Leading Article

Current Concepts of Estrogen-Regulated Growth Hormone Secretion in Postmenopausal Women

JOHANNES D VELDHUIS

General Clinical Research Center, Mayo School of Graduate Medical Education,
Mayo Clinic, Rochester, MN, USA

Epidemiological investigations correlate organic growth hormone (GH) deficiency (hyposomatotropism) with decreased insulin sensitivity, dyslipidemia, increased cardiovascular mortality, intra-abdominal adiposity, sarcopenia, osteopenia, diminished quality of life, and possibly reduced cognitive function [1,2]. Aging increases the prevalence of these signs and symptoms and is associated with an exponential decline in systemic GH and insulin-like growth factor-I (IGF-I) concentrations, beginning at the transition between late puberty and early adulthood [3–5]. However, maximally stimulated GH secretion, GH elimination kinetics, and the capability of GH to drive hepatic IGF-I production are preserved in the older individual [6–9]. Thus, aging appears to impair the normal regulation of GH secretion, rather than irreversibly reducing the capacity to secrete GH or blocking the actions of GH on the liver.

Visceral adiposity, increasing age, low sex-steroid hormone concentrations, and limited aerobic capacity are major predictors of decreased GH availability [4,10–12]. Substantial weight loss and prolonged aerobic training can double 24 h-integrated GH concentrations in the young adult, but may be less effective in the older individual. However, supplementation with estradiol or an aromatizable androgen (e.g. testosterone) stimulates GH production several-fold in both aging and hypogonadal adults [10,11].
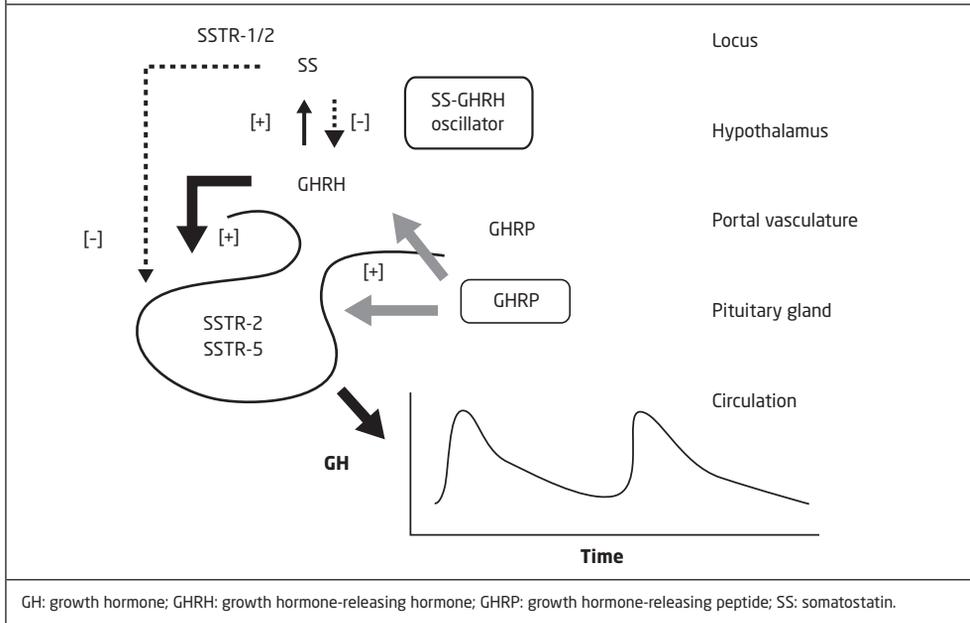
Estrogens are primary agonists, inasmuch as the formation of estradiol from testosterone contributes significantly to the gonadal-steroid drive of GH secretion. Significant advances have been made in elucidating some of the basic mechanisms that mediate estrogenic stimulation of the human somatotropic axis in older individuals, and these are discussed here.

