recent ultrasensitive chemiluminescence assays and an ELISA for GH (sensitivities of approximately 0.001–0.005 $\mu\text{g/l}$) can measure serum GH concentrations uniformly in normal and hyposomatotropic adults. Such assays document consistently small amounts of apparently basal (inter-pulse) GH release (7, 8, 11, 12), and suggest differential regulation of pulsatile versus basal GH secretion; e.g. testosterone is a positive, and obesity or age a negative, statistical determinant of GH secretory pulse mass, but not basal GH secretion (7); organic hypopituitarism is marked by preferential attenuation of GH secretory burst mass (11); and the total daily pulsatile GH secretion rate correlates positively with serum insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-1 concentrations, whereas basal GH secretion correlates inversely with IGFBP-3 concentrations (7).

Despite the foregoing assay advances, the neuroendocrine mechanisms underlying the hyposomatotropism accompanying (relative) obesity, aging, and hypoandrogenemia are poorly understood. Among possible pathophysiologies are somatostatin excess, growth hormone-releasing hormone (GHRH) deficiency and/or other hypothalamo–pituitary disturbances. In testing for GHRH deficiency, somatotrophic responses to single and repetitive GHRH stimuli have been inconsistent, namely blunted or normal in aging and/or obese humans (10, 13–16). In addition, whether GHRH infusion amplifies the basal versus pulsatile components of GH secretion in normal, obese, relatively hypoandrogenemic, and/or aging individuals is not known.

We hypothesized that an i.v. pulsatile GHRH stimulus administered experimentally for several days would obviate some of the limitations of earlier single-dose, twice-daily, or continuous subcutaneous GHRH treatments, and help affirm or refute an hypothesis of GHRH deficiency or insensitivity in aging, obesity, or (physiological) hypoandrogenemia. If GHRH deficiency were the sole factor pertinent to the hyposomatotropism of adiposity, aging, and/or relative hypoandrogenemia, we predicted that sustained pulsatile GHRH drive would reduce or eliminate the strongly negative correlations otherwise observed between GH secretory activity and one or more of these key variables (7, 8).

To test an hypothesis of isolated GHRH deficiency in aging and/or obesity, we investigated a diverse cohort of healthy men (ages 18–66 years, percentage total body fat 12–37%, and serum total testosterone 220–710 ng/dl) at baseline (saline) and on day 3 of a 72-h pulsatile i.v. GHRH infusion in randomly ordered sessions. We assessed by regression analysis the impact of age, testosterone, and percentage body fat on exogenous GHRH-driven GH secretory measures, as well as on serum concentrations of IGF-I, IGFBP-3, IGFBP-1, and insulin.

Materials and methods

Human subjects

Nineteen healthy men were recruited over a range of ages (18–66 years), body mass indices (18–39 kg/m^2), and percentage total body fat (12–37%, as determined by underwater weighing). Baseline data have been reported earlier (7). After provision of written informed consent approved by the Human Investigation Committees of the University of Virginia and the Salem VA Medical Center, volunteers were admitted to the General Clinical Research Center the night prior to blood sampling. A detailed history and physical examination were performed. No subject had any acute or chronic weight change, known disease, medication or drug use, strenuous exercise within 24 h, recent transmeridian travel, or disruption of sleep–wake patterns. Screening blood measurements of hematological, renal and hepatic function, and metabolic indices were normal.

Each volunteer was studied twice at an interval of four or more weeks, and the order of study randomly assigned to no drug (control) or 0.33 $\mu\text{g/kg}$ GHRH(1–29) NH_2 (Geref, Serono Laboratories, Randolph, MA, USA) diluted in bacteriostatic water for i.v. bolus (1 min) infusion every 90 min for 72 h. A second i.v. catheter was placed in a contralateral forearm vein at 0600 h on the second day of the control admission and on the third day of GHRH treatment for blood sampling (1.5 ml/sample) at 10-min intervals for 24 h. Subjects received isocaloric meals at 0830, 1200, and 1800 h clocktimes with no intervening snacks or caffeine-containing beverages. Volunteers were awake and allowed to ambulate in the study room with bathroom privileges until 2300 h clocktime. Although sleep was not monitored, lights were extinguished at 2300 h.

Underwater weighing was used to calculate the percentage body fat, as described earlier (9). Body mass index (BMI) was computed as the subject's weight in kilograms divided by his height in meters squared.

