Model-projected mechanistic bases for sex differences in growth hormone regulation in humans

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1Division of Endocrinology and Metabolism, Department of Internal Medicine, School of Medicine, University of Virginia, Charlottesville, Virginia; and 2 Tulane University Health Sciences Center, Medicine/Endocrinology, New Orleans, Louisiana; and 3 Endocrine Research Unit, Mayo Medical and Graduate Schools, General Clinical Research Center, Mayo Clinic, Rochester, Minnesota

Farhy LS, Bowers CY, Veldhuis JD. Model-projected mechanistic bases for sex differences in growth hormone regulation in humans. Am J Physiol Regul Integr Comp Physiol 292: R1577–R1593, 2007. First published December 21, 2006; doi:10.1152/ajpregu.00584.2006.—Models of physiological systems facilitate rational experimental design, inference, and prediction. A recent construct of regulated growth hormone (GH) secretion interlinks the actions of GH-releasing hormone (GHRH), somatostatin (SRIF), and GH secretagogues (GHS) with GH feedback in the rat (Farhy LS, Veldhuis JD. Am J Physiol Regul Integr Comp Physiol 288: R1649–R1663, 2005). In contrast, no comparable formalism exists to explicate GH dynamics in any other species. The present analyses explore whether a unifying model structure can represent species- and sex-defined distinctions in the human and rodent. The consensus principle that GHRH and GHS synergize in vivo but not in vitro was explicable by assuming that GHS 1) evokes GHRH release from the brain, 2) opposes inhibition by SRIF both in the hypothalamus and on the pituitary gland, and 3) stimulates pituitary GH release directly and additively with GHRH. The gender-selective principle that GH pulses are larger and more irregular in women than men was conferrable by way of 4) higher GHRH potency and 5) greater GHS efficacy. The overall construct predicts GHRH/GHS synergy in the human only in the presence of SRIF when the brain-pituitary nexus is intact, larger and more irregular GH pulses in women, and observed gender differences in feedback by GH and the single and paired actions of GHRH, GHS, and SRIF. The proposed model platform should enhance the framing and interpretation of novel clinical hypotheses and create a basis for interspecies generalization of GH-axis regulation.

feedback; somatotropin; peptides; growth hormone-releasing hormone; growth hormone secretagogues; somatostatin

NEUROENDOCRINE SYSTEMS TYPICALLY communicate via intermittent (pulsatile) rather than continuous (steady state) signal exchange. Hormone pulses convey significant information to target tissues, as illustrated in the case of gonadotropin-releasing hormone, parathormone, adrenocorticotropin, and growth hormone (GH) (119). For example, pulsatile and continuous GH secretion patterns dictate adult body size, inducible hepatic enzymes, lipoprotein metabolism, muscle IGF-I expression, and insulin sensitivity (47, 129).

Episodic hormone secretion is governed by integrative feedback mechanisms. Pulsatile GH secretion is stimulated by GH-releasing hormone (GHRH), potentiated by ghrelin (a GH secretagogue; GHS), and inhibited by somatostatin (SRIF). GH feeds back via cognate receptors in the brain to regulate each of the GHRH (inhibited), SRIF (stimulated), and GHS receptors (repressed) (9, 12–14, 23, 25, 34, 76, 83, 85, 86, 90, 114, 138). Transgenic laboratory models and sporadic mutations in the mouse and human establish the importance of each of these four signals in directing GH secretion (43, 48, 70, 92, 100, 108, 115).

Physiological regulation proceeds via repeated incremental adjustments toward homeostasis. The complexity of neuroendocrine systems has motivated objective representations of interactive mechanisms, such as those directing pulsatile GH secretion in the rat (14, 37–41, 74, 91, 112, 133). In contradistinction, no formalism exists to explicate how GHRH, SRIF, GHS, and GH jointly control GH dynamics in other species, including the human. This deficit in the field is significant, because model constructs provide a complementary means to affirm, reject, or revise experimental intuition, frame novel testable hypotheses, and parse the basis of unexpected outcomes.

The pathophysiology of GH secretion varies among species such as human, monkey, sheep, pig, hamster, guinea pig, and rat (reviewed in Refs. 47, 84, 129). For example, the human and rat differ completely in sex-related GH pulsatility, wherein 1) GH pulse frequency is the same in women and men but is significantly higher in the female than the male animal (26, 28, 75); and 2) GH pulse amplitude is two-fold greater in women than in men but is 30-fold larger in the male than the female rodent (14, 118, 130). On the other hand, GH secretory patterns are less regular (more disorderly) in the female than the male in both species (45, 53, 94). The physiological bases of such species- and sex-related distinctions remain unknown. To begin to address this integrative issue, our present analyses explore whether a minimal feedback structure with distinguishable pathway strengths can explicate both species- and sex-related differences.

METHODS

No animal or human experiments were conducted, and the study is institutional review board exempt.

Existing Core Construct in Rats

The GH feedback construct in the rat assumes three principles of physiological control, as inferred experimentally: 1) GH pulse renewal arises from interactions among GHRH, SRIF, and GH feed-
back; 2) GHS acts as an amplifier of the basic network; and 3) GH feedback evokes greater SRIF outflow in the male than the female animal (39–41).

The complex GH pulse-renewal process in the adult male and female rat is envisioned as arising from two feedback loops. First, GH feedback induces periventricular nuclear SRIF (SRIFPeV) outflow. SRIF, in turn, inhibits GHRH neurons in the arcuate nucleus (ArC) transsynaptically and blocks GH release after its delivery to the pituitary via the hypophysial-portal microcirculation. This time-delayed mechanism evokes distinct volleys of GH secretion every 2.2–2.5 h in the male animal. Second, within the mediobasal hypothalamus, SRIFArC rapidly inhibits GHRH neurons and GHRH neurons rapidly stimulate SRIFArC neurons. This reciprocity generates higher-frequency GH oscillations within volleys in the male and independently of volleys in the female (40). The proposed functional connectivity among GHRH, SRIF, and GH is in accordance with experimental data (1, 22, 25, 29, 43, 54, 59, 71, 72, 77, 103, 111, 114, 136).

