Complex regulation of GH autofeedback under dual-peptide drive: studies under a pharmacological GH and sex steroid clamp

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Veldhuis JD, Erickson D, Miles JM, Bowers CY. Complex regulation of GH autofeedback under dual-peptide drive: studies under a pharmacological GH and sex steroid clamp. Am J Physiol Endocrinol Metab 300: E1158–E1165, 2011. First published April 5, 2011; doi:10.1152/ajpendo.00054.2011.—To test the postulate that sex difference, sex steroids, and peptidyl secretagogues control GH autofeedback, 11 healthy postmenopausal women and 14 older men were each given 1) a single iv pulse of GH to enforce negative feedback and 2) continuous iv infusion of saline vs. combined GHRH/GHRP-2 to drive feedback escape during pharmacological estradiol (E2; women) or testosterone (T; men) supplementation vs. placebo in a double-blind, prospectively randomized crossover design. By three-way ANCOVA, sex difference, sex hormone treatment, peptide stimulation, and placebo/saline responses (covariate) controlled total (integrated) GH recovery during feedback (each P < 0.001). Both sex steroid milieu (P = 0.019) and dual-peptide stimulation (P < 0.001) determined nadir (maximally feedback-suppressed) GH concentrations. E2/T exposure elevated nadir GH concentrations during saline infusion (P = 0.003), whereas dual-peptide infusion did so independently of T/E2 and sex difference (P = 0.001). All three of sex difference (P = 0.001), sex steroid treatment (P = 0.005), and double-peptide stimulation (P < 0.001) augmented recovery of peak (maximally feedback-escaped) GH concentrations. Peak GH responses to dual-peptide 1 agonists were greater in women than in men (P = 0.016). E2/T augmented peak GH recovery during saline infusion (P < 0.001). Approximate entropy analysis corroborated independent effects of sex steroid treatment (P = 0.012) and peptide infusion (P < 0.001) on GH regularity. In summary, sex difference, sex steroid supplementation, and combined peptide drive influenced nadir, peak, and entropic measurements of GH release under controlled negative feedback. To the degree that the pharmacological sex steroid, GH, and dual-clamp drive provide prephysiological regulatory insights, these outcomes suggest major determinants of pulsatile GH secretion in the feedback domain.

GROWTH HORMONE (GH) is secreted primarily in pulses that mediate anabolic tissue effects (17). Pulses of GH secretion are controlled in turn by the hypothalamic peptides GH-releasing hormone (GHRH) and somatostatin (SS) and by the hypothalamic-gastro-pancreatic peptide ghrelin [a GH-releasing peptide (GHRP)] (4, 12, 23, 36). Somatostatin inhibits, whereas GHRH and GHRP stimulate, GH release (13, 16, 25). The complex interplay between inhibition and stimulation is achieved by feedback and feedforward connections (7, 10, 22, 26, 32, 38). In particular, GH and possibly GHRH itself impose negative feedback by initiating hypothalamic outflow of SS, which transsynaptically quenches neuronal GHRH secretion and transportally inhibits both GHRH and GHRP action at the pituitary gland (1, 5, 9, 22, 29).

Viewed from a regulatory perspective, feedback and feedforward are dynamically counterbalanced processes. According to this concept, disruption of either regulatory limb would impair physiological GH pulsatility (25, 27, 34). Indeed, in Laron syndrome, GH receptor defects result in massive outpouring of GH due to feedback failure (49). Conversely, experimentally induced GH excess causes hypothalamic overexpression of SS and underexpression of GHRH genes (33, 35). Despite these important physiological tenets, the primary factors that regulate feedback-dependent control of pulsatile GH secretion are not well understood. In particular, whereas sex difference and sex steroids are dominant determinants of GHRH and GHRP feedforward (17), their impact on feedback regulation remains largely unexplored. To address this fundamental issue, the present study introduces an experimental paradigm of GH feedback-regulated pulsatile GH secretion, where the principal independent variables are sex difference, sex steroid supplementation, and attempted rescue from feedback inhibition by dual-peptide (GHRH/GHRP-2) drive.

