Relative effects of estrogen, age, and visceral fat on pulsatile growth hormone secretion in healthy women

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Departments of 1Internal Medicine and 2Obstetrics and Gynecology, Endocrine Research Unit, Clinical Translational Research Unit, Mayo Medical and Graduate Schools of Medicine, Mayo Clinic, Rochester, Minnesota; and 3Endocrine Division, Department of Medicine, Tulane University Health Sciences Center, New Orleans, Louisiana

Submitted 6 April 2009; accepted in final form 26 May 2009

Veldhuis JD, Hudson SB, Erickson D, Bailey JN, Reynolds GA, Bowers CY. Relative effects of estrogen, age, and visceral fat on pulsatile growth hormone secretion in healthy women. Am J Physiol Endocrinol Metab 297: E367–E374, 2009. First published May 26, 2009; doi:10.1152/ajpendo.00230.2009.—Growth hormone (GH) secretion is subject to complex regulation. How pre- and postmenopausal age (PRE, POST), estradiol (E2) availability, and abdominal visceral fat (AVF) jointly affect peptidyl-secretagogue drive of GH secretion is not known. To this end, healthy PRE (n = 20) and POST (n = 22) women underwent a low- vs. high-E2 clamp before receiving a continuous intravenous infusion of GH-releasing hormone (GHRH) or GH-releasing peptide (GHRP-2). According to analysis of covariance, PRE and POST women achieved age-independent hypo- and eustrogenemia under respective low- and high-E2 clamps. All four of age (P < 0.001), E2 status (P = 0.006), secretagogue type (P < 0.001), and an age × peptide interaction (P = 0.014) controlled pulsatile GH secretion. Independently of E2 status, POST women had lower GH responses to both GHRH (P = 0.028) and GHRP-2 (P < 0.001) than PRE women. Independently of age, GHRP-2 was more stimulatory than GHRH during low E2 (P = 0.011) and high E2 (P < 0.001). Stepwise forward-selection multivariate analysis revealed that computerized tomographic estimates of AVF explained 22% of the variability in GHRH action (P = 0.002), whereas age and E2 together explained 60% of the variability in GHRP-2 drive (P < 0.001). These data establish that age, estrogen status, and AVF are triple covariates of continuous peptide-secretagogue drive of pulsatile GH secretion in women. Each factor must be controlled for to allow valid comparisons of GH-axis activity.

Growth hormone (GH) secretion is predominantly (>85%) pulsatile in healthy young adults (20). The first day of infant life, Tanner stages IV-V of puberty, and the late-follicular phase of the menstrual cycle are associated with severalfold augmentation of the mass (size) of GH secretory bursts with no evident changes in GH secretory-burst frequency or GH half-life (54). Conversely, midchildhood, hypogonadism in young adults, older age, low aerobic capacity, and adiposity are accompanied by a marked diminution in pulsatile GH production (8, 17, 29, 30, 33, 44, 55).

Although the factors that control basal (nonpulsatile) GH secretion are not well known, pulsatile GH secretion is stimulated by fasting, aromatizable androgens, and estradiol (E2) but attenuated by factors associated with food intake, abdominal visceral fat (AVF), and aging (11, 19, 23, 29, 31, 39, 44, 45, 48). From a mechanistic vantage, the size of GH secretory bursts is determined by specific peptide signals that mediate feedforward [GH-releasing hormone (GHRH), GH-releasing peptide (GHRP)-ghrelin] and feedback [GH, insulin-like growth factor 1 (IGF-1), and somatostatin (SS)] (3, 5, 7, 9, 18, 22, 38, 42, 47). In this context, what remains difficult to parse includes 1) which particular secretagogues are affected by age, estrogen, and adiposity; 2) the relative contributions that age, sex steroids, and AVF make to the regulation of GH secretion; 3) the degree to which the same three covariates interact pairwise or altogether to determine GH production; and 4) the differential impact of age, sex hormones, and AVF on pulsatile vis-à-vis basal GH secretion.