GHS is viewed as amplifying GH output via four complementary mechanisms: 1) direct stimulation of somatotrope GH release; 2) opposition to SRIF’s inhibition of ArC GHRH neurons and 3) opposition to SRIF’s inhibition of somatotrope GH release; and 4) stimulation of GH secretion from ArC neurons (41, 129). The model allowance for greater GH feedback-evoked SRIFPeV outflow in the male than female rat accounts for attenuated efficacy of consecutive GHS pulses in the male animal and explains reduced pulsatile GH secretion in female GHS receptor knockdown mice (41, 108, 116).

Specific Features of Regulated GH Secretion in Humans

Control of GH pulse size and number in humans. Hypothalamic-pituitary portal blood cannot be sampled directly in the human. However, significant indirect clinical data exist regarding the control of GH pulse amplitude and frequency. Hypothalamic GHRH outflow maintains GH pulse size, because infusion of a GHRH receptor antagonist and spontaneous mutations of the GHRH receptor reduce the amplitude of GH pulses in men and women (8, 57, 100). However, GHRH pulses do not drive the basic pulse renewal process, inasmuch as GH pulse frequency is normal in healthy subjects receiving a continuous GHRH infusion, as well as in uninfused patients harboring a truncational mutation of the GHRH receptor (35, 58, 100). These data permit the hypothesis that putative GHRH-SRIF oscillations arise via neurotransmitter pathways that require GHRH neurons but not the GHRH receptor (40, 41). Continuously infused GHS also selectively amplifies GH pulse size without altering frequency (55, 105, 127). Conversely, systemically delivered SRIF and GH feedback-induced SRIF release repress GH pulse amplitude and inhibit stimulation by GHRH > GHS > combined GHRH/GHS (4, 6, 21, 56, 81). Therefore, GHRH, GHS, and SRIF all regulate GH secretory burst size, whereas neither the GHRH receptor nor systemically available secretagogues determine GH pulse frequency in the human.

Gender contrasts in GH pulsatility in humans. The amplitude of GH pulses is twofold larger in women than in men, but GH pulse frequency does not differ by sex (118, 130, 135). Administration of L-arginine, a putative inhibitor of hypothalamic SRIF release (2, 46), stimulates GH secretion more in women than in men (82, 133), suggesting greater baseline SRIF restraint in women. In addition, women compared with men exhibit 1) greater feedback-induced absolute and fractional decrements in GH concentrations and greater subsequent reboundlike GH secretion (124, 127), 2) more irregular (less reproducible) patterns of GH release as quantified by higher approximate entropy (ApEn) (94), and 3) higher GH peaks after combined intravenous infusion of the paired GH secretogogues GHRH/L-arginine and GHRP/L-arginine but not necessarily GHRH/GHRP (109). Other analyses demonstrate that administration of estradiol compared with placebo J increases the potencies but not the efficacies of both GHRH and SRIF (20, 122), 2) enhances stimulation by natural (ghrelin) and synthetic (GHRP-2) GHS (3, 125), and 3) heightens reboundlike GH secretion after negative feedback induced by a pulse of recombinant human (rh) GH (120). In contrast, elimination kinetics of GH do not differ in men and women (104, 121). The foregoing regulatory characteristics are highlighted schematically in Fig. 1.

Construct of GHRH, SRIF, GHS, and GH Network in Humans

There is no a priori evidence that basic neuroanatomic connections differ in the human and rat (47, 129). Thus a core construct may be postulated in the human that comprises four peptides and five primary regulatory nodes [GHS (ghrelin), GH, GHRH, SRIFArC, and SRIFPeV]. These nodes are interconnected functionally as introduced and justified in a recent model for the rat system (39–41) (see Existing Core Construct in Rats above).

The proposed equation system defines SRIF as a noncompetitive inhibitor of both GHRH neurons and pituitary somatotropes. Specifically, the construct assumes that SRIF blocks the release of both GHRH and neurotransmitters from GHRH neurons and inhibits basal, GHRH-stimulated, and GHS-stimulated exocytosis of GH by pituitary cells (13, 36, 117). Withdrawal of inhibition by SRIF evokes brief reboundlike secretion of neuronal GHGH and pituitary GH (25, 76, 112). GHS stimulates pituitary GH secretion directly and additively with GHRH, antagonizes hypothalamic (combined PeV and ArC derived) SRIF restraint on GHRH neurons, and opposes SRIF’s inhibition of GH release from the pituitary gland but does not alter secretion of SRIF into portal blood. According to these conditions, GHRH neurons are inhibited by total SRIF (from PeV and ArC) and stimulated by GHS. The model concepts are encapsulated in a set of coupled delayed ordinary nonlinear differential equations. Interactions are rendered via Hill functions, wherein the exponents ni represent sensitivity (steepness) of responses. Rate constants of elimination are given by ki for i = 1, 2, 3, 4. Potencies of the five core signals are given by ti for i = 1, 2, . . . , 5. The four ki, parameters denote efficacies of indicated inputs. The rates of change of concentrations of plasma GH, SRIFPeV, SRIFArC, and released GHRH are then given as
The hypothesis tested is that given this core model structure, physiologically relevant system parameters exist that predict distinct GH dynamics in vivo and women that differ from those inferred in the rat (41).

Model Assumptions and System Parameters in Humans

Elimination rates and delay times. The model assumes a mean GH half-life of 14 min in men and women (121). Nominal half-lives of portal-venous SRIF and GHRH are taken to be 1.5 and 1 min, respectively. The two feedback delay times, \( \tau_1 \) and \( \tau_2 \) in Eqs. 2 and 3, were estimated empirically to permit intravolley GH oscillations every 30–45 min and distinct volleys of GH pulses every 90–110 min (51, 118, 130) (see Table 1).

Secretion rates. The maximal physiological secretion rate of GH, \( k_{1,t} \), corresponded arbitrarily to a stimulated GH peak concentration of 540 ng/ml (47, 129). The secretion rates of SRIF and GHRH are unknown and thus were functionally scaled.

Hill coefficients. Because of the lack of experimental data, these parameters were rescaled from model values in the rat (41).

Model-related condition that GHRH and GHS synergize in vivo but not in vitro. The construct defined by parameters were rescaled from model values in the rat (41). In vivo behavior is approximated by the complete model comprising Eqs. 1–4. Infusion of maximally stimulatory doses of GHRH and/or GHS would marginalize any effects of endogenous GHRH or GHS. Assume that a maximally effective GHRH stimulus amplifies the effect of the GHRH-containing term analogously by \( b \)-fold (from 1/1 to 1) and that of the GH and SRIF-containing term by \( c \)-fold (from 1/c to 1). The expected increase in GH secretion due to combined maximal stimulation is then \( abc(1 + g_0)/(b + a + g_0) \)-fold. On the other hand, simple addition of the individual effects of GHRH and GHS would give a \( bct/(1 + a + g_0) \)-fold increase. Therefore, in vivo synergy (a more than additive combined effect) requires that \( 1 + g_0/b/c + 1/a < 1 \). This condition is satisfied by the parameter values we used (see Table 1). However, note that if, in vivo, a GHS infusion can stimulate hypothalamic GHRH outflow, which is maximally effective, the synergy may be lost (discussion).

For in vitro experiments not involving SRIF, the rightmost term containing SRIF in Eq. 1 equals unity, and the rate of change of GH concentrations becomes

\[
\frac{\text{d}G}{\text{d}t} = -k_1G + k_{1,t} \left( \frac{\text{GHRT}_1}{\text{GHRT}_1 + 1 + \frac{\text{GHSRT}_1}{\text{GRT}_1 + 1}} \right) \end{equation}

The combined in vitro effects of GHRH and ghrelin will be additive, not synergistic. For example, assume that maximally effective concentrations of GHRH and GHS increase the GH-containing term by \( a \)-fold (from 1/1 to 1) and the GH-containing term by \( b \)-fold (from 1/b to 1) in Eq. 6. The combined GH response is then \( a + g_0 + a + g_0 \)-fold. In contrast, simple summation of the individual GH-augmenting effects of GHRH and GHS predicts a \( \frac{a + b + a + g_0}{a + b + a + g_0} \)-fold increase. Thus the in vitro response defined by Eq. 6 will be less than additive for all positive real nonzero values of \( a, b, \) and \( g \).

Note that in this work, the term “synergy” means “more than additive,” which includes consequences of nonlinearities in the dose-response interactions. If one accepts the more rigorous definition “supra-additive response to hormones/peptides, both of which are given individually at maximal doses,” then neither the human system nor the female model produce synergy.

Human gender-related differences. GH secretion is greater in women than men. Differences in women compared with men were represented as follows:

1) GH peaks are twice as great. Women have higher GH potent (denoted in the model by a 12% increase in the GH potency parameter, \( \tau_1 \), in Eq. 1) and greater GH efficacy (confessed in the model by 2.85-fold greater opposition by GHS of SRIF’s inhibition at the pituitary, expressed in the GH feedforward parameter, \( g_1 \), in Eq. 5).

2) The GH response to combined GHRP-2/L-arginine drive is twice as great (3, 109). Estrogenized women exhibit higher GH efficacy (in the model assumed to be due to a 50% increase in GHS action on the pituitary, \( g_0 \), in Eq. 1), and a heightened response to L-arginine (82), a putative inhibitor of SRIF outflow [translated in the model as an 80% (women) vs. 33% (men) reduction by L-arginine in GH-induced and basal SRIF outflow from PeV, Eq. 2].

3) The GH responses to combined GHRH/L-arginine are twice as great (109). The effect follows from the fact that women have higher GH potency (item 1) and greater capacity of L-arginine to stimulate GH secretion by blocking SRIF than men (item 2) (44, 47, 82, 129).

4) The responses to combined GHRH/GHS are comparable (109). No gender-related parameter changes were explored.

5) Fractional and absolute feedback suppression of GH concentrations and subsequent reboundlike GH release are greater in women (127). Accentuated feedback was explicated via items 2 and 3 and heightened rebound-like GH secretion via increased feedforward in item 1.

Nominal values of interactive constants defined by the foregoing principles are summarized in Table 1. Partial parameter sensitivity analysis was performed by ApEn analysis as indicated below in Parameter Sensitivity Analysis and DISCUSSION.

Physiological Interpretation of Model Equations and Parameters

The four model equations (Eqs. 1–4) describe the dynamics of the concentration of GH, GHRH, SRIF_{PeV}, and SRIF_{AcC}. Each equation approximates the assumption that the secretion of one hormone is regulated by the concentration of the other system hormones in a dose-dependent fashion. The specific interactions are shown in Fig. 1, and the model is a mathematical approximation of these regulatory assumptions. Table 2 summarizes the physiological meaning of all model parameters.
The following equation to the core system of...

\[
\frac{dC}{dt} + k_{r_1}C = \left( k_{r_2} + k_{r_3} \right) \cdot C
\]

DRIVEN SRIFPeV outflow in latency for GHRH neurons to stimulate SRIFArC neurons. Physiological interpretation of parameters in the GH feedback construct.

