Estradiol regulates GH-releasing peptide’s interactions with GH-releasing hormone and somatostatin in postmenopausal women

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Abstract

Objective: Estrogen stimulates pulsatile secretion of GH, via mechanisms that are largely unknown. An untested hypothesis is that estradiol (E₂) drives GH secretion by amplifying interactions among GH-releasing hormone (GHRH), somatostatin (SS), and GH-releasing peptide (GHRP).

Design: The design comprised double-blind randomized prospective administration of transdermal E₂ vs placebo to healthy postmenopausal women (n = 24) followed by pulsatile GHRH or SS infusions for 13 h overnight with or without continuous GHRP2 stimulation.

Methods: End points were mean concentrations, deconvolved secretion, and approximate entropy (ApEn; a regularity measure) of GH.

Results: By generalized ANOVA models, it was observed that E₂ vs placebo supplementation: i) augmented mean (13-h) GH concentrations (P = 0.023), GHRH-induced pulsatile GH secretion over the first 3 h (P = 0.0085) and pulsatile GH secretion over the next 10 h (P = 0.054); ii) increased GHRP-modulated (P = 0.022) and SS-modulated (P < 0.001) GH ApEn; and iii) did not amplify GHRH/GHRP synergy during pulsatile GH secretion. By linear regression, E₂ concentrations were found to be positively correlated with GH secretion during GHRP2 infusion (P = 0.022), whereas BMI was found to be negatively correlated with GH secretion during GHRH (P = 0.006) and combined GHRH/GHRP (P = 0.015) stimulation. E₂ and BMI jointly determined triple (combined L-arginine, GHRH, and GHRP2) stimulation of GH secretion after saline (R² = 0.44 and P = 0.003) and pulsatile GHRH (R² = 0.39 and P = 0.013) infusions.

Conclusion: In summary, in postmenopausal women, E₂ supplementation augments the amount (mass) and alters the pattern (regularity) of GH secretion via interactions among GHRH, SS, GHRP, and BMI. These outcomes introduce a more complex model of E₂ supplementation in coordinating GH secretion in aging women.

Introduction

Decreased growth hormone (GH) secretion during aging, hypogonadism, and obesity was recognized almost four decades ago (1, 2, 3, 4). Low GH concentrations, in turn, are associated with insulin resistance, dyslipidemia, cardiovascular mortality, sarcopenia, osteopenia, possible cognitive impairment, and reduced quality of life. Supplementation of estradiol (E₂) in girls or women and that of an aromatizable androgen in boys or men stimulate GH production and alter the regularity of GH secretion patterns (2, 3, 5). The importance of E₂ is inferable in both sexes,
since an ER antagonist lowers GH concentrations in women and men and an aromatase inhibitor blocks GH responses to testosterone in men (2, 3, 6). Nonetheless, the precise clinical mechanisms mediating estrogen’s augmentation of GH secretion remain largely unknown.

The effects of gonadal estrogen on the GH axis have been investigated in animal models by ovariectomy and transgenic silencing of the Erα (ESR1) and aromatase genes (2, 3, 7). In rats, E2 controls hypothalamic GH-releasing hormone (GHRH) neurons, hypothalamic receptors for GH-releasing peptide (GHRP), pituitary receptors for GHRH and GHRP, somatostatin (SS) receptor subtypes 2 and 5, and gastric ghrelin (native GHRP) expression (2, 3, 7). The various effects of E2 on GHRH, SS, and ghrelin secretion and action in animal models strongly suggest the estrogenic control of multiple peptide pathways. However, to date, available clinical studies of E2 action have evaluated primarily single-peptide effects, rather than their interactions. To address this major limitation, the present study combined: i) experimental imposition of young-adult E2 concentrations in postmenopausal women; ii) individual and combined infusions of hypothalamic peptides (GHRH, SS, and GHRP) for 13 h overnight; iii) deconvolution (secretion-based) and approximate entropy (ApEn, a pattern-regularity measure) analyses of GH concentration-time series; and iv) triple stimulation of GH secretion with l-arginine and GHRH/GHRP to estimate maximal (pharmacological) pituitary GH secretory capacity. The end points are important, because burst-like (pulsatile) GH secretion and ApEn-quantified secretion regularity are physiologically supervised modes of GH secretion (2, 3, 8). The postulate was that both the amount and pattern of GH secretion are jointly specified by E2, GHRH, GHRP/ghrelin, and SS, along with BMI as a surrogate of relative adiposity.