GH assay

An ultrasensitive chemiluminescence assay (Nichols Laboratories, San Juan Capistrano, CA, USA) with robotic automation (7, 8) was used to assay GH in all 145 serum samples from any given subject together to eliminate interassay variability. Assay sensitivity (four standard deviations (s.d.) above the zero dose tube) was 0.005 $\mu\text{g/l}$. No serum GH measurements fell below 0.005 $\mu\text{g/l}$, and not more than 5% of the measurements were below 0.030 $\mu\text{g/l}$. The within-assay coefficients of variation ranged from 4.5% to 13%. Within-sample s.d. were recalculated in each series as a dose-dependent power function of the 145 sample GH concentrations (for deconvolution analysis see below). Serum concentrations of IGF-I, IGFBP-1, and IGFBP-3 were assayed by RIA in 24-h pools (17).

Deconvolution analysis

A multiparameter deconvolution technique was applied to quantitate GH secretion from the 24-h serum GH concentration profiles (5–8, 18, 19). Preliminary fitting used waveform-independent deconvolution (PULSE2) (20), assuming a nominal GH secretory burst half-duration of 14–18 min and GH half-life of 16–18 min, as measured earlier (18). Basal GH secretion (PULSE2) approximated the lowest 4–6% of all serum GH measurements in that individual. These estimates were then used by multiparameter deconvolution analysis (DECONV) to quantify: GH secretory burst frequency (number of pulses per 24 h), amplitude (maximal rate of GH secretion within a pulse, $\mu\text{g/l/min}$), mass (integral or mass, $\mu\text{g/l}$, of the calculated GH secretory pulse), half-duration (duration in min of a secretory episode at half its maximal amplitude), basal GH secretion rate ($\mu\text{g/l/min}$, basal amount of hormone secreted per unit distribution volume per unit time), and GH half-life (min). The daily pulsatile GH production rate is the product of the mean GH secretory burst mass and frequency, and the daily basal GH secretion rate the product of the mean basal secretion rate ($\mu\text{g/l/min}$) and the duration of sampling (1440 min). The percentage pulsatile GH release is the ratio (expressed as a percentage) of daily pulsatile GH release to the grand total of pulsatile plus basal GH secretion.

Twenty-four-hour variations in calculated GH secretory burst mass were evaluated by cosinor analysis (21).

Statistical analysis

Data are presented as the mean $\pm$ S.E.M. ($n = 19$). Paired Student's *t*-testing (two-tailed) was used, and confirmed by Wilcoxon nonparametric testing, to appraise the within-subject effects of GHRH treatment. To evaluate age-, adiposity-, and sex-steroid hormone-dependent changes in GH secretory measures in response to GHRH infusions, univariate linear or exponential regression analyses were employed (7). When more than five regressions were carried out, a protected *P* value of 0.01 or less was utilized.

Results

Overall GH responses

Pulsatile i.v. GHRH infusion stimulated GH release in all 19 subjects regardless of age and percentage body fat, as illustrated in Fig. 1. GHRH treatment increased the 24-h mean serum GH concentration (control vs GHRH) (0.30 ± 0.11 vs $2.4 \pm 0.4 \mu\text{g/l}$; $P = 0.0001$), and its integrated value (427 ± 153 vs $3400 \pm 540 \mu\text{g/l/min}$; $P = 0.0001$). Pulsatile GHRH injections also significantly altered the following parameters: frequency of GH secretory bursts (control vs GHRH) (11 ± 0.5 vs

17 ± 0.3 ; $P < 0.01$), GH secretory burst half-duration (24 ± 2.4 vs 4.4 ± 0.72 min; $P < 0.01$), GH secretory burst amplitude (0.05 ± 0.01 vs $2.1 \pm 0.5 \mu\text{g/l/min}$; $P < 0.01$), GH secretory burst mass (1.1 ± 0.4 vs $5.9 \pm 1.0 \mu\text{g/l}$; $P < 0.01$), total daily pulsatile GH secretory rate (21 ± 9.5 vs $97 \pm 17 \mu\text{g/l/day}$; $P < 0.01$), and grand total (basal and pulsatile) daily GH production rate (22 ± 10 vs $100 \pm 18 \mu\text{g/l/day}$; $P < 0.01$) (Fig. 2). GHRH augmented both pulsatile ($21 \pm 9.5 \mu\text{g/l/day}$ ($82 \pm 5\%$ of total) vs $97 \pm 17 \mu\text{g/l/day}$ ($96 \pm 1\%$ of total)) and basal (0.66 ± 0.09 vs $3.1 \pm 0.72 \mu\text{g/l/min}$) GH secretion (Fig. 3). GHRH treatment also slightly, but consistently, prolonged GH half-life (16.9 ± 0.9 vs 20.5 ± 1.3 min; $P < 0.01$).