L-arginine was simulated by dividing the right-hand term for GH-im...imulation of Peptide Infusions

Table 1. Summary of core interactive constants in the GH feedback construct

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value Description</th>
<th>Physiological Interpretation</th>
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<tbody>
<tr>
<td>( k_1 )</td>
<td>3 h⁻¹</td>
<td></td>
</tr>
<tr>
<td>( k_{r_1} )</td>
<td>600 ng ml⁻¹ h⁻¹</td>
<td></td>
</tr>
<tr>
<td>( k_{r_2} )</td>
<td>2,200 pg ml⁻¹ h⁻¹</td>
<td></td>
</tr>
<tr>
<td>( k_{r_3} )</td>
<td>20,400 pg ml⁻¹ h⁻¹</td>
<td></td>
</tr>
<tr>
<td>( k_{r_4} )</td>
<td>900 pg ml⁻¹ h⁻¹</td>
<td></td>
</tr>
<tr>
<td>( \text{basal} )</td>
<td>63,000 pg ml⁻¹ h⁻¹</td>
<td></td>
</tr>
<tr>
<td>( t_5 )</td>
<td>10 ng/ml</td>
<td></td>
</tr>
<tr>
<td>( n_s )</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>( D_1 )</td>
<td>0.82 h</td>
<td></td>
</tr>
<tr>
<td>( t_2 )</td>
<td>10 pg/ml</td>
<td></td>
</tr>
<tr>
<td>( n_s )</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>( t_4 )</td>
<td>28 pg/ml</td>
<td></td>
</tr>
<tr>
<td>( n_s )</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>( t_1 )</td>
<td>357 pg/ml (women)</td>
<td></td>
</tr>
<tr>
<td>( n_s )</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>( D_2 )</td>
<td>0.2 h</td>
<td></td>
</tr>
<tr>
<td>( t_3 )</td>
<td>400 pg/ml</td>
<td></td>
</tr>
<tr>
<td>( n_s )</td>
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<td>( t_{5,0} )</td>
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<tr>
<td>( n_{s,0} )</td>
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<tr>
<td>( n_{s,1} )</td>
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<tr>
<td>( t_{5,2} )</td>
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</tr>
<tr>
<td>( n_{s,2} )</td>
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Table 2. Physiological interpretation of parameters in the GH feedback construct

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mathematical Description</th>
<th>Physiological Interpretation</th>
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<tbody>
<tr>
<td>( k_i )</td>
<td>Elimination rate</td>
<td></td>
</tr>
<tr>
<td>( k_{r_i} )</td>
<td>Secretion rates</td>
<td></td>
</tr>
<tr>
<td>( S_{\text{basal}} )</td>
<td>Basal secretion rate</td>
<td></td>
</tr>
<tr>
<td>( t_{\text{onset}} )</td>
<td>Thresholds and Hill coefficients</td>
<td></td>
</tr>
<tr>
<td>( D_1, D_2 )</td>
<td>Delays</td>
<td></td>
</tr>
<tr>
<td>( \text{GHS} )</td>
<td>Ghrelin concentration</td>
<td></td>
</tr>
<tr>
<td>( g_0, g_1, g_2 )</td>
<td>Scaling constants</td>
<td></td>
</tr>
</tbody>
</table>
duration = 0.2 h, and \( k_{ghr} = 1 \text{ h}^{-1} \), and then dividing the right-most term in Eq. 2 by \((1 + ghr)\). This formulation assumes suppression of SRIF_{PeV} secretion by l-arginine infusion.

4) Continuous combined infusion of GHRH and GHS was implemented as described in schema 1 and 2 with a duration = 3 h and a magnitude of \( C = 3,000 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1} \) for GHRH and \( C = 2,500 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1} \) for GHS.

**Numerical Procedures and Initial Conditions**

Model equations were integrated using a Runge-Kutta 4 algorithm (Berkeley Madonna software, version 8.0.1). A constant value for the unknown delayed functions on the required time interval was assumed to generate appropriate initial conditions. Infusion simulations were performed after generating 50-h run-in profiles, thereby marginalizing any impact of initial conditions. Random changes in the initial conditions were used to verify that simulation results were robust. Simulations were calculated at discrete intervals of 0.01 h in most cases (for technical reasons, we used a step of 1 min for the ApEn analysis).

**Parameter Sensitivity Analysis**

Parameter sensitivity was assessed in relation to the ApEn statistic (93), a regularity measure that strongly discriminates male and female patterns of GH output in both the human and rat (45, 94). ApEn values are higher in women than in men, signifying reduced regularity (increased disorderliness). The threshold \((r)\) was 0.2 SD, and the pattern range \((m)\) was 1 for ApEn calculations performed using simulated 10-min GH time series over 24 h, as validated earlier (95). No nycthemeral rhythmicity in GH release was superimposed. We computed changes in the ApEn values corresponding to deviations from the control values of selected model parameters (half-lives, secretion rates, and delays). The parameters were varied in a way that the resulting GH model output remains pulsatile in both men and women.

**RESULTS**

**Human Reference Model Output**

Figure 2 illustrates concomitant GH, GHRH, SRIF_{ArC}, and SRIF_{PeV} concentration-time series predicted by the foregoing construct. Predicted GH pulse frequency was gender independent, but pulse amplitude was twofold higher in women \((right)\) than in men \((left)\). These outcomes are consistent with the postulate that a suitable core model structure can render distinct pulse renewal properties in the female and male on a species-defined basis.

Simulated GH responses to paired secretagogue infusions are summarized in Fig. 3. The plots give projected effects of combined bolus l-arginine/GHS injection at 52:00 h \((top)\), bolus l-arginine/GHRH injection at 52:00 h \((middle)\), and continuous 3-h GHRH/GHS infusion \((bottom)\) beginning at 52:00 h in men \((left)\) and women \((right)\). The construct forecasts twofold larger GH peaks in women than in men given l-arginine/GHRH and or l-arginine/GHS, with a minimal \((30\%)\) sex difference during combined continuous stimulation with GHRH and GHS. The model-generated results are consistent with published clinical data (109, 129).

**Feedback Actions of GH**

The feedback effect of injected GH is greater \((defined\) by a fractional or absolute decrement in GH concentrations) and postinhibitory rebound-like GH release is higher in women than in men (124, 127). Plausible bases for the sex differences were evaluated by simulating feedback by either of two doses of infused rhGH. GH infusion was defined in Eq. 3 as \( ghr(t) \) with onset = 52:00 h, duration = 0.2 h, \( k_{ghr} = 2.1 \text{ h}^{-1} \), and a magnitude of \( C = 120 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1} \) or \( C = 280 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1} \). The elimination parameter, \( k_{ghr} \), assumes that the feedback action \((functional\) effect) of infused GH is \(\sim 1.5\)-fold longer than its plasma half-life (107, 127, 134). Figure 4 illustrates model-developed responses to the low \((top)\) and high \((bottom)\) GH feedback dose in men \((left)\) and women \((right)\). The higher feedback signal prolonged the duration of GH suppression in both sexes. Feedback-enforced decrements \((absolute\) and fractional) and postinhibitory rebound-like increments in GH concentrations are greater in women than in men, consistent with clinical outcomes (98, 124, 127).

**Individual vs. Combined Administration of GHRH and GHS**

In vivo synergy between GHRH and GHS. Model responses to individual and combined administration of GHRH and GHS were evaluated using the following parameters: for GHRH, onset = 52:00 h, duration = 0.2 h, \( k_{ghr} = 15 \text{ h}^{-1} \), and \( C = 90,000 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1} \); for GHS, onset = 52:00 h, duration = 0.2 h, \( k_{ghr} = 15 \text{ h}^{-1} \), and \( C = 7,000 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1} \). The term...
**GHRH** was added to GHRH in Eq. 4 of the core construct. Analogously, **ghr**GHS was added to GHS in Eq. 1 and **ghr(t)/10** to GHS in Eq. 4. As illustrated in Fig. 5A, the model forecasts in vivo synergy between (supra-additive effects of) GHRH and GHS in both sexes and a larger GH response to GHS in women than in men (15, 17, 18). GHRH/GHS synergy also was rendered for a minimally stimulatory dose of GHS (defined by $C_{H11005}/H18528 g/ml/H110021/h$) and a near maximally effective dose of GHRH ($C_{H11005}/H18528 20,000 pg/ml/H110021/h$) (Fig. 5B).

Simulations next explored the hypothesis that unequal SRIF outflow to the pituitary gland determines momentary variability in synergy between GHS and GHRH. In the model, maximal SRIF outflow occurs during interpulse nadirs and minimal SRIF outflow during GH volleys. To address the variability postulate, we simulated administration of a fixed bolus injection of GHRH ($C = 12,000 pg/ml^{-1} \cdot h^{-1}$), GHS ($C = 1,600 pg/ml^{-1} \cdot h^{-1}$), or both during a volley and a trough phase of the GH pulse renewal cycle. Figure 6 shows predicted GH pulse profiles in this setting. Model-based outcomes were greater synergy during increased SRIF outflow (trough period) in both sexes in terms of the degree of increase of the response to combined vs. the sum of individual secretagogues.

**Lack of in vitro synergy between GHRH and GHS.** Model-defined responses to the stimulus paradigm given in Fig. 5A were tested under simulated in vitro conditions by nullifying endogenous GHRH, SRIFPeV, and GHS inputs in Eq. 4 so that GH release is stimulated only by exogenous GHRH and GHS. Under these conditions (METHODS), there is no synergy (Fig. 7).

**Rebound GH Secretion After a SRIF Withdrawal**

Figure 8 summarizes the outcome of simulated intravenous infusion of SRIF in men (left) and women (right) over the interval from 52:00 h until 56:00 h. Systemic SRIF was assumed to act solely on the pituitary gland (no access to the CNS) (top) or, as in the case of octreotide, on both the pituitary and hypothalamus with partial (middle) or full access (bottom) to CNS. SRIF infusion was simulated with the following parameters: onset = 52:00 h, duration = 4 h, $k_{SRIF} = 25 h^{-1}$, and $C = 2,000 pg/ml^{-1} \cdot h^{-1}$. The term $ghrSRIF$ was added to $SRIF_{PeV}$ in Eq. 1 (full access to the pituitary), and the term $\alpha \cdot ghrSRIF$ was added to $SRIF_{PeV} + SRIF_{ArC}$ in Eq. 4, with parameter $\alpha$ modeling variable access to the CNS. Minimal
partial access to the CNS was modeled with $\alpha = 0.1$ and full access with $\alpha = 1$. The model predicts that in both sexes, full access vs. no access to the hypothalamus will elicit higher rebound GH release. However, it is possible that some limited access will actually restrict the response, since it will decrease the GHRH amplitude but will be insufficient to evoke a large rebound hypothalamic GHRH release (Fig. 8, middle).