**Subjects and methods**

**Study design and volunteers**

The study protocol was approved by the Mayo Institutional Review Board (IRB) and reviewed by the U.S. Food and Drug Administration. This was a double-blind, placebo-controlled, prospectively randomized study of 24 healthy, community-based, ambulatory women conducted in the Center for Translational Science Activities (CTSA). The participants were admitted to the CTSA by 1530 h on the evening of the study. To limit nutritional confounds, a standardized meal (33 kJ/kg of 50% carbohydrate, 20% protein, and 30% fat) was served at 1600 h. The subjects then remained fasting, alcohol abstinent, and caffeine free until noon the next day. Vigorous exercise and daytime sleep were not allowed. Bilateral cannulas were placed in forearm veins at or before 1900 h to permit concurrent overnight blood sampling and peptide infusions.

**Infusion and sampling paradigm**

Each subject participated in a total of six (6) separate randomly ordered double-blind overnight consecutive 13- and 3-h (total 16-h) infusion sessions (2000 h until noon the next day) scheduled at least 48 h apart in the

**E2 treatment**

E2 supplementation was via daily transdermal E2 patches (Novartis) at escalating increasing doses, viz. 0.05 mg/day (184 nmol) for the first 4 days, 0.10 mg/day (368 nmol) for the next 4 days, and 0.15 mg/day (552 nmol) for 20 additional days (9). Overnight infusion studies were initiated after at least 4 days on the highest (0.15 mg) E2 dose. Medroxyprogesterone acetate (5 mg; Pharmacia and Upjohn, New York, NY, USA) was given orally daily for 12 days after E2 supplementation, when all infusion/sampling sessions were completed.

**Sampling schedule**

The participants were admitted to the CTSA by 1530 h on the evening of the study. To limit nutritional confounds, a standardized meal (33 kJ/kg of 50% carbohydrate, 20% protein, and 30% fat) was served at 1600 h. The subjects then remained fasting, alcohol abstinent, and caffeine free until noon the next day. Vigorous exercise and daytime sleep were not allowed. Bilateral cannulas were placed in forearm veins at or before 1900 h to permit concurrent overnight blood sampling and peptide infusions.
CTSA (Fig. 1). Three infusion sessions comprised continuous i.v. saline infusions (30 ml/h) and the other three sessions comprised continuous i.v. GHRP2 infusions (1 μg/kg per h) for 13 h (2000–0900 h). Based upon prior dose–response curves obtained in older women (10, 11, 12), bolus i.v. saline (Sal; 1 ml), GHRH (1.0 μg/kg), or SS (0.67 μg/kg) pulses were superimposed upon the continuous saline or GHRP2 infusions every 90 min for a total of nine pulses during the inclusive time window 2000–0850 h. GHRP2 was obtained from Kaken Pharmaceuticals (Tokyo, Japan), and GHRH and SS were obtained from Bachem Laboratories (Torrance, CA, USA).

Immediately after the 13-h overnight pulsatile peptide infusions, a triple secretagogue was administered, followed by three more hours of sampling. This was done to test maximal (pharmacological) GH release. The triple stimulus comprised sequential i.v. infusion of L-arginine (30 g continuously from 0900 to 0930 h). This was immediately followed by combined bolus injections of GHRH and GHRP2 (both i.v. at 1.0 μg/kg) at 0930 h. This combination has been used by others (8).

Blood (0.75 ml/sample) was withdrawn every 10 min for 16 h (2000 h–noon) from all 24 subjects during all the six sessions. Sleep was not assessed in this study. Serum that was separated was frozen at −70 °C until assay.

Assays
GH concentrations were determined as a batch of 582 samples/subject via a robotics-assisted two-site monoclonal immunoenzymatic chemiluminescence assay (sensitivity 0.010 μg/l). The assay standard was 22 kDa recombinant human GH. Inter-assay coefficients of variation (CV) for GH concentrations of 3.4 and 12 μg/l were 7.9 and 6.3% respectively. Intra-assay CV values for GH concentrations of 1.1 and 20 μg/l were 4.9 and 4.5% respectively. No values were <0.020 μg/l. Screening thyroid-stimulating hormone (TSH), prolactin, luteinizing hormone (LH), and FSH concentrations were quantified by an automated chemiluminescence assay (ACS 180; Bayer), using as standards recombinant TSH and prolactin and the First and Second International Gonadotropin Reference Preparations. Liquid chromatography–tandem mass spectrometry was used to quantify E2 and testosterone concentrations in serum samples collected at 0800 h at screening and at each of the six (6) CRU visits (13). SHBG, insulin-like growth factor 1 (IGF1), IGF-binding protein 1 (IGFBP1), and IGFBP3 concentrations were assayed by an immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX, USA).

Analytical methods
Deconvolution analysis was applied to the initial 3-h (2000–2250 h inclusive) and the next 10-h (2300–0900 h) windows of the 13 h of 10-min sampling sessions during peptide/saline infusions, as well as to the final 3 h (0900 h–noon) of 10-min sampling sessions during the triple stimulus. The goal was to estimate basal (nonpulsatile) and pulsatile (burst-like) GH secretion and thereby total GH secretion. The first 3-h GH responses to peptide infusions (2000–2250 h) were used to estimate readily releasable GH (14). GH secretion was quantified using published biexponential GH-elimination kinetics (viz. 3.5- and 20.8-min half-lives (15)). The Matlab model is conditioned mathematically on a priori identification of candidate sets of pulse-onset times using an image boundary-detection technique. Pulse number is optimized by the Akaike information criterion (16). Both sensitivity and specificity are 93%.
Approximate entropy

ApEn (1, 20%) was used as a scale- and model-independent statistic to quantify the relative orderliness (regularity) vs disorderliness (irregularity). Higher ApEn denotes greater relative randomness (irregularity) of the secretion process. Mathematical models and clinical experiments establish that greater irregularity signifies decreased feedback control with high sensitivity and specificity (both >90%) (17).

Biostatistical analysis

The experimental design was consistent with the general principles of a split-plot design. The whole plots were the primary treatments of E2 or placebo. Within each condition, the participants received six different infusions (GHRP2 vs Sal infusion crossed with Sal, GHRH, or SS infusion). The nesting of the treatments within patients was addressed statistically by means of a mixed fixed- and random-effects model using SAS PROC MIXED. Main effects for each level of the split-plot design along with their two- and three-variable interactions were modeled as fixed effects. The random effect consisted of a random participant (blocking) factor. To limit heterogeneity of variance, parameters were first log-transformed. Model-based means were compared with the Tukey–Kramer post hoc correction factor. The degrees of freedom for the fixed effects were estimated using the Kenward–Roger method. Experiment-wise (adjusted) P values < 0.05 were considered statistically significant, except for multiple comparisons when P < 0.01 (protected) was used (18).

Rapid (3-h) GH responses ▶ The response observed in the first 3 h of each 13-h peptide/saline infusion session was arbitrarily considered an immediate or rapid response. Analysis of 3-h pulsatile GH secretion revealed strong effects of SS suppression was 0.5 to 3.2-fold saline; the effect of GHRP2 was 8.5 to 2.2-fold saline; and the effect of SS suppression was 0.5 to 0.11-fold saline (5, 10, 11, 12).

Results

Subject characteristics at baseline screening

Epidemiological and baseline endocrine data are given in Table 1. Absolute age range for the 24 participants was 50–76 years. There was no age or BMI difference in the randomized groups (protected P < 0.01 for multiple tests). Total testosterone, E2, LH, FSH, prolactin, SHBG, and TSH concentrations were all normal for age (Mayo Medical Reference Laboratories, Rochester, MN, USA). After E2 vs Pl supplementation, E2 (P < 0.001) and mean GH concentrations during Sal/Sal infusions (P = 0.023) and SHBG concentrations (P < 0.01) increased, whereas IGF1, IGFBP1, and IGFBP3 concentrations did not change significantly (Table 2).

