Differential pulsatile secretagogue control of GH secretion in healthy men

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Norman C, Miles J, Bowers CY, Veldhuis JD. Differential pulsatile secretagogue control of GH secretion in healthy men. Am J Physiol Regul Integr Comp Physiol 304: R712–R719, 2013. First published March 13, 2013; doi:10.1152/ajpregu.00069.2013.—Pulsatile growth hormone (GH) secretion putatively reflects integrated regulation by GH-releasing hormone (GHRH), somatostatin (SST), and GH-releasing peptide (GHRP). GHRH and SST secretion is itself pulsatile. However, how GHRH and SST pulses act along with GHRP to jointly determine pulsatile GH secretion is unclear. Moreover, how testosterone (T) modulates such interactions is unknown. These queries were assessed in a prospectively randomized, placebo-controlled double-blind cohort comprising 26 healthy older men randomized to testosterone (T) vs. placebo supplementation. Pulses of GHRH, SST, or saline were infused intravenously at 90-min intervals for 13 h, along with either continuous saline or ghrelin analog (GHRP-2). The train of pulses was followed by a triple stimulus (combined L-arginine, GHRH, and GHRP-2) to estimate near-maximal GH secretion over a final 3 h. Testosterone vs. placebo supplementation doubled pulsatile GH secretion during GHRH pulses combined with continuous saline (GHRH/saline) (P < 0.01). Pulsatile GH secretion correlated positively with T concentrations (270–1,170 ng/dl) in the 26 men during saline pulses/saline (P = 0.015, R2 = 0.24), GHRH pulses/saline (P = 0.020, R2 = 0.22), and combined GHRH pulses/GHRP-2 (P = 0.016, R2 = 0.25) infusions. Basal nonpulsatile GH secretion correlated with T during saline pulses/GHRP-2 drive (P = 0.020, R2 = 0.16). By regression analysis, pulsatile GH secretion varied negatively with body mass index (BMI) during saline/GHRP-2 infusion (P = 0.001, R2 = 0.36), as well as after the triple stimulus preceded by GHRH/GHRP-2 (P = 0.013, R2 = 0.23). Mean (10-h) GH concentrations under GHRP-2 were predicted jointly by estradiol (positively) and BMI (negatively) (P < 0.001, R2 = 0.520). These data indicate that estradiol, T, and BMI control pulsatile secretagogue-specific GH-regulatory mechanisms in older men.

androgen; estrogen; growth hormone; human; pulsatile; secretion

GH is secreted at a low basal rate with superimposed secretory bursts (pulses). In healthy individuals, pulses account for the majority (>85%) of 24-h GH release (14). Pathophysiological regulation of GH output is achieved by amplifying or attenuating GH secretory-burst size rather than by modulating pulse frequency (13, 39). Burst size is increased by GH-releasing hormone (GHRH), decreased by somatostatin (SST), and synergistically augmented in vivo by combined stimulation with GHRH and GH-releasing peptide (GHRP, also known as GH secretagogue), like ghrelin (5, 7, 31). Major secondary regulators include sex, gonadal sex steroids, visceral fat, pregnancy, puberty, aging, exercise, sleep, amino and fatty acids, glucose, fasting, core body temperature, insulin, IGF-I, and GH feedback (3, 13, 24, 36, 39). Although the precise mechanisms integrating these multiple effectors are poorly understood, overall GH regulation requires the core peptides, GHRH, SST, and GHRP (9).

Dynamic interactions among key GH-regulating peptides remain largely unstudied. Indeed, in most investigations only one peptide is infused at a time (39). Usually, a single bolus injection is used, thereby precluding direct estimation of physiologically pulsatile and basal (nonpulsatile) GH secretion. Alternatively, GH-regulating peptides are given continuously, despite the fact that physiological patterns of GHRH and SST release into hypothalamo-pituitary portal blood are significantly pulsatile in the rat, pig, monkey, and sheep (13). Whether hypothalamic and/or intrapituitary ghrelin release is pulsatile is not known (17), but continuous ghrelin/GHRP infusion sustains pulsatile GH secretion in older men and women for 1–3 mo (4). This background raises the question how pulsatile GHRH and SST signals interact with nearly continuous GHRP stimulation. Because testosterone (T), estradiol (E2) and relative adiposity (BMI, body mass index) influence GH output, a corollary issue is how sex steroids and BMI tune GH responses to defined multipeptide interactions.