**DISCUSSION**

Understanding the mechanisms controlling a specific endocrine axis is often challenged by the high complexity of the system. Apparent behavior is typically a result of the convoluted interplay among multiple hormones and neuroregulators, which limit intuitive reconstruction of control mechanisms. In this regard, mathematical models can aid intuition and add to one’s ability to dissect a particular endocrine mechanism and explore the consistency of various regulatory hypotheses. In this work we follow a minimal model approach in an effort to identify the minimal possible system components that could explain selected properties of the GH axis in the human. This approach can generate testable predictions to experimentally support, modify, or reject the underlying regulatory model. We build on previous experience in constructing minimal GH models in the rodent, which included basic features of GH secretion, such as the existence of a high-frequency (45–90 min) GH rhythmicity in the male and female, additional 3.3 hourly periodicity in the male, higher amplitude and lower baseline in the male, and less orderliness in the female, while explaining important experimental observations. The present analyses show that a minimal feedback model simulating time-delayed nonlinear interactions among GHRH, SRIF, GHS (ghrelin), and GH can explain in vivo synergy between GHS and GHRH, while embodying complex sex differences in GH pulsatility in humans opposite to those in the rat (7, 17, 41, 52, 129). The model condition required for synergy (supra-additive effect of combined stimuli) is that GHS directly oppose SRIF’s inhibition at the pituitary level. This condition is fulfilled in mammals (19, 31, 32, 68, 97, 110, 137). Conversely, the formulation correctly excludes direct in vitro GHS/GHRH synergy and in vivo inhibition by GHS of PeV SRIF release (15, 50, 129). Testable predictions of this construction are that in vivo synergy would be abolished if either 1) GHRH or 2) GHS did not stimulate somatotropes and 3) SRIF did not inhibit pituitary GH release directly. The first prediction is realized in humans and rats administered a GHRH receptor antagonist, as well as in patients and mice harboring inactivating mutations of the GHRH receptor (43, 49, 87, 100). Testing the second prediction would require pituitary-selective disruption of the GHS receptor, which has not been described. The third prediction is fulfilled in vitro inasmuch as SRIF is not present and in vivo when structural hypothalamicpituitary disconnection occurs (42, 96, 101). Isolated disruption of pituitary SRIF receptors has not been reported. However, indirect experimental data indicate that anesthesia (putatively by restricting hypothalamic SRIF release) and passive immunization to SRIF (by muting pituitary inhibition) attenuate in vivo synergy between GHS and GHRH (16, 27). The foregoing congruence with empirical data is necessary albeit not sufficient to prove model validity.

Model simulations established that prominent male/female differences in GH secretion in the human and rat can be
conferred by a shared core structure of peptide interactions. In particular, sex-specific contrasts were explicable through intra- and interspecies differences in 1) GH feedback-induced SRIF outflow from PeV to the pituitary gland and to GHRH neurons in Arc and 2) potencies and efficacies of GHRH, SRIF, and GHS. Whether a comparable strategy for model generalization would apply to other species is not yet ascertainable. However, to the extent that neurophysiological connections within the hypothalamopituitary unit are consistent among mammals (47, 129), a general model would allow one to explore both species- and sex-defined regulatory mechanisms. The accompanying analyses suggest that framing a unifying model should be possible whenever one is able to define (at a minimum) relative potencies and efficacies of GHRH, SRIF, and GHS and sex-specific predominance of GH feedback.

An assumption of the construct developed is that GH feeds back on the CNS via its receptor to induce PeV SRIF outflow (9, 22, 23, 25, 29, 43, 77, 103, 114). The molecular mechanisms may involve GH receptor-mediated increases in STAT5b (signal transducer and activator of transcription) gene expression chronically and STAT5b phosphorylation acutely (11). The physiological consequences of SRIFPeV outflow include brief repression of Arc GHRH neurons and somatotrope GH release, followed by reboundlike release of GHRH and GH (14, 67, 76, 83, 99). The precise neurophysiological basis for SRIF-induced neuronal GHRH rebound is not clear. However, partial silencing of the hypothalamic subtype 1 SRIF receptor (SSTR-1) but not SSTR-2 in the adult male rat diminishes GH pulsatility markedly (66), whereas transgenic knockdown of whole body SSTR-2 blunts GH autoregulation in male mice (138). Because neither study included sex comparisons, the precise roles of SSTR isofoms in mediating sex differences in the rodent remain unclear. The existence of subpopulations of somatotropes with distinguishable responses to SSTR-1/2 and SSTR-5 agonists in some species could allow for pituitary-selective mechanisms of sexually dimorphic GH regulation (73). The present general platform offers a basis for incorporating such additional complexity of physiological control.

Actions of GHS represented in the model are inferable from in vivo and in vitro experiments in the mouse, rat, sheep, and human (129). The bases are as follows. First, transgenic knockdown of neuronal GHS receptors in female mice and systemic administration of a GHS-receptor antagonist in the male rat indicate that GHS amplifies the size of GH pulses without accelerating their frequency (24, 108, 139). The same inference applies to GH pulses monitored during continuous GHS infusions in humans (17, 55, 58, 106). The relevance of GHS-receptor activity in the human was recognized in a recent study of short children harboring mutations in GHS-receptor signaling (88). Second, data in the ram indicate that a large dose of GHS promptly evokes GHRH release into portal blood, consistent with proposed pathways (50). Third, administration of GHS stimulates GH secretion directly in vitro two- to fourfold (129) and comparably in vivo in patients with inactivating

