Synergy of L-arginine and growth hormone (GH)-releasing peptide-2 (GHRP-2) stimulation of GH release: Influence of gender

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Abstract:

We test the hypotheses that *1*) growth hormone (GH)-releasing peptide-2 (G) synergizes with l-arginine (A), a compound putatively achieving selective somatostatin withdrawal and *2*) gender modulates this synergy on GH secretion. To these ends, 18 young healthy volunteers (9 men and 9 early follicular phase women) each received separate morning intravenous infusions of saline (S) or A (30 g over 30 min) or G (1 μg/kg) or both, in randomly assigned order. Blood was sampled at 10-min intervals for later chemiluminescence assay of serum GH concentrations. Analysis of covariance revealed that the preinjection (basal) serum GH concentrations significantly determined secretagogue responsiveness and that sex ($P = 0.02$) and stimulus type ($P < 0.001$) determined the slope of this relationship. Nested ANOVA applied to log-transformed measures of GH release showed that gender determines *1*) basal rates of GH secretion, *2*) the magnitude of the GH secretory response to A, *3*) the rapidity of attaining the GH maximum, and*4*) the magnitude or fold (but not absolute) elevation in GH secretion above preinjection basal, as driven by the combination of A and G. In contrast, the emergence of the G and A synergy is sex independent. We conclude that gender modulates key facets of basal and A/G-stimulated GH secretion in young adults.

Keywords: male | female | pituitary | somatotropin | regulation | endocrine

Article:

Gender differences in basal (unstimulated) pulsatile growth hormone (GH) secretion are recognized in experimental animals and throughout the human lifetime (24, 28). Recent clinical studies have revealed other distinctions in the neuroendocrine control of GH release in men and women (55). For instance, Pincus et al. (40) reported that men exhibit more orderly patterns of GH release than women. Analogous gender differences are evident in the patterns of GH release

in rats (28, 36). Further laboratory investigations have demonstrated differential regulation of hypothalamic GH-releasing hormone (GHRH) and somatostatin gene expression in male and female rodents (4). Distinguishable mechanisms of GH release in men and women thus are likely to embody sex-specific neuromodulatory inputs to the hypothalamus and/or pituitary gland. Gender differences also exist in GH responses to certain discrete physiological and pharmacological stimuli (17,27). However, there is only a limited understanding of the exact neuroendocrine mechanisms that drive differential GH release in men and women (53).

l-Arginine (A) is a widely employed provocative test to evaluate the GH axis. Although the mechanism by which A releases GH in the human is not known definitively, on the basis of some, but not all studies, in the rat, a current proposition is that A stimulates GH release via withdrawal of hypothalamic somatostatin (2, 22,23). Early studies by Merimee et al. (34) reported that women manifest greater GH release in response to A stimulation than men and that this difference is due to estrogen exposure. Penelva et al. (37) reported that pyridostigmine, which also increases GH release at least in part via somatostatin withdrawal, slightly enhanced the GH secretory response to GH-related peptide (GHRP)-6. GHRP-2 (G) is a novel potent non-GHRH peptidyl stimulus for GH release in both men and women (7). Whereas some studies have suggested gender differences in GH responses to GHRP, others have not (6, 9, 37). One recent analysis suggested that there is no gender variation in maximal GHRP-stimulated GH release but rather a heightened sensitivity in women to low G doses (7).

In vivo in the rat and human, combined infusions of GHRH and GHRP exert synergistic rather than additive actions. Whereas the precise mechanistic basis for this synergism has not been elucidated, the two effectors clearly activate different cellular signaling pathways (1, 14). Moreover, each of these peptidyl agonists can partially restrain the inhibitory effects of somatostatin (24).

The current clinical study investigates the ability of A and G to act synergistically in healthy young adults and tests the hypothesis that the synergy is gender dependent.

METHODS

Clinical protocol. Eighteen healthy subjects [9 men (means ± SE) (age = 25 ± 1.5 yr, height = 178 ± 1.0 cm, wt = 75.1 ± 1.8 kg, total body fat = 14.5 ± 1.9%) and 9 women (age = 25 ± 1.0 yr, height = 169 ± 2.0 cm, wt = 66.5 ± 3.1 kg, total body fat = 22.6 ± 1.8%)] participated in the present study. All volunteers underwent a detailed screening medical history and complete physical examination and provided written informed consent as approved by the Human Investigation Committee at the University of Virginia. Subjects were not taking any medications or hormones and were moderate habitual exercisers (20–30 min of aerobic exercise, 3 or 4 times/wk).

Body density was measured by hydrostatic weighing (30). Residual lung volume was measured by oxygen dilution (62). Each subject was weighed in air on an Accu-weigh beam scale accurate to 0.1 kg and again underwater on a Chatillon autopsy scale accurate to 10 g. Percentage body fat was calculated using the equation of Brozek et al. (11).

Subjects were admitted overnight to the General Clinical Research Center on four separate occasions (at rest). Admissions were scheduled at least 2 days apart and followed the study-specific randomization schedule based on gender, which was produced by SAS (Proc Plan). Women were studied during the early follicular phase (*days 2–8*) of the menstrual cycle. Volunteers received a standardized constant meal, based on body weight, at 1700 the evening before the study. Caloric content of the meal was calculated as 0.33 × 37 kcal/kg for females and 0.33 × 38.5 kcal/kg for males, which included an activity factor for moderate activity (19). The nutrient composition of the meal was fixed at 55% carbohydrate, 30% fat, and 15% protein. After volunteers fasted overnight, venous cannulas were placed in contralateral forearm veins at 0500 and blood samples were withdrawn at 10-min intervals from 0600 to 1200. At 0600 on each admission, blood samples were obtained for later measurements of serum insulin-like growth factor (IGF)-1, total and free testosterone, and estradiol concentrations. At 0730, an intravenous infusion of either A (30 g in 300 ml) or saline (S; 300 ml) was given over 30 min. At 0800, an intravenous bolus of either G (1 μg/kg) or S was given. Subjects rested quietly in their rooms during the studies.

Assays. GH concentrations in all serum samples (0600–1200) were measured using a recently validated ultrasensitive (0.005 μg/l threshold) chemiluminescence-based assay (Nichols, San Juan Capistrano, CA) