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Human GH pulsatility: An ensemble property regulated by age and gender

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ABSTRACT. Age and gender impact the full repertoire of neurohormone systems, including most prominently the somatotropic, gonadotropic and lactotropic axes. For example, daily GH production is approximately 2-fold higher in young women than men and varies by 20-fold by sexual developmental status and age. Deconvolution estimates of 24-h GH secretion rates exceed 1200 μg/m^2 in adolescents and fall below 60 μg/m^2 in aged individuals. The present overview highlights plausible factors driving such lifetime variations in GH availability, i.e., estrogen, aromatizable androgen, hypothalamic peptides and negative feedback by GH and IGF-I. In view of the daunting complexity of potential neuromodulatory signals, we underline the utility of conceptualizing a simplified three-peptide regulatory ensemble of GHRH, GHRP (ghrelin) and somatostatin. The foregoing signals act as individual and conjoint mediators of adaptive GH control. Regulation is enforced at 3-fold complementary time scales, which embrace pulsatile (burst-like), entropic (orderly) and 24-h rhythmic (nycthemeral) modes of GH release. This unifying platform offers a convergent perspective of multivalent control of GH outflow.

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DYNAMIC (TIME-SENSITIVE) MODES OF HYPOTHALAMIC-PITUITARY REGULATION

Endocrine glands typically communicate with remote target glands via intermittent signal exchange (1). In the case of the growth-promoting and metabolism-modulating somatotropic axis, core signals include multiple neuropeptides released by the hypothalamus, GH secreted by the anterior pituitary gland, blood-borne metabolites, systemic hormones, IGF-I and cognate binding proteins synthesized in the central nervous system (CNS), adenohypophysis and a host of somatic cells (2). From a heuristic point of view, fluctuating GH concentrations exhibit pulsatile (briefly episodic), low-entropic (feedback-enforced orderly) and 24-h rhythmic patterns (Fig. 1). GH release is nycthemeral, but not unequivocally circadian, i.e., Zeitgeber phase-setting, free-running under temporal isolation and temperature-compensated in poikilothermic species (3). The term nycthemeral rhythmicity is more appropriate for diurnally varying GH secretion until definitive circadian data become available (3, 4). Below, we discuss the pulsatile and entropic modes of regulated GH release.

Feedback and feedforward control

An emergent concept in neuroendocrine systems is that homeostasis is achieved via incremental feedback and feedforward signaling (2, 5-8). Interglandular signal exchange embraces: a) variable time delays inherent in hormone synthesis, secretion, delivery and action; b) non-linear dose-response properties; c) stochastic (random) factors of both biological and technical origin; d) simultaneous modulation by external (e.g. nutrition) and internal (e.g. suprachiasmatic nuclear) signals (9, 10). Figure 2 illustrates some stochastic and deterministic mechanisms that mediate homeostasis. Mathematical formalism aids in quantitating such time-sensitive and signal-adaptive dynamics, which are difficult to visualize intuitively (5, 6, 11, 12).

Key-words: Somatotropin, sex steroid, aging.

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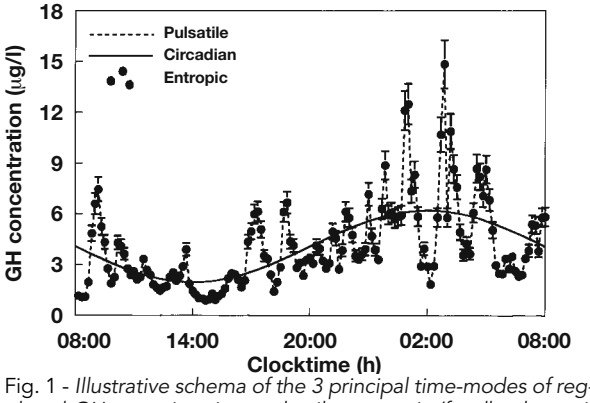

Fig. 1 - *Illustrative schema of the 3 principal time-modes of regulated GH secretion, i.e., pulsatile, entropic (feedback-sensitive) and nycthemeral (circadian-like). Composite control is endowed by time-delayed, non-linear, dose-dependent and stochastically modulated feedback and feedforward signal exchange (5, 6).*

Episodic or pulsatile GH release

Pulsatile GH secretion evolves as a succession of randomly timed and variable-amplitude volleys composed of individual discrete secretory bursts (13-17). Nycthemeral factors drive 24-h variations in GH pulse amplitude and (in lesser measure) frequency (13, 15, 16, 18, 19). Fasting and sleep markedly amplify GH peak height and elevate event frequency moderately consistent with preponderantly amplitude-dependent control of this axis (14, 20).

Secretory-burst mass is a derived estimate of the unobserved amount of hormone secreted into the bloodstream per unit distribution volume within a given pulse (e.g. μg/l GH) (1, 21, 22). The mass of GH released per burst fluctuates across the day and night from as little as 0.3-1.0 μg in the awake, calorically replete older adult to as much as 80-130 μg in the asleep, fasting neonate and adolescent (15, 17, 23-28). In contradistinction, GH pulse frequency averages 14 to 18 prominent events/day independently of gender, hormonal milieu, physical fitness, age or developmental status (15, 16, 18, 19, 25, 29, 30). Thus, the absolute range of daily GH secretion per unit surface area (m^2) is <60 μg in the elderly and >1200 μg in adolescents (assuming a 7% GH distribution volume) (2). In the latter regard, puberty amplifies the mass of GH secreted within each pulse by 3- to 11-fold (15, 17) (Fig. 3). Estrogen, aromatizable androgen, physical fitness and systemic IGF-I depletion likewise augment GH secretory-burst amplitude by several fold (19, 25, 29-36). Indeed, all primary secretagogues drive secretory-burst GH size selectively (37-41).

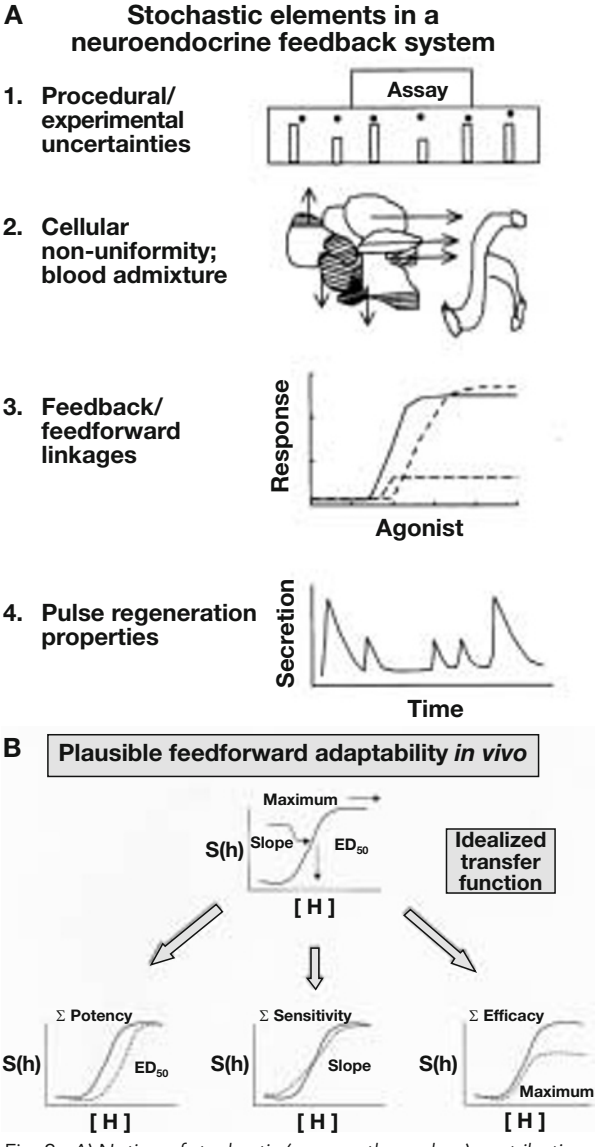

Fig. 2 - *A) Notion of stochastic (apparently random) contributions to serial hormone measurements. B) Illustrative feedforward adaptability of an implicit agonist (effector)-response (secretion) interface. Dose-response modulation includes adjustment of agonist potency and efficacy as well as target-cell sensitivity.*

GH kinetics

The plasma half-life of GH averages 14-18 min (extreme range 8-25 min), and is unrelated to gender, stage of the menstrual cycle, androgen or estrogen status, pubertal stage or age (42-45). On the other hand, short-term estradiol repletion increases the distribution volume and metabolic clearance rate (MCR) of GH by approximately 30% within-subject [recalculated from data in (46)]. The indistin-

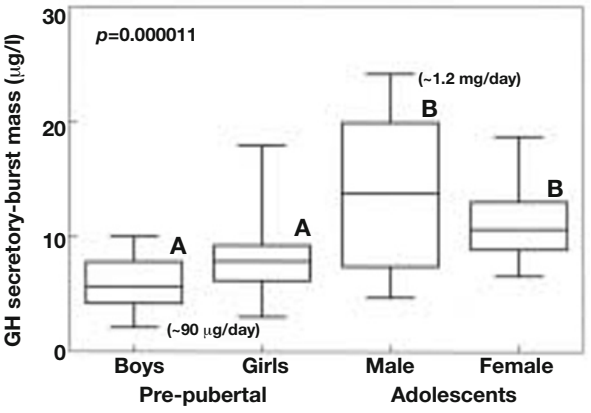

Fig. 3 - *Pubertal augmentation of GH secretory-burst mass (μg/l). The latter was assessed by deconvolution analysis of serum GH concentration time series collected at 5-min intervals for 12 h and assayed by ultrasensitive immunochemiluminesce assay (17). The extreme range of GH secretion rates is noted in parentheses.*

guishable half-life in estradiol-deficient and replete individuals signifies that estrogen does not control the rate of exit of GH from the circulation.

The estradiol-induced increase in GH distribution space implies that estrogen stimulates (total-body) GH secretion by up to 1.3-fold more than would be inferable from concentration measurements (47). This effect is small compared with the 1.8- to 2.2-fold augmentation of 24-h GH secretion. Visceral obesity mimics the effects of estrogen, and for unknown reasons accelerates the removal of GH from plasma (thus reducing GH half-life) (42-44, 48, 49). Whether GH-binding protein concentrations in tissue fluids determines physiological efflux of GH from plasma is not known (42, 50-52). However, GH receptors do not greatly influence GH kinetics, inasmuch as administration of a 3000-fold excess of a selective GH-receptor antagonist peptide (pegvisomant) does not alter any of 5 independent measures of GH elimination in humans (52).

Analytical issues

Statistical reconstruction of underlying GH secretory-burst waveform (and, hence, mass) is now possible under several assumptions. The family of estimation procedures is termed deconvolution analysis (22, 53). One analytical approach entails quantitating burst-like episodes of time-delimited GH secretion (21, 54). This model predicts that approximately 10^{15} molecules (10 nmol) of GH are released into the circulation in a single burst. Based on statistical mechanics, one would expect secretory rates for this number of molecules to vary smoothly, so that initial (granule-prestored) distribution of release rates would be approximately Gaussian over time (Fig. 4). Direct cavernous-vein sampling in the human and sheep indicate that this *a priori* approximation is realistic (55, 56). Such reasoning applies more strictly to purely random (stochastic) processes, such as the discharge of readily releasable hormone molecules by a topographically dispersed set of cells (57). Time-delayed *de novo* biosynthesis of GH, encapsulation of protein molecules into exocytotic secretory vesicles, vectoral granule movement to the membrane, and docking, fusion and discharge of GH contents confer deterministic skewness to the evolving secretory burst (11, 58). Thus, more recent representations of a secretory event predict a variably asymmetric (right tail-extended) burst, which can be represented by the generalized γ-density function (11, 57, 58). The Gaussian model provides a practical approximation (and *de facto* mathematical subset) of the generalized γ density under less frequent sampling conditions.

Available estimates indicate that 50% of an underlying GH secretory burst unfolds over 8 to 14 min, unlike the 60-100 min duration of a resultant GH concentration peak. Prolongation of the pulse in the circulation is due to delayed elimination of secreted GH molecules. Thus, precise quantitation of true GH secretory-burst shape would require successive measurements every 1 to 3 min (e.g. 5 or more observations per event) (22, 54, 59). This concept is illustrated by the analysis of 30-sec or 5-min sampling protocols in healthy young men or patients with acromegaly, respectively (13, 14, 60).

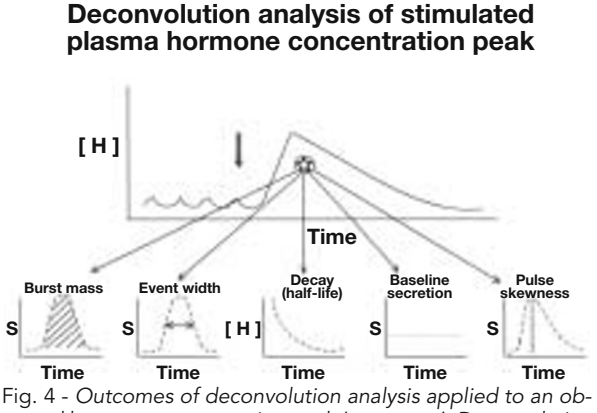

Fig. 4 - *Outcomes of deconvolution analysis applied to an observed hormone concentration peak (top center). Deconvolution quantitates the apparent mass (bottom, left-to-right) and width (duration, min) of the underlying secretory burst; exponential decay (elimination) of secreted molecules; baseline (time-invariant, nonpulsatile) secretion; and variable-waveform shape.*

Pattern regularity

On a more rapid time scale of minutes, neurohormone release is controlled by repeated incremental, deterministic adjustments enforced by physiological feedforward and feedback with superimposed stochastic effects (60, 61). This perspective applies to any adaptive network, such as the somatotropic, gonadotropic or corticotropic axis that maintains mean homeostasis (6, 12, 62-64). As shown in Figure 5, regularity or orderliness on a more subtle (sample-by-sample) time scale can be quantitated *inter alia* by the approximate entropy (ApEn) statistic (61, 65, 66). According to concepts of ensemble or network control, adaptively interlinked systems maintain quantifiable subpattern regularity or orderliness (low process randomness). Loss or gain of integrative control respectively disrupts or enhances regularity (25, 62, 67). Less regular patterns of hormone secretion by pituitary tumors exemplify this principle, wherein tumoral autonomy blunts feedback and feedforward (corrective) adjustments. Thus, quantitation by ApEn (randomness score) identifies marked secretory disorderliness of GH, ACTH, prolactin and insulin release in acromegaly, Cushing's disease, prolactinoma and aging, respectively (60, 68-71). Validation of ApEn applications to short data series indicates that 12-24 consecutive measurements of serum GH (at midnight) and ACTH (in the morning) concentrations are sufficient to discriminate tumoral from physiological sample-by-sample regularity with >90% sensitivity and >90% specificity (62).

Factors driving altered feedback-dependent regularity

Gender, age and pubertal status determine the quantifiable orderliness (ApEn) of GH release (34, 35, 61). Secretory irregularity is greater in adolescent girls and young or older women than in male counterparts of comparable ages (17, 72). Such distinctions denote unequal within-axis feedback control by gender. Within gender, pattern reproducibility further depends upon sex steroid availability (25, 34). For example, a longitudinal appraisal of GH ApEn transpubertally in healthy boys documented that marked deterioration of regularity emerges 0-4 months before maximal linear height velocity is attained (73). Surgical castration and GnRH analog-induced gonadotropin downregulation in the juvenile rat impose graded distinctions in GH secretory regularity in the adult animal, thereby demonstrating that gonadal sex-steroid hormones imprint feedback control (74). However, feedback and feedforward interactions in the GH axis are by no means fixed at sexual maturity in the human or rodent. Estrogen and testosterone (but not 5α-dihydrotestosterone or stanozolol) administration in children and adults will elevate GH ApEn (induce greater secretory randomness), which signifies prominent adaptations in feedback and/or feedforward control (18, 19, 25, 32, 75). Analogously, a single sc injection of testosterone in the adult ovariectomized rat will induce a masculine-like pattern of more regular GH release (lower ApEn) within 48 h (76).

In the more subtle context of healthy aging, regularity estimates unveil attenuation of the orderliness of each of GH, LH, ACTH and insulin in men and women (2, 40, 64, 69, 77-79). Assessment of the joint synchrony (cross-ApEn) of coupled hormone releases further establishes aging-related deterioration of feedback-linked hormone release, i.e., decreased coordinate LH-FSH, LH-testosterone, LH-prolactin, ACTH-cortisol and glucose-insulin secretory patterns (64, 69, 70, 79-84). Certain body-compositional correlates, such as visceral adiposity, also forecast feedback disruption (elevated ApEn) within the GH axis at any given range (40, 48, 85).

Validation of feedback quantitation

The emergent precept that greater subpattern randomness (orderliness loss) signifies alterations in regulatory linkages in a coupled network has been validated by way of: a) mathematically reductionistic (coupled stochastic) numerical systems; b) clinical interventional paradigms, comprising: 1) fixed feedforward enforced by iv infusion of GHRH, GHRP-2 and GnRH, 2) withdrawal of feedback signals to GH, ACTH and LH output by pharmacological depletion of downstream products, IGF-I, cortisol and testosterone, and 3) accentuation of feedback by constant iv infusion of testosterone, IGF-I or somatostatin; c) formalized models of ensemble neuroendocrine control (5, 11, 12, 65-67, 86-90); d) demonstrated reversibility of secretory irregularity by surgical cure of autonomous endocrine tumors (above).

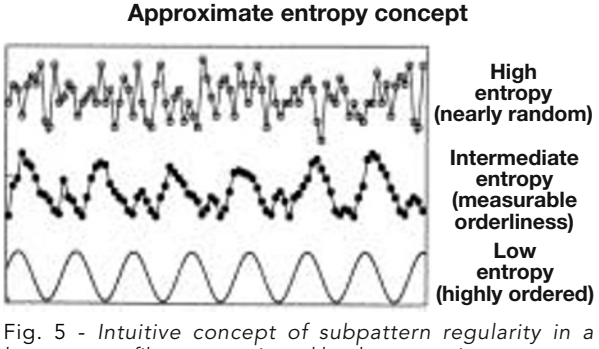

Fig. 5 - *Intuitive concept of subpattern regularity in a hormone profile, as quantitated by the approximate entropy (ApEn) statistic.*

TRIPEPTIDYL MODEL OF GH NEUROREGULATION

Multiple effectors modulate GH secretion singly and interactively. Important regulatory inputs include (non-exclusively) direct somatotrope inhibition by systemic free fatty acids; hypothalamic signaling via the 3 pivotal neuropeptides, GHRH, GHRP/ghrelin and somatostatin; and hypothalamo-pituitary feedback by GH and IGF-I (2). Inasmuch as the full repertoire of potential agonists and antagonists of GH synthesis and/or release is formidable, we proposed a simplified tripeptidyl model that embodies convergent (final-common) signaling by the ensemble of GHRH, GHRP and somatostatin (2, 28, 33, 34, 56, 91) (Fig. 6). The ensemble concept specifically encapsulates reciprocal interactions among GHRH, GHRP/ghrelin and somatostatin and autonegative feedback by GH and IGF-I. The central thesis is that no single peptide acts in isolation. Rather, GH secretion is governed by recurrent, incremental, concentration-dependent, time-varying homologous and heterologous interactions among members of the axis (5-8).

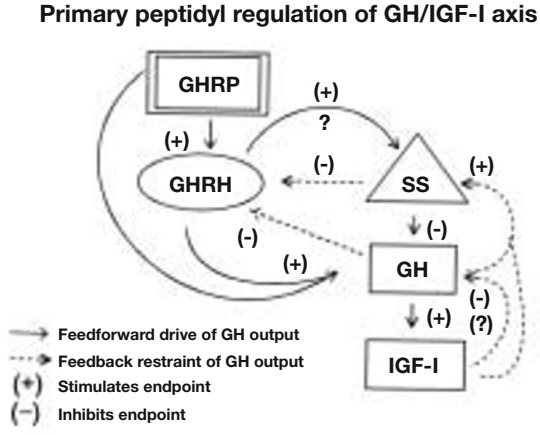

Fig. 6 - *Principal feedback (interrupted, -) and feedforward (solid, +) connections among GHRH, GHRP, somatostatin, GH and IGF-I.*

Genetic silencing of individual signaling pathways establishes that GHRH feedforward, GHRP/ghrelin drive and somatostatin inhibition are crucial components of the core GH/IGF-I regulatory network. In particular, in the human, rare mutation of the GHRH-receptor gene reduces GH secretory-burst mass (and thereby daily GH secretion) by >30 fold, but does not alter GH pulse frequency or extinguish 24-h rhythmicity (92) (Fig. 7). As predicted by ApEn-based regularity concepts, interruption of GHRH-receptor signaling disrupts GH subpattern irregularity profoundly (>5 SDs from normal). In the mouse, transgenic repression of the hypothalamic GHRP (ghrelin) receptor diminishes food intake, reduces visceral fat mass and retards juvenile somatic growth in both sexes, but suppresses GH and IGF-I concentrations and

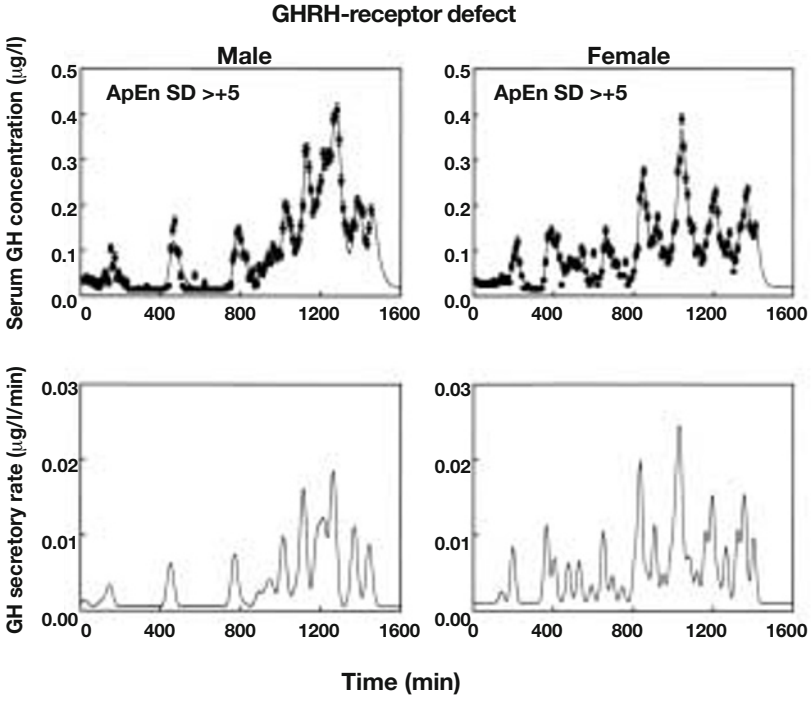

Fig. 7 - *Twenty-four h serum GH concentrations (upper) and deconvolved GH secretory rates (lower) in 2 young adults harboring a truncational mutation of the GHRH-receptor gene. Salient findings include: a) marked deterioration of pattern regularity (elevated approximate entropy, ApEn); b) preservation of discrete pulsatility; c) normal daily GH event frequency; d) profound reduction in GH secretory-burst mass; and e) retention of nycthemeral rhythmicity. Modified from (92).*

GH secretory patterns only in the female animal (93). Additional studies will be needed to define whether the latter distinction reflects a true gender contrast or unknown experimental factors (e.g. transgene dosage or strain background). Transgenic quenching of somatostatin-gene expression feminizes GH secretory patterns and hepatic sex-specific gene transcription. Lastly, loss-of-function mutations of the GH receptor and a single clinical example of IGF-I gene deletion establish the role of GH and IGF-I in feedback control (2).

Tripeptide control is mediated by homologous and heterologous effector-receptor interactions (Fig. 6). For example, GHRH stimulates pituitary ghrelin gene expression; intrahypothalamic somatostatin represses GHRH release; and ghrelin synergizes with GHRH and antagonizes somatostatin action. Wherever studied in detail, such interactions are dynamic, i.e., time-sensitive, dose-dependent, gender-modulated and developmentally regulated.

ESTROGENIC MODULATION OF TRIPEPTIDYL CONTROL

Clinical studies establish that estrogen is a dominant positive determinant of pulsatile GH secretion (1, 10, 34, 45, 94). Oral estradiol administration (in doses designed to emulate concentrations typical of the late follicular phase of the menstrual cycle) doubles GH secretory-burst mass and the (daily) GH secretion rate in ovariprival girls and women (18, 25, 95). The oral route is not singular in stimulatory efficacy, since review of extensive literature shows that oral, higher-dose transdermal, intranasal, im, iv and intravaginal delivery of diverse estrogens in women and oral administration of diethylstilbestrol and im injection of estradiol polyphosphate in men elevate GH concentrations and reduce or do not alter systemic IGF-I concentrations in all age groups examined (33, 34, 91, 96). In contrast, states of endogenous estrogen repletion, such as the late follicular phase of the normal or (exogenously) induced menstrual cycle and puberty in girls, are marked by concurrent increments in GH and IGF-I concentrations (10, 75, 97). The basis for the latter disparity is not fully explained. One clue may be that (oral) estradiol administration elevates IGF-binding protein-1 (IGFBP-1) concentrations (98), which would predictably reduce free IGF-I availability further and unleash GH secretion by feedback withdrawal. Indeed, experimental (non-estrogen-dependent) suppression of plasma IGF-I concentrations by pharmacological blockade of GH-receptor function doubles pulsatile (and basal) GH secretion in young adults (36). A second observation is that systemic estrogen administration does not stimulate (hepatic) IGFBP-3 production despite clearly augmenting GH drive (46, 98). This response dissociation is consistent with relative (hepatic) resistance to GH action, as inferred following high-dose estradiol exposure in the rat and rabbit (2, 39). A third evident distinction is that only (endogenous) estrogen secretion is characterized by concomitant ovarian production of androgen and (in the luteal phase) progesterone. In this regard, combined supplementation with estrogen and a synthetic progestin elevates both GH and IGF-I concentrations (2, 33, 34, 91). However, synthetic progestins in these contexts could act via the androgen and/or progesterone receptor.

Estrogen selectively stimulates GH secretory-burst mass and, thereby, elevates the incremental and absolute height of serum GH concentrations (16, 18, 25, 95). According to the foregoing simplified 3-peptide model, estrogen's amplification of GH secretory-burst mass would denote modulation of single or conjoint signaling by GHRH, GHRP/ghrelin, somatostatin and/or GH and IGF-I-enforced negative feedback (Fig. 2). Indeed, recent mechanistic experiments establish that estradiol repletion impacts all 5 regulatory mechanisms (41, 46, 99-102).

GHRH

In the rodent, estrogen represses the expression of GHRH peptide and the GHRH receptor (34). Inferences concerning sex-steroid control of exogenous GHRH feedforward drive in the human have been less consistent (96). We reasoned that earlier discrepancies may reflect variable cohort selection, choice of GHRH dose, concomitant somatostatin outflow and method of analysis (91, 94). As complementary strategies to address the impact of estradiol on (exogenous) GHRH action, we have utilized: a) continuous 24-h infusion of rh GHRH-1, 44-amide at a near-maximally effective dose to explore maximal agonist actions; b) acute L-arginine pretreatment to limit endogenous somatostatin inhibition during separate-day, randomly ordered, single-bolus injection of a 300-fold dose range of biosynthetic GHRH-1,44-amide in a within-subject crossover design; c) baseline-corrected and kinetically adjusted (deconvolution quantitation of) GH secretory-burst mass (μg/l of distribution volume) (21, 38, 40, 100).

Figure 8 illustrates 24-h serum GH concentration profiles monitored after randomly ordered placebo and estradiol supplementation along with constant iv infusion of saline or rh GHRH-1,44-amide (1 μg/kg/h). Notably, continuous near-maximal GHRH stimulation drives pulsatile and entropic GH secretion markedly, and estrogen repletion fails to

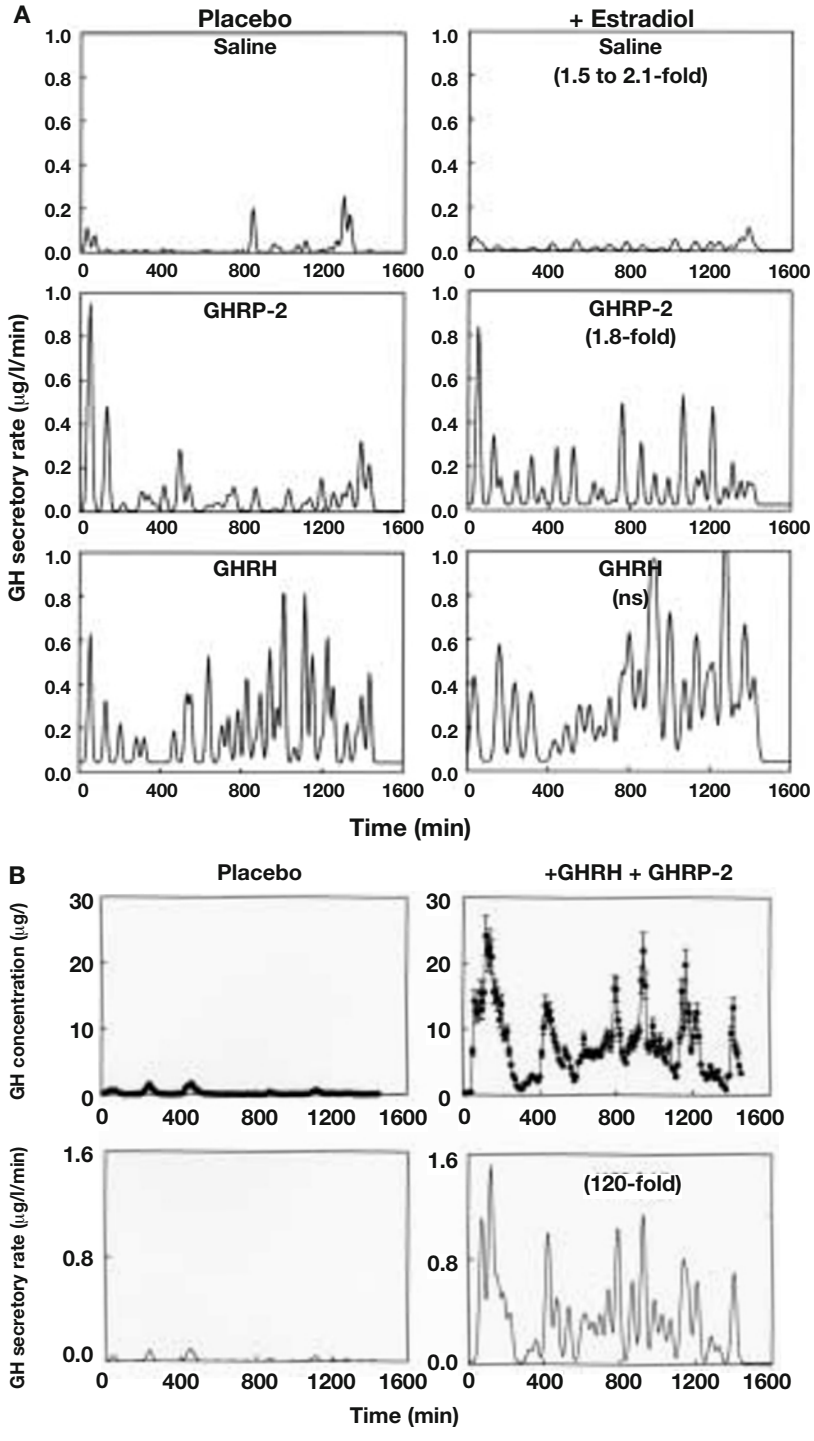

Fig. 8 - *A) Impact of continuous iv infusion of GHRP-2 or GHRH (1 μg/kg/h) during placebo and oral estradiol replacement on GH secretion rates in a healthy postmenopausal woman. The fold notation defines the ratio of the effect of estradiol over that of placebo. Combined from (38, 98). Figure 7 for data format. B) One hundred and twenty-fold stimulatory effect of combined continuous iv infusion of GHRH and GHRP-2 (each 1 μg/kg/h) over basal GH secretion in a postmenopausal volunteer withdrawn from estrogen. Dual secretagogue drive maintains a normal frequency of GH pulses, thereby indicating that signals other than GHRH and GHRP (alone or combined) mediate the GH pulse-renewal process. Adapted from data in (39).*

affect either response (38). Bolus injection of single GHRH pulses in the somatostatin-withdrawn context augments GH secretory-burst mass dose-dependently (100). In this context, estrogen supplementation does not augment the maximal effect of GHRH, but amplifies: a) pituitary sensitivity to GHRH (defined by 2-fold elevation of the maximal slope of the dose-response function); b) GHRH po-

tency (identified by a 53% reduction in the half-maximally effective dose, ED_{50} of GHRH) (38, 100). The foregoing observations offer an explanation for some of the reported disparities in estrogenic modulation of GHRH action, inasmuch as: a) estradiol does not facilitate a maximal GHRH stimulus; b) estradiol potentiates a submaximally effective (physiologically attainable) GHRH signal; c) L-arginine pretreatment may be required to limit GH secretory-response variability to GHRH injection. Whether testosterone heightens GHRH feedforward by analogous sensitization is not known.

GHRP (ghrelin)

Continuous iv infusion of a high dose of GHRP-2 (1 μg/kg/h) for 24 h markedly amplifies all 3 of pulsatile, entropic and nycthemerally rhythmic GH secretions (37) (Fig. 8). This tripartite neurosecretory response emulates that induced by GHRH as well as that observed in normal midpuberty in boys and girls (15, 17, 28, 38, 73).

Administration of estrogen or testosterone augments apparent efficacy of synthetic GHRPs (103-105). The term "apparent efficacy" is appropriate at present, since a GHRP-2 dose-response analysis indicates that an unequivocally maximal secretory response is not demonstrable following injection of the highest evaluable dose (3 μg/kg) (41) (Fig. 9). Supplementation with estradiol potentiated the stimulatory effect of the last dose by 1.8-fold. Comparable studies will be important to conduct with the endogenous GHRP-receptor ligand, ghrelin. Moreover, the mechanisms mediating this striking action of estrogen will be meritorious to dissect (33, 41). A plausible clinical hypothesis is that estradiol induces the expression of hypothalamo-pituitary GHRP receptors, as reported by *in vitro* transcriptional assay (106, 107).

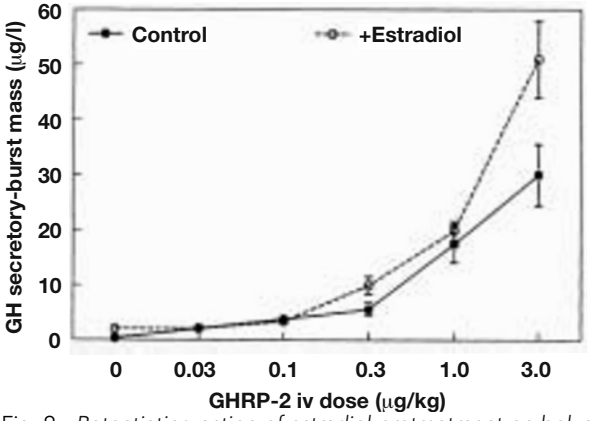

Fig. 9 - *Potentiating action of estradiol pretreatment on bolus iv GHRP-2-stimulated GH secretory-burst mass in 11 healthy postmenopausal women. Modified from (41).*

Somatostatin

The impact of sex steroids on somatostatin synthesis, release and action is complex and incompletely understood. In the rodent, testosterone or 5α-dihydrotestosterone induces, and orchidectomy represses, periventricular-nuclear somatostatin gene expression. In the rat, estradiol does not mimic the androgenic effect consistently (2). On the other hand, estradiol enhances submaximal L-arginine-stimulated GH release in the human (100). Assuming that this amino acid reduces hypothalamic outflow of somatostatin, the latter effect would denote that estrogen does not act exclusively by withdrawing somatostatin restraint. However, whether estrogen alters somatostatin release in the human is not known. According to a tripeptidyl regulatory model, muting of somatostatinergic inhibition would further amplify (non-maximal) GH secretion driven by a combined GHRH/GHRP stimulus (34). We tested this concept by simultaneous infusion of GHRH and GHRP-2 (1 μg/kg/h each) for 24 h in post-menopausal women during (randomly ordered) estrogen withdrawal and supplementation. In this unique setting, estradiol increases daily total, pulsatile and basal (non-pulsatile) GH secretion significantly by within-subject paired comparison (39) (Fig. 8). These data, if corroborated, would indicate that estradiol can repress hypothalamic somatostatin release or directly stimulate somatotropes (33). In addition, in the same setting, estradiol elevates GH ApEn and 24-h rhythmic GH release (technically defined by the mesor of the nycthemeral rhythm) (38). Mechanistically, such outcomes could reflect facilitation of GHRP action (38), enhancement of GHRH drive (100), disinhibition of GH autofeedback (46) and/or antagonism of somatostatin restraint (99).

In relation to somatostatin action, a recent clinical study disclosed that estradiol supplementation in postmenopausal women reduces the inhibitory potency (ID_{50}) of and blunts somatotrope sensitivity to somatostatin, but does not attenuate suppressive efficacy (99) (Fig. 10). The latter distinction is important, since it affirms estrogenic determination of physiological rather than pharmacological effects of somatostatin.

IMPACT OF AROMATIZABLE ANDROGEN

Testosterone repletion in puberty, hypogonadal boys and older men stimulates GH secretory-burst mass, increases pattern irregularity (ApEn) and heightens 24-h rhythmicity (19, 25, 29, 31, 32).

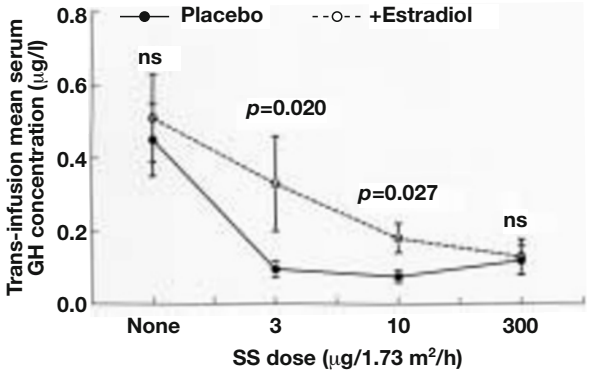

Fig. 10 - *Estrogen relieves submaximal inhibition of GH release otherwise enforced by iv infusion of increasing doses of somatostatin (SS) in postmenopausal volunteers.* p- *values denote within-subject statistical contrasts (*p=ns *defines* p>0.05*). Modified from (99).*

These effects are not observed in eugonadal young men even in the face of pharmacological serum total testosterone concentrations (e.g., 1000 to 1500 ng/dl) (19, 75). Other studies illustrate certain salient differences between the actions of exogenous testosterone and estrogen: a) only testosterone significantly (and consistently) increases plasma total IGF-I concentrations (34, 35); b) testosterone elevates underlying basal (non-pulsatile) GH release; c) oral estradiol, but not parenteral testosterone, increases serum IGFBP-1 concentrations (19, 41). Testosterone-dependent stimulation of IGF-I production requires augmentation of GH secretion, but is independent of ageor genotype (2, 34). In contrast, neither estrogen alone nor a non-aromatizable androgen (such as 5α-dihydrotestosterone, fluoxymesterone or stanozolol) uniformly elevates IGF-I concentrations (25, 75). One plausible hypothesis to account for distinguishable effects of systemic estrogen and testosterone on IGF-I production, basal GH secretion and IGFBP-1 concentrations is that *in situ* aromatization of testosterone to estradiol (but not systemic delivery of estrogen) directs specific signaling pathways by attaining high local concentrations (33, 34, 94).

GH-DEPENDENT AUTOFEEDBACK

Impact of estrogen

Compared with the male, the adult female rat is resistant (but not insensitive) to GH autonegative feedback (2). In the human, gender and pubertal status impact GH autofeedback in a unique fashion. In particular, a single iv pulse of rh GH inhibits GHRH-stimulated and spontaneous pulsatile GH secretion consistently (by 50-70%) in pre-, mid- and post-pubertal boys, young women, postmenopausal individuals, and young and older men (28, 46, 101). Inhibition of spontaneous and exercise-stimulated GH secretion in young women and men is proportionate to dose of recombinant human (rh) GH over the range 1, 3 and 10 μg/kg (101). Gender comparisons reveal that men exhibit greater sensitivity to autofeedback inhibition than women, antipodal to the sex difference in the rodent (2). On the other hand, estradiol administration in postmenopausal women blunts GH-enforced negative feedback specifically on the GHRP-2 stimulus (but not on basal, exercise or GHRH-induced GH release) (46). Further complexity of sex-steroid control is evident in the recent clinical finding that administration of estradiol accentuates inhibition by rh IGF-I of spontaneous and GHRH-stimulated GH secretion (102). A plausible but unproven central mechanism for the last observation is the experimental capability of estrogen to upregulate expression of the pituitary Type 1 IGF receptor (108).

Impact of puberty

Puberty is remarkable by way of combined elevations in GH and IGF-I production (15, 17, 29). This circumstance also prevails in the late follicular phase of menstrual cycle and in pathological hypersomatotropism (60, 109, 110). Simultaneously increased secretion of a trophic hormone and the feedback product would point mechanistically to: a) autonomous feedforward drive (e.g., by fixed secretagogue infusion or unregulated hormone release by an endocrine tumor); b) impairment of central reception of the feedback signal (e.g., due to a genetic GH-receptor defect); c) attenuation of the central inhibitory response-effector mechanism, despite a normal feedback signal (e.g., transgenic silencing of somatostatin receptor subtype 2); d) disruption of the feedback signal proper (e.g., mutation of the GH protein). Plausible testable bases for the simultaneous increase in GH and IGF-I concentrations in puberty are heightened hypothalamic drive and blunted IGF-I and/or GH-dependent autonegative feedback. Investigation of these conjectures in healthy boys yielded 3 novel insights. First, a single pulse of rh GH inhibits GHRH-stimulated GH release equivalently in all 3 of prepubertal boys, midpubertal boys and young men (28). This outcome signifies comparable rh GH-induced somatostatin outflow across adolescent development. Secondly, infused GH suppresses spontaneous GH secretion both fractionally (%) and decrementally (μg/l) more in midpuberty than in the child or adult. This mode of autofeedback could create marked downswings after GH pulses. Moreover, in view of

inferentially equivalent GH-enforced somatostatin outflow, the later feature in mid-adolescence may denote greater feedback susceptibility of hypothalamic GHRH (and possibly ghrelin) release. And, thirdly, the time-course of recovery of GH secretion after maximal suppression is several-fold more rapid in midpuberty than in childhood or adulthood (Fig. 11). Accelerated resurgence of GH secretion after feedback repression could underlie prompt upswings in GH concentrations after an endogenous pulse. The basis for robust escape of GH pulsatility in mid-puberty may include an abbreviated duration of somatostatin outflow and/or heightened secretagogue drive.