METHODOLOGY

Human subjects. Eleven postmenopausal women and 14 older men of similar age were recruited for study. Each of the 25 volunteers completed four 8-h study sessions, two after placebo and two after sex steroid supplementation in randomized order. Healthy postmenopausal women and older men (age range 50–80 yr) provided voluntary, written, informed witnessed consent, which was approved by Mayo Institutional Review Board and reviewed by the FDA. Reimbursement was for time spent in the study. Involvement included safety screening, blood sampling, saline or peptide infusion, and followup safety evaluation. Volunteers were healthy, ambulatory, unmedicated postmenopausal women with no spontaneous menstrual cycles for >2 yr, serum FSH >50 IU/l [1st International Reference Preparation (IRP)], LH >20 U/l (2nd IRP), and estradiol <20 pg/ml. Criteria for inclusion were mental competence, 21 kg/m2 ≤ BMI < 35 kg/m2, prestudy hematocrit >38%, normal screening safety data, and provision of written informed consent. Exclusion criteria were any recent (5 biological half-lives) use of psycho- or neuroaffective drugs or sex hormones, allergies to any of the compounds used, psychiatric illness (e.g., psychosis, depression, anorexia nervosa), substance abuse, acute or chronic systemic illness, hypothalamic-pituitary disease, recent transmeridian travel (exceeding 3 time zones within 10 days), acute weight loss or gain (>3 kg change in 2 wk), poorly controlled hypertension, VLDL-predominant hyperlipidemia (triglycerides >300 mg/dl), arterial thrombosis, stroke or myocardial ischemia, venous thrombophlebitis, pulmonary embolism, estrogen-responsive neoplasms of...
the breast, uterus, or other organs, undiagnosed vaginal bleeding or endometrial hyperplasia, and prostatic disease.

Subjects were screened by way of medical history, physical examination, and blood chemistries. Hematological, metabolic, hepatorenal (liver enzymes, creatinine), and endocrine [TSH, LH, FSH, estradiol (E2), testosterone (T), and prolactin] measurements were expected for age.

Experiment paradigm. Volunteers were studied in the morning fasting after ingesting a standardized meal at 1800 the night before. Two randomly ordered study sessions were performed after placebo (Pl) and two after sex steroid supplementation. There was a 6-wk washout between the sex steroid treatments. Women received Pl capsules or 1.0 mg of 17β-estradiol (E2) orally twice daily for 21 days, and men received saline or T enanthate 200 mg im on days 1, 8, and 15. Sampling/peptide infusion sessions were carried out ≈2 days apart during the time window of 11–21 days (day 1 was the onset of Pl or T/E2 treatment).

Each study session comprised blood sampling every 10 min for 8 h beginning at 0800, a single intravenous (iv) pulse of GH 1 μg/kg over 6 min at 0830–0836, and continuous iv infusion of saline (20 ml/h) or combined GHRH/GHRP-2 (both peptides together at the same constant rate: 1 μg·kg⁻¹·h⁻¹) from 0800 to 1600. The goal was to enforce negative feedback (with the iv pulse of GH) and then rescue inhibition (with saline or the 2 peptides) in the unmodified and sex steroid-supplemented milieus.

Assays. GH was measured in duplicate in each serum sample using an ultrasensitive robotics-automated assay system (DXI Beckman) exactly as described (45). The standard was 22,000 Dalton recombinant human GH. Sensitivity was 0.01 pg/ml at three standard deviations above the zero-dose tubes. Calibrating standards were run in triplicate. No dilution was required. Over the GH ranges observed here, median intra-assay and interassay coefficients of variation were 4.5 and 6.8%, respectively. No more than 1 sample/100 was undetectable in this system in the present studies. Cross-reactivity with 20,000 Dalton GH was <5% and with recombinant GH-binding protein <1%.

Screening hormone concentrations were assayed by immunologically based assays, as described earlier (21). Infusion-associated 0800 T and E2 were quantified by tandem mass spectrometry, as reported (28). Free T and bioavailable T levels were calculated exactly as described in the Appendix of Ref. 37.

Analysis. The primary end points were total (5.5-h integrated) and three-point moving-average nadir (lowest) and peak (highest) GH concentrations over the time interval beginning 120 min (7.5 half-lives) after bolus GH injection. A secondary outcome was approximate entropy (ApEn), which is a model-free measurement of relative feedforward/feedback strength (30). Higher ApEn denotes greater irregularity of subpatterns in a time series, which in turn signifies relative feedback loss (48).