Table 1 Baseline epidemiological and endocrine data: pre-randomization (fasting 0800-h values). Data are means ± S.E.M. (median, range).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre-randomization (fasting 0800-h values)</th>
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<tbody>
<tr>
<td></td>
<td>Subsequent placebo exposure (n = 14)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>66 ± 1.5 (66) (55–76)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26 ± 0.85 (26) (22–30)</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13 ± 0.22 (13) (12–15)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.3 ± 0.05 (4.3) (3.9–4.6)</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>0.83 ± 0.13 (0.58) (0.26–1.8)</td>
</tr>
<tr>
<td>E2 (pmol/l)</td>
<td>69 ± 12 (69) (39–128)</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>25 ± 2.6 (26) (12–44)</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>88 ± 13 (75) (41–171)</td>
</tr>
<tr>
<td>Prolactin (µg/l)</td>
<td>6.1 ± 0.74 (5.5) (3–11)</td>
</tr>
<tr>
<td>SHBG (nm)</td>
<td>53 ± 5.5 (48) (31–98)</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>1.8 ± 0.22 (1.6) (0.5–3.2)</td>
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between GHRP2 and GHRH (effect). There was a prominent amplifying interaction responses (blue lines) in all four major conditions: Pl (no green in Fig. 2. GHRH pulses elicited marked pulsatile GH interval (2300–0900 h) under saline infusion are shown in SHBG (nM) 42
IGF1 (nmol/l) 21
IGFBP3 (mg/l) 3.6
infusions with or without E2 supplementation was maxi-
mally stimulatory. SS pulses were inhibitory (red lines) only
in the presence of Pl/GHRP2 and E2/GHRP2. Deconvolu-
tion analysis revealed significant treatment effects on 10-h pulsatile GH secretion. Figure 3 shows box-and-whisker
ranges (solid dots)) for the 12 arms: six with Pl supple-
mentation (left panel) and six with E2 supplementation
(right panel). Statistical main effects existed for E2
(P=0.054), GHRP2 (P<0.0001), and GHRH (vs saline and
SS; P<0.0001) (Table 3, column 2). SS vs Sal infusion had no effect. There was a prominent amplifying interaction between GHRP2 and GHRH (P<0.0001) and a nonsignifi-
cantly trending potentiating interaction between E2 and
GHRP2 (P=0.059) for 10-h pulsatile GH secretion.

Table 2 Baseline epidemiological and endocrine data: post-
randomization (mean of six visits per subject). Data are means ±
S.E.M. (median, range).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Post-randomization (mean of six visits per subject)</th>
<th>During placebo exposure</th>
<th>During E2 exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2 (pmol/l)</td>
<td>66 ± 13 (58)</td>
<td>450 ± 110 (355)*</td>
<td></td>
</tr>
<tr>
<td>IGFBP1 (μg/l)</td>
<td>35 ± 4.6 (28)</td>
<td>43 ± 6.1 (42)</td>
<td></td>
</tr>
<tr>
<td>IGFBP3 (mg/l)</td>
<td>3.6 ± 0.71 (3.5)</td>
<td>3.2 ± 0.23 (3.1)</td>
<td></td>
</tr>
<tr>
<td>IGFl (nmol/l)</td>
<td>21 ± 2.6 (18)</td>
<td>17 ± 2.0 (16)</td>
<td></td>
</tr>
<tr>
<td>SHBG (nM)</td>
<td>42 ± 4.3 (39)</td>
<td>93 ± 17 (91)*</td>
<td></td>
</tr>
<tr>
<td>GH (μg/l)</td>
<td>0.48 ± 0.073 (0.42)</td>
<td>0.88 ± 0.16 (0.72)*</td>
<td></td>
</tr>
</tbody>
</table>

Protected P<0.01 vs placebo.
*Mean of 13-h sampling during Sal/Sal infusions (P=0.023 for Pl vs E2 exposure).