Mechanisms of GH control

Clinical studies have combined specific high-sensitivity GH assays, intensive blood-sampling schedules, innovative peptidyl infusions, validated analytical methods, and model-assisted formulations to dissect the mechanisms of GH regulation in health and disease [13]. Such strategies establish that the proximate basis for low GH concentrations in aging individuals is a reduction in the mass of GH secreted per burst (defined as μg of GH released per unit distribution volume per pulse), with minimal or no change in the basal secretion, pulse frequency, or plasma half-life of GH [4,5,10,11]. The insight that aging selectively reduces GH secretory-burst mass has led to recent investigations focusing on the mechanisms that generate and sustain high-amplitude GH pulses in the healthy young adult [14].

Collectively, data from laboratory and clinical investigations have indicated that coordinated interactions among systemic, hypothalamic, and pituitary peptides and sex steroids jointly govern the amount of GH

Figure 1. Schematic illustration of ensemble regulation of GH secretion by the primary peptides GHRH, SS, GHRP/ghrelin, and GH. Stimulatory inputs are marked by continuous arrows and +, and feedback inhibitory pathways by interrupted arrows and – symbols. GH, as well as insulin-like growth factor-I (not shown), enforces negative feedback by evoking SS release and repressing GHRH secretion. SSTR-2 and SSTR-5 denote two subtypes of SS receptors in the pituitary glands. SS-receptor subtypes 1 and 2 mediate intrahypothalamic inhibition of GHRH neurons.

GH: growth hormone; GHRH: growth hormone-releasing hormone; GHRP: growth hormone-releasing peptide; SS: somatostatin.

secreted per burst [13,15]. However, the species selectivity of sex-steroid action makes inferences gained in the rat, mouse, pig, and sheep illustrative of, rather than definitive to, the human. For example, estrogens suppress, while non-aromatizable androgens augment, GH pulse height in the rat, but exert opposite effects in the human [12]. Nonetheless, a fundamental inference in all species is that GH-releasing hormone (GHRH, a 41- and 44-amino acid peptide), the GH-releasing peptide (GHRP) ghrelin (28-amino acid peptide), and somatostatin (14-amino acid peptide) constitute a core ensemble of potent GH regulators. Feedback inhibition of GH secretion by systemic IGF-I and GH appears to maintain normal pulsatility by inducing the inhibitor, somatostatin, and repressing the stimulator, GHRH (**Figure 1**). GHRH and somatostatin are produced in discrete hypothalamic nuclei and released into pituitary-portal blood. Ghrelin peptide and gene transcripts are localized predominantly

(>70%) in the gastric fundus, as well as in diencephalic neurons and the pituitary gland [16]. Whether ghrelin is released into hypothalamo-pituitary portal blood under normal physiological conditions is not yet known.

Estrogenic and androgenic stimulation of GH release

The estradiol-enriched preovulatory phase of the menstrual cycle correlates with a doubling of GH concentration due to a doubling of pulsatile GH secretion [17]. Greater estrogen availability predicts higher mean GH concentrations and larger GH pulses in pre- and postmenopausal women compared with age-matched men [18–20]. In interventional studies, estrogen supplementation in girls with Turner syndrome, postmenopausal women, male-to-female transsexual patients, and men with prostatic cancer increased GH concentrations by 1.8–3.3-fold [2,10,12,21,22]. The route of estrogen delivery is not crucial,

Figure 2. Sex-steroid supplementation augments the size (mass) of GH secretory bursts in healthy older men and women [10,11].