To examine these questions, the present study utilizes an experimental paradigm of continuous intravenous infusion of a submaximally stimulatory amount of GHRH or GHRP-2 combined with experimentally controlled eu- and hypoestrogenemia in premenopausal (PRE) and postmenopausal (POST) women with varying degrees of relative adiposity. Indirect markers of E2 action were also measured to verify estrogenic effects.

METHODS

Subjects. Volunteers provided written informed consent and were compensated for time spent in the study according to Mayo Institutional Review Board-approved guidelines. The studies were approved by the Mayo Institutional Review Board. No woman had received birth control pills or hormone replacement for at least 4 wk before the first leuprolide injection. Exclusion criteria were any history, symptoms, or signs of ischemic or occlusive arteriovenous events; hepatic, renal, cardiac, pulmonary, malignant, or infectious disease; untreated triglyceride-predominant hyperlipidemia; untreated cholelithiasis; known or suspected breast neoplasm; acute illness; anemia (hemoglobin <11.8 g/dl); ongoing psychiatric treatment; concurrent drug or alcohol abuse; use of neuroactive drugs, such as antidepressant, antihypertensive, or anticonvulsant agents; >3 kg weight change in 6 wk; nightshift work; and unwillingness or inability to provide informed consent. Inclusion criteria were community-living, consenting, informed healthy women ages 18–30 or 50–80 yr. PRE status was defined by cyclic menses with a history of normal puberty. Pregnancy was excluded by blood human chorionic gonadotropin measurement. POST status was defined by amenorrhea for at least 2 yr, follicle-stimulating hormone (FSH) >45 IU/l, luteinizing hormone (LH) >20 IU/l, and E2 ≤35 pg/ml (multiply by 3.68 for pmol/l).

Computerized axial tomography at L4–L5 was used to estimate AVF, as described earlier (12). Clinical protocol. The study was a prospectively randomized, parallel-cohort comparison of the effects of age on secretagogue vs.

somatotropin; growth hormone-releasing hormone; growth hormone secretion; growth hormone-releasing peptide; human; estrogen

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saline stimulation of GH secretion during controlled estrogen depletion vs. repletion. To achieve estrogen depletion, depot leuprolide acetate (3.75 mg im) was administered two times 3 wk apart (13). Leuprolide was given to both POST and PRE subjects to obviate any potential confounding by the downregulation regimen. The first injection was given in young volunteers within 8 days of menses onset and 48 h of a negative blood pregnancy test, and in older women three or more weeks after withdrawal of any estrogen supplements. Graded transdermal E2 repletion via a medicated patch was accomplished on an outpatient basis, starting on the day of the second leuprolide injection (day 0). A placebo patch was provided by the manufacturer, identical to the active patch except lacking E2. The transdermal E2 dose was 0.05 mg/day, and then increased every 4 days to 0.10 mg, 0.15 mg, and 0.20 mg/day [Estraderm (Novartis, Basel, Switzerland)]. The highest E2 dose (0.2 mg/day) was administered for 12 days (days 12–23 inclusive). The transdermal paradigm was designed to elevate serum E2 concentrations into the late-follicular range of 115–165 pg/ml (12, 13). At the end of the study, micronized progesterone (100 mg orally) was administered for 12 days to women with an intact uterus according to standards of good medical care.

Infusion schedule. Two randomly ordered, separate-day intravenous infusion sessions were undertaken at least 48 h apart in fasting subjects. Infusion studies were performed on days 17–23 of transdermal E2 or placebo patches. At 1800 the night before study, volunteers received a standardized outpatient meal of 8 kcal/kg distributed as 20% protein, 50% carbohydrate, and 30% fat. Subjects then remained fasting overnight and until the end of sampling. At 0700 the next morning, two intravenous catheters were placed in (contralateral) forearm veins to allow simultaneous secretagogue infusion and blood sampling (1 ml) every 10 min for 6 h from 0800 to 1400. The infusion sessions comprised intravenous saline (20 ml/h) from 0800 to 1000, followed by GHRH or GHRP-2 delivered continuously for 4 h from 1000 to 1400 at a constant rate of 0.33 

Deconvolution analysis. GH concentration time series (all 6 h) were analyzed using a recently developed automated deconvolution method, which was mathematically verified by direct statistical proof and empirically validated using hypothalamo-pituitary sampling and simulated pulsatile time series (6, 25). The Matlab-based algorithm first defends the data and normalizes concentrations to the unit interval [0, 1] (24). Second, the program creates multiple successive potential pulse-time sets, each containing one fewer burst via a smoothing process (a nonlinear adaptation of the heat-diffusion equation). Third, a maximum-likelihood expectation estimation method computes all secretion and elimination parameters simultaneously conditional on each of the candidate pulse-time sets. Deconvolution parameters comprise basal secretion (β0), two half-lives (α1, α2), secretory-burst mass (ν0, ν1), random effects on burst mass (σλ), procedural/measurement error (σε), and a three-parameter flexible Gamma secretory-burst waveform (β3, β2, β1). The fast half-life was represented as 3.5 min constituting 37% of the decay amplitude and the slow half-life as 20.8 min (15). Statistical model selection was performed to distinguish among the independently framed fits of the multiple candidate pulse-time sets using the Akaike information criterion (1). Outcomes evaluated here were basal and pulsatile GH secretion (concentration units/session), mass secreted per burst (concentration units), and waveform shape (mode, or time delay to maximal secretion after objectively estimated burst onset, min).

Statistical methods. The design was a prospectively randomized, double-masked parallel-cohort comparison of the effects of age and E2 on specific secretagogue actions. There were two age groups, two estrogen states, and two secretagogues. For the primary outcome of pulsatile GH secretion during either GHRH or GHRP-2 infusion, prestudy power analyses predicted >85% statistical power to detect a 30% difference due to age in either the low- or the high-E2 milieu by unpaired two-tailed Student’s t-test if a total of 40 subjects completed the study (35).

The effects of age, estrogen status, secretagogue type, and their interactions were evaluated using a three-way (age, estrogen, secretagogue) least-squares general-linear ANCOVA model (57). The covariate was the mean 2-h prestimulus baseline GH concentration in each subject. Departure of the variance-covariance matrix from compound symmetry was adjusted for using the Huynh-Feldt statistic. Wilk’s lambda was applied to evaluate the significance of paired (and triple) interactions between (among) age, E2 status, and secretagogue type. According to the null hypothesis, age stratum, E2 condition, and secretagogue type do not individually or jointly (interactively) determine GH secretion. Results were considered significant for experimentwise P < 0.05. Post hoc contrasts were made using Tukey’s honestly significantly different (HSD) test (16).

Regression. Stepwise forward-selection multivariate linear regression analysis was used to relate pulsatile (and basal) GH secretion to age, E2 concentration, and/or AVF in the combined cohorts (n = 42 subjects). Computations were made using SYSTAT Version 11 (Point Richmond, CA).

RESULTS

Subject characteristics. All 42 women completed both infusion sessions. Enrollment comprised 20 healthy PRE subjects with an age range of 18–29 yr and 22 POST volunteers with an age range of 55–74 yr [Supplemental Table 1 (Supplemental material for this article can be found on the American Journal of Physiology: Endocrinology and Metabolism website)]. Body mass index in PRE ranged from 18 to 30 and in POST from 19 to 32 kg/m2 (P = not significant). AVF estimated by computed tomography scan was higher in POST than PRE volunteers (P < 0.01).

Baseline hormone values. Baseline screening (outpatient) concentrations of LH and FSH (measured at 0750 fasting
before leuprolide injection) in POST women were higher and E2 lower than corresponding values in PRE women (each \( P < 0.001 \) for age effect) (supplemental Table 1). Concentrations of total T were 33% \( (P = 0.004) \), IGF-I 52% \( (P < 0.001) \), prolactin 36% \( (P = 0.008) \), albumin 6.5% \( (P < 0.001) \), and IGFBP-3 16% \( (P = 0.002) \) lower in POST than PRE subjects. In contrast, IGFBP-1 was 77% higher in POST than PRE individuals \( (P = 0.030) \).