Sustained (10-h) responses to peptide infusions ► The response observed in the last 10 h of each 13-h infusion session was considered a sustained response. Median 10-min GH concentrations (Pl, n=14; E2, n=10) over this interval (2300–0900 h) under saline infusion are shown in green in Fig. 2. GHRH pulses elicited marked pulsatile GH responses (blue lines) in all four major conditions: Pl (no E2)/Sal, E2/Sal, Pl/GHRP2, and E2/GHRP2. The unique combination of pulses of GHRH and continuous GHRP2 infusions with or without E2 supplementation was maxi-
mally stimulatory. SS pulses were inhibitory (red lines) only
in the presence of Pl/GHRP2 and E2/GHRP2. Deconvolu-
tion analysis revealed significant treatment effects on 10-h pulsatile GH secretion. Figure 3 shows box-and-whisker plots (medians, interquartile ranges, 95% CIs, and extreme ranges (solid dots)) for the 12 arms: six with Pl supple-
mentation (left panel) and six with E2 supplementation
(right panel). Statistical main effects existed for E2
(P=0.054), GHRP2 (P<0.0001), and GHRH (vs saline and
SS; P<0.0001) (Table 3, column 2). SS vs Sal infusion had no effect. There was a prominent amplifying interaction between GHRP2 and GHRH (P<0.0001) and a nonsignifi-
cantly trending potentiating interaction between E2 and
GHRP2 (P=0.059) for 10-h pulsatile GH secretion.

ApEn (degree of irregularity)
The ApEn of GH secretion over the sustained 10-h period
of saline or peptide infusions (2300–0900 h) revealed main
effects of E2 (P=0.028), continuous GHRP2 (P<0.0001),
pulsed GHRH (P<0.0001), and pulsed SS (P=0.013) to increase ApEn (Table 3, column 3). In particular, E2 vs Pl supplementation elevated GH ApEn under Sal/SS (P<0.001) and GHRP2/Sal (P<0.01) infusions, strongly suggesting decreased feedback. Moreover, there were significant interactions between E2 and GHRP2 (P=0.022), between E2 and GHRH (P=0.019), and among E2, GHRP2, and GHRH/SS (P=0.027) in increasing ApEn.

Triple-stimulus effects
A triple stimulus of consecutive L-arginine and combined GHRP2/GHRH infusion was used to evaluate pituitary GH secretory capacity at the end of the 13-h pulsatile peptide infusion session (Fig. 4). Prior 13-h pulsatile GHR
infusion potentiated pulsatile GH secretion after the triple
stimulus (P=0.0015), whether or not E2 was administered
(blue lines in the top two panels). Concomitant GHRP2
infusion (blue lines, bottom two panels) unexpectedly
suppressed (P<0.0001) the GH effect (bottom two panels).
Statistical outcomes are summarized in column 4 of
Table 3. Specifically, E2 supplementation increased the
GH-inhibitory effect of GHRP2 on GHRH’s priming of
pulsatile GH secretion (P=0.049). E2 supplementation did
not affect median GH concentrations after GHRH infu-
sion, but it did increase the simple peak GH concentration
(arithmetic maximum) response to the triple stimulus
following 13-h Sal/SS (P<0.01), GHRP2/Sal (P<0.05), and
GHRP2/GHRH (P<0.05) infusions. Comparable median
levels but higher peak values after GHRH pretreatment
probably reflect the fact that peak values occur at non-
uniform time points after the triple stimulus.

Table 3 Pulsatile GH secretion and ApEn in 24 women. Splitplot analysis with main effects and interactions.