E_2: estradiol; GH: growth hormone; PI: placebo; Te: testosterone.
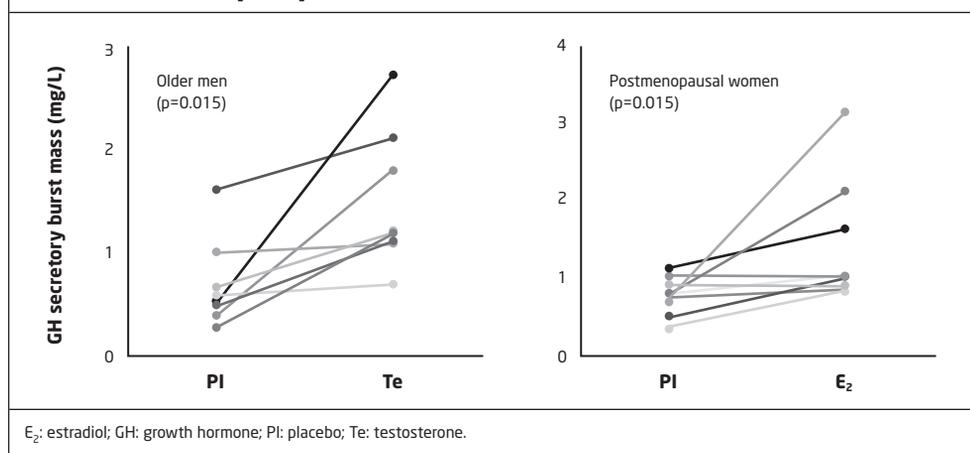
with intravenous, intramuscular, oral, intravaginal, intranasal, or higher-dose transdermal estradiol administration stimulating GH secretion [10,12,21–23]. The aromatizable androgen, testosterone, acts analogously [11].

Current analytical methods indicate that the gender distinction in GH concentrations and the stimulatory effects of estradiol and testosterone on GH production arise from the 2-fold augmentation of GH secretory-burst mass (**Figure 2**) [10,11]. This has motivated clinical studies to identify the mechanisms by which estrogen and androgen promote greater GH release within each pulse and, thereby, over 24 h. One goal of such investigations is to create a scientific platform for developing novel strategies to obviate hyposo matotropism, employing steroidal and nonsteroidal interventions, in both aging and hypogonadal individuals. Nonsteroidal interventions are of increasing importance in a subset of estrogen-deficient women who may be at increased risk of neoplastic, cerebro- and cardiovascular, thrombophlebitic, and cholestatic disease [24].

## Concept of ensemble-peptide control of GH secretion

Estrogen and testosterone selectively increase, whereas aging and hypogonadism primarily reduce, pulsatile GH production. This mechanistic insight is important since burst-like GH secretion constitutes the majority (88–94%) of total daily GH output [4,5,10,11,19,20]. Recent advances establish that GH pulse frequency does not vary during the course of development and aging; this leaves GH secretory-burst mass as the principal determinant of pulsatile GH secretion. Because the ensemble of GHRH, somatostatin, GHRP/ghrelin, GH, and IGF-I collectively control GH pulse size, a growing challenge is to visualize how mechanistic interactions govern the generation of GH pulses [13]. This issue has prompted the development of simplified biomathematical models to assist clinical interpretation [15]. Current analytical constructs indicate that consensus linkages among GHRH, somatostatin, and GH/IGF-I, along with the amplifying actions of GHRP/ghrelin, are necessary and sufficient to confer self-renewing, high-amplitude GH secretory bursts. Aging and estrogen appear to modulate each of these key peptidyl pathways.

Multisite actions of estradiol in the human

Clinical experiments have unveiled distinct hypothalamo-pituitary actions of estradiol that contribute to the amplification of GH secretory-burst mass. In principle, testosterone could drive pulsatile GH