Statistics. Primary comparisons were made via three-way analysis of covariance (ANCOVA) of ln-transformed measurements (50). Logarithmic transformation was applied to minimize the dispersion of residual variance among groups. The three categorical factors were sex difference (male or female), sex steroid supplementation (placebo vs. T/E2), and peptide infusion (saline vs. GHRH/GHRP-2). Interactive terms were estimated for sex difference × treatment, sex difference × peptide, and treatment × peptide as well as an overall interaction among all three main factors. The covariate was the intrasubject measurement on the placebo/saline day. Model parameters were estimated by way of residual maximum likelihood, and the variance-covariance matrix was modeled in the compound symmetry form. ANCOVA allows valid statistical estimation in the face of possible autocorrelation due to the repeated-measures design (24). Post hoc Tukey’s honestly significantly different test was utilized at experiment-wise two-tailed P < 0.05 for multiple comparisons of means. Linear regression was employed to evaluate effects of E2, body mass index (BMI), or IGF-I in the combined cohorts (assuming protected P < 0.01). Analyses used Systat (Richmond, CA) version 11.0.

Data in the tables are presented as the geometric mean ± SD and elsewhere ± SE.

RESULTS

Mean ± SE (range) age was 58 ± 3.0 (52–74) yr in women and 55 ± 2.6 (45–74) yr in men (P = 0.435). Women had a lower BMI (25 ± 0.88 kg/m²) than men (28 ± 0.82 kg/m²) (P = 0.023). Baseline endocrine data are given in Supplemental Table S1 (Supplemental Material for this article is available online at the AJP-Endocrinology and Metabolism website). All values were expected for age, including total T and calculated free and bioavailable T concentrations in men. Screening (RIA) measurements of E2, LH, and FSH in women corroborated menopausal status. Baseline T concentrations were lower in women than in men and conversely for sex hormone-binding globulin and IGF-binding protein (IGFBP)-1. Untreated prolactin, IGF-I, and IGFBP-3 were similar by sex.

Treatment effects on sex steroid concentrations (mass spectrometry) and IGF-I-related measurements are given in Table 1. E2 rose significantly in both women and men given sex steroid, with comparable posttreatment values. IGF-I concentrations were lower, whereas sex hormone-binding globulin and IGFBP-1 levels were higher in sex-hormone-supplemented women than in men (each P < 0.01). IGFBP-3 values were comparable by sex and unaffected by E2 or T treatment (P = 0.64).

Figure 1 depicts mean (± SE) 10-min GH concentration time series obtained over 8 h in the 11 women and 14 men. Visual inspection revealed strong negative feedback by injected GH during continuous saline infusion and marked rescue by GHRH/GHRP-2 infusion. Peak injected GH concentrations (μg/l) on saline infusion days occurred 10 min after the bolus

Table 1. Pl and sex hormone treatment data

<table>
<thead>
<tr>
<th></th>
<th>PI Women (n = 11)</th>
<th>E2 Women (n = 11)</th>
<th>PI Men (n = 14)</th>
<th>T Men (n = 14)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol, pg/ml</td>
<td>9.9 ± 1.2a</td>
<td>114 ± 61b</td>
<td>27 ± 6.6a</td>
<td>83 ± 28b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IGFBP-1, μg/l</td>
<td>34 ± 11a</td>
<td>48 ± 22a</td>
<td>17 ± 8.3a</td>
<td>18 ± 10a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IGFBP-3, mg/l</td>
<td>3.8 ± 0.71a</td>
<td>3.4 ± 0.77a</td>
<td>3.8 ± 0.84a</td>
<td>3.5 ± 1.0a</td>
<td>0.64</td>
</tr>
<tr>
<td>IGF-I, μg/l</td>
<td>164 ± 71ab</td>
<td>134 ± 52ae</td>
<td>211 ± 77ab</td>
<td>238 ± 83b</td>
<td>0.009</td>
</tr>
<tr>
<td>SHBG, nmol/l</td>
<td>48 ± 11a</td>
<td>92 ± 34a</td>
<td>31 ± 13a</td>
<td>24 ± 9.7a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T, ng/dl</td>
<td>ND</td>
<td>ND</td>
<td>384 ± 109a</td>
<td>1,114 ± 255b</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± SD. Pl, placebo; E2, estradiol; T, testosterone; IGFBP-1 and -3, IGF-binding protein-1 and -3, respectively; SHBG, sex hormone-binding globulin; ND, not determined. Superscripted letters (a–c) were assigned by post hoc Tukey’s honestly significantly different (HSD) test. Unshared superscripted letters indicate P < 0.05 between groups. T and E2 were determined here by mass spectrometry. *IGF-I for E2 women vs. Pl men, P = 0.056 by post hoc Tukey’s HSD test.
To evaluate the role of peptide secretagogues in feedback control, saline or combined GH-releasing hormone (GHRH)/GH-releasing peptide-2 (GHRP-2) (1 μg·kg⁻¹·h⁻¹) was infused continuously (0600–1400). Data are from 11 women and 14 men.