<table>
<thead>
<tr>
<th>Main effects</th>
<th>Pulsatile (acute/3 h)</th>
<th>Pulsatile (sustained/ 10 h)</th>
<th>ApEn (irregularity)</th>
<th>Pulsatile after triple stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>0.0085</td>
<td>0.054</td>
<td>0.028</td>
<td>0.92</td>
</tr>
<tr>
<td>GHRP2</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pulsed peptides</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GHRH/SS</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.001</td>
<td>0.0015</td>
</tr>
<tr>
<td>GHRH vs Sal</td>
<td>0.63</td>
<td>0.95</td>
<td>0.013</td>
<td>0.32</td>
</tr>
<tr>
<td>GHRH vs SS</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.012</td>
<td>0.091</td>
</tr>
<tr>
<td>Interactions</td>
<td>E2 × GHRP2</td>
<td>0.61</td>
<td>0.059</td>
<td>0.022</td>
</tr>
<tr>
<td>E2 × GHRH/SS/SS</td>
<td>0.45</td>
<td>0.17</td>
<td>0.019</td>
<td>0.10</td>
</tr>
<tr>
<td>GHRP2 × GHRH/SS/SS</td>
<td>0.091</td>
<td>&lt;0.0001</td>
<td>0.31</td>
<td>0.55</td>
</tr>
<tr>
<td>All three factors</td>
<td>0.77</td>
<td>0.67</td>
<td>0.027</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Data are P values for main effects and interactions.
The present clinical investigation in postmenopausal women unveils the major effects of E2 supplementation on GH secretory dynamics driven by an ensemble of GH-regulating peptides, viz. repetitive i.v. pulses of GHRH, SS, and saline with or without continuous GHRP stimulation. Peptide doses were chosen based on prior peptide dose–response curves obtained in older women (10, 11, 12). The salient effects of E2 were as follows: i) amplification of rapid (initial 3-h) pulsatile GH secretion during overnight pulsatile Sal/GHRH stimulation (P=0.0085), with a strong analogous trend for E2 to augment prolonged (10-h) pulsatile GH secretion during GHRP2 drive (P=0.059), signifying a greater mass of GH released per burst; ii) elevation of GH ApEn during GHRP2 (P=0.022) and SS (P<0.001) infusions, thus suggesting decreased feedback effects under E2 supplementation; and iii) opposition in an E2 concentration-dependent manner the negative effect of BMI on the GH secretory response to a massive triple stimulus. The last point becomes relevant for interpreting pharmacological testing of GH reserve capacity (8), suggesting that
both BMI and E2 administration may alter the cutoffs for a maximal GH response at least in older women.

GH regulation is important in aging, in as much as small increments in GH availability in older adults decrease visceral adiposity, diastolic blood pressure, and LDL concentrations and increase lean body mass (19). Pulsatile secretion is a key locus of physiological GH regulation by the hypothalamo-pituitary GHRH, SS, and GHRP (2, 20). In turn, GH pulses specifically control liver and muscle gene expression, mediate GH feedback on brain, and enhance lipolysis in fat (3). Thus, tissue responses to GH peaks may be more significant than those to mean/median GH concentrations per se, especially in relation to growth and anabolism (3). The generation of GH pulses is believed to require SS, GHRH, and GHRP/ghrelin. First, SS withdrawal seems necessary before GHRH pulses can initiate prominent GH secretory bursts (3, 20, 21). Second, GHRH is essential for stimulating both GH release and GH synthesis (2). SS and GHRH act in concert, since SS restraints exocytosis (but not synthesis) of GH, thus allowing the accumulation of releasable GH stores under ongoing GHRH-stimulated GH synthesis (14). Third, whereas GHRP does not augment GH synthesis, it markedly potentiates GHRH-evoked GH release, thus serving as an in vivo amplifier of GH pulsatility (3, 22, 23, 24). We tested the amplifying interaction between GHRH and GHRP by infusing GHRH pulses every 90 min for 13 h along with saline or GHRP2. GHRP2 and a physiologically pulsatile GHRH stimulus were strongly synergistic over 10 h independently of E2 availability. The importance of GHRH for maximal GHRP drive has been established in patients with inactivating mutations of the GHRH receptor (21) or hypothalamic-pituitary disconnection (23). Thus, a working model of GH pulsatility would include SS as a determinant of the timing and size of GH secretory bursts and ghrelin/GHRP and GHRH as individual and synergistic amplifiers of the size of GH pulses (20, 25, 26, 27, 28).

SS is a powerful inhibitor of GH release. In the presence of E2, repeated SS pulses increased GH ApEn (Table 3), a statistical measure of the relative randomness, irregularity, or disorderliness of secretory patterns (29). The ability of E2 and SS to increase GH ApEn would be consistent with the ability of E2 to attenuate GH and IGF1 feedback and SS potency, as well as to enhance submaximal GHRH and GHRP stimulation in women (3, 10, 23, 30). Nonetheless, SS pulses at the dose used in the present study in women had no detectable effect on GH ApEn in an earlier study in older men in the presence and absence of supplemental testosterone (31). This raises the question whether pituitary sensitivity to SS differs by gender in humans.