Figure 3. Distinct mechanisms by which E_2 promotes pulsatile GH secretion in postmenopausal women. Estrogen decreases the inhibitory potency of SS and increases the stimulatory potency of GHRH, heightens feedback inhibition by rh-insulin-like growth factor-I (A, left to right), facilitates stimulation by GHRP-2 (a ghrelin analogue) in the absence or presence of negative feedback by a pulse of rhGH, and augments post-SS rebound-like GH release that is mediated via endogenous GHRH outflow (B, left to right).
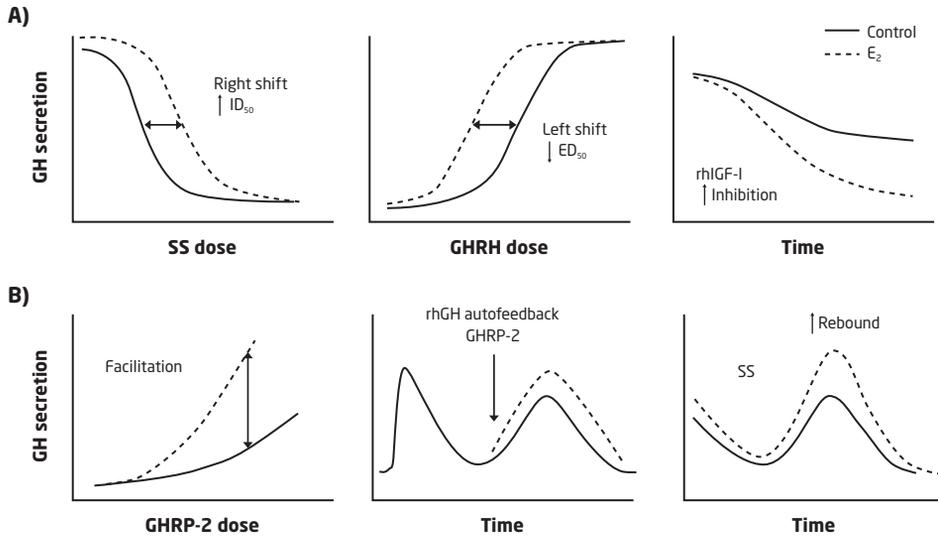
E_2: estradiol; GH: growth hormone; GHRH: growth hormone-releasing hormone; GHRP: growth hormone-releasing peptide; rh: recombinant human; SS: somatostatin.

secretion via analogous mechanisms, although few data are available that directly test this postulate.

In summary, short-term estradiol supplementation in postmenopausal women has the following effects [25–33]:

- Enhances the stimulatory potency of exogenous GHRH pulses.
- Attenuates the inhibitory potency of infused somatostatin.
- Potentiates stimulation by GHRP-2, which is a synthetic analog of ghrelin.
- Mutes negative feedback by a pulse of GH.
- Amplifies post-somatostatin rebound-like release of GH.
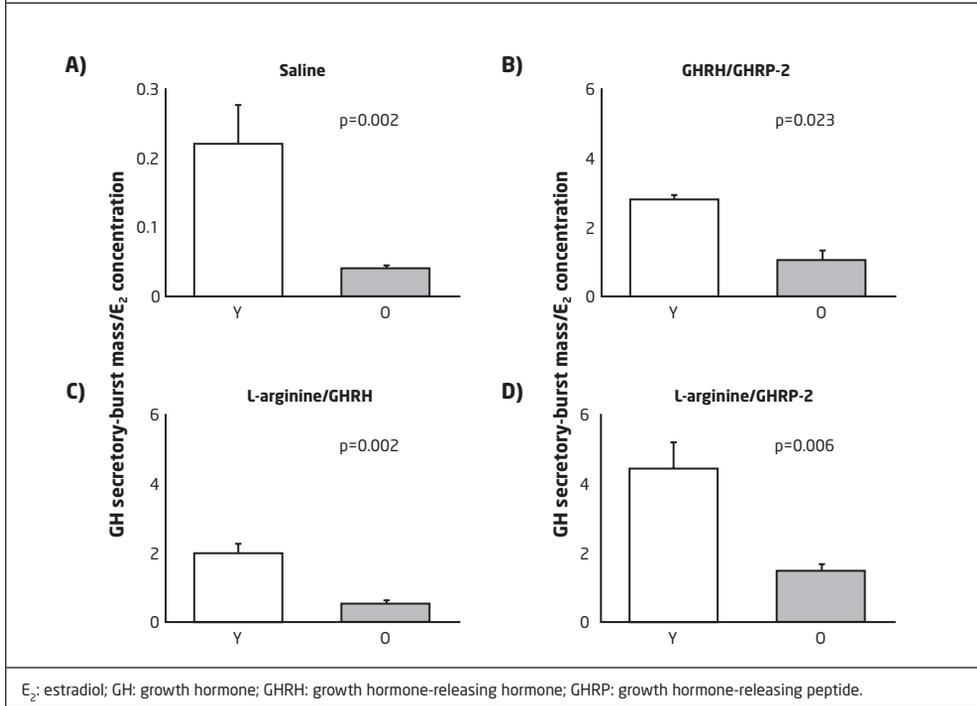- Accentuates inhibition of GH secretion by infused IGF-I.