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Fig. 5. Model projection of in vivo synergy between GHRH and GHS. Data represent the individual effects of GHRH (top) and GHS (middle) compared with their combined action (bottom) in men (left) and women (right). Doses tested include supramaximal GHRH and submaximal GHRP-2 (A) and maximal GHRH and minimal effective GHRP-2 (B).
Fig. 6. Simulated GH and SRIF time series in women and men, wherein saline (reference) or GHS and/or GHRH were infused by bolus during a peak (volley) or a trough period of the GH renewal cycle.
mutations of the GHRH receptor, thus defining GHRH-independent effects (49, 100). A submaximal GHS stimulus synergizes with a maximally effective GHRH stimulus in the rat and human, indicating that GHS-induced GHRH release is not required (although it may occur) to explain synergy (7, 17, 52) (see below). Moreover, the efficacy of GHS greatly exceeds that of GHRH in the human and monkey, thus establishing that GHS does not act solely by releasing GHRH (3, 78). Fourth, GHS does not reduce SRIF outflow into portal blood but opposes two significant (submaximal) actions of SRIF, namely, repression of ArC gene expression and direct inhibition of somatotrope secretion (19, 34, 36, 50, 102). These collective inferences are embedded in a four-part mechanistic model, in which GHS evokes GHRH secretion from ArC, opposes SRIF’s inhibition of GHRH neurons, antagonizes SRIF action on the pituitary, and stimulates pituitary somatotrope GH secretion additively with GHRH (see Eqs. 1 and 4, METHODS). Exploratory sensitivity analyses (Table 3) are consistent with bounded roles for each of these four mechanisms in mediating GHS’s augmentation of GH pulse size. What cannot be determined from available in vitro and in vivo experiments and current simulation studies are the relative contributions of the foregoing individual regulatory mechanisms. In addition, model structure does not exclude the operation of unknown systemic, pituitary, or hypothalamic factors that might potentiate or transduce synergistic effects of submaximally stimulatory GHS and maximally stimulatory GHRH signals.

The sexual dimorphism of GH secretion patterns is opposite in the human and rat. Although the bases for such distinctions are not known, we demonstrate that a common structural model can explicate sex differences in GH regulation in the two species. Clinical data motivated evaluation of a finite set of pathway differences in women compared with men: 1) greater GH feedback-evoked SRIF outflow from PeV to both ArC and the pituitary gland (the opposite of the female-male contrast in the rat), 2) higher GHRH potency (opposite from the rat) and greater pituitary GHS efficacy in women (similar to the sex difference in the rat), 3) lower pituitary SRIF potency in women than in men (opposite to in vitro data in the female and male rodent), and 4) comparable GH kinetics in the two sexes (similar to other species) (41, 47, 80, 104, 118, 121, 129, 130). The first gender difference explains greater fractional feedback suppression of GH concentrations in women than in men (124, 127). The second and third pathway properties reflect clinical data from intravenous peptide dose-response studies conducted with and without estradiol supplementation (17, 20, 120, 122). Comparable GH kinetics have been verified directly (121).

How androgens impact the dose-dependent effects of GHRH, SRIF, and GHS in humans is unknown. However, testosterone supplementation augments reboundlike GH secretion after GH feedback in adults (98, 123) and potentiates stimulation by GHS in children but not adults (10, 69, 126). Thus the extent to which apparent sex-related differences in GH secretion patterns reflect the effects of testosterone vis-à-vis estradiol cannot be defined as yet in humans.

The approximate entropy statistic, ApEn, was used as a sensitive model-free measure of the relative regularity or orderliness of GH secretory patterns (93). Orderliness monitors feedback coordination within an interlinked system. Studies in the human and rat demonstrate greater irregularity of GH patterns in the female than male and in puberty than adulthood (45, 94, 131). The ApEn analysis of the reference model profiles was consistent with these observations and showed that women have higher ApEn values compared with men (1.05 vs. 0.91). To analyze the dependence of the ApEn values on the choice of parameters, we computed the changes in the ApEn values corresponding to deviations from the nominal (control) values of selected individual model parameters (half-lives, secretion rates, and delays; Table 3). For each tested parameter, two values below and two values above the nominal value were chosen with a goal to maintain reasonable pulsatile GH profiles in both men and women. The extreme values (double down and up arrows in Table 3) correspond to values for which the GH profile subjectively loses its basic properties (pulsatility, multiphase character of volleys, unrealistic increase or decrease in GH amplitude) in the model for either men or women. The intermediate values (single down and up arrows in Table 3) were chosen in the middle, between the extreme, and the nominal control values. The results indicate that the orderliness of the GH dynamics varies with the parameter choice, and various combinations can guarantee the desired greater orderliness in men. In the reference female model, because of the specific relationships between the model parameters (primarily the delays), the mechanism that regulates the volley recurrence repeats itself every other volley, whereas in men, the mechanism is the same at every volley, which explains the sex difference in ApEn.

The core construct was used to explore mechanism(s) mediating sex differences in GH responses to both single (above)
and paired secretagogues, namely, GHS, GHRH, and L-arginine. L-Arginine is construed to be an inhibitor of SRIF outflow, because it overcomes negative feedback by systemically infused GH (1, 2, 23, 46, 47, 90). Under this assumption, model-assisted predictions are that women compared with men have greater L-arginine-relieved SRIF outflow from PeV to both ArC and the pituitary gland, as well as greater GHS-mediated opposition to SRIF’s inhibition on the pituitary. The proposed mechanisms are concordant with clinical studies showing that L-arginine is more stimulatory in women than in men (82); GHS induces larger GH pulses in women than in men and in pubertal girls than in boys (10, 69, 128); triple administration of GHS, GHRH, and L-arginine abolishes the sex difference in GH secretion (5); female sex and estrogen supplementation amplify reboundlike GH secretion after infusion of a GH pulse or injection of SRIF (120, 124); testosterone supplementation does not potentiate maximal individual or combined GHS and GHRH drive (126); and octreotide, a synthetic SRIF, inhibits the individual more than the combined actions of GHS and GHRH more than their combined effects (6, 56).

An important model-based prediction addresses the varying potential of ghrelin and GHRH to act in synergy at different concentration levels. As the parameters of the model are chosen, the GHRH neurons are capable of secreting GHRH at amplitudes much higher than the ED50 of GHRH at the pituitary (1,600 vs. 400 pg/ml). This does not occur under normal conditions but can happen (briefly) under appropriate stimulation by ghrelin. This means that if the ghrelin/GHS stimulus is very high (in the hypothalamus), it will cause maximal endogenous release of GHRH. Thus GHRH receptors at the pituitary and the GH secretion become limiting when one combines exogenous GHS and GHRH, eliminating synergy. However, some interaction will be visible, since exogenous GHS cannot completely oppose SRIF in the hypothalamus. Figure 9 shows the model response to a very high GHRP stimulus. In contrast to Fig. 5 (low GHS), at very high GHRP, the model predicts complete loss of synergy in women and significant reduction in men.