The present clinical study examines combined pulsatile (GHRH, SST) and continuous (GHRP) peptide regulation of fasting basal and pulsatile GH secretion in 3, 13, 24, 36, 39. Although the precise mechanisms integrating these multiple effectors are poorly understood, overall GH regulation requires the core peptides, GHRH, SST, and GHRP (9).

The present clinical study examines combined pulsatile (GHRH, SST) and continuous (GHRP) peptide regulation of fasting basal and pulsatile GH secretion and 2) near-maximal acute GH release. Specifically, GHRH or SST was injected repeatedly over 13 h as bolus pulses consistent with their short half-lives of 1.1 to 4.8 min (29, 32) superimposed upon constant infusion of saline or a ghrelin analog (GHRP-2). To evaluate possible effects on apparent pituitary GH-secretory reserve, the pulsatile protocol was followed by an acute triple stimulus comprising sequential L-arginine (to putatively limit hypothalamic SST release) and delayed simultaneous GHRH and GHRP-2 injections (1, 2, 11, 12). To create a graded range of systemic T (and E2) concentrations, healthy older men were pretreated with either intramuscular saline (placebo) or T before the peptide-infusion studies. The composite strategy allowed stepwise regression of basal and pulsatile GH secretion measures on T, E2, and BMI. Older men were studied, because exogenous T supplementation stimulates GH secretion more in older than young men (10). Women were not studied here. The primary hypothesis was that T (or E2) potentiates, whereas BMI attenuates, basal and pulsatile GH production driven by repetitive GHRH pulses and continuous GHRP stimulation without affecting near-maximal acute GH secretion.
MATERIALS AND METHODS

Study Design and Volunteers

This was a double-blind, placebo-controlled, prospectively randomized study of 26 healthy, community-based, ambulatory men. Procedures were conducted in the Mayo Clinical Translational Science Center-Clinical Research Unit (CRU). Recruitment was confined to middle-aged or older men (allowable age range 45–80 yr). Eligible subjects were randomized to sex-steroid supplementation to middle-aged and older men (allowable age range 45–80 yr).

Participants signed witnessed, voluntary informed consent, provided a detailed medical history, and underwent a screening physical examination as outpatients. The protocol was approved by the Mayo Institutional Review Board and was reviewed by the U.S. Food and Drug Administration. Biochemical testing was performed to ensure normal hepatic, renal, hematological, metabolic, and endocrine function before admission to the study. Exclusion criteria comprised hypogonadism; hyperthyroidism; hyperprolactinemia; concurrent use of neuroactive medications or sex hormones; acute or chronic systemic illness; diabetes mellitus; weight loss (>2 kg) in last 3 mo; systemic inflammatory disease; abnormal medical history, physical examination or biochemical screening data; >3 time-zone transmeridian travel in last week; shift-work schedule; concurrent involvement in any other study; drug or alcohol abuse; hemoglobin <12.0 g/dl; thrombotic arterial disease (stroke, transient ischemic attack, myocardial infarction, angina), pulmonary embolus or thrombophlebitis; history or suspicion of cancer or neoplasm (except for basal cell carcinoma if localized and treated surgically); prostatic disease (elevated prostate-specific antigen, indeterminate nodule or mass, carcinoma, obstructive uropathy); and anticoagulant use (due to intramuscular testosterone injections).