Total GH feedback escape (recovery) was estimated by the 5.5-h area under the curve (time-integrated GH concentration) beginning at 1030 (120 min after the iv GH pulse). Figure 2 shows main effects of sex (P < 0.001), peptide (P < 0.001), and treatment (P < 0.001) on post-feedback-integrated GH concentrations (overall 3-way ANCOVA model P < 0.001, covariate P < 0.001). Exploratory multivariate analysis showed that the sex effect persisted (P < 0.01) after BMI was included. For combined responses to saline and GHRH/GHRP-2, women receiving E2 treatment manifested greater total GH recovery than when receiving Pl (P = 0.002) and than men during T (P = 0.003) as well as Pl (P = 0.001) and T (P = 0.001) treatment (Fig. 2, top). Viewed analogously across both infusions, T had no main effect (P = 0.171). Considering the combined placebo/sex steroid milieu, women exhibited greater total GH recovery than men during saline infusion (P = 0.021; Fig. 2, middle). In contrast, sex difference did not influence marked effects of GHRH/GHRP-2 (women vs. men P = 0.109; peptide effects per se P < 0.001 in both sexes). Sex steroid (E2/T) treatment stimulated GH recovery during saline (P < 0.001) and not 2-peptide (P = 0.996) infusions (Fig. 2, bottom). Thus, dual-peptide stimulation markedly increased GH recovery in women without or with E2 (both P < 0.001) and likewise in men without or with T (both P < 0.001). There were no sex differences (P > 0.60; Fig. 2, middle and bottom). The strong treatment × peptide interaction (P < 0.001; Fig. 2, bottom) reflected the fact that, during saline infusion, both E2 (P < 0.001) and T (P = 0.031) enhanced total GH recovery over respective saline/placebo controls by Tukey’s post hoc multiple comparison test. During Pl exposure, sex difference did not influence GH recovery under saline (P = 0.982) or 2-peptide stimulation (P = 0.842). However, women given E2 treatment had a greater response to saline than men given T (P = 0.036). This sex difference was lost during GHRH/GHRP-2 stimulation (P = 0.610). In view of marked regulation of total GH recovery during feedback, we next asked how sex difference, peptide, and treatment selectively determined nadir and peak GH concentrations.

![Graphs showing mean GH concentration profiles](image-url)

Fig. 1. Mean (± SE) 8-h growth hormone (GH) concentration profiles obtained by sampling blood every 10 min from 0600 to 1400 fasting. A single pulse of GH (1 μg/kg) was injected intravenously at 0630 to enforce negative feedback. To evaluate the role of peptide secretagogues in feedback control, saline or combined GH-releasing hormone (GHRH)/GH-releasing peptide-2 (GHRP-2) (1 μg·kg⁻¹·h⁻¹) was infused continuously (0600–1400). Data are from 11 women and 14 men.

![Graphs showing total integrated GH escape](image-url)

Fig. 2. Total integrated GH escape during autonegative feedback along with saline or GHRH/GHRP-2 infusion in 11 women and 14 men. Subjects were pretreated with placebo (Pl), estradiol (E2), or testosterone (T) (see METHODOLOGY). Data are results of 3-way analysis of covariance (ANCOVA) to assess main and interactive effects of sex difference (women or men), infusion type (saline or combined GHRH/GHRP-2), and sex steroid treatment (E2 or T vs. placebo) on feedback-suppressed 5.5-h integrated GH concentrations. Overall, P < 0.001 for the ANCOVA model, with covariate P < 0.001. Means with different letters differ significantly as main effects. Data are geometric means ± SE.