Pulsatile GHRH infusions augmented the serum (24-h pool) concentrations of IGF-I (control vs GHRH) (261 ± 33 vs $436 \pm 37 \mu\text{g/l}$; $P < 0.05$), IGFBP-3 (3320 ± 107 vs $4320 \pm 114 \mu\text{g/l}$; $P < 0.05$), glucose (5.2 ± 0.37 vs 6.1 ± 0.56 mmol/l; $P = 0.002$) and insulin (45 ± 13 vs 79 ± 17 IU/l; $P = 0.0002$), and reduced the mean serum concentration of IGFBP-1 (16 ± 1.2 vs $14 \pm 0.9 \mu\text{g/l}$; $P = 0.02$) (Fig. 4). On the other hand, GHRH injections did not alter the (24-h pooled) serum concentrations of testosterone (15.6 ± 1.4 vs 16.2 ± 1.4 nmol/l; $P > 0.05$) or estradiol (144 ± 10 vs 133 ± 10 pmol/l; $P > 0.05$).

Effect(s) of age, percentage body fat, or serum testosterone concentration on responses to GHRH

During pulsatile infusions of GHRH, mean (24-h) serum concentrations of GH remained negatively correlated to age and relative adiposity, defined either by BMI or percentage body fat (Table 1 and Fig. 5). Age and body fat were correlated negatively to both the absolute and incremental (difference between post-GHRH and basal) mean 24-h serum GH concentration, GH production rate, and mass of GH secreted per burst. In contrast, the regression of serum testosterone on GHRH-stimulated (absolute) serum GH concentration did not attain significance. However, serum testosterone correlated strongly and positively with GHRH-stimulated absolute or incremental (total) GH production rate or incremental serum GH concentration (Table 1 and Fig. 5).

Figure 6 presents the individually observed ($n = 19$ men) basal, incremental, and GHRH-stimulated values of (mean 24-h) GH secretory burst mass, given as a function of age (top panel), serum total testosterone concentration (second panel), BMI (third panel), and percentage body fat (bottom panel).

Figure 7 depicts the individual basal, incremental, and GHRH-stimulated serum IGF-I concentrations in relation to age, percentage body fat, BMI and serum testosterone concentrations. Correlation coefficients for corresponding regressions are given in Table 1. Serum IGF-I concentrations (incremental and absolute) were

Figure 2 Summary of specific GH secretory parameters and the total daily GH production rate basally (control) and in response to i.v. pulsatile GHRH ($0.33 \mu\text{g/kg}$) administration over 72 h in 19 men of varying ages and body compositions. Data are means $\pm$ s.e.m. $P < 0.01$ for all paired comparisons shown.

statistically independent of percentage body fat during GHRH administration, whereas GHRH-stimulated incremental (but not absolute) serum IGF-I levels correlated negatively with age and BMI, and positively with serum testosterone concentrations ($P = 0.051$ for $R = +0.454$). Estradiol levels did not correlate with any of the foregoing GH-axis responses to GHRH.

Figure 8 shows the relationships of the ratios of serum IGF-I to GH pulse mass (during placebo or GHRH

treatment) to percentage body fat (top panel), testosterone (middle panel), and age (bottom panel). The basal (non-GHRH treatment) ratio of serum IGF-I to GH burst mass correlated, respectively, negatively ($R = -0.623$, $P = 0.006$) and positively ($R = +0.724$, $P = 0.007$) with testosterone and age. On the other hand, percentage body fat correlated positively with serum IGF-I/GH burst mass during GHRH treatment ($R = +0.784$, $P = 0.0002$).