Interventions

Each subject participated in a total of six separate randomly ordered overnight 16-h infusion sessions scheduled at least 72 h apart in the inpatient CRU beginning 10 days after the first intramuscular saline or T injection: Fig. 1. In each man, three types of pulsatile-peptide infusions were performed during continuous intravenous saline or T injection: Placebo (saline) injections (top left, top right). Each subject then underwent a total of six prospectively randomized, double-blind, placebo-controlled separate overnight 16-h study sessions scheduled at least 72 h apart. Three studies comprised 13-h continuous intravenous saline (placebo) and 3 others 13-h continuous intravenous growth hormone-releasing peptide (GHRP-2; a ghrelin analog) infusions starting at 2000 in the evening until 0900 the next morning (middle). During each continuous infusion, 1-min bolus pulses of saline, growth hormone-releasing hormone (GHRH; 1 µg/kg) or somatostatin (SST; 0.67 µg/kg bolus) were infused every 90 min for a total of nine pulses (left bottom). Immediately thereafter, a triple stimulus was applied consisting of sequential infusion of l-arginine (30 g iv) continuously from 0900 to 0930 and then combined bolus injection of GHRP-2 and GHRH (both 1 µg/kg iv at 0930 right bottom). Repetitive GH sampling was performed every 10 min for a total of 16 h from 2000 until 1200. Thirteen men were randomized to testosterone (T) and 13 others to placebo pretreatment.

Assays

Concentrations of GH were determined as a batch of 582 samples/subject via robotics-assisted, two-site monoclonal immune-enzymatic chemiluminescence assay (sensitivity 0.010 µg/l) (38). The assay standard was 22-kDa recombinant human GH. Interassay coefficients of variation (CV) for GH concentrations of 3.4 and 12 µg/l were 7.9 and 6.3%, respectively. Intraassay CVs at 1.1 and 20 µg/l were 4.9 and 4.5%, respectively. No values were less than 0.020 µg/l. Screening thyroid-stimulating hormone (TSH), prolactin, leutinizing hormone (LH), and follicle-stimulating hormone (FSH) concentrations were quantified by automated chemiluminescence assay (ACS 180; Bayer, Norwood, MA), using as standards recombinant TSH and prolactin and the First and Second International Gonadotropin Reference Preparations. Procedural sensitivities for TSH, prolactin, LH, and FSH were 0.01 mIU/l, 2.0 µg/l, and 0.2 and 0.4 IU/l, respectively. Liquid-chromatography tandem mass spectrometry was used to quantify E2 and T in serum samples collected at 0800 for screening, as well as before starting the 16-h sampling during each of the six CRU visits (20). Sex hormone-binding globulin, IGF-I, IGF-binding protein (IGFBP)-1 and IGFBP-3 concentrations were assayed by immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX) as reported earlier (15). Sample values from the start of the six CRU visits were first averaged in each man, since the CV across six visits was <15%.

Analytical Methods

Deconvolution analysis was applied to the last 10 h (2300–0900) of the 13 h of 10-min sampling during peptide/saline infusions, as well as to the final 3.0 h (0900–1200) of sampling during the triple infusions followed by a triple stimulus. Volunteers were randomized to receive intramuscular testosterone (T) or placebo (saline) injections (top left, top right). Each subject then underwent a total of six prospectively randomized, double-blind, placebo-controlled separate overnight 16-h study sessions scheduled at least 72 h apart. Three studies comprised 13-h continuous intravenous saline (placebo) and 3 others 13-h continuous intravenous growth hormone-releasing peptide (GHRP-2; a ghrelin analog) infusions starting at 2000 in the evening until 0900 the next morning (middle). During each continuous infusion, 1-min bolus pulses of saline, growth hormone-releasing hormone (GHRH; 1 µg/kg) or somatostatin (SST; 0.67 µg/kg bolus) were infused every 90 min for a total of nine pulses (left bottom). Immediately thereafter, a triple stimulus was applied consisting of sequential infusion of l-arginine (30 g iv) continuously from 0900 to 0930 and then combined bolus injection of GHRP-2 and GHRH (both 1 µg/kg iv at 0930 right bottom). Repetitive GH sampling was performed every 10 min for a total of 16 h from 2000 until 1200. Thirteen men were randomized to testosterone (T) and 13 others to placebo pretreatment.

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stimulus to estimate basal (nonpulsatile) and pulsatile GH secretion. The first 3-h responses to peptide infusions were considered “startup effects” and not analyzed further (37). Pulsatile GH secretion was defined as the summed mass of GH (µg) secreted in bursts per unit distribution volume (liters). The deconvolution methodology simultaneously estimates basal secretion, secretory burst mass, and secretory-burst shape using published biexponential GH-elimination kinetics [namely, 3.5- and 18-min half-lives (8)]. The model is conditioned mathematically on a priori identification of candidate sets of pulse-onset times using an image boundary-detection technique (18). Pulse number is optimized by the Akaike information criterion (19). Sensitivity and specificity are both 93% (37).

Biostatistical Analysis

The experimental design was consistent with the general principles of a split plot design. The whole plots were the primary treatment of T or placebo. Within this condition, participants received six different infusions (ghrelin vs. saline crossed with saline, GHRH, or SST). The nesting of the treatments within patients was addressed statistically by means of a random-effects model using SAS PROC MIXED. Main effects for each level of the split plot design along with their interactions were modeled as fixed effects. An F test was used to test and remove the three-way interaction term from the model. The random effect consisted of a random participant (blocking) factor. Model-based means were computed from the estimated parameters with the Tukey-Kramer post hoc correction factor. The degrees of freedom for the fixed effects were estimated using the Kenwood-Rodger method (22). Adjusted P values less than 0.05 were considered statistically significant. Analyses were conducted using the SAS System, v 9.3 (Cary, NC).

Significant main effects were confirmed by 3-way ANCOVA (2 × 3 × 2 factors) using the saline/saline response as the covariate, as described in detail earlier (15). Post hoc analysis used Tukey’s honestly significantly difference (HSD) test (40). Pilot data indicated that GHRP-2 synergizes with GHRH to augment the latter effect by 2.2 ± 0.59 (SD) fold. Under this assumption, power was >90% to detect a unit SD difference at P < 0.05 with 26 men for one-tailed comparison of a stimulatory T vs. placebo effect (25).

Backward stepwise-elimination linear regression was performed to identify the independent or joint contributions of T or E₂ concentrations and/or BMI in modulating GH production. Overall experiment-wise, P < 0.05 was construed as significant.

RESULTS

Subject characteristics. The two cohorts of healthy men randomly assigned to T supplementation (n = 13) vs. placebo (n = 13) were comparable in age (59 ± 7.7 vs. 64 ± 11 yr, P = 0.26), and BMI (29 ± 3.3 vs. 28 ± 2.1 kg/m², P = 0.45). Screening (prestudy) T concentrations were normal for age (>240 ng/dl, Mayo Medical Laboratories), namely mean 395 ± 178 (mean ± SD), median 369, range (251–679) ng/dl. Hormonal data in the T and placebo cohort averaged across all six CRU visits in each subject included IGF-I (190 ± 65 vs. 160 ±

Fig. 2. GH concentrations during continuous saline/GHRP-2 infusions with superimposed saline, GHRH, and SST pulses. Each panel represents 10-min GH concentrations over the last 10 h (2300–0900) of a 13-h continuous infusion of GHRP-2 (A and C) or saline (B and D). Studies were conducted after supplementation with testosterone (T) (A and B) or placebo (C and D). Nine consecutive 1-min bolus saline (continuous line), GHRH (interrupted line), or SST (dotted line) pulses were superimposed as one bolus every 90 min. Data are the geometric mean in each subgroup of n = 13.
As expected, T and E² were significantly higher in the T
0.86), and IGFBP-3 (2.9
0.001) in the T and placebo groups (P
0.001) independently of T or SST. T vs. placebo supplementation
doubled pulsatile GH secretion under GHRH pulses (P < 0.01). The absence of other dichotomous T/placebo
effects raised the possibility that T action is graded, rather than threshold-like, and thus is better assessed by regression analysis.

