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Sex-Steroid Modulation of Growth Hormone (GH) Secretory Control

Three-Peptide Ensemble Regulation Under Dual Feedback Restraint by GH and IGF-I

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Technical, genetic, and clinical developments have unveiled a burgeoning array of novel effectors of GH secretion. The present appraisal of central neuroregulatory components of the somatotropic axis highlights a simplifying concept of ensemble control by the final common peptides, GH-releasing hormone (GHRH), GH-releasing peptide(s) (GHRP, ghrelin), and somatostatin. These potent signals act individually, antagonistically, and synergistically to direct pulsatile GH secretion. GHRH, GHRP/ghrelin, and somatostatin further adapt to autonegative feedback by GH and IGF-I. Estradiol modulates the impact of each of the primary peptidyl inputs; viz.: (i) enhances submaximally effective feedforward by discrete pulses of (injected) recombinant human GHRH-1,44-amide (as defined by increased agonistic potency and pituitary sensitivity); (ii) potentiates the submaximally stimulatory effects of GHRP-2, a hexapeptidyl mimetic of ghrelin; (iii) blunts dose-dependent inhibition of fasting GH secretion by somatostatin-14; and (iv) relieves rhGH-enforced negative feedback on GHRP-2 (but not on basal, exercise, or GHRH)-stimulated GH secretion. The foregoing estrogenic activities collectively augment GH secretory burst mass by amplifying feedforward (via both GHRH and GHRP) and attenuating feedback (imposed by somatotatin and GH). Whether testosterone fully mimics the foregoing mechanistic actions of estradiol is not known.

In conclusion, the present conceptual platform of tripeptide-directed feedforward and GH/IGF-I-mediated feedback should aid in unraveling some of the complex regulatory dynamics targeted by sex-steroid hormones.

Key Words: Sex steroids; puberty; aging; somatotropin; IGF-I; feedback; GHRH; somatostatin; GHRP.

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Introduction

Multiple regulatory signals impact GH secretion under in vitro and in vivo conditions *(1)*. Gene-silencing experiments illustrate that many effectors act convergently on three pivotal peptides—GHRH, GHRP/ghrelin, and somatostatin *(2–6)*. This peptidyl trilogy also mediates time-delimited autonegative feedback by GH and IGF-I. Sex-steroid hormones further determine the individual, combined, and interactive effects of GHRH, GHRP/ghrelin, and somatostatin, as exemplified in studies of puberty and the effects of estrogen and testosterone repletion in older adults *(7–13)*. To accommodate implicit network-like properties of the GH–IGF-I axis, we propose an integrative notion of reciprocal signaling linkages among GHRH, GHRP, and somatostatin (Fig. 1). Established connections are bidirectional in topography, positive and negative in effect, and homologous and heterologous in receptor dependency *(1,2,14,15)*.

Overall Impact of Sex Steroids on GH Secretion

Body composition, physical fitness, gender, stage of sexual development, age, sleep, exercise, and systemic disease *inter alia* govern GH availability in the human. Sex-steroid hormones mediate many of the neuroregulatory distinctions associated with gender. In overview, recent clinical investigations establish that estrogen specifically facilitates feedforward drive by GHRH and GHRP, and, conversely, overcomes feedback restraint by somatostatin and GH *(16–21)*. The aggregate effect of these fourfold actions is amplification of pulsatile and total daily GH secretion.

Testosterone (an aromatizable androgen) drives the secretion of both GH and IGF-I in hypogonadal boys and men, albeit not in eugonadal adults *(6,8,10,11,13,22)*. Nonaromatizable androgen fails to reproduce these actions in the human (unlike in the rodent). Exogenous estrogen differs from testosterone by not affecting or by lowering IGF-I concentrations, except when administered with a synthetic progestin *(1–5,14,23,24)*. At higher doses, estrogens suppress systemic IGF-I concentrations independently of the route of external delivery (oral, transdermal, intravenous, intramuscular, intravaginal, and intranasal), type of estrogen,

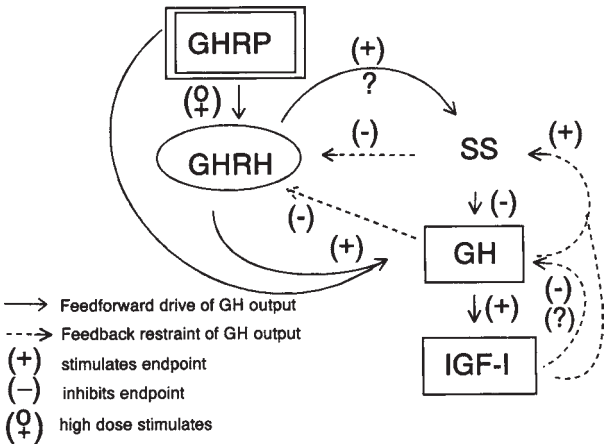

Fig. 1. Concept of a network-like interaction (ensemble) among GHRH, GHRP/ghrelin, and somatostatin under feedback modulation by (hypothalamo-pituitary-systemic) GH and IGF-I. (Unpublished schema.)

age of the recipient, genotypic sex, and presence of the ovary or testis *(1,3,5)*. Administered estradiol but not testosterone elevates the IGFBP-1 concentration, which would predictably lower free IGF-I availability further *(13,18,20)*. Such distinctions reject the facile hypothesis that testosterone acts on the GH–IGF-I axis exclusively after aromatization to estrogen. On the other hand, mixed estrogen-receptor antagonists and agonists impede testosterone's stimulation of GH secretion in young men *(1,6)*. A potentially unifying hypothesis is that estrogen-receptor subtype-specific mechanisms are modulated unequally by androgen, estrogen, mixed agonists, and/or *in situ* metabolites of testosterone. Studies with a pure antiestrogen would aid in making this type of distinction.

Augmentation of GH Pulse Amplitude as the Dominant Mechanism of Peptidyl Secretagogue and Inhibitor Action

Serum GH concentrations are determined conjointly by the frequency and amplitude (and hence the mass) of underlying secretory bursts, half-life, distribution volume, and basal (nonpulsatile) secretion *(25–28)*. Clinical and laboratory experiments indicate that adaptations in GH secretory-burst mass mediate prominent physiological transitions (e.g., exercise, fasting, sleep) and neonatal, pubertal, and lifetime adaptations in total GH production *(1,5,7,29,30)*. Sex steroids, peptide secretagogues, and somatostatin control burst mass expressly; e.g., estradiol, testosterone, GHRH, and GHRP-2 augment by 6- to 12-fold and somatostatin suppresses by > 90% the amount of GH (μg) released per unit distribution volume (L) within an individual pulse *(13, 21,23,31–33)*. Figure 2 illustrates the 3- to 10-fold within-gender and the greater intercohort range of spontaneous GH secretory burst mass achieved in mid-to-late puberty compared with childhood in boys and girls.

Secretion measures are expressed per unit hormone distribution volume. Other studies have verified that puberty does not alter the (directly measured) distribution volume, metabolic clearance rate (MCR), or plasma half-life of GH *(9,29,30)*. However, in a rigorous within-subject comparison (four randomly ordered placebo and estrogen-repletion studies in each of eight volunteers), oral estradiol supplementation increased the distribution volume and the MCR of rhGH by 20–30% (data recalculated from ref. *17*). On the other hand, the plasma half-life of GH is independent of estrogen replacement, pubertal status, stage of the menstrual cycle, and gender *(9,17,23,34–36)*. Therefore, oral estrogen does not control the exit of GH from the vascular space into interstitial fluid, but does accelerate its extravascular total-body disposal. Visceral obesity acts similarly, albeit also by undetermined primary mechanisms *(37,38)*. Indeed, estradiol downregulates the hepatic GH receptor, and both estrogen and intraabdominal obesity upregulate GHBP production *(1)*, but neither effect would explicate increased GH extraction. For example, profound GH-receptor blockade imposed pharmacologically does not alter whole-body GH kinetics in men or women *(39)*, and selectively elevated plasma GHBP availability may reduce the MCR of GH *(40)*. However, if estradiol should induce the accumulation of GHBP in interstitial fluid as well as in blood, then trapping of GH molecules in proximity to tissue degradation sites could facilitate extravascular GH distribution and disposal. In principle, retention of GHBP in organ spaces in the hyperinsulinemic patient with visceral obesity or older individual could offer an analogous (testable) mechanism for the 10–30% acceleration of GH MCR in these contexts *(1,37,38)*.