Figure 1 Illustration of the 24-h profiles of serum GH concentrations and calculated GH secretory rates before (left column) and during the last day of 3 days of pulsatile i.v. GHRH administration (right column) at a dose of $0.33 \mu\text{g/kg}$ every 90 min in three men of different ages and body compositions. Subpanels are paired vertically and horizontally. The top subpanels in each vertical pair represent serum GH concentrations in each man measured by chemiluminescence assay in blood samples collected at 10-min intervals over a period of 24 h. The continuous curves through the observed serum GH levels are predicted by deconvolution analysis (see Materials and methods). The matching lower subpanels in each man depict the corresponding deconvolution-calculated GH secretory events and any basal GH secretion as a function of time. Note different y-axis scales to accommodate the wide range of serum GH concentrations attained. Insets show expanded axis views. Ages were (top-to-bottom pairs) 18, 31, and 57 years, and percentages of total body fat were 12, 31, and 37 in the 3 men whose GH data are shown here.

Figure 3 Total daily GH secretory rates with the respective contributions of pulsatile and basal (non pulsatile) GH secretory components before and during the last 24 h of 72-h i.v. administration of GHRH pulses at 90-min intervals in 19 men of differing ages and body compositions. Data are means $\pm$ S.E.M. $P < 0.01$ for all paired comparisons shown.

GH nyctohemeral rhythmicity

As shown in Fig. 9, cosinor analysis revealed diurnal (24-h) rhythmicity of GH secretory burst mass both basally and during pulsatile GHRH infusions. There were no significant differences in the mean (95% confidence interval) times of the circadian acrophases, namely, 1031 (841–1236) min vs 1112 (1009–1217) min after 0800 h ($P = \text{not significant}$) for the basal and the GHRH-infusion sessions respectively. However, GHRH infusions significantly increased the amplitude (1.8 (0.31–3.3) vs 4.7 (2.6–6.8) $\mu\text{g/l}$; $P < 0.05$) and mesor (1.8 (0.78–2.9) vs 6.2 (4.7–7.9) $\mu\text{g/l}$; $P < 0.01$) of the 24-h variation in GH secretory burst mass.

Discussion

To test a hypothesis of isolated GHRH deficiency or GHRH resistance in aging and/or obesity, we applied an

experimental paradigm of unvarying i.v. pulsatile GHRH infusions over 72 h in a diverse cohort of healthy men ($n = 19$) of varying ages (span, 18–66 years) and degrees of adiposity (range of percentage total body fat, 12–37%). Three days of an experimentally fixed GHRH ‘clamp’ stimulated both pulsatile and interpeak basal GH secretion, and elevated serum IGF-I, IGFBP-3, and insulin concentrations, while lowering IGFBP-1 levels in each study subject. This is consistent with but not proof of (partial) GHRH deficiency. However, despite uninterrupted i.v. infusions of GHRH every 90 min for 72 h, mean 24-h serum GH concentrations remained strongly negatively determined statistically by age, BMI, and percentage body fat, which is consistent with, among other considerations, relative GHRH insensitivity. These major inverse relationships applied also to the daily pulsatile GH secretory rate, and the mass of GH secreted per burst, and were evident from

Figure 4 Effect of 72-h pulsatile i.v. administration of GHRH every 90 min on serum concentrations of IGF-I and two IGF-I binding proteins (IGFBP-1 and IGFBP-3) in 19 men of variable ages and body compositions. Data are means $\pm$ S.E.M. $P < 0.05$ for all paired comparisons shown, except $P = 0.02$ for the IGFBP-1 comparison.

Table 1 Effects of age, body composition (BMI and percentage body fat), and serum testosterone concentrations on GH and IGF-I response to 3 days of pulsatile GHRH infusions.

Physiological variable	Serum GH concentration			GH production rate			Mass of GH secreted burst			Plasma IGF-I						
	Incremental		Absolute	Incremental		Absolute	Incremental		Absolute	Incremental		Absolute				
	R	P		R	P		R	P		R	P					
	R	P	R	P	R	P	R	P	R	P	R	P				
Age	-0.62	0.005	-0.667	0.0018	-0.60	0.007	-0.598	0.0068	-0.60	0.007	-0.701	<0.001	-0.627	0.004	-0.38	NS
BMI	-0.70	0.0008	-0.825	<0.001	-0.65	0.003	-0.645	0.0029	-0.7	0.0008	-0.820	<0.001	-0.708	<0.001	-0.37	NS
Percentage body fat	0.87	<0.0001	-0.798	<0.001	-0.80	0.0001	-0.803	<0.001	-0.63	0.007	-0.817	<0.001	-0.352	NS	+0.08	NS
Serum testosterone	0.39	NS	+0.545	0.016	0.34	NS	+0.311	NS	0.34	NS	+0.532	0.019	+0.454	0.051	+0.34	NS