**Triple Stimulus-Mediated GH Secretion**

Under the triple stimulus (l-arginine, GHRH, and GHRP-2), median GH concentrations, pulsatile and basal GH secretion (µg/l1-3.0 h1) and mass of GH secreted per burst were similar for T vs. placebo administration. Three-hour pulsatile results are given in Supplemental Appendix Table S2B, **bottom**. By GLM analysis, there was a strong negative effect of prior 13-h GHRP vs. saline infusions on median 3-h GH concentrations and pulsatile GH-secretion responses to the triple stimulus (P < 0.001; see Fig. 4). The difference was significant for GHRP-2 associated with pulses of saline, GHRH, or SST in the presence (P < 0.01) and absence (P < 0.01) of T supplementation compared with the non-GHRP-

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<tr>
<th>Pulsatile</th>
<th>Basal</th>
<th>Total</th>
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<tr>
<td>Overall P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>Overall R²</td>
<td>0.394</td>
<td>0.482</td>
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**Main effects**

| T | 0.238 | 0.092 | 0.116 |
| GHRP | <0.001 | <0.001 | <0.001 |
| Peptide differences | <0.001 | <0.001 | <0.001 |
| GHRH vs. SST | <0.001 | <0.001 | <0.001 |
| GHRH vs. saline | <0.001 | <0.001 | <0.001 |
| SST vs. saline | 0.833 | 0.99 | 0.619 |

**Triple Stimulus Analysis**

| Overall P | <0.001 | <0.001 | <0.001 |
| Overall R² | 0.203 | 0.105 | 0.211 |

**GH Secretion During Continuous GHRP-2 and Saline Infusions in T-Supplemented and Placebo Groups**

Figure 2 depicts 10-min GH-concentration time series over the last 10 h of the 13-h continuous infusions of GHRP-2 or saline with superposed pulses of saline or GHRH or SST in the 13 men given T and 13 others given placebo. Fig. 2, A and C, depict T vs. placebo (top and bottom) outcomes during GHRP-2 infusion, and the Fig. 2, B and D, shows T vs. placebo outcomes (top and bottom) during saline infusion. On the basis of general linear model (GLM) analysis and Tukey’s HSD post hoc comparisons, continuous GHRP-2 compared with saline infusion augmented 10-h median GH concentrations, basal GH secretion, pulsatile GH secretion, and the mass of GH secreted per burst in all three paired conditions (GHRP-2/saline vs. saline/saline; GHRP-2/GHRH vs. saline/GHRH; and GHRP-2/SST vs. saline/SST) (P < 0.001) (Table 1, **top**). GHRH exerted a greater effect than either saline (P < 0.001) or SST (P < 0.001). There were no main differences in 10-h pulsatile GH secretion between T and placebo supplementation (P = 0.467) or between SST and saline infusion (P = 0.501) (see Supplemental Appendix Table S2A, **top**). GHRP-2 had overall synergistic effects with GHRH (interactive effect P < 0.01 for both with T and without T). The degree of synergy was no different in the T and placebo groups (P = 0.491). Compared with non-GHRP controls, the mean effect size (95% confidence intervals) of GHRP-2 was 89 (60–118) for pulsatile GH and 105 (82–130) µg·l⁻¹·10 h⁻¹ for total GH secretion.

To visualize 10-h pulsatile GH secretion among all peptide-infusion conditions, box-and-whisker plots of median, interquartile range, 95% confidence intervals, and absolute range are presented in Fig. 3. The attendant GLM analysis allows one to assess differences between any pair of peptide-infusion types for both T (**left** 6 columns) and placebo (**right** 6 columns) supplementation. There was a marked GHRP-2 effect (P < 0.001) independently of T or SST. T vs. placebo supplementation doubled pulsatile GH secretion under GHRH pulses (P < 0.01). The absence of other dichotomous T/placebo effects raised the possibility that T action is graded, rather than threshold-like, and thus is better assessed by regression analysis.