Estrogenic Control of Tripeptidyl Inputs

GHRH

In the rodent, estrogen represses hypothalamic GHRH and hypophysial GHRH-receptor gene expression *(5,14)*. In the human, the impact of estradiol on acute GHRH action has been controversial. We reasoned that reported discrepancies may reflect any of (i) variable somatostatin outflow within and among subjects at the moment of GHRH injection; (ii) nonuniform interstudy choice of a near-maximal versus threshold GHRH stimulus, thus confounding interpretation of agonist efficacy and potency and somatotrope sensitivity; (iii) unequal elevation or depression of the preinjection baseline GH concentration; and (iv) lack of quantitation of (kinetically normalized) GH secretory-burst mass. To address these issues, we appraised the effects of short-term estradiol repletion on (exogenous) GHRH action via two distinct study paradigms in healthy postmenopausal women. First, continuous intravenous (iv) infusion of rhGHRH-1,44-amide at a near-maximally effective dose (1 μg/kg/h)

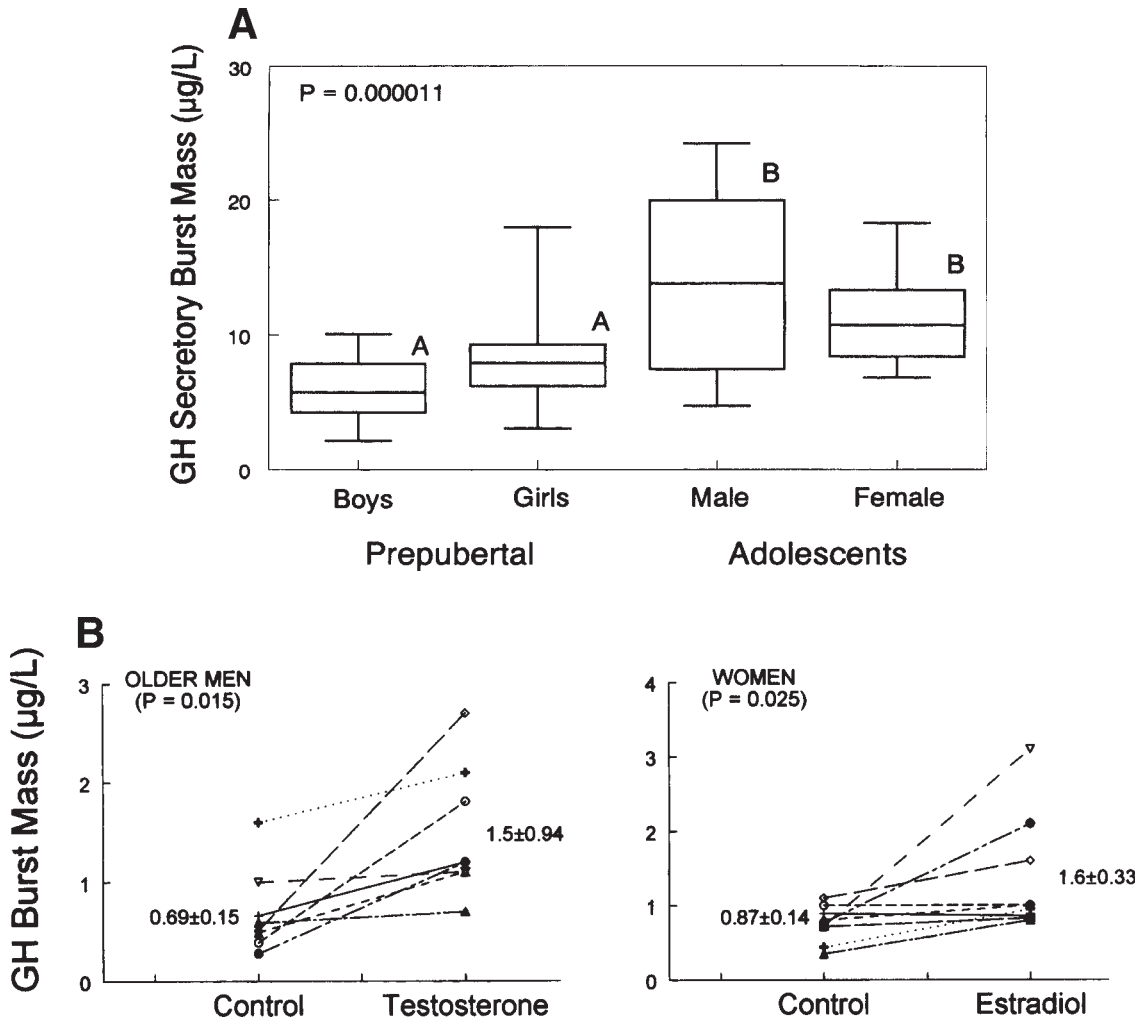

Fig. 2. (**A**) Impact of puberty in boys and girls on the mass of GH secreted per burst (µ/gL). Box-and-whisker plots capture the extreme range, interquartile limits, and median value. The mass secreted per burst is the time integral of the underlying pituitary secretory event, which drives the observed peak in the serum hormone concentration. GH kinetics, secretory-burst frequency, and duration do not change across puberty. Reprinted from ref. *30*. (**B**) Testosterone and estradiol supplementation specifically augment GH secretory-burst mass in the relatively gonadoprival older male and postmenopausal woman. Data representation combined from refs. *13* and *23*.

stimulated each of pulsatile, nonpulsatile (basal), entropic (feedback-sensitive), and 24-h rhythmic GH secretion markedly (by 8- to 11-fold) (Fig. 3). Supplementation with estradiol-17 beta (1 mg twice daily orally) in this setting exerted no detectable further effect over that elicited by GHRH alone *(18)*. And, second, in a paradigm designed to limit variability of concurrent somatostatin release, we injected L-arginine immediately prior to single iv pulses of rhGHRH-1,44-amide spanning a 100-fold dose range on separate randomly ordered mornings in the fasting state following placebo and estrogen exposure *(18,41)*. Estradiol replacement in this setting failed to augment the GH secretory response to the highest two (pharmacological) GHRH stimuli, but significantly amplified: (a) pituitary *sensitivity* to GHRH (mirrored by a twofold increase in the maximal positive slope of the dose-response function) and (b) GHRH *potency* (reflected in a 50% reduction in the half-maximally effective stimulatory dose, ED_{50}) *(42)*. The key role of GHRH in the human is verified by the >30-fold selective reduction in GH secretory burst mass in rare patients with inactivating mutations of the cognate receptor (Fig. 4A,B). The notion of burst mass is illustrated in Fig. 4C.

GHRP/Ghrelin

Molecular silencing of the GHRP receptor by transgenically targeted expression of the cognate antisense oligonucleotide to the hypothalamus limits appetite and retards somatic growth in the male and female mouse, but reduces concentrations of GH and IGF-I only in the female animal *(43)*. In another (dwarf) transgenic model, GH autofeedback