**Leuprolide/E2 (or placebo) clamp.** Indirect markers of E2 action were also measured in serum collected at 0750 fasting (10 min) before beginning saline/peptide infusions on the first of the two inpatient study sessions to verify estrogenic effects. Leuprolide suppressed LH concentrations to comparable values \( (P = 0.087) \) in PRE and POST women given placebo or E2 (grand mean for \( n = 42 \), 1.0 ± 0.20 IU/l) but reduced FSH (IU/l) concentrations to lower values in PRE + E2 (0.61 ± 1.2) than in the other three groups \( (P < 0.001) \) and to lower values in both PRE − E2 (2.8 ± 0.47) and POST + E2 (2.0 ± 0.43) than in POST − E2 (8.2 ± 1.2) \( (P < 0.001) \) (Fig. 1, top). Our earlier demonstration that an estrogen receptor (ER)-antagonist, which does not cross the blood-brain barrier, elevates FSH secretion during exogenous E2 supplementation in older women permits the hypothesis that E2 in part directly inhibits FSH secretion. The present data showing that E2 suppresses FSH concentrations further in the presence of leuprolide would be consistent with partial direct (gonadotropin-releasing hormone-independent) pituitary inhibition by exogenous E2.

During the leuprolide clamp, SHBG did not vary among the four cohorts \( (P = 0.077) \), grand mean 59 ± 9.4 nmol/l). Serum albumin (g/dl) was lower in POST − E2 (4.0 ± 0.77) than in both PRE + E2 and POST + E2 (mean 4.5 ± 0.065) but not than in PRE − E2 (4.3 ± 0.097) \( (P < 0.001) \). Total E2, free E2, and bioavailable (bio-) E2 were comparable in PRE + E2 and POST + E2 women, thus verifying efficacy of the high-E2 clamp (Fig. 1, middle). Likewise, E2 levels were similar in the low-E2 clamp in PRE − E2 and POST − E2 individuals. Each

**Fig. 1.** Influences of premenopausal (PRE) and postmenopausal (POST) status on hormone concentrations (Conc) during leuprolide/placebo and leuprolide/estradiol (E2) clamp in 42 women. Top: luteinizing hormone (LH), follicle-stimulating hormone (FSH), sex hormone-binding globulin (SHBG), and albumin concentrations. Middle: growth hormone (GH), insulin-like growth factor (IGF)-I, IGF-binding protein (IGFBP)-I, and IGFBP-3. Bottom: E2 (left) and T (right) moieties. \( P \) values above each cluster of 4 columns were determined by 1-way ANOVA. Data are means ± SE \( (n = 10 \) in each cohort, except POST-E2 where \( n = 12 \)). Bio, bioavailable. Means with different (unshared) alphabetic superscripts differ significantly by Tukey’s honestly significantly different (HSD) post hoc multiple-comparison test at experiment-wise \( P < 0.05 \). Thus A differs from BC and C but not from AB.
Age, E₂ and Peptide Secretagogue Determine Pulsatile GH Sec

![Graph showing pulsatile GH secretion](image)

Fig. 2. Triple influences of age (PRE vs. POST status), E₂ clamp (low vs. high), and peptide secretagogue [GHRH vs. GHRP-2] on pulsatile GH secretion (Sec; µg·l⁻¹·h⁻¹) in 42 women. Format is described in Fig. 1. Means with unshared alphabetic superscripts differ significantly by multiple-comparison post hoc Tukey’s HSD test.

The GHRP-2 and the GHRH effect in PRE women, but only amplified the GHRP-2 effect by 2.5-fold and the GHRH effect by 1.25-fold in POST women.