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Three-way ANCOVA of nadir GH concentrations identified overall $P < 0.001$ for the mixed-effects model ($\chi^2 = 0.887$) and $P < 0.001$ for the covariate (Fig. 3). Sex difference had no overall effect on GH nadirs ($P = 0.407$; Fig. 3, top). Specifically, saline ($P = 0.086$) and peptide ($P = 0.620$) effects were respectively nonsignificant as a trend and comparable between sexes (Fig. 3, middle). There were significant main effects of peptide infusion ($P < 0.001$) and sex steroid treatment ($P = 0.019$) (Fig. 3, middle and bottom). Whereas sex difference × treatment had a nonsignificant trend to interact ($P = 0.074$; Fig. 3, top), sex difference and peptide ($P = 0.012$) as well as treatment and peptide ($P = 0.007$) interacted strongly (Fig. 3, middle and bottom). This was due to a stimulatory effect of $E_2$ ($P = 0.031$) in women but not $T$ in men ($P = 0.972$) over $P_I$ (Fig. 3, top). The peptide effect was due to marked and comparable elevation of GH nadirs during GHRH/GHRP-2 infusion in women ($P < 0.001$) and men ($P < 0.001$) compared with saline (Fig. 3, middle) whether $P_I$ or $E_2/T$ was administered (Fig. 3, bottom). Moreover, $T/E_2$ treatment elevated nadir GH concentrations during saline infusion ($P = 0.003$) but not 2-peptide stimulation ($P = 0.994$) (Fig. 3, bottom). By Tukey’s multiple comparison test, sex difference × peptide and treatment × peptide interactions existed because $E_2$ in particular increased nadir GH concentrations during saline ($P = 0.014$) but not 2-peptide infusion ($P = 0.999$). In contradistinction, $T$ had no effect during either saline or peptide infusions (both $P > 0.987$). Thus, $E_2$ selectively elevates nadir GH concentrations during controlled negative feedback under endogenous GHRH/ghrelin (but not exogenous GHRH/GHRP-2) drive.

Peak (escape) GH concentrations during negative feedback were also analyzed by three-way ANCOVA. The overall model and covariate were significant (both $P < 0.001$, $\chi^2 = 0.808$). There were prominent main effects of sex difference ($P = 0.001$; Fig. 4, top), peptide ($P < 0.001$; Fig. 4, middle), and sex steroid treatment ($P = 0.005$; Fig. 4, bottom). The main sex effect was explained by higher peak GH values in women given $E_2$ than in men given $P_I$ ($P < 0.001$) or $T$ ($P = 0.013$; Fig. 4, top) and by higher peak GH response to GHRH/GHRP-2 in women than in men ($P = 0.016$) (Fig. 4, middle). There was a nonsignificant trend for an $E_2$ effect over $P_I$ in women ($P = 0.058$; Fig. 4, top). The categorical peptide effect was attributable to marked stimulation by GHRH/GHRP-2 in both sexes ($P < 0.001$ compared with saline, both without and with $E_2/T$ supplementation; Fig. 4, middle and bottom). The primary treatment effect was due to stimulation by $E_2/T$ ($P < 0.001$ over $P_I$) during saline but not GHRH/GHRP-2 ($P = 0.996$) infusion (Fig. 4, bottom). The concomitant × peptide interaction ($P = 0.002$) was accounted for by augmentation of peak GH recovery by $E_2/T$ during saline ($P < 0.001$) but not double-peptide infusion ($P = 0.996$) in the combined-sex groups. By Tukey’s post hoc honestly significantly different test, $E_2$ in women ($P = 0.012$) but not $T$ in men ($P = 0.249$) infused with saline elevated peak GH recovery compared with matching placebo condition. Dual-peptide stimulation elimi-
nated the capability of E2/T to further elevate peak GH levels (P = 0.999; Fig. 4, bottom).

GH ApEn (irregularity) was used as a model-free measurement of relative feedforward/feedback strength (see Methodology). By three-way ANCOVA, overall model \( P \) was \( \leq 0.001 \) with \( r^2 = 0.384 \) and covariate \( P \) was \( 0.019 \). There were categorical effects of peptide \( (P = 0.001) \) and treatment \( (P = 0.012) \) but not sex difference \( (P = 0.687; \text{Fig. } 5) \). There was a nonsignificant trend for a treatment-by-peptide interaction \( (P = 0.093) \). When considering combined saline/dual-peptide effects (Fig. 5, top), T compared with Pl selectively elevated GH ApEn in men \( (P = 0.025) \). E2 had no such effect in women \( (P = 0.823) \). For the combined sexes, GHRH/GHRP-2 vs. saline stimulated GH ApEn in the Pl \( (P < 0.001) \) and the E2/T \( (P = 0.026) \) settings (Fig. 5, middle). Whereas E2/T treatment increased ApEn during saline infusion \( (P = 0.018) \), E2/T did not alter ApEn during dual-peptide infusion \( (P = 0.927) \) (Fig. 5, bottom). By post hoc Tukey’s test, in women, neither individual E2 nor double-peptide stimulation raised GH ApEn over that of Pl/saline \( (P = 0.003) \). In contrast, T but not E2 selectively augments GH ApEn during negative feedback. The exact bases for the dual-peptide infusion distinctly augment ApEn during negative feedback.