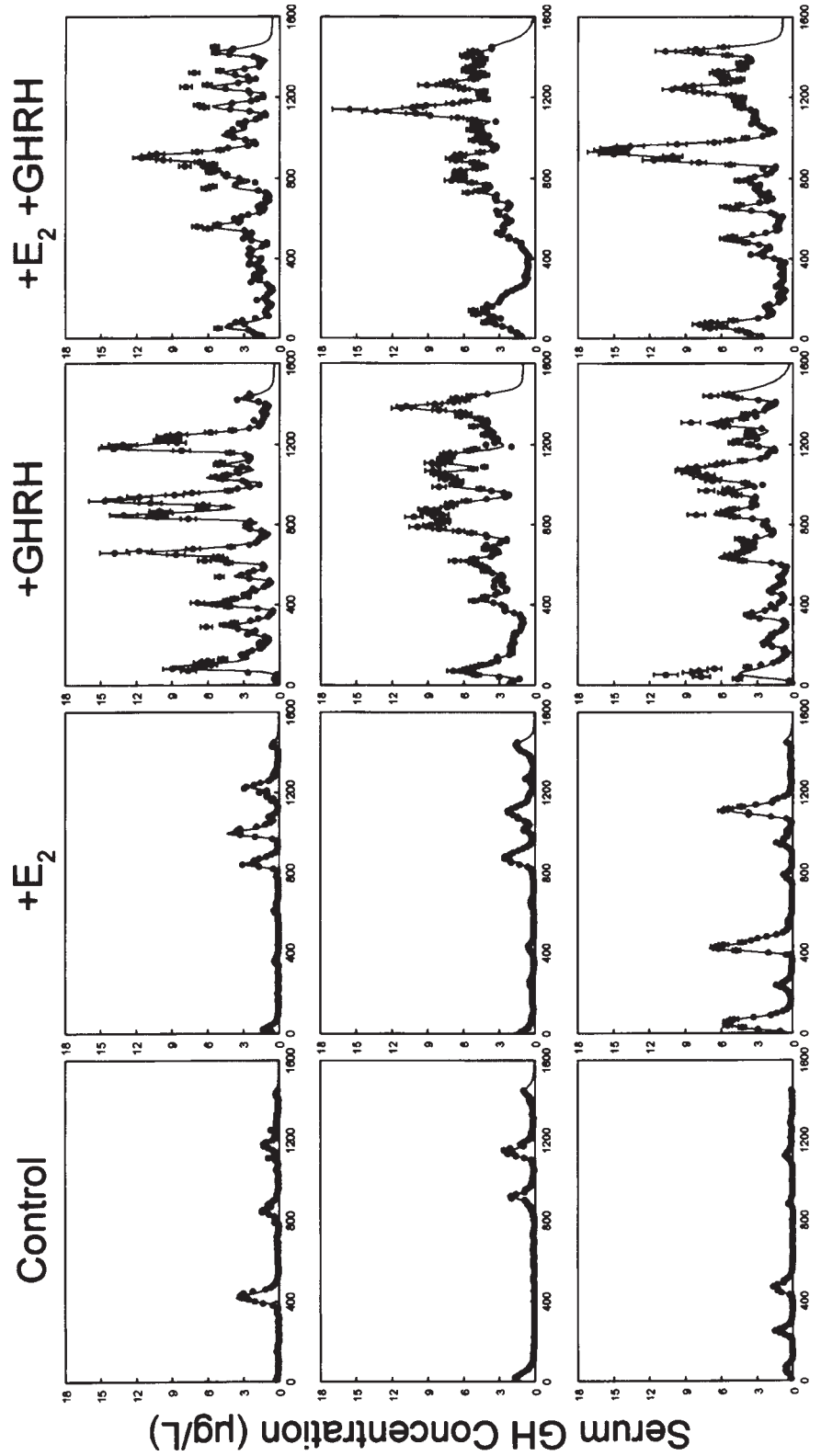

Fig. 3. Impact of constant 24-h iv infusion of saline or a near-maximally stimulatory dose (1 μg/kg/h) of rhGHRH-1,44-amide on pulsatile GH release monitored by sampling blood every 10 min after placebo versus estradiol replacement.

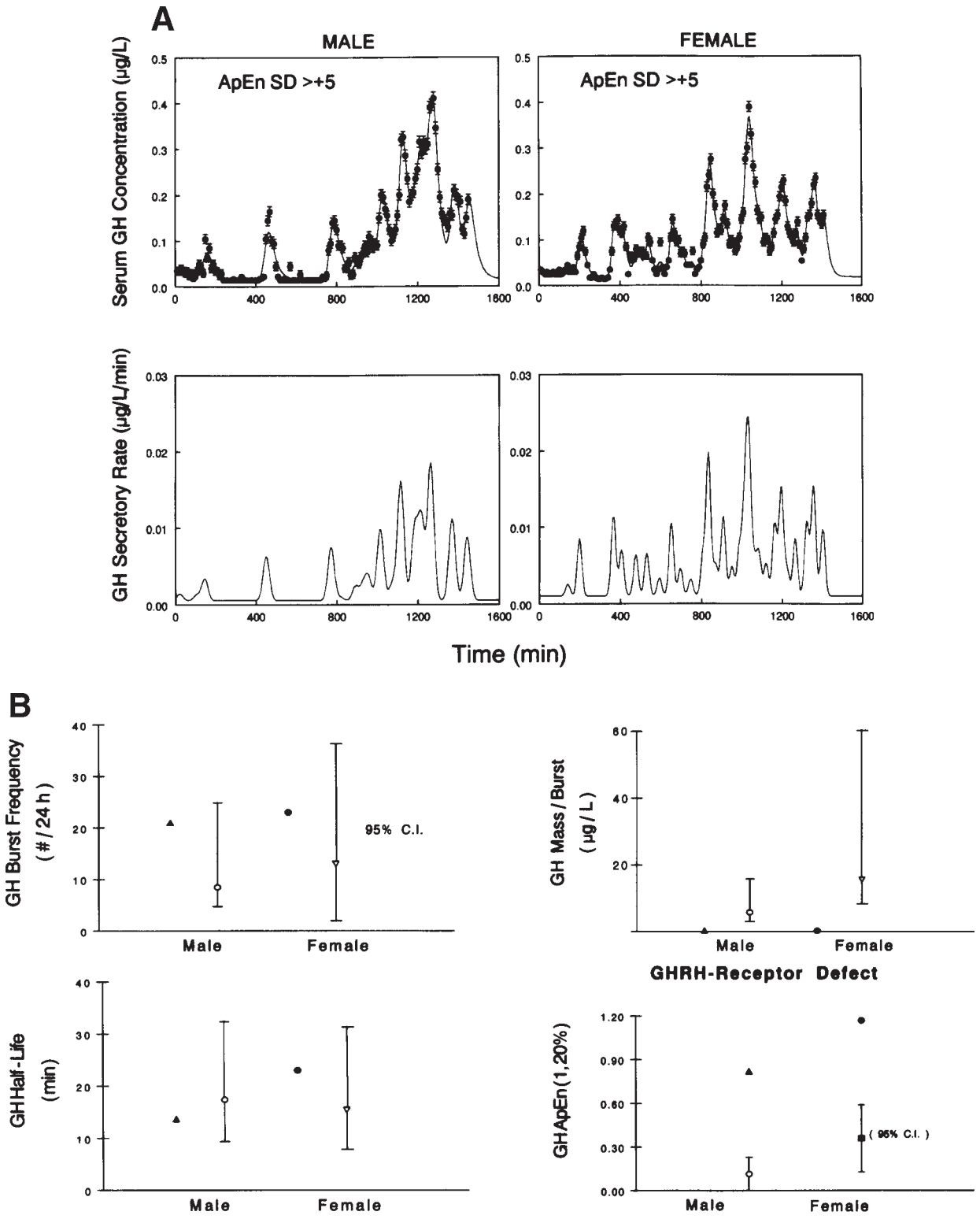

Fig. 4. (**A**) Profound (> 30-fold) reduction in the height of serum GH concentration peaks (top) and the calculated mass of underlying GH secretory bursts (bottom) in two young-adult siblings harboring a homozygous truncational mutation of the GHRH-receptor gene. Pulsatility ($p < 10^{-6}$) and 24-h rhythmicity ($p < 10^{-3}$) of GH secretion are unequivocally retained in an ultrahigh-sensitivity immunofluorometric assay. In these subjects, a bolus of GHRP-2 stimulates GH release by approximately threefold, but GHRH is inactive. The daily GH pulse frequency is normal, which denotes that factors other than GHRH receptor-dependent signaling mechanisms maintain *de facto* pulse generation: (**B**) The GH half-life is normal, but basal GH secretion and the mean (24-h) serum GH concentration are reduced markedly. High ApEn (approximate entropy) of GH release (> 5 standard deviations above expectation) defines marked departure from minute-by-minute (subpulsatile) orderliness of feedback/feedforward control. The latter irregularity of the release process arises by way of functional disruption of the GHRH receptor, which mediates adaptive feedforward drive of more orderly patterns of GH release. Adapted from ref. *69*.