Another model-assisted prediction addresses the possibility of direct effect of ghrelin on GHRH-secreting neurons in the CNS (we note that the initial model assumed that the GHRH-stimulating action of ghrelin was exerted exclusively by opposing SRIF in the ArC). In the current formulation, the direct action is required to be able to reproduce a 30% reduction in GH amplitude observed in the ghrelin receptor knockout mice if the knockout is isolated to GHRH neurons (108).
simulations showed that 30% reduction could be achieved by decreasing the secretion rate of GHRH by 8%. To incorporate direct action into the model, we replace the current Eq. 4 by

\[ \text{GHRH} = -k_1 \text{GHRH} + k_{p3} \left[ \frac{0.92 + 0.08}{1 + (\text{GHS}/T)^n} \right] \]

where \( T = 8 \), and the slope \( n = 4 \). All other parameters remain the same.

The low value of \( T \) means that changes in typical endogenous ghrelin concentrations will cause changes in GHRH secretion. Consequently, in its direct action on the GHRH neurons, ghrelin is about 500 times more potent than when it opposes SRIF at the same neurons. However, an increase in hypothalamic ghrelin above its typical levels will not cause substantial increase in GHRH outflow, and blocking the GHS receptors on these neurons will decrease GH amplitude by 30%. Therefore, the maximal response to the direct effect would be limited. This simulation explains GHRH/ghrelin synergy at low but not maximal ghrelin drive in the human (129).

Caveats include the following considerations. Model realizations are necessary but not sufficient to verify proposed relationships in biological systems. Reliable model performance does not exclude the possible importance of unmodeled or unknown pathways. The narrow range of parameter sensitivity observed in some cases indicates that further model refinement is needed as well. Because the present construct encapsulates acute dynamics of GH regulation, further model terms are necessary to explicate chronic adaptations due to aging, disease, and sustained over- or underexpression of GH, GHRH, SRIF, and GHS peptides or their receptors (47, 65, 70, 79, 129). The sources of GHS acting on the pituitary and hypothalamus are not separately specified presently but could include, minimally, the hypothalamus, pituitary, and stomach (30, 63, 64). Other collateral pathways, such as proopiomelanocortin and neuropeptide Y neurons, regulated by GHS are not contained in the model (33, 89). Last, sex differences inferred in the human GH axis are assumed to reflect the dissimilar sex-steroid milieus, but other factors are not thereby excluded. A potential limitation is that the model is not completely validated, and some parameters are functionally determined, since Hill coefficients or delay times within the hypo-

### Table 3. ApEn values for deviations from the nominal (control) values of selected individual model parameters

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Value</th>
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<th>ApEn women</th>
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ApEn, approximate entropy values. See text for details.
thalamus cannot be determined directly at local interface sites in the human. However, we note that the parameter determination was performed under the restrictions described in Model Assumptions and System Parameters in Humans, and the ApEn partial parameter sensitivity analysis indicated that individual numerical choices are not highly constraining to general model performance.

In summary, a minimal network model of dose-responsive (nonlinear) time-delayed interactions among GHRH, SRIF, GHS, and GH feedback establishes a general platform for representing experimentally verified differences in GH dynamics in the rat and human. The construct predicts 1) comparable GH pulse frequency in women and men and 2) larger GH pulses, more irregular GH patterns (higher ApEn), greater negative feedback, heightened reboundlike GH secretion, and accentuated synergy between SRIF withdrawal and GHS feedforward in women compared with men. The present unification of GH models in the human and rat offers precedence for developing generalizable regulatory constructs in other endocrine, metabolic, and physiological systems.

Perspectives

The primary anatomic locations, receptors, and individual actions of many physiological effectors are known. Nonetheless, network-level feedback and feedforward relationships are not so readily established. The challenge arises because experimental isolation of any one regulatory locus or connecting pathway definitionally disrupts ongoing interactions within the system as a whole. Accordingly, model-assisted simulation of signal exchange provides an integrative tool to examine complex dynamics that are difficult to visualize intuitively. Intuitive reconstruction of network properties is challenging, given unequal time delays, nonlinear dose-response properties, combined feedback and feedforward effects, and stochastic variability (60, 61). The last element has not yet been incorporated systematically into models of GH dynamics, given limited understanding of the proximate sources of unexplained variability in this system. A further need in the field is to develop analytical capabilities from basic simulation models. One technical impasse in the human GH axis is that at least three important unobserved signals (GHRH, SRIF, and GHS) exist that act on a single monitored output (GH). At present, modeling capabilities are restricted to estimating only one unknown signal in a three-node system (62). Last, individual models must be recognized as neither exclusive nor complete. In this regard, Fig. 10 illustrates several other ways to formu-

Fig. 9. Model prediction of loss of in vivo synergy between GHRH and GHS at maximally effective GHS concentrations.

Fig. 10. Unexplored models of GH pulse renewal based on inferred neuroanatomic connectivity. SSTR, SRIF receptor.
late the GH pulse renewal process that can be inferred from published experimental data but are different from the connectivity proposed in this work. Note that in these models the high-frequency pulsatility arises from a putative negative (auto) feedback of SRIF on its own release. Therefore, regulatory and integrative models should be construed as existing along a pathway of evolving utility, wherein significant advances require concomitant structural definition of the system, interventional experiments, model synthesis, and revision (91, 112).

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