R = R value (correlation coefficient); P = probability value (alpha); NS = not significant ($P > 0.05$). Incremental, difference between value during GHRH infusion and the control (basal) study. Absolute, measured concentration or secretory value during GHRH infusions.

Figure 5 Linear regressions of age, body mass index, percentage body fat, and total serum testosterone concentrations on the mean 24-h serum GH concentrations during pulsatile i.v. infusion of GHRH over 72 h in 19 men. All regressions are $P < 0.02$. Exact statistical values for the individual correlations are given in Table 1.

the perspectives of both absolute and incremental GH secretory responses. Thus, somatotrope (GH) responsiveness to exogenous GHRH drive is clearly attenuated by increasing age and relative adiposity, suggesting relative resistance to GHRH, lack of GH co-secretagogues, and/or excessive GHRH antagonism (e.g. due to heightened somatostatin tone).

Both greater age and percentage body fat are associated with markedly reduced spontaneous pulsatile GH release (7). Given such baseline relative hyposomatotropism, the consistent amplification of 24-h GH secretion in all 19 men during pulsatile GHRH stimulation indicates significant (but incomplete) preservation of somatotrophic secretory capability in older relatively hypoandrogenemic, and/or more obese individuals. Earlier studies have recorded attenuated GH secretion following a single GHRH challenge in obesity and aging (10, 15, 16), albeit not always (10, 13). In a study of 50 men ranging in age from 21 to 86 years and with BMIs of 20 to 29 kg/m², Pavlou *et al.* discerned no impact of age on peak GH response following a single morning i.v. injection of GHRH (1 µg/kg), in contrast to a markedly negative effect of BMI (13). Corpas *et al.* observed similar peak serum GH concentrations after bolus administration of GHRH in a study of 9 young (22–33 years) versus 10 older (60–78 years) men (BMI: 22–29 kg/m²) (10).

To avoid the well known moment-to-moment variability in the GH secretory response to a single stimulus of GHRH, we here used 72-h pulsatile infusions of GHRH, and 24-h blood sampling. We then evaluated by linear

regression analysis any impact of age, BMI, or percentage body fat, as well as serum testosterone concentrations, on absolute (and incremental) 24-h GH secretory responses to GHRH. Even under unvarying GHRH drive, increased age and adiposity correlated negatively, and testosterone positively, with daily GH secretory activity. Thus, partially GHRH-independent or GHRH-resistant hyposomatotropism exists in aging and obesity. Conversely, testosterone appears to enhance pituitary GHRH actions. Various GHRH-independent or GHRH-resistant pathophysiological mechanisms are possible, and might include: (i) imperfect stimulation of pituitary cells by 3 days of pulsatile GHRH infusion alone, due to the choice of infusion dose or schedule; (ii) partial somatotrope-cell failure; (iii) increased hypothalamic somatostatin secretion and/or action; and/or (iv) accentuated GH or IGF-I feedback inhibition of the GH axis. The last mentioned is unlikely, at least in aging, since older subjects if anything are less sensitive to IGF-I's inhibition of GH secretion (22). Since older and obese humans can respond with significantly augmented pulsatile GH secretion and serum IGF-I concentrations to treatment with oral nonpeptidyl analogs of growth hormone-releasing peptide (GHRP) (23), an additional relevant hypothesis in aging and/or obesity is deficiency of a putative endogenous GH co-secretagogue, such as a GHRP-like agonist, whose identify is not yet established.