Figure 3 presents ANCOVA outcomes from the perspective of pairwise-factor effects. Combining responses to both peptide and peptide secretagogues, age (P < 0.001) and E₂ stratum (P = 0.006), determined pulsatile GH secretion (top). Whereas age and E₂ did not interact, PRE + E₂ was associated with greater pulsatile GH secretion under peptide drive than both POST + E₂ and POST − E₂ (P < 0.001 by Tukey’s HSD test). PRE − E₂ responses were also higher than POST − E₂ responses (P < 0.001). Considered independently of E₂ status, age (P < 0.001), peptide type (P < 0.001), and their interaction (P = 0.014) controlled pulsatile GH secretion (middle). The PRE + GHRP-2 response exceeded that of PRE + GHRH, POST + GHRH, and POST + GHRP-2 (each P < 0.001). In addition, PRE + GHRH was greater than POST + GHRH (P = 0.028). The significant interaction term (P = 0.014) reflected conjoint effects of age and peptide types in defining pulsatile GH secretion. When viewed independently of age, peptide secretagogue (P < 0.001) and E₂ level (P = 0.006) individually (but

of total T, free T and bio T was decreased in POST − E₂ compared with PRE − E₂ (P = 0.002, P = 0.023, P = 0.011, respectively). There were no differences between PRE and POST T moieties (total, free, bio) during E₂ addback. In addition, total, free, and bio T were similar in POST + E₂ and POST − E₂, whereas total T in PRE + E₂ exceeded that in POST − E₂ (P < 0.01). Therefore, POST women had lower T values than PRE women in the low-E₂ but not in the high-E₂ milieu.

GH, IGF-1, IGFBP-1, and IGFBP-3 during the leuprolide clamp tended to retain relative POST/PRE differences observed at baseline before the clamp (Fig. 1, bottom). In contrast, total and active ghrelin concentrations did not differ among the four study groups (respective grand means: 985 ± 98 and 16 ± 1.8 pg/ml).

Pulsatile GH secretion. Three-way ANCOVA revealed that pulsatile GH secretion was statistically determined by each of age (P < 0.001), estrogen (P = 0.006), and secretagogue (P < 0.001) (Supplemental Table 2). The covariate (presecretagogue 2-h saline-infused mean GH concentration) was a determinant of pulsatile-GH responses (P = 0.002). Age and peptide type interacted significantly (P = 0.014). Primary outcomes of post hoc Tukey’s HSD multiple-comparisons testing at experiment-wise P < 0.05 were as follows: 1) the highest pulsatile GH secretion occurred in PRE + E₂ given GHRP-2 compared with all seven other conditions, except PRE − E₂ given GHRP-2; 2) numerically lowest pulsatile GH secretion emerged in three of four POST conditions (GHRP-2 + E₂ excepted) and in PRE − E₂ during GHRH infusion; and 3) intermediate pulsatile GH output characterized POST + E₂ + GHRP-2, PRE − E₂ + GHRP-2, and PRE − E₂ + GHRH (Fig. 2). For all 42 subjects, the descending numerical rank order of potency (assuming PRE + E₂ + GHRP-2 = 130 ± 15 µg·l⁻¹·h⁻¹ = 100%) was as follows: PRE − E₂ + GHRP-2 49%, PRE + E₂ + GHRH 37%, POST + E₂ + GHRP-2 32%, and PRE − E₂ + GHRH 19% with POST + E₂ + GHRH 15%, POST − E₂ + GHRP-2 13%, and POST − E₂ + GHRH 12%. Thus E₂ doubled both
not interactively) modulated pulsatile GH secretion (bottom). GHRP-2 + E2 stimulated pulsatile GH secretion more than each of GHRH - E2 (P < 0.001), GHRH + E2 (P < 0.001), and GHRP-2 - E2 (P = 0.027). The collective data establish strong effects of age, E2 status, and secretagogue type as well as an interaction between age and secretagogue type in the determination of pulsatile GH secretion in women.

**Basal GH secretion.** Based upon two-way ANCOVA, basal (nonpulsatile) GH secretion was statistically determined by age (P = 0.016) and E2 status (P = 0.005), but not their interaction (P = 0.39). The main contrast was PRE + E2 > POST - E2 (P = 0.002). Secondly, basal GH secretion in PRE + E2 tended to be greater than in PRE - E2 (P = 0.054).

**Secretory-burst waveform (mode).** The mode of GH secretory bursts (time delay from burst onset to peak secretion rate) was invariant of secretagogue type (global mode 20 ± 1.7 min), according to three-way ANCOVA. There was an age × E2 interaction during GHRH and GHRP-2 stimulation (P = 0.020), wherein E2 supplementation in POST only was associated with more extended GH secretory bursts (mode 22.4 ± 1.2 min) than placebo addback (mode 17.6 ± 0.93 min) (P = 0.007) (Fig. 4).