**Fig. 3.** Deconvolution estimates of 10-h pulsatile GH secretion for all 12 interventions. Box-and-whisker plots are shown after T (**left**) and placebo (**right**) supplementation during continuous GHRP-2 (**columns** 1–3 and 7–9) or saline (**columns** 4–6 and 10–12) infusion. Bolus pulses of saline (designated by -GHRH/-SST), GHRH (+GHRH/-SST), and SST (-GHRH/+SST) were superimposed. Data show the median, interquartile range, 95% confidence intervals, and extreme range (individual dots). GLM analysis with partially repeated measures was applied to evaluate the main effects of T vs. placebo treatment, continuous GHRP-2 vs. saline infusion, and bolus peptide type. Overall P was <0.001. A, B, C, D Box plots with unique (unshared) alphabetic letters differ by post hoc Tukey’s multiple-comparison HSD test. Thus, GHRH is potentiated by T [compare **columns** 5 and 11, where B (GHRH + T) is different from both A (GHRP) and from C (GHRH – T)].

70 µg/l, P = 0.27), IGFBP-1 (31 ± 13 vs. 30 ± 15 µg/l, P = 0.86), and IGFBP-3 (2.9 ± 0.6 vs. 3.1 ± 0.5 µg/ml, P = 0.62). As expected, T and E² were significantly higher in the T supplementation than the placebo group (T: 898 ± 191 vs. 488 ± 171 ng/dl, P < 0.001 and E²: 65 ± 2.0 vs. 28 ± 5.4 pg/ml, P < 0.001). Individual values in all 26 subjects are given in Supplemental Appendix Table S1 on the journal’s website.

### Table 1. Ten-hour profiles and triple stimulus analysis

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<th>Pulsatile</th>
<th>Basal</th>
<th>Total</th>
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Overall P: <0.001; Overall R²: 0.394; Main effects: T: 0.238; GHRP: <0.001; Peptide differences: <0.001; GHRH vs. SST: <0.001; GHRH vs. saline: <0.001; SST vs. saline: 0.833; Gamma: 0.99; 0.619; General linear model statistical-analysis shows P values for main effects.
infused controls (Table 1, bottom). The overall (negative) GHRP-2 effect size (95% confidence intervals) was 87 (51–123) for pulsatile and 99 (68–130) μg·L⁻¹·3 h⁻¹ for total GH secretion. The decrease was explained fully by a corresponding decrease in GH secretory-burst mass (not shown). There were no effects of prior SST or GHRH infusion (see Supplemental Appendix Table S2B).

Stepwise Linear Regression Analysis

Ten-hour GH secretion. Since GLM analyses indicated that SST pulses at the dose used did not inhibit GH production, stepwise backward-elimination multivariate linear regression was performed in the saline/saline, saline/GHRH, GHRP-2/saline, and GHRP-2/GHRH subgroups only. Dependent variables were 10-h basal, pulsatile, or total GH secretion, and independent variables were BMI, T, and E2. Testosterone alone positively determined GH output during the 10-h pulsatile saline/peptide infusion, as follows: 1) total (pulsatile plus basal) GH secretion during saline/saline (R² = 0.18, P = 0.03) and saline/GHRH (R² = 0.28, P = 0.0045); 2) pulsatile GH secretion during saline/saline (R² = 0.24, P = 0.015), saline/GHRH (R² = 0.22, P = 0.02), and GHRP-2/GHRH (R² = 0.25, P = 0.016) (see Fig. 5; 3) basal GH secretion during GHRP-2/saline (R² = 0.16, P = 0.02); Supplemental Appendix Table S3A; and 4) 10-h mean GH concentration during GHRP-2/GHRH (R² = 0.46, P = 0.001).

BMI alone emerged as a strong negative correlate of J) total 10-h GH secretion in both the GHRP-2/saline (R² = 0.34, P = 0.002) and GHRP-2/GHRH (R² = 0.27, P = 0.006) subgroups; 2) pulsatile GH secretion in the GHRP-2/saline (R² = 0.36, P = 0.001) subgroup; and 3) basal GH in GHRP-2/GHRH (R² = 0.23, P = 0.01) subgroup.