This set of well-defined mechanisms is illustrated in **Figure 3**. From the collective evidence, it is likely that estradiol regulates interactions among GHRH, somatostatin, ghrelin, GH, and IGF-I. The aggregate effect is to augment pulsatile (and thereby total) daily GH production.

Investigation of ensemble mechanisms

A consequence of multipeptide control of pituitary-hormone secretion is that modulation of any given regulatory locus will also alter the output of interconnected sites [13]. The latter feature makes clinical inference difficult. For example, the finding that estradiol amplifies GH secretory-burst mass following a submaximal GHRH stimulus could in principle signify that estrogen upregulates somatotrope GHRH receptors, reduces negative feedback by systemic GH, attenuates inhibition by somatostatin, augments GHRH-releasable GH stores,

Figure 4. Despite identical E_2 concentrations, postmenopausal women (O) secrete 55-80% less GH in individual secretory bursts than young women (Y) when fasting (A) and in response to each secretagogue pair studied (as indicated in B–D) [37]. Y-axis values are GH secretory-burst mass normalized to experimentally controlled E_2 concentrations (µg/L)/(pg/mL).

E_2: estradiol; GH: growth hormone; GHRH: growth hormone-releasing hormone; GHRP: growth hormone-releasing peptide.

and/or potentiates the action of ghrelin, which alone stimulates GH secretion and synergizes with GHRH [32,34,35]. Therefore, interpreting precisely how estradiol directs multipeptide regulation cannot be easily deduced from any single experiment. Recent studies address this important issue through several new investigative strategies [29,33,36]:

- Simultaneous infusion of two peptides in order to unmask estradiol-dependent control of the heterotypic (non-infused) peptide.
- Feedback induction of hypothalamic somatostatin outflow by exogenous GH, injected prior to peptide infusion, in order to detect estradiol-regulated somatostatin restraint.
- Continuous GHRP stimulation, alone and combined with GHRH, in order to discern estradiol-modulated two-peptide interactions.

Because L-arginine has been shown to reduce somatostatin outflow in experimental animal models, secretagogue infusions occasionally utilize L-arginine combined with GHRH or GHRP-2 (**Figure 4**) [37]. In the experiment shown in Figure 4, young and older women initially received leuprolide to suppress ovarian estrogen secretion, and then received graded transdermal estradiol supplementation to "clamp" circulating estradiol concentrations equivalently in the two age groups. Fasting and secretagogue-stimulated pulsatile GH secretion rates were significantly reduced in postmenopausal compared with premenopausal women, despite comparable estradiol concentrations. Thus, age, rather than estrogen availability alone, reduces GH secretion in healthy individuals. Model-based analyses predict that impaired two-peptide stimulation in the elderly cohort reflects diminished endogenous drive by GHRH and ghrelin,

and heightened inhibition by hypothalamic somatostatin [15,38].

Investigative challenges

Elucidating the basis of complex physiological adaptations (e.g. those associated with aging) and pituitary responses to multipeptide interventions depends, in part, upon the availability, reliability, and incisiveness of analytical tools. Technical challenges include using serial GH concentrations to estimate regulatory endpoints, such as simultaneous feedback and feedforward within an axis, the timing and mass of underlying GH secretory bursts, constitutive GH release, biexponential elimination kinetics, and random variability in the data associated with sample collection, processing, and assay [15,38]. Recent biomathematical advances address some of these fundamental issues, as illustrated by clinical studies of the somatotropic, thyrotropic, corticotropic, and gonadotropic axes [15,26,38,39].