**Regression analyses.** Univariate regression analysis revealed strongly negative effects of AVF on GHRH- and GHRP-2-stimulated pulsatile GH secretion (respective r² = 0.22, P = 0.0015 and r² = 0.28, P = 0.0004) (Fig. 5, top). Because age was a correlate of AVF (r² = 0.29, P < 0.001), we next used stepwise forward-selection multivariate regression to distinguish possible individual and joint effects of age, AVF, and E2. In this analysis, neither age nor E2 remained significant, but there was still a negative correlation between GHRH-stimulated pulsatile GH secretion and AVF (multivariate r² = 0.60, P < 0.001) (Fig. 5, bottom). In addition, age (P = 0.037) and E2 (P = 0.039) together determined basal (nonpulsatile) GH secretion (multivariate r² = 0.22, P = 0.0088) (bottom).

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**Fig. 4.** Negative influence of abdominal visceral fat (AVF) on pulsatile GH secretion during 4-h GHRH infusion (top) and 4-h GHRP-2 infusion (bottom). P and r² values are univariate estimates (n = 42 subjects) based on Pearson’s correlation coefficient.

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**Fig. 5.** Three-dimensional plots depicting the joint linear effects of age and E2 concentrations on pulsatile GH secretion (top) and basal (nonpulsatile) GH secretion (bottom) in 42 women. Numerical values are multivariate P and r², and partial (age or E2) P values with corresponding slope estimates. The 4 subgroups of women studied are defined by the indicated symbol types.
DISCUSSION

The combination of leuprolide plus transdermal E2 or placebo addback successfully enforced high- and low-estrogen milieus. This paradigm clearly demonstrated that E2 addback in POST women is unable to achieve the values of fasting GH, IGF-I, and IGFBP-3 concentrations demonstrated in E2-treated PRE women. Because GH administration in young and older adults does generate comparable IGF-I responses (54), these outcomes favor the interpretation that lower IGF-I and IGFBP-3 levels in POST than PRE women are due to lower GH drive per se. Lesser GH output in older than young women is unexpected in view of their lower IGF-I concentrations, which should decrease negative feedback and stimulate more GH secretion (47). Thus we propose that either IGF-I feedback efficacy is higher in POST than PRE women (an unstudied question) and/or that peptide-secretagogue drive of GH secretion is impaired in POST women. In examining the latter consideration, the present study disclosed that secretagogue type, PRE vs. POST menopausal status, E2 milieu, and AVF all determine pulsatile GH secretion in healthy women. In the case of GHRH stimulation, AVF was the dominant-negative regulator explaining 22% of response variability among the 42 women studied. In contrast, in the case of GHRP-2 stimulation, menopausal status and age emerged together as strong negative and positive modulators, respectively, which jointly accounted for 60% of GH-response variability.

Two-way ANCOVA demonstrated that E2 enhances the effects of both peptides studied (P = 0.006). The question arises how estrogen increases stimulation by GHRH and GHRP-2. Because the infused dose of GHRH was submaximally stimulatory (METHODS), in principle, E2 could augment exogenous GH secretion by: 1) decreasing hypothalamic SS release to (or action on) the pituitary gland; 2) increasing hypothalamic GHRH secretion to or actions of GHRH on somatotropes; and 3) amplifying the presumptive potentiating interaction between GHRH and endogenous ghrelin. A dose-response study in POST women showed that E2 does decrease the inhibitory potency (but not efficacy) of infused SS (4). Another dose-response analysis indicated that E2 doubles the stimulatory potency of infused GHRH (50). Whether E2 additionally reduces hypothalamic SS secretion and/or increases hypothalamic GHRH secretion in humans is not yet known. Indirect evidence points to a facilitative effect of E2 on endogenous GHRH release (46). Animal models allow for both possibilities, but neither has been proven by direct hypothalamic portal-venous sampling (20, 54). Moreover, although E2 enhances submaximal ghrelin action in women (52), no studies have yet tested whether E2 can augment the interaction between exogenous GHRH and endogenous ghrelin (22). Finally, E2 supplementation increases overnight acetylated ghrelin concentrations, but not values measured at 0800 (36), as confirmed here in both POST and PRE women.