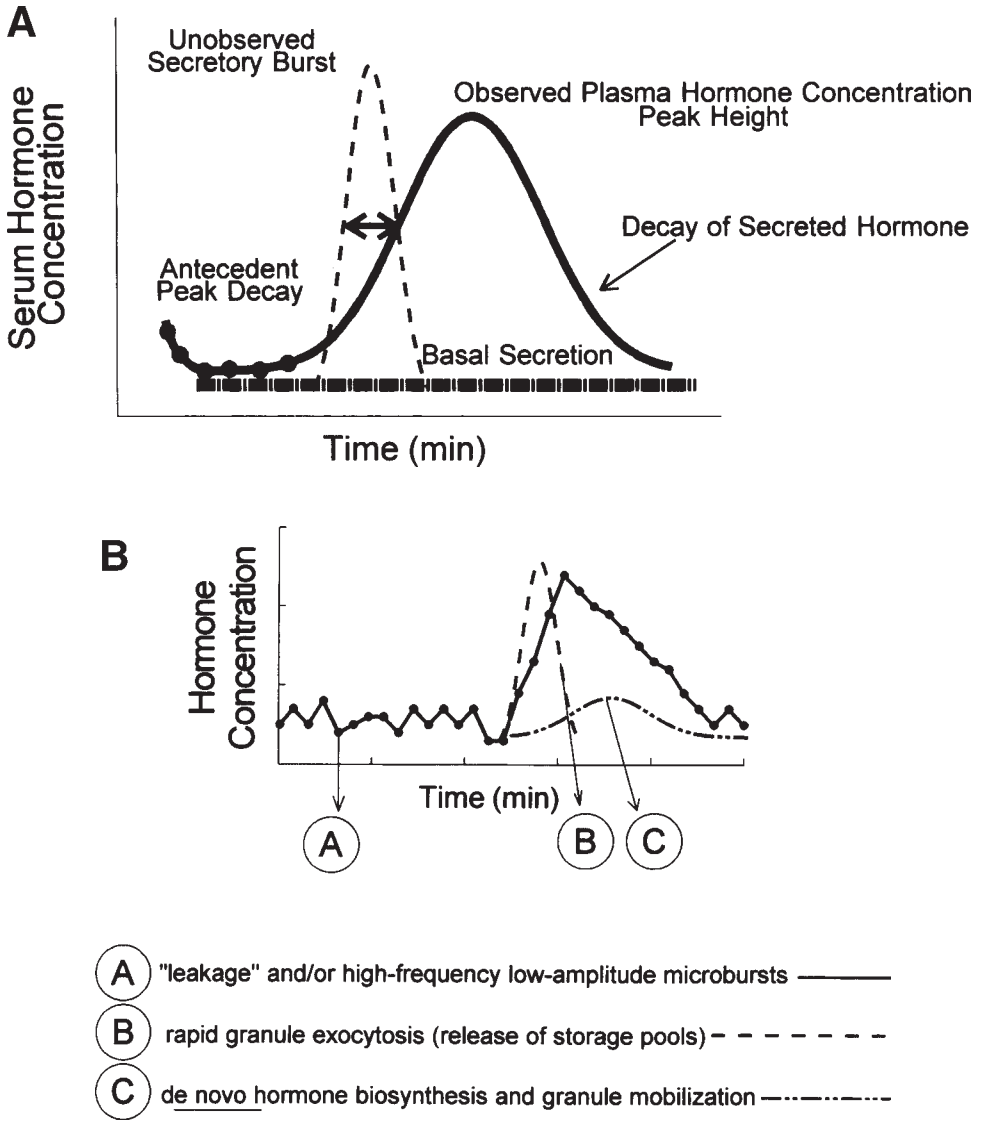

Fig. 4C. Concept of deconvolution analysis (unraveling or dissecting) of a hormone concentration peak into major underlying secretion features and half-life: (**A**) Schematic illustration of cellular basis for admixed basal and pulsatile hormone outflow (**B**).

repression of the GHRP-receptor gene in the ventromedial nucleus is relatively spared in the adult female compared with male *(44)*. Relative feedback muting in the female rodent could reflect the ability of estradiol to stimulate transcriptional activity of the GHRP receptor gene promoter *in vitro* and to repress expression of the hypothalamic GH-receptor gene *in vivo (45–47)*.

The pubertal sex-steroid milieu, female gender in the adolescent, and short-term supplementation of estrogen or testosterone (but not of a synthetic anabolic steroid) augments GHRP action by 1.5- to 2-fold in prepubertal children *(47–52)*. However, the maximal action of a synthetic GHRP-receptor agonist (true efficacy) has not been determined clinically; for example, in postmenopausal women, the maximal GHRP-2 dose utilized to date of 3 μg/kg evokes nearly two-fold more GH secretion (mass/burst) than a bolus dose of 1 μg/kg *(32)*. Figure 4 schematizes the concept of analytically estimating the burst-like component of GH release. Estradiol replacement enhances GH secretion after the highest GHRP-2 stimulus by 1.8-fold (Fig. 5). The latter facilitative effect could be explained, if estrogen in fact induces the GHRP receptor in the CNS and pituitary gland *in vivo* (see above). This inference follows, inasmuch as GHRP-dependent mechanisms in the rodent are known to (a) oppose certain intrahypothalamic somatostatinergic effects; (b) induce arcuate-nucleus GHRH release; and (c) stimulate GH secretion by somatotrope cells directly *(1,2)*.

Continuous iv infusion of a near-maximally effective dose of GHRP-2 (1 μg/kg/h) in estrogen-withdrawn postmenopausal women stimulates each of pulsatile, nonpulsatile (basal), entropic (pattern-irregular and feedback-sensitive), and 24-h rhythmic GH secretion by 6- to 7-fold and elevates

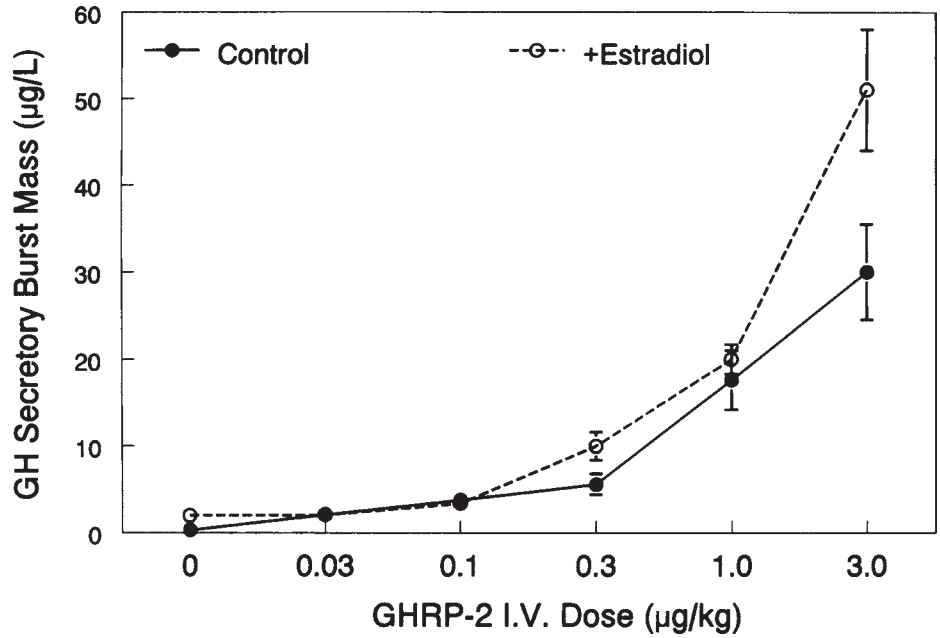

Fig. 5. Estradiol amplifies the dose-responsive stimulation of GH secretion by GHRP-2, a synthetic ghrelin-like agonist, in postmenopausal women. Adapted from ref. *32*.

the serum total IGF-I concentration over saline by approximately 110 µg/L within 24 h *(53)* (Fig. 6). The foregoing neuroendocrine phenotype closely emulates that of normal mid- and late puberty in both genders *(29,30,54)*. Simultaneous infusion of GHRH and GHRP-2 (each at a rate of 1 µg/kg/h) reproduces these dynamics, while amplifying the 24-h GH secretion rate by up to 120-fold (Fig. 7). Collectively, these experimental insights suggest (but do not prove) that enhanced GHRH and ghrelin feedforward drives high-amplitude pulsatile GH secretion in puberty.

Somatostatin

How sex steroids control somatostatin synthesis, release, and action remains incompletely understood at the hypothalamic and pituitary levels. In the rodent, sexual maturation, testosterone, and 5-alpha-dihydrotestosterone stimulate periventricular–nuclear somatostatin gene expression in the male or ovariectomized female *(1)*. On the other hand, estradiol does not uniformly induce this gene in either sex, except in the first 5 d neonatally *(55,56)*. In the anterior pituitary gland, sex-steroid hormones both upregulate and downregulate transcripts of specific somatostatin-receptor subtypes (SSTRs). For example, *in vitro* and/or *in vivo* exposure to estradiol induces hypophysial SSRT-2 and SSTR-3 and represses SSTR-1 and SSTR-5, and testosterone increases SSTR-2 and SSTR-5 gene expression *(57,58)*. Integrative concepts are needed to forecast the overall regulatory impact of such complex adaptive control.

Recent clinical experiments indicate that estrogen replacement reduces the suppressive potency (ID_{50}), but not the efficacy (maximal inhibitory effect), of infused somatostatin-14 *(16)* (Fig. 7). The latter results illustrate the present challenge of mechanistic inference in the ensemble context. In fact, estradiol-induced attenuation of (exogenous) somatostatin-enforced inhibition of pulsatile GH secretion is concordant mechanistically with suppression of SSTR-1 and SSTR-5 gene expression in somatotrope cells and/or facilitation of GHRH and GHRP feedforward drive.