Results of the present 'GHRH clamp' argue against the *a priori* hypothesis of an isolated GHRH deficiency in aging and obesity. However, an interpretation of a partial

or non-exclusive diminution in (endogenous) GHRH action is consistent with reported near-normalization of blunted GH secretion in older men administered GHRH intermittently or continuously over 12 to 14 days (10). On the other hand, GH secretion failed to rise in obese or older subjects treated once daily with GHRH for 15 days

(24, 25). Once daily GHRH treatment for 6 weeks also did not normalize serum IGF-I or IGFBP-3 concentrations in older men (26). Conversely, in obese subjects, GH secretion rose following both acute (single dose) and prolonged (repetitive doses over a 14-day period) GHRH administration, especially when combined with dietary restriction or fasting (16), weight loss (27), arginine (28), pyridostigmine (29), or GHRP-6 (30). Importantly, the foregoing treatments are believed to withdraw somatostatin (31), thus favoring our inference of at least dual defects in obesity. Analogously, in aging, GH secretion can be amplified many fold if GHRH is infused in combination with putatively somatostatin-antagonizing agents, e.g. L-arginine and/or the GH-releasing peptide, hexarelin (25, 32, 33).

Although we cannot prove that a fully reconstituting GHRH stimulus is achieved by the present 72-h i.v. peptide infusions every 90 min, this paradigm elicits near-physiological ultradian and nyctohemeral GH pulsatility (34). Assuming, furthermore, that preserved maximal somatotrope secretory capacity is preserved in obese and older individuals ((10), and above), the persisting negative regressions of GH secretory output on age and obesity during GHRH infusions suggests GHRH receptor insufficiency, relative lack of GH co-secretagogues, and/or excessive somatostatin release. Our data do not distinguish among these considerations. While studies in the rat or dog are not necessarily definitive to the human, experimental evidence in these species points to both decreased GHRH and increased somatostatinergic activity in older animals (e.g. references 35–37). A recent clinical paradigm of rebound GH secretion after somatostatin withdrawal also points to (partial) GHRH deficiency in aging (38).

Exogenous GHRH-stimulated GH pulsatility continued to follow a well defined 24-h rhythmicity, which is akin to the preserved nyctohemeral rhythmicity of serum GH concentrations reported recently in young men (39). Such diurnal variations during an unchanging GHRH stimulus suggest to us (but do not prove) concomitant 24-h non-uniformity in the release of somatostatin and/or co-secretagogues of GH. These considerations would also explain ongoing pulsatile GH release during continuous GHRH infusions (40). Since the amplitude and mesor (but not the acrophase) of the 24-h rhythm in GH secretory burst mass increased (by 2.5- and 3.5-fold respectively) during GHRH infusion, we infer that the magnitude but not the timing of day–night GH rhythmicity is modulated by GHRH. Timing appears to be GHRH independent. Thus, whether somatostatin and/or various GH co-secretagogues control the timing of 24-h GH rhythms remains plausible but unknown.

Unexpectedly, repeated pulsatile injections of GHRH evoked GH secretory bursts of abbreviated duration. In young men, exercise above the lactate threshold also induces short GH secretory pulses (41). We speculate

Figure 6 GH secretory burst mass basally (placebo, control, (Δ)) and in GHRH-treated ($\bullet$) volunteers in relation to age, serum total testosterone concentrations, BMI, and percentage body fat in 19 men. GHRH ($0.33 \mu\text{g/kg}$) was administered i.v. every 90 min for 3 days. Vertical lines connect control and GHRH treatment sessions in each individual. (To convert serum testosterone concentrations in ng/dl to nmol/l multiply by 0.035.)

Figure 7 Serum IGF-I concentrations before (basal, placebo, (Δ)) and during ($\bullet$) pulsatile i.v. GHRH treatment for 3 days as a function of age, percentage body fat, and serum total testosterone concentrations in 19 men. Study sessions were randomly ordered, and are shown with a connecting vertical line. (To convert testosterone values in ng/dl to nmol/l multiply by 0.035.)

that in these two settings either somatotrope cells release pre-stored GH immediately and briefly, and/or that hypothalamic somatostatin secretion is stimulated by GHRH, GH, or IGF-I auto-feedback, which thereby terminates GH release rapidly (31).

We found a small but reproducible increase in the calculated GH half-life during GHRH infusions. This most likely reflects the inverse relationship between the metabolic clearance rate of GH and circulating GH concentrations (42). Alternatively, an increase in serum GH-binding protein levels (not measured here) during GHRH infusions could in principle prolong GH half-life (43).