Significance of pulsatile and basal GH outflow to target tissues

In the experimental animal, GH regulates gene expression, in part, by inducing *in situ* IGF-I production and modifying local IGF-binding protein (IGFBP) availability [13,39]. GH promotes synthesis of IGF-I in the liver and kidneys, and systemic secretion of IGF-I by the same organs [40]. In addition, GH may act directly to stimulate lipolysis, pre-chondrocyte proliferation, growth of erythroid precursors, hepatic synthesis of the acid-label subunit and low-density lipoprotein receptor, and hormone and drug metabolism [40–42]. Both local and systemic IGF-I are physiologically important, because marked (>65%) depletion of systemic IGF-I concentrations, achieved in transgenic mice, does not restrict neonatal or pubertal somatic growth, but impairs carbohydrate balance and reduces bone density in the adult animal [40,43].
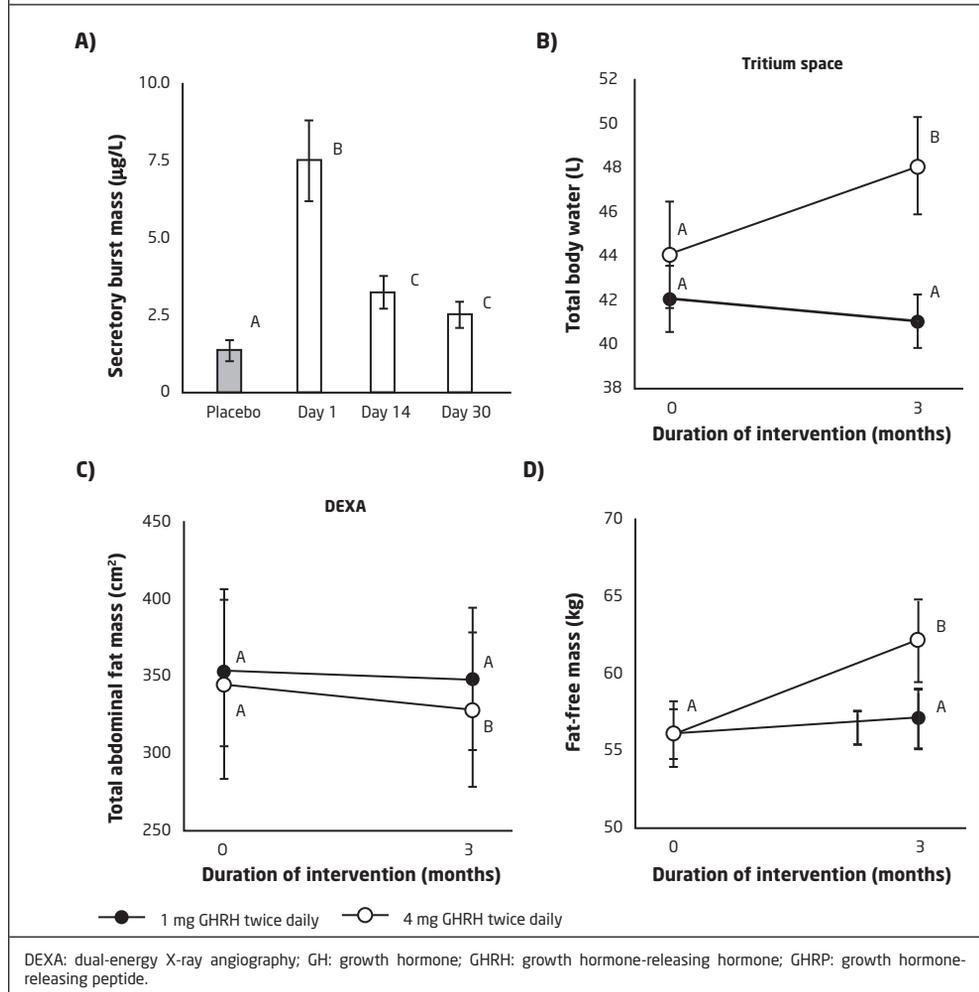
Sex-steroid hormones govern the actions of available GH, IGF-I, and IGFBPs in diverse target tissues [44]. For example, GH and estrogen modulate the production, turnover, and signaling of central nervous system IGF-I, which can enhance neurogenesis, cytodifferentiation, and memory in animal models [45]. Thus, an emerging notion is that GH and sex steroids provide conjoined control of tissue-specific IGF-I synthesis, secretion, binding, action, and degradation [12]. An implication of this is that interventions designed to elevate systemic GH concentrations would enhance physiological GH-dependent IGF-I synthesis, and thereby oppose some of the catabolic consequences of aging. At the cellular level, estrogens act via an array of full-length and truncated cognate receptors, which mediate gene repression and induction as well as rapid, membrane-dependent signaling cascades. Among the genes induced is a suppressor of cytokine (and thereby also GH) signaling (SOCS). These mechanisms are discussed elsewhere [46,47].