The estrogen-enriched milieu of the preovulatory phase of the menstrual cycle in young women and estradiol replacement in postmenopausal individuals enhances L-arginine-stimulated GH secretion *(1,41)*. Inasmuch as this amino acid will restrict hypothalamic somatostatin release *in vitro*, further amplification by estradiol of L-arginine stimulation would forecast facilitation of other pathways, such as feedforward drive by GHRH and/or GHRP. Both predictions are endorsed by independent analyses showing that estradiol potentiates submaximal stimulation of GH secretion by GHRH and GHRP-2 *(32,41)*.

Endogenous peptide release in the human is more challenging to calibrate than exogenous peptide action. As one indirect strategy to estimate in vivo somatostatinergic outflow, we quantitated the incremental effect of estradiol supplementation over placebo on pulsatile GH secretion under constant iv infusion of both GHRH and GHRP-2 *(21)*. According to a tripeptidyl regulatory model, estrogenic amplification of GH secretion under fixed feedforward by dual agonists delivered at near-maximally effective doses would signify muting of somatostatinergic restraint, facilitation of GHRP-2 feedforward, or induction of a novel GHRH-

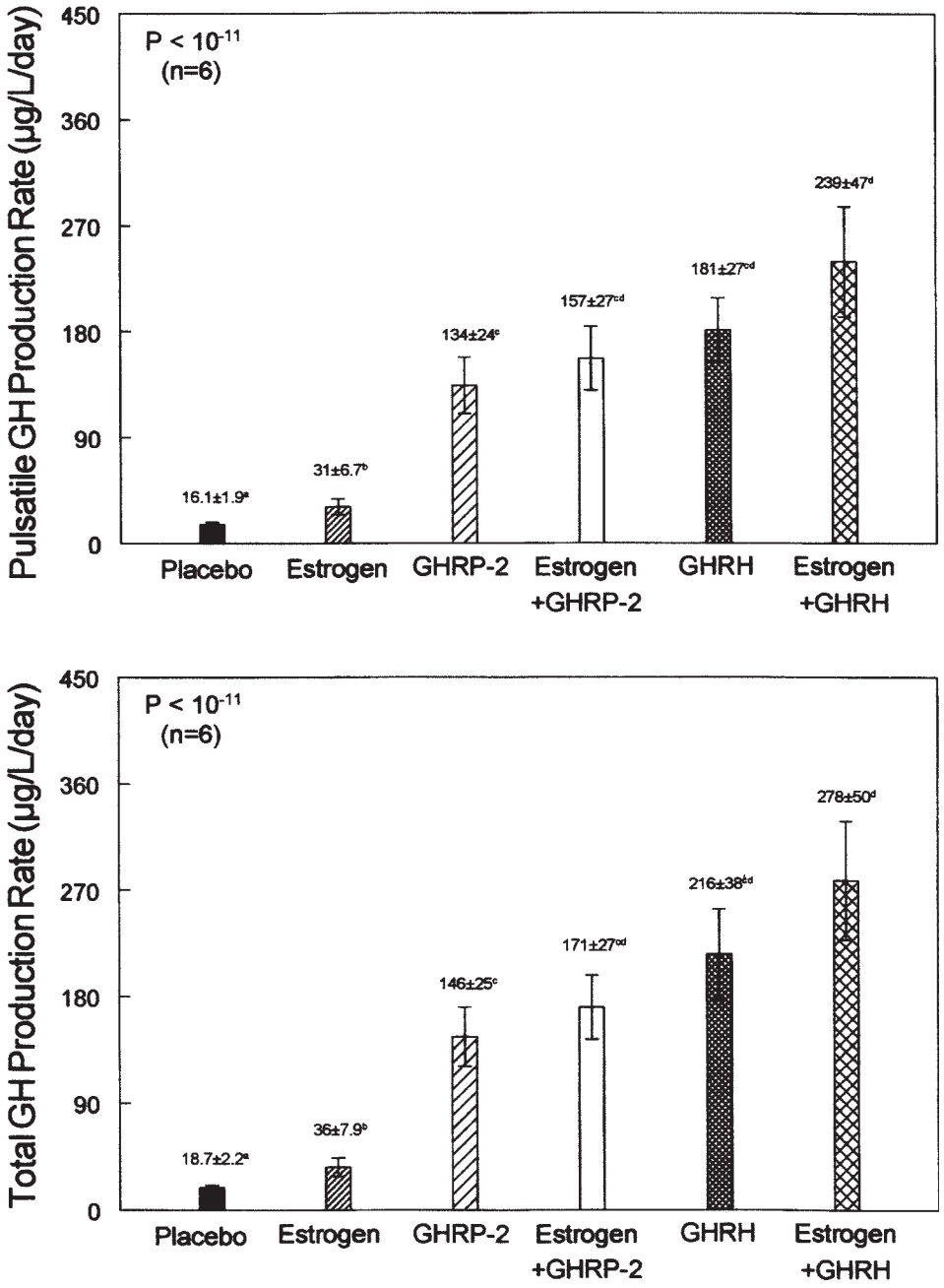

Fig. 6. Daily pulsatile (top) and total (pulsatile plus basal, bottom) GH secretion rates driven by constant infusion of GHRP-2 with and without estrogen repletion. Adapted from refs. *18* and *20*.

GHRP-2 interaction. Enhanced GHRH action is not relevant, because estrogen does not alter maximal (acute) GHRH stimulation of GH release *(18–20)*. In this construct, estradiol repletion augments feedforward by the two-peptide clamp on several specific endpoints of GH secretion *(21)*. These endpoints are (i) feedback-dependent irregularity (approximate entropy) of GH release; (ii) the basal (nonpulsatile) rate of GH secretion; and (iii) the 24-h rhythmicity of GH output (the mesor of the nyctohemeral rhythm) *(21)*. Such actions could reflect estrogenic repression of somatostatin outflow or pituitary somatostatin receptor expression (see above), enhancement of GHRP-2 stimulation, and blunting of GH and/or IGF-I-mediated autoinhibition of GHRP-2 stimulation *(16–18,41)*.

Total plasma IGF-I concentrations decline in response to exogenous estrogen supplementation, but not in the estrogen-enriched milieu of the late follicular phase *(3,5,59)*. IGFBP-1 concentrations rise by nearly twofold during estra-