The triple-secretagogue paradigm has been suggested as a probe of age-independent GH release (8). In the present study, we used it to rule out possible ceiling effects of the double-peptide clamps. The average peak GH concentration after the triple stimulus in the present study was 339 ± 53 (146–648 range) µg/l after overnight pulsatile Sal/GHRH infusions. This excluded the downregulation of GH secretion under the overnight GHRH clamp, rather showing the GHRH priming effect. In the combined group of 24 women, E2 concentrations positively determined the peak GH response to the triple stimulus after exposure to Sal/Sal and Sal/GHRH, indicating a selective influence of E2 on GHRH’s priming of acute GH secretion.

When continuous GHRP2 stimulation was combined with a train of GHRH pulses, GHRH’s potentiating effect on the acute triple stimulus was suppressed by more than 50%. This was unexpected. The attenuation of the triple-secretagogue effect by GHRP2 could be due, in principle, to homologous GHRP receptor desensitization or heterologous GHRP/GHRH receptor downregulation, as reported in animal models (32). Since heterologous GHRH/GHRP receptor upregulation also occurs, our results introduce the clinical question: what determines GHRH/GHRP up- vs downregulation? The present data further indicate that E2 increases the negative effect of GHRP2 on GHRH’s amplification of the triple stimulus. Since E2 elevates GH levels, high GH concentrations may have exerted negative feedback by hypothalamic SS (20). Nonetheless, L-arginine, as
part of the triple stimulus used in the present study, would be predicted to mute GH negative feedback (2, 3, 33).

The significance of this study is that target tissues responded to circulating GH in a pulse-defined and concentration-dependent fashion (2). Complexity arises, since E2 can both inhibit and augment GH’s actions on target tissues (e.g. on liver, brain, breast, fat, or bone) (34). The tendency of IGF1 levels to decrease during E2 administration, especially orally, may constitute another mechanism for amplified pulsatile GH secretion beyond that of muting dose-dependent IGF1 feedback actions (30). The joint capabilities of E2 to drive pulsatile patterns of GH production and to modulate GH’s actions on target tissues should confer precise metabolic and anabolic effects. This may be particularly important clinically in otherwise estrogen-deprived women, who are secondarily relatively GH deficient as well.

BMI was a major and consistently negative determinant of total (pulsatile plus basal) GH secretion during pulsatile Sal/GHRH infusions and after triple-secretagogue stimulation. During overnight pulsatile GHRH infusion, BMI (negatively) accounted for 29% of the variance in total GH secretion. The negative and positive effects of BMI and E2 together explained 44% of inter-subject variability in triple stimulus-driven total GH secretion after Sal/Sal infusions. BMI also interacted with age reciprocally in controlling 46% of the variance in the triple-stimulus effect after combined GHRH/GHRP priming. Thus, even pharmacological multi-stimuli tests of GH secretory reserve are damped by relative obesity. Further studies are needed to test the reproducibility of, and elucidate the exact bases for, these unique interactions.

Limitations include the ultimate need to: elucidate the dose dependence and long-term sustainability of E2 effects on multiple peptide-regulated GH secretion; examine a wide range of pulsed SS and GHRH doses; assess possible effects on sleep as well as GH; and extend the short-term (weeks) paradigm to long-term (months) hormone-replacement regimens.

In summary, controlled E2 supplementation in postmenopausal women exerts positive multipathway effects on GHRH/GHRP/SS-regulated GH secretion. E2 also counteracts the suppressive effect of BMI on stimulated GH secretion. These outcomes suggest multiple loci of estrogenic regulation of the adult female GH axis, viewed as an ensemble of GHRH, GHRP/ghrelin, and SS signals.

Declaration of interest
Dr Dana Erickson is on the Advisory board for Ipsen. Otherwise, the authors have nothing to disclose.

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Author contribution statement
C Norman carried out the experiments, drafted the manuscript, and approved the final version of the manuscript; N L Rolle, D Erickson, and J M Miles carried out the experiments and approved the final version of the manuscript; C Y Bowers was responsible for the conception and design of the research, interpreted the results of the experiments, and approved the final version of the manuscript; J D Veldhuis was responsible for the conception and design of the research, carried out the experiments, analyzed the data, interpreted the results of the experiments, prepared the figures, revised the manuscript, and approved the final version of the manuscript.

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