EXPLORATORY linear regression was used to test the hypothesis that BMI, IGF-I, or E2 concentrations determine feedback recovery in the combined cohorts. Figure 6 shows that increasing BMI in the Pl/saline setting reduced nadir GH concentrations at \( P = 0.0035 \) and \( r^2 = 0.31 \) (assuming protected correlational, \( P < 0.01 \)). During combined E2/T and dual-peptide drive, higher IGF-I concentrations correlated with lower peak GH recovery at a trend level for \( P < 0.01 \) \( (P = 0.023, r^2 = 0.23; \text{Fig. } 7) \).

DISCUSSION

The present analyses unveil distinct individual and interactive effects of sex steroid supplementation, sex difference, and dual-secretagogue stimulation in a paradigm of experimentally controlled GH feedback. Although both E2 and T stimulate pulsatile GH secretion in the absence of a feedback clamp (17), the present data show that primarily E2 elevates total, nadir, and peak GH concentrations when GH feedback is imposed experimentally. In contrast, T but not E2 selectively augments GH ApEn during negative feedback. The exact bases for the

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Fig. 5. GH approximate entropy (ApEn) as a model-free measurement of relative feedforward/feedback signal strength. Data are presented as described in Fig. 3.

Fig. 6. Inverse relationship between body mass index and nadir GH concentrations monitored during experimental negative feedback in the placebo saline setting. Data are from 25 adults.

Fig. 7. Linear regression of peak GH concentrations attained during feedback recovery on serum IGF-I concentrations. Data were obtained during E2 supplementation in women \( (n = 11) \) and T supplementation in men \( (n = 14) \) along with dual-peptide infusion.
specific actions of E2 in women and T in men in this setting are not known. Whereas IGF-I concentrations did not differ in men and women receiving placebo, IGF-I concentrations were lower and IGFBP-1 concentrations higher in women than in men supplemented with sex steroids (Table 1). Although serum E2 concentrations were similar by sex during E2/T supplementation, presumptively lower free IGF-I levels in sex steroid-treated women than in men (42) may be relevant if free IGF-I concentrations contribute to negative feedback (8). In relation to androgen, higher GH ApEn under T administration has been recognized in prepubertal boys treated with T but not dihydrotestosterone (44), suggesting a role for aromatization of T in mediating the increase in ApEn (17).

A salient finding was that combined peptide infusion significantly elevated total, nadir, and peak GH concentrations despite the imposition of GH feedback. In this setting, E2 supplementation no longer significantly rescued feedback-suppressed GH secretion. Hypothalamic GH receptors mediate GH autofeedback in experimental animals by signaling periventricular nucleus SS release (46). Periventricular SS-ergic nerves terminating in the arcuate nucleus confer direct inhibition of neuronal GHRH secretion, whereas those terminating on hypothalamo-pituitary portal vessels enforce suppression of somatotrope exocytosis (10, 14, 20). The infusion of GHRH/GHRP-2 during negative feedback would effectively replace any feedback-induced deficiency in portal GH release and possibly pituitary ghrelin availability. Thus, the capability of E2 to amplify GH secretion during saline but not GHRH/GHRP-2 infusion could indicate that E2 increases hypothalamic release or action of endogenous GHRH and/or hypothalamo-pituitary availability of or sensitivity to endogenous GHRP (ghrelin) or blunts inhibitory feedback by endogenous GH, possibly via SS-mediated pathways (46). Reduced SS outflow to the pituitary would not necessarily explain loss of the E2 effect under two-peptide stimulation, because putative SS withdrawal by other means potentiates joint stimulation by GHRH/GHRP-2 in humans (3). Small effects on SS outflow cannot be excluded because the degree to which pharmacological GHRH/GHRP-2 drive might overwhelm a lesser effect of E2 on endogenous SS is not yet known.