An additional novel observation is that pulsatile GHRH infusions augment interpulse basal GH secretion. Although the origin of basal (non pulsatile) GH secretion is not known, we speculate that each pulse of GHRH evokes an immediate release of pre-stored GH (detected as a peak), followed by delayed augmentation of GH stores and interpulse (basal) GH secretion. Other

Figure 8 Impact of percentage total body fat (top panel), serum testosterone concentration (middle panel), and age (bottom panel) on the ratio of serum IGF-I to GH secretory burst mass. Data are basal (control, (Δ)) and the third day of pulsatile i.v. GHRH stimulation ($\bullet$) for $n = 19$ men (see Materials and methods). Significant linear regressions are depicted by the continuous lines, for which the individual correlation coefficients and P values are given in the Results section. (To convert testosterone levels in ng/dl to nmol/l multiply by 0.035.)

considerations include basal GH secretion that is 'constitutive', partially GHRH dependent, and/or conditional on the overall GH production rate. Although pulsatile GHRH stimulation increased (absolute) basal GH secretion rates, it preferentially amplified the pulsatile component of total GH release. Thus, expressed as a fraction of total 24-h GH secretion, pulsatile GH secretion rose significantly during GHRH treatment, namely from 82% to 96%. This response is like that observed in puberty and after sex steroid treatment, both of which magnify GH pulse mass (44, 45), thus suggesting that accentuated GHRH drive underlies pubertal activation of the GH axis. In addition, the disorderliness (approximate entropy) of GH release rose in the present study in all 19 men receiving GHRH infusions, consistent with similar changes in puberty (31, 46).

Figure 9 Nyctohemeral rhythmicity of deconvolution-calculated GH secretory burst mass in 19 men basally (upper panel, control) and on the third day of pulsatile i.v. GHRH (lower panel) infusions at 90-min intervals for a period of 72 h. The cosinor fits of the data assume 24-h periodicity. GHRH treatment significantly increased the mesor (mean) and amplitude of the 24-h rhythmicity in GH secretory burst mass, but did not alter the acrophase (timing of maximal value) (see Results).

GHRH-stimulated GH release was biologically active, given significant concomitant rises in serum glucose, insulin, IGF-I and IGF-BP-3, with a reciprocal fall in IGF-BP-1 concentrations. Age and BMI, but not percentage body fat, correlated negatively, and testosterone positively, with incremental serum IGF-I responses to GHRH infusion. The basis for the foregoing distinction between BMI and percentage body fat could reflect the relevance of topographic distribution of fat stores (e.g. visceral versus subcutaneous fat depots) to pituitary responses to GHRH infusions and/or tissue IGF-I responses to GH (47). Indeed, we observed in obesity that GHRH infusions augment serum IGF-I concentrations even in the face of attenuated pituitary GH secretion, suggesting normal or enhanced tissue IGF-I responses to GH. This hypothesis would coincide with: (a) increased ultrafiltratable (free) IGF-I concentrations in obesity; relatively high IGF-I levels may in turn contribute by way of feedback to the evident hyposomatotropinemia of obesity (48), and (b) an increasing ratio of serum IGF to GH secretory burst mass in more obese men during GHRH stimulation (present data). Conversely, the falling ratio of serum IGF-I/GH secretory burst mass with higher serum testosterone concentrations in the untreated (basal) state suggests partial hepatic resistance to GH action due to testosterone, as

inferred during (oral) estrogen treatment (23, 31). Lastly, the positive correlation of the ratio of serum IGF-I to GH burst mass with age in the basal (non GHRH-treated) state indicates apparently enhanced (hepatic) tissue IGF-I responsiveness to low basal (unstimulated) GH levels in aging. When exogenous GHRH is administered, however, the serum IGF-I/GH burst mass ratio no longer bears any relationship to age, which allows the hypothesis that experimentally increased pulsatile GH secretion augments IGF-I production similarly in older and young individuals. Such observations suggest that the impact of age on tissue IGF-I generation depends in part on GH concentration, i.e. at low serum GH concentrations, tissue IGF-I release is enhanced by increasing age, whereas at higher GH concentrations, tissue IGF-I responses are age independent. In contrast, higher serum GH concentrations (e.g. driven here by exogenous GHRH infusions) unmask an enhancing effect of percentage total body fat on circulating total IGF-I levels. *In vivo* GH dose-response studies ultimately will be required to test this hypothesized notion of unequal tissue sensitivities to GH in obese versus aging men.

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