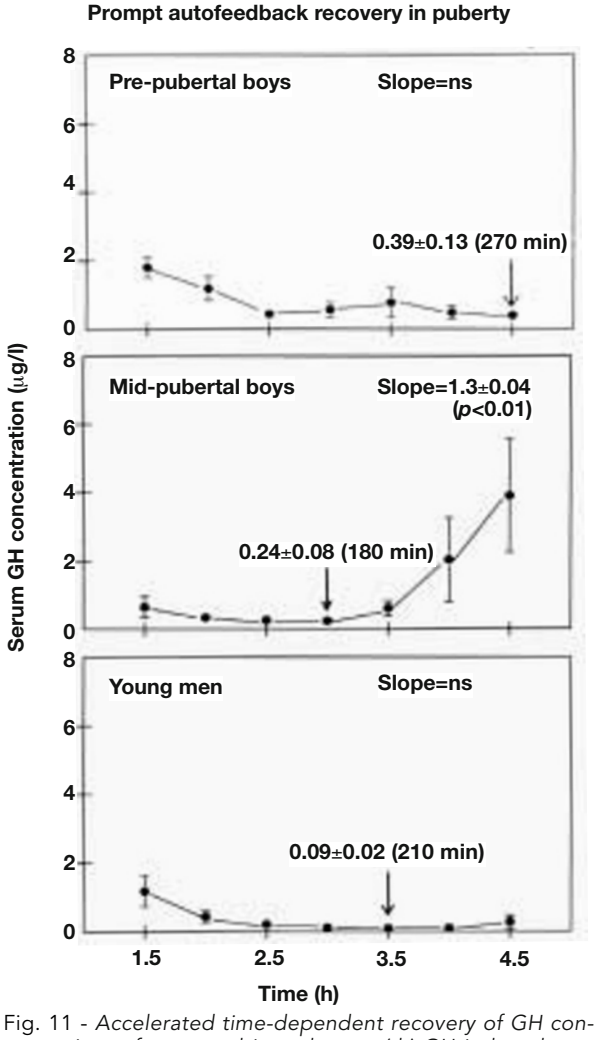

Fig. 11 - *Accelerated time-dependent recovery of GH concentrations after recombinant human (rh) GH-induced suppression in mid-pubertal (middle) compared with pre-pubertal (top) boys and young men (bottom). Rh GH was injected at time 0 as a 3 μg/kg 6-min iv bolus. GH concentrations are the 30-min mean of 10-min data (±SEM, no.=6 subjects). The value and the time of the absolute nadir are marked. The slope (±SEM) of GH recovery is significantly nonzero in the midpubertal study cohort only. Revised from data in (28).*

FEEDBACK RESTRAINT BY SYSTEMIC IGF-I CONCENTRATIONS

Systemic IGF-I depletion typically unleashes GH secretion, as observed in fasting, anorexia nervosa, malabsorption, GH-receptor defects and rare deletion of the IGF-I gene (2). However, the metabolic complexity of these conditions limits the facile conclusion that IGF-I deprivation *per se* stimulates pulsatile GH release. More explicitly, however, administration of a highly selective GH-receptor antagonist peptide (pegvisomant) suppresses serum (total) IGF-I by 30% within 72 h and stimulates pulsatile (and basal) GH secretion by 1.8-fold in healthy young men and women (36, 52) (Fig. 12). There is no evident gender difference in the GH response, but larger study cohorts will be needed to verify this inference. The mechanism of feedback unleashing by this oligopegylated protein analog is not definitively inferable as yet. For example, GH-receptor blockade and IGF-I depletion both occur in this setting, either or both of which changes may induce GH secretion. Native GH molecules enter human cerebrospinal fluid, and act centrally via the cognate receptor to mediate autonegative feedback (2). Whether oligope-

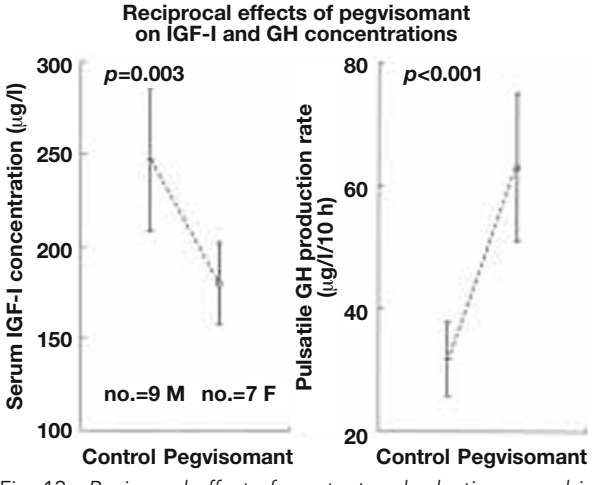

Fig. 12 - *Reciprocal effect of a potent and selective recombinant human GH-receptor antagonist (pegvisomant) to suppress systemic total IGF-I concentrations and unleash pulsatile GH secretion. A double-monoclonal immunofluorometric assay (IFA) was applied to sera collected every 10 min overnight during the time window 64-72 h after saline or pegvisomant injection (1 mg/kg). The IFA measures GH concentrations without inference by pegvisomant. A separate IFA was used to quantitate 3000-fold higher serum concentrations of pegvisomant than GH (52). Values are the mean±SEM in 16 healthy young adults (9 men and 7 women). Adapted from data in (36).*

gylated drug or a sparing amount of depegylated protein does likewise is not established clinically. In addition, large proteins, such as horseradish peroxidase, accumulate in the external median eminence and arcuate (but not periventricular) nucleus rapidly after systemic injection. The latter point is significant in that the GH receptor is expressed on inhibitory somatostatinergic and NPYergic neurons in the arcuate nucleus (2).

CONCLUSIONS

A basic tripeptidyl model of interactive control provides a platform for examining how gender and age impact GHRH, GHRP/ghrelin and somatostatinergic pathways and GH and IGF-I-dependent feedback. The present review highlights the utility of this construct in beginning to parse the complex mechanisms that mediate interactive control.

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