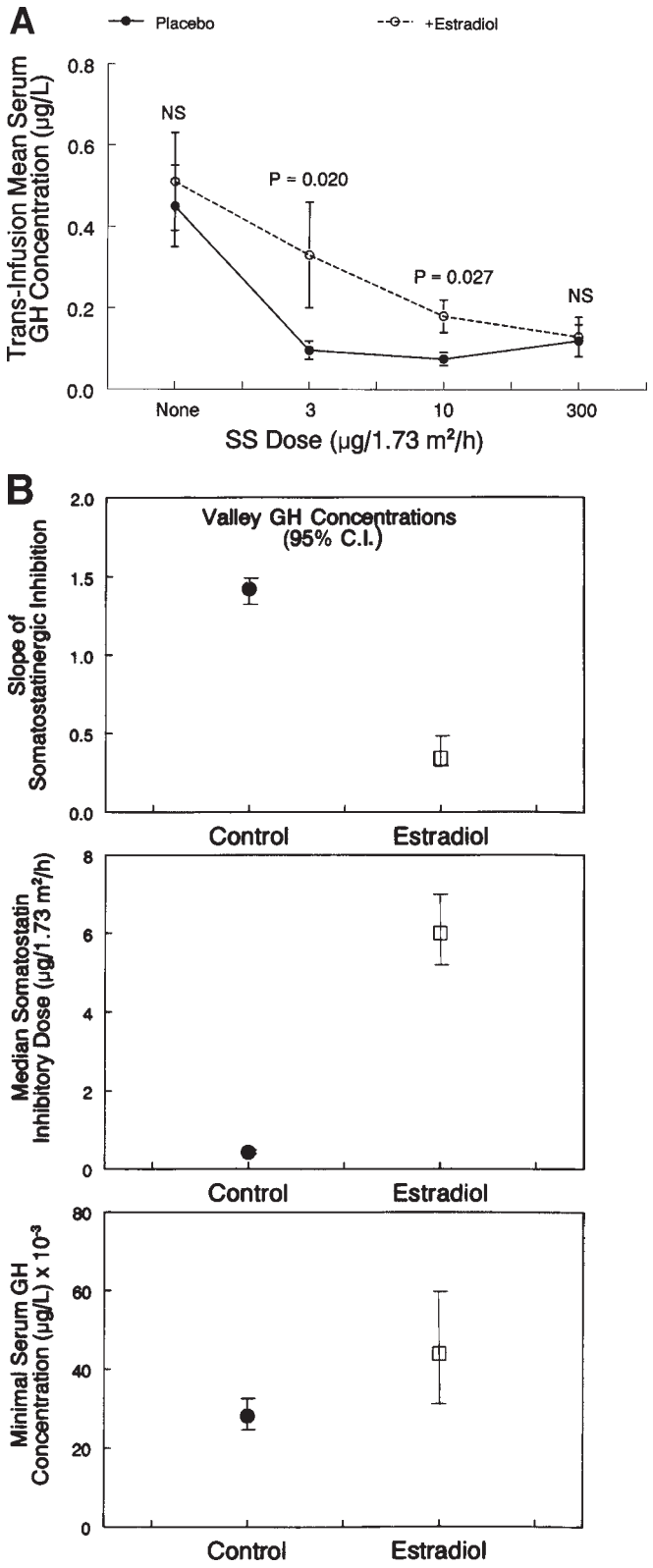

Fig. 7. (**A**) Estradiol supplementation mutes the dose-dependent inhibitory effect of continuously infused somatostatin-14 on pulsatile GH secretion in ovariprival women studied in the fasting state on separate, randomly ordered mornings. (**B**) Estrogen reduces the slope (top) and median dose (middle), but not the maximal suppressive effect of intravenously delivered somatostatin-14 on serum GH concentrations. Reprinted from ref. *16*.

diol supplementation, which would also reduce free IGF-I availability. IGF-I depletion would putatively unleash GH secretion further by feedback withdrawal *(18,20)*. Thus, an important (unresolved) mechanistic query is whether, and if so the extent to which, IGF-I feedback attenuation mediates estrogenic stimulation of GH production. A corollary physiological issue is the nature of factors associated with endogenous estrogens that sustain combined elevations of GH and IGF-I concentrations *(2)*.

Integration of Three-Peptide Control

In a minimal tripeptidyl model, simultaneous facilitation of GHRH and GHRP/ghrelin stimulation and attenuation of somatostatin inhibition would selectively augment the mass of GH secreted per burst *(1)* (Fig. 1). Amplitude-specific secretory control typifies all estrogen-enriched states, including normal puberty in girls (and boys) and the late follicular phase of the menstrual cycle *(1,3,5,14,15,24, 29,30,36,60)*. In contrast, few physiological factors appear to govern the frequency of GH pulse generation beyond fasting and sleep *(36,61–65)*. Rather, self-renewing automaticity of pulsatile GH release may be mediated via intrahypothalamic and pituitary–hypothalamic autoregulation *(66,67)*. The basic (unknown) pulsing mechanism operates independently of a functional GHRH receptor (but not necessarily neurotransmitters released by hypothalamic GHRHergic neurons) and independently of the *systemic* concentration of GHRH, GHRP, or both *(20,21,68,69)*. For example, single or combined continuous infusions of GHRH/GHRP-2 do not alter daily GH pulse frequency, but stimulate secretory-burst mass and basal release multifold.

Feedback Control

GH Autonegative Feedback

A single pulse of rhGH represses GH secretion rapidly (within 2 h) and consistently (by 50–70%) in pre-, mid-, and postpubertal boys, young women, and postmenopausal individuals *(9,17,70)*. Inhibition of GH secretion is dose-dependent across 1, 3, and 10 µg/kg in young adults, in which population premenopausal women exhibited greater sensitivity to feedback inhibition than comparably aged men *(70)*. The latter clinical data, if confirmed, differ from observations in the adult rodent, wherein the male rat manifests greater autoinhibition than the female *(1)*. Estradiol supplementation in postmenopausal women significantly blunts acute rhGH-induced inhibition of GHRP-2-stimulated (but not of basal, GHRH-, or exercise-induced) GH secretion *(17)* (Fig. 8). The specificity and implications of estrogen-inducible relief of autofeedback will be important to explore further.

The somatotropic axis in puberty is remarkable by way of the simultaneous rise in GH and IGF-I production *(30)*. Concurrently elevated GH and IGF-I output also prevails in the late follicular phase of menstrual cycle (see above) and pathologically in active acromegaly *(59,71,72)*. Simul-

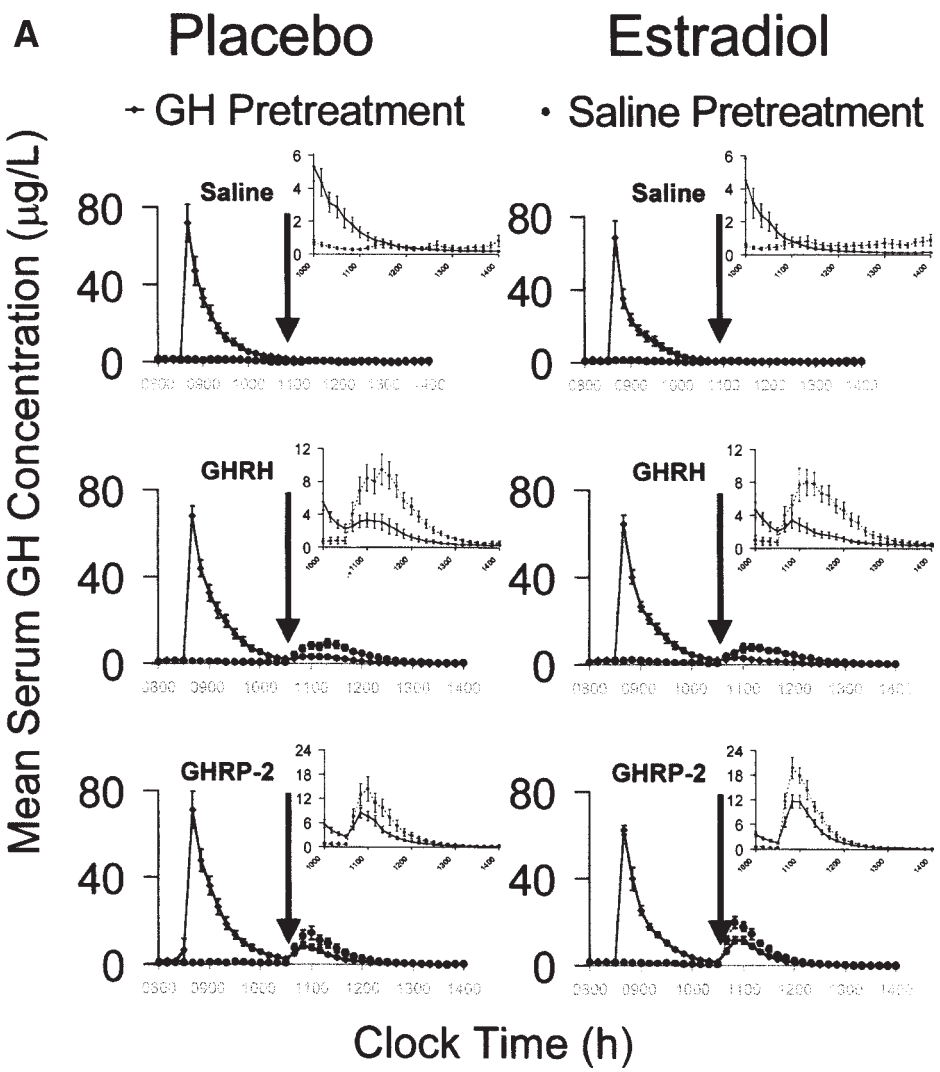

Fig. 8. (**A**) GH concentration profiles depicting acute autofeedback by a single pulse of rhGH (10 μg/kg iv over 6 min) on basal (saline), GHRH (1 μg/kg iv bolus) and GHRP-2 (1 μg/kg)-stimulated GH release in postmenopausal women (cohort mean ± SEM).

taneously increased secretion of a trophic hormone and product points mechanistically to autonomy of feedforward (e.g., in the ectopic GHRH syndrome or by a somatotropinoma) and/or selective failure of negative feedback (e.g., in experimentally imposed silencing of the CNS GH receptor) *(1)*.