GHRPs stimulate GHRH secretion and oppose brain actions of SS, but they do not impede SS release into portal blood in animals (12, 15, 18). If the same is true in humans, then the dual-peptide infusion paradigm used here should unmask primarily the effects of feedback-dependent endogenous SS release and exogenous peptide actions on the pituitary gland. We found that neither T nor E2 increased the combined efficacy of GHRH/GHRP-2 stimulation under GH feedback. Peptidyl efficacy was also not augmented in feedforward dose response studies performed during E2 vs. placebo exposure (2, 40, 41). Unchanged efficacy of dual-peptide drive in an estrogen-rich milieu during GH autofeedback would speak against any major estrogen-induced alteration in periventricular SS outflow to the pituitary gland.

ApEn provided an independent scale-invariant measurement of feedback adaptations (31, 48). This model-free metric quantifies altered process randomness with high sensitivity and specificity (both >90%). Process randomness in the GH system increases (yielding increased ApEn) under the low-feedback effects of IGF-I depletion during fasting (19). Analogously, relative reductions in feedback (inhibition) compared with feedforward (stimulation) elevate GH ApEn in puberty and during GHRH and/or GHRP-2 (or ghrelin) stimulation (6, 11, 40, 47). The present analyses further show that, under exogenously imposed negative feedback, combined GHRH/GHRP-2 infusion still increases GH ApEn in women and men (both P < 0.001). Neither E2 nor T administration potentiated the dual-peptide effect. These data provide further evidence against sex steroidial augmentation of GHRH/GHRP-2 efficacy under feedback restraint. On the other hand, in men, T supplementation elevated ApEn during GH feedback and saline infusion (i.e., under endogenous GHRH/ghrelin drive). In women, E2 did not have this effect. A plausible explanation is that T (or locally produced E2 in the hypothalamos-pituitary unit) antagonizes systemic IGF-I feedback (39) and/or potentiates endogenous GHRH/ghrelin action (43). These mechanisms require further study.

Exploratory linear regression analysis disclosed a strong inverse relationship between nadir GH recovery and BMI only in the saline/placebo setting. The negative association explained 31% of the variance in nadir GH concentrations in the combined cohorts. The exact basis for the putative effect of greater relative adiposity on nadir GH levels is not known, although adipocytokines might be involved (46). A somewhat weaker inverse association (r² = 0.23) emerged between dual-peptide stimulated peak GH concentrations under sex steroid supplementation and total IGF-I concentrations. This negative relationship has not been recognized in the GH feedback setting. It would be consistent with a combined role for GH and IGF-I in negative feedback control.

Caveats include the relatively small overall cohort size (n = 25), although 100 visits of 8-h sampling were analyzed. Thus, our conclusions require confirmation. The individual effects of GHRH and GHRP-2 were not evaluable in the dual-peptide model instituted here. Future studies could examine this as well as the visceral adiposity-, age-, and E2/T dose-related effects on GHRH/GHRP’s stimulatory effects on GH secretion in feedback-inhibited contexts. For example, small effects of age within the range of 45 to 74 yr and/or of BMI between the strata of 21–25 and 26–30 kg/m² might have been overlooked in a cohort of only 25 subjects. The present results cannot be transposed directly to physiological regulation of GH secretion by sex steroids in older adults given the pharmacological GH and sex steroid clamp imposed for experimental reasons. Thus, the insights gained can be regarded as prephysiological or preclinical.

In conclusion, E2 supplementation and dual-peptide stimulation in older adults elevate variously total (integrated), nadir (suppressed), and peak (recovering) GH release during experimentally controlled negative feedback. Sex difference also influences peak GH recovery during negative feedback. Under combined peptide (GHRH/GHRP) stimulation, effects of sex steroid and sex difference were not observable. An independent objective measure of relative feedforward/feedback strength, ApEn, corroborates the key role inferred for dual-peptide drive in feedback regulation. To the degree that negative feedback counterbalances feedforward stimulation, these data introduce key factors that may be relevant in modulating the pulsatile secretion of GH in the feedback domain under joint secretagogue drive. Whether regional or whole body adiposity interacts specifically with sex difference, sex ste-
roids, and combined GHRH/GHRP drive under feedback is not known.

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DISCLOSURES
The authors have no conflict of interest to disclose.

REFERENCES

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