Ten-hour mean GH concentrations in the GHRP-2/saline subgroup were modulated jointly by two independent variables, namely BMI, negatively (P = 0.012), and E₂, positively (P = 0.001) (overall R² = 0.52, P < 0.001).

Triple stimulus-mediated GH secretion. Triple-stimulus-mediated 3-h GH secretion was independent of BMI after 13-h pretreatment with saline/saline and saline/GHRH (see Supplemental Appendix Table S3B). However, BMI negatively predicted total GH secretion after 13-h pretreatment with GHRP-2/saline (R² = 0.18, P = 0.029) and GHRP-2/GHRH (R² = 0.21, P = 0.018). Neither T nor E₂ was related to triple-stimulus effects after any of the four pulsatile-infusion types.

**DISCUSSION**

Analyses of GH secretion in a novel combined pulsatile and continuous peptide-clamp model in 26 healthy older men revealed for the first time that short-term T supplementation doubles pulsatile GHRH drive of overnight GH secretion. Multivariate regression unveiled several additional new insights: 1) systemic T concentrations extending continuously across the low-normal to pharmacological range positively predict pulsatile GH secretion during each of saline infusion, pulsatile GHRH stimulation, and GHRP/GHRH synergism; 2) T levels are positively associated with basal (nonpulsatile) GH secretion under GHRP-2 drive; 3) BMI alone negatively predicts both pulsatile GH secretion during GHRP stimulation and basal GH secretion during combined GHRP/GHRH stimulation; 4) mean GH concentrations during continuous GHRP stimulation are controlled jointly by E₂ (positively) and BMI (negatively); 5) near-maximal triple stimulus-induced GH secretion is unaffected by T or E₂ concentrations or BMI in the control (saline/saline) setting, but is negatively influenced by BMI when preceded by GHRP-2 stimulation; and 6) a train of GHRH pulses preceding the triple stimulus strongly potentiates triply stimulated GH secretion, unless GHRP-2 is delivered concomitantly. Together, these data identify strong regulatory effects on single and combined peptide actions of T, E₂, and BMI in healthy older men.

A multivariate regression strategy disclosed that continuously varying T concentrations, rather than a placebo vs. T treatment dichotomy or an arbitrary all-or-none threshold T concentration, correlate with overnight pulsatile GH secretion driven by endogenous peptides (saline), GHRH alone and GHRH/GHRP synergy. Specifically, stepwise backward-elimination multivariate regression analysis revealed that T per se specifies 22–25% of the variability in pulsatile GH secretion during saline, saline/pulsatile GHRH, and GHRP/pulsatile GHRH stimulation. In contrast, in the case of GHRP-2 infused alone, BMI was the dominant (negative) determinant of pulsatile GH secretion. BMI also negatively determined basal GH secretion under GHRP/GHRH drive. Thus, there is both pulsatile secretagogue selectivity and sex-steroid specificity in regulating basal and pulsatile GH secretion in men. The distinction in analysis pathways used (multivariate regression compared with dichotomous T vs. placebo treatment) provides a possible basis for several negative studies reported for T vs. placebo administration in men (13, 39).

An unexpected finding was that the dose of SST used here, which inhibits GH secretion by ~50% in postmenopausal women (6), did not suppress fasting or triple stimulus-induced GH in older men. This outcome raises the possibility of a sex
difference in the inhibitory potency of SST. Dose-response studies would be required to address this possibility.