Stepwise forward-selection multivariate regression analysis disclosed that POST vs. PRE status and E2 availability together explain 60% of overall intersubject variability in continuous GHRP-2-stimulated pulsatile GH secretion. By univariate regression analysis, AVF was a prominent negative predictor of GHRP-2 action (r² = 0.28, P = 0.0004), but this effect vanished in stepwise forward-selection regression analysis probably because of the close positive association between AVF and age (r² = 0.29, P < 0.001). Of the four variables studied (age, estrogen, secretagogue type, and visceral fat), visceral fat gives the weakest form of evidence, because its contribution was assessed by correlations rather than manipulations of body fat. Precisely how age or a low-E2 status impairs hypothalamic-pituitary responsiveness to GHRP-2 is not established (20, 54). However, the strong positive correlation between GHRP-2-stimulated pulsatile GH secretion and E2 concentrations (P < 0.001) is explicable by the capability of E2 to potentiate GHRP/ghrelin drive (2, 52). Potentiation might occur by estrogenic upregulation of the expression of the ghrelin receptor, which is subject to in vitro transcriptional activation by E2 (37). Model-based simulations using experimental data obtained in the rat further suggest the mechanistic hypotheses that E2 may reduce SS’s inhibitory effects on the pituitary gland (26, 56) and/or augment arcuate-nucleus GHRH outflow (41). The notions arise because E2 downregulates the type 5 SS receptor (10, 26) and ER-α is expressed in GHRH neurons (40). Stimulation of endogenous GHRH release by E2 would be predicted to potentiate exogenous GHRP-2 actions (14).

Greater relative responsiveness to GHRP-2 than GH in older than young women was observed in the presence of E2. This was unexpected because brain GHRP-receptor expression is reportedly decreased in older humans (34). A possible explanation is that SSergic opposition declines more than GHRP responsivity in aging. This postulate is testable.

Little is known about the regulation of basal (unstimulated, nonpulsatile) GH secretion, except that knock-out of the type 1 SS receptor increases basal (unstimulated) GH secretion in vitro (28) and that acromegaly is marked by elevated basal GH secretion in vivo (21). In the present analysis, E2 enhanced and age-repressed basal GH release. Estrogen can stimulate GH secretion by pituitary cells in vitro and by ectopic pituitary tissue in vivo (23, 27), thus allowing the postulate of direct E2 drive of GH synthesis. How age decreases basal GH secretion is less clear. Possibilities involve increased SS outflow and/or increased systemic concentrations of putative GH-inhibiting cytokines, adipokines, free fatty acids, and/or insulin (54). Albeit statistically significant, basal GH secretion only represented 6–18% of total GH secretion.

Deconvolution analysis corroborated an earlier finding that E2 is able to prolong GHRH-stimulated (albeit not GHRP-stimulated) GH secretory-burst duration in POST women (51). Extended release of GH within individual secretory bursts could reflect a reduction in hypothalamic outflow of, or pituitary inhibition by, SS (54). Whereas the first point remains indeterminate (20, 54), a clinical dose-response study inferred that exogenous E2 does diminish the inhibitory potency of infused SS in POST women (4).

Caveats include the absence of data currently available on the dose-dependency of estrogenic effects; the possibility that leuprolide itself might influence GH secretion in some manner; and the need to extend the duration of low- and high-E2 clamps, replicate outcomes in larger cohorts of women, and assess similar mechanisms longitudinally.

In summary, a leuprolide-clamp paradigm maintains total, bioavailable, and free E2 concentrations at comparably low or high levels in PRE and POST women. Based on stepwise forward-selection multivariate analysis, AVF primarily determines pulsatile GH responses to continuous GHRH stimula-
tion, whereas age and E2 together principally control pulsatile GH responses to continuous GHRP-2 drive. These three co-

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