Clinical implications

In principle, the adverse consequences of diminished GH availability in aging adults could be overcome by supplementation with recombinant human GH [48,49]. However, there are no definitive safety data that justify long-term administration of GH in healthy older individuals. Injection of high doses of GH in certain clinical settings (such as protracted critical illness) can increase mortality rates, and excessive GH replacement in hypopituitary adults may elicit fluid retention, arthralgias, carpal tunnel syndrome, and (rarely) benign intracranial hypertension [50,51]. Therefore, complementary approaches include unraveling the primary mechanisms that regulate GH secretion and mimicking physiological control by steroid-independent and steroid-dependent means. Two such approaches have been assessed (**Figure 5**). The first approach showed that continuous subcutaneous infusion of GHRP-2 initially quadruples and then maintains 2-fold elevation of GH concentrations in older adults [36]. Another study demonstrated the capability of twice daily injections of GHRH for 3 months to increase total body water, decrease total abdominal fat, and elevate fat-free mass in healthy aging men [52]. In the latter study, stair-climbing and level-walking

Figure 5. Continuous subcutaneous infusion of GHRP-2, a ghrelin analogue, elevates GH secretion 4-fold in 24 h and 2-fold after 2 and 4 weeks in older adults (A; $p < 10^{-4}$) [36]. Twice-daily subcutaneous injection of recombinant human GHRH for 3 months in older men increases total body water (B; $p = 0.021$), reduces total abdominal fat (C; $p < 0.05$), and increases fat-free mass (D) [52].

DEXA: dual-energy X-ray angiography; GH: growth hormone; GHRH: growth hormone-releasing hormone; GHRP: growth hormone-releasing peptide.

performance also improved significantly after 3 months.

Conclusion

Tripartite depletion of systemic GH, IGF-I, and sex steroids in healthy postmenopausal women and older men is accompanied by an increased prevalence of clinical, hormonal, biochemical, and structural features of frailty with an attendant increased risk of physical disability and reduced quality of life. Supplementation with estradiol or testosterone augments GH secretion in both genders.

Fundamental mechanisms subserving the stimulatory actions of estrogen and androgen on the hypothalamo-pituitary control of GH secretion are being unraveled. Primary interactions involve the pivotal regulatory peptides GHRH, ghrelin/GHRP, and somatostatin, as well as feedback by GH and IGF-I. Far less is known about the testosterone-mediated mechanisms of stimulation, with the exception that some of its somatotropic effects are mediated following its aromatization to estradiol. Two recent interventional studies have indicated

that GHRH or GHRP administration for 1–3 months can increase both GH and IGF-I production by approximately 2-fold and modify body composition in older adults [36,52]. Continuing advances in this field should aid in the ultimate development of selective peptidyl, steroidal, nonpeptidyl, and nonsteroidal interventions that facilitate GH secretion in aging and hypogonadal adults.

Address for correspondence: JD Veldhuis, Division of Endocrinology and Metabolism, Department of Internal Medicine, General Clinical Research Center, Mayo School of Graduate Medical Education, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA. Email: veldhuis.johannes@mayo.edu

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