Testosterone supplementation in 50% of the subjects in concert with expected intersubject variability in endogenous T and E\(_2\) levels yielded a graded range of not only T but also E\(_2\) concentrations (23–116 pg/ml). By stepwise regression, BMI (negatively) and E\(_2\) (positively) together determined mean 10-h GH concentrations during continuous GHRP-2 infusion. The joint regression was highly significant (\(P < 0.001\)), explaining 52% of the variability in mean GH. Whether the same combined influences operate in young men or in women of any age is unknown. Although further studies will be required to confirm the strongly positive effect of E\(_2\) on GHRP-2 drive in older men studied here (\(R^2 = 0.46, P < 0.001\)), estrogen is able to augment expression of the human GHRP receptor at least in vitro (26) and the murine GHRP receptor in vivo (41). This mechanism could also explain the strongly positive univariate effects of E\(_2\) on GHRP-2 drive (\(P = 0.0009\)). The last effect probably reflects the GHRH potency-effects of E\(_2\) on GHRP-2 drive (\(P = 0.0024\)), and pulsatile GHRH stimulation (\(P = 0.0009\)). The last effect probably reflects the GHRH potency-enhancing effect of E\(_2\) recognized earlier in women (35).

Corresponding univariate T correlations existed (namely \(P = 0.0015, P = 0.0090, P = 0.037\)). Notably, multivariate regression showed that T concentrations could fully explain and replace the univariate effects of E\(_2\). The predominance of the systemic T effect over E\(_2\) could suggest that local hypothalamo-pituitary aromatization of T to E\(_2\) acting via ER-\(\alpha\) mediates GH drive, and/or that other T or dihydroxytryptamine (DHT) metabolites mediate stimulation (e.g., via ER-\(\beta\)) (34, 41, 42). For example, E\(_2\) is able to repress pituitary SSTR-5 (thus disinhibiting GH secretion), stimulate ER-containing hypothalamic GHRH neurons and pituitary somatotropes cells, and upregulate brain-pituitary GHRP receptors (16, 21, 26, 30, 42). In contrast, GH-stimulating actions of T or 5a-DHT acting directly via the androgen receptor have not been affirmed in humans (13, 39).

The capability of triple secretagogues (L-arginine, GHRH, and GHRP-2) to elicit near-maximal pulsatile GH production acutely was amplified significantly by prior priming with GHRH pulses. The priming effect of GHRH pulses was independent of T, E\(_2\), and BMI, yet was completely abrogated by prior (13 h) GHRP stimulation. A hypothetical explanation is that GHRP exposure, by augmenting pulsatile GH secretion and elevating total IGF-I concentrations (4), heightens negative feedback. In fact, both GH and IGF-I decrease GHRH-stimulated GH secretion (1, 12, 28). A difference in the present paradigm is that GHRP only opposed the potentiating effect of GHRH pulses on a triple stimulus. Thus, further studies will be needed to unravel the mechanisms of GHRP/GHRH interactions. Nonetheless, the capability of GHRH pulses to double the triple-stimulus effect suggests a possible sequential peptide-infusion strategy for testing maximal pituitary reserve
nor E2 concentrations determine the near-maximal GH secretion.

Caveats include the relatively narrow age range studied here with no octogenarians; the ultimate need to selectively block androgen or estrogen receptors or aromatase activity in further studies; the desirability of eventually extending cohort size to verify interactions among key effectors, as inferred here; and the potential value of later assessing the time course of various T actions on GH secretion.

Perspectives and Significance

Under defined secretagogue drive, systemic T concentrations explain about 25% of the variability in overnight basal and pulsatile GH secretion driven by repeated pulses of saline or GHRH alone or combined with sustained GHRP stimulation in healthy older men. In aging men, BMI and E2 together also determine mean GH concentrations under GHRP stimulation. Whether comparable principles operate in women is not known. How puberty affects pulsatile-secretagogue selectivity also remains to be studied. However, the emerging regulatory hypothesis is that T, E2, and body composition modulate basal and pulsatile modes of GH secretion via pulsatile secretagogue-selective mechanisms.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: C.N., J.M.M., C.Y.B., and J.D.V. performed experiments; C.N., C.Y.B., and J.D.V. interpreted results of experiments; C.N. prepared figures; C.N. drafted manuscript; C.N., J.M.M., C.Y.B., and J.D.V. approved final version of manuscript; I.M.M., C.Y.B., and J.D.V. edited and revised manuscript; C.Y.B. and J.D.V. conception and design of research; J.D.V. analyzed data.

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