Plausible hypotheses for jointly enhanced outpouring of GH and IGF-I at the time of puberty are sustained GHRH/GHRP drive and/or reduced IGF-I and/or GH-dependent autonegative feedback *(1)*. Investigation of the latter postulate in healthy boys revealed equivalent rhGH-induced inhibition of submaximal GHRH-stimulated GH release, but accentuated fractional and decremental suppression of spontaneous GH release in midpuberty compared with prepuberty and young adulthood *(9)*. The first observation of comparable GH-induced inhibition of GHRH stimulation suggests that a GH pulse evokes a similar amount of somatostatin release before, during, and after puberty. This is because somatostatin outflow is the primary mediator of GH-induced autorepression *(1)*. The second finding of heightened (percentage and absolute) GH-dependent suppression of endogenously driven GH secretion in midpuberty could denote unique vulnerability of hypothalamic (feedforward) secretagogues, such as GHRH or GHRP, to inhibition by GH at this time in sexual development *(9,66,67)*. Large pulses of GH emerge in this context due to (a) the resultant sharp downswings in GH release and (b) the subsequently accelerated escape of GH secretion from the inhibitory nadir (Fig. 9). The latter novel dynamic might signify a reduced duration (albeit not lesser peak amount) of somatostatin outflow following a GH pulse and/or heightened secretagogue drive. These mechanistic considerations are not distinguishable at present.

Feedback Restraint by IGF-I

The inhibitory efficacy of exogenously infused rhIGF-I is unknown in older adults *(73)*. In fact, the only available study employed successive within-session escalation of the

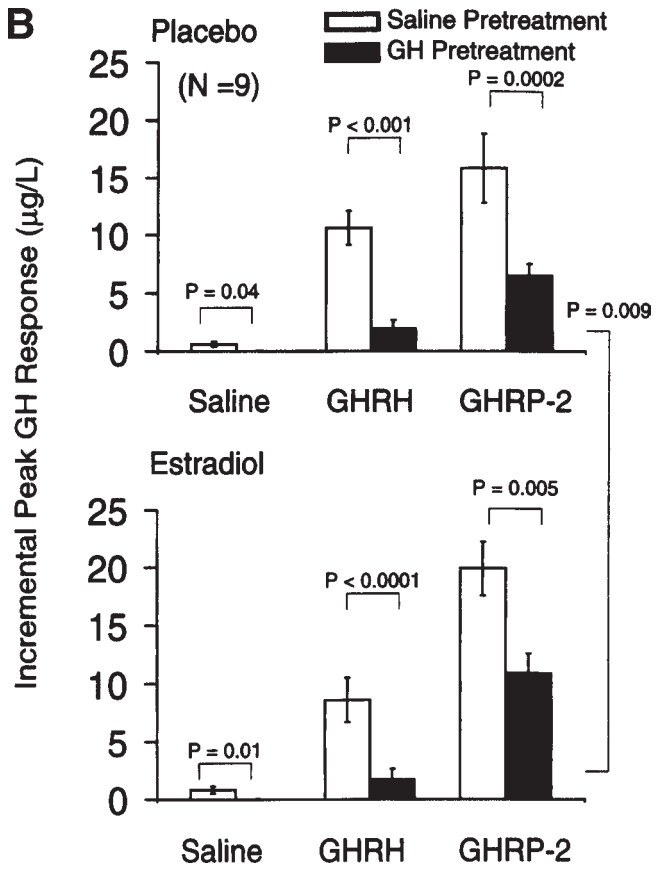

Fig. 8. (**B**) Estradiol relieves autonegative feedback on GHRP-2 (but not basal or GHRH)-stimulated GH secretion in postmenopausal individuals. Reprinted from ref. *17*.

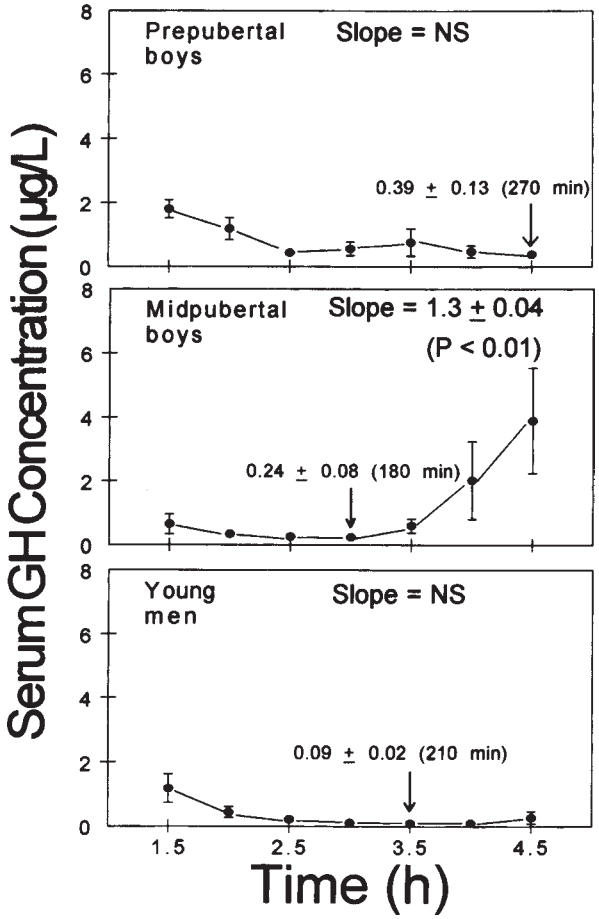

Fig. 9. Accelerated time-dependent escape of endogenous GH output after rhGH-enforced autofeedback in normal midpubertal boys. A single 6-min iv pulse of rhGH was infused at time zero (*x* axis). No secretagogue was injected, so as to monitor spontaneous axis recovery. Numerical values are absolute nadir (and the matching times post-rhGH injection) GH concentrations and the rates (slopes) of recovery of GH concentrations over time after the observed nadir. Data are the mean ± SEM (N = 5 boys/group). Adapted from ref. *9*.

rhIGF-I dose *(73)*, which sequential design could confound valid interpretation. For example, (low) initial IGF-I concentrations may either upregulate or downregulate subsequent responses, as recognized widely in other IGF-I target organs. Randomly ordered and separate-day infusions would have greater epistemic merit in this respect.

Constant iv delivery of a fixed dose of rhIGF-I has unmasked an apparent gender difference, wherein young women manifest lesser suppression of GH concentrations than men *(74)*. This inference differs from the finding of greater IGF-I-dependent repression of disorderly GH release in women than men in the same study (Fig. 10). In counterpoint, short-term estradiol replacement in postmenopausal women nearly doubles the inhibitory effect of continuous iv infusion of rhIGF-I (10 µg/kg/h) on pulsatile and GHRH-stimulated GH secretion *(75)*. Accentuated feedback by IGF-I in this clinical milieu might reflect the reported ability of estradiol to induce the type-I IGF receptor and/or IGFBP-2 in the pituitary gland of the experimental animal *(76)*.

The role of systemic IGF-I availability in GH feedback was explored recently by administration of a highly selective GH-receptor antagonist peptide, pegvisomant, to deplete circulating IGF-I. In this analysis, a single dose of pegvisomant (1 mg/kg sc) lowered the total serum IGF-I concentrations by 34% within 72 h and elevated pulsatile GH secretion by 1.8-fold in 16 healthy young men and women *(77)* (Fig. 11). Stimulation of GH secretion is not a technical artifact, because the directly measured distribution volume and the half-life of GH are unaffected by the drug *(39)*. There was no evident gender difference in feedback unleashing, but larger study cohorts will be needed to verify this inference. GH concentrations also increase under long-term pegvisomant therapy in successfully treated (IGF-I-deficient) patients with acromegaly *(78)*.

In the rodent, genetic silencing of the CNS GH receptor stimulates GH secretion by muting the autofeedback drive of somatostatin secretion *(79,80)*. Native GH penetrates human

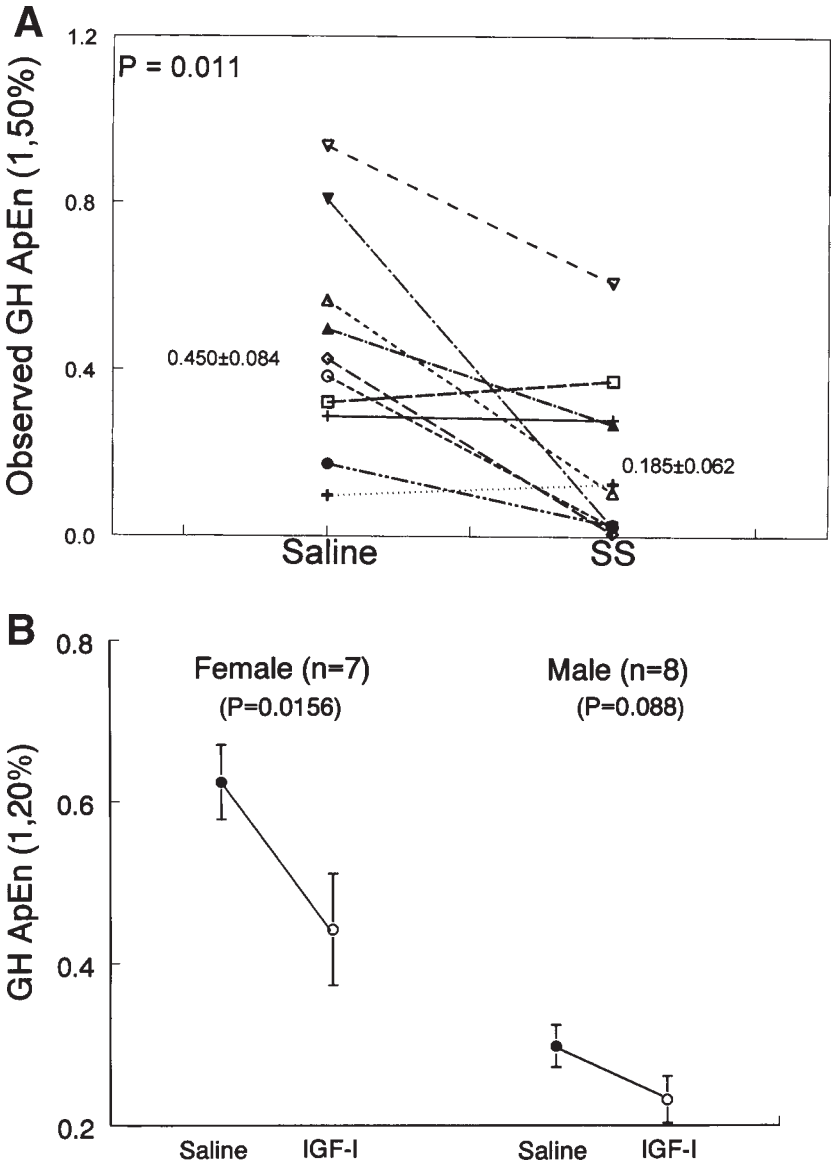

Fig. 10. Feedback restraint enhances GH secretory regularity. ApEn, the approximate entropy statistic, quantitates graded irregularity or pattern disruption. Infusion of somatostatin-14 (SS) in postmenopausal women (**A**) or of rhIGF-I in young women and men (**B**) heightens secretory orderliness (lowers ApEn). Adapted from ref. *82*.

cerebrospinal fluid dose-dependently, and presumptively acts in the CNS to enforce autoinhibition *(1,81)*. Whether a small percentage of nonpegylated or oligopegylated GH-receptor antagonist behaves comparably is not established clinically. In addition, intravenously delivered proteins as large as horseradish peroxidase accumulate rapidly in the median eminence of the hypothalamus, where GH receptors are expressed in arcuate-nucleus NPYergic neurons that inhibit GH secretion *(1)*. Accordingly, further investigations will be needed to clarify the precise site(s) of GH and IGF-I feedback control, and the exact loci of pegvisomant-induced disinhibition of GH output.

Summary

GH secretion and action are subject to dynamic, time-varying, and multifactorially controlled regulation by an array of discrete effectors, including sex-steroid hormones. We highlight a simplified tripartite ensemble of key neuroendocrine peptides subject to feedback modulation by GH and IGF-I (Fig. 1). This conceptual platform underscores distinct clinical actions of estradiol (and, therefore, possibly testos-terone as an aromatizable androgen) on each of GHRH, GHRP, and somatostatin inputs and on GH and IGF-I auto-inhibition (Fig. 12).

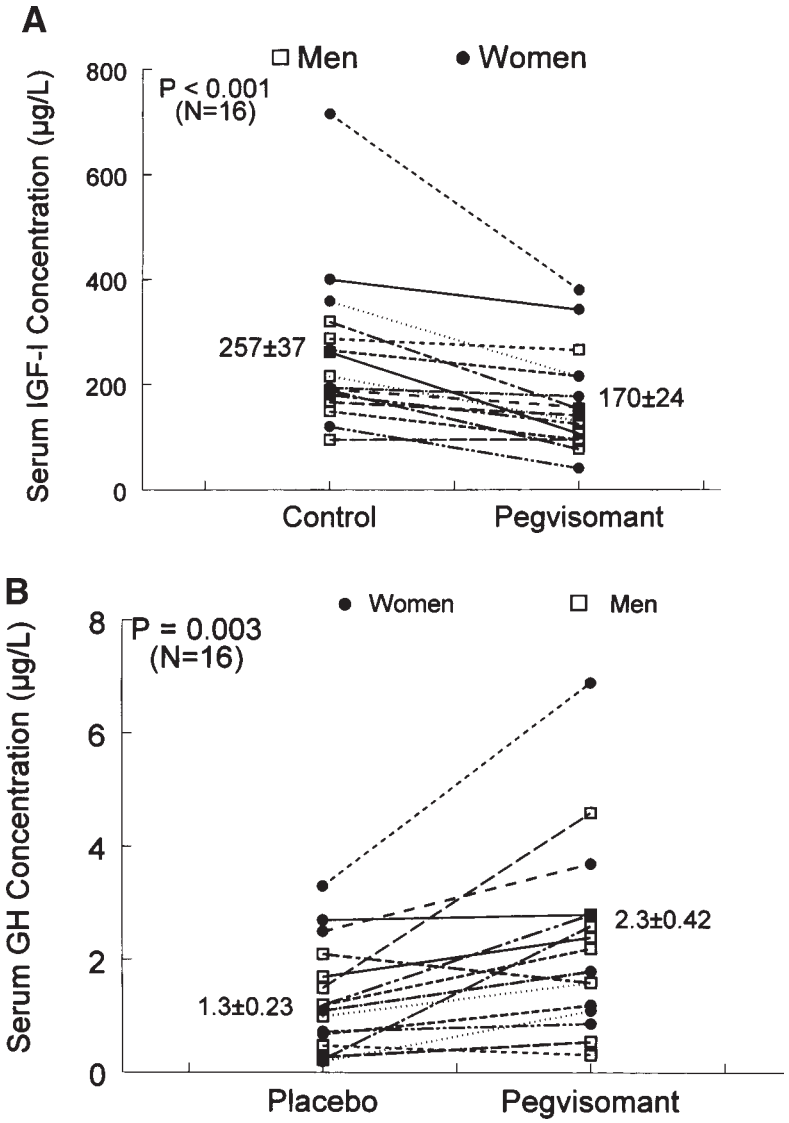

Fig. 11. Pegvisomant-induced suppression of total peripheral IGF-I concentrations (**A**) and augmentation of overnight (10-h mean) pulsatile GH secretion (**B**). Data are individual values demarcated by gender in young men (n = 9) and women (n = 7). Pegvisomant is a recombinant (nine amino acid mutated) GH molecule that selectively binds to and disables GH receptor-dependent cellular signaling. A single injection of drug (1 mg/kg sc) or placebo was given 72 h earlier in a prospective, double-blind, within-subject crossover design. GH was assayed by double-monoclonal immunofluorometry, which does not cross-react with the modified GH protein, pegvisomant. Adapted from ref. *77*.

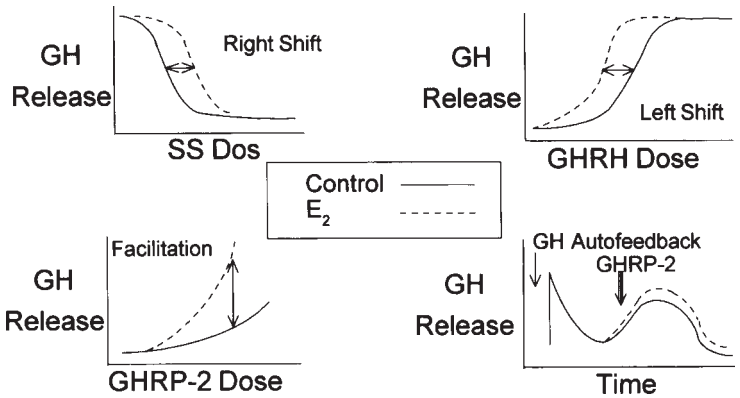

Fig. 12. Précis of estrogen-dependent mechanisms that serve to augment pulsatile GH secretion in the gonadoprival woman given a late follicular-phase (oral) dose of estradiol-17 beta for 5 d or more. Unpublished line drawing compiling data from refs. *16–18,20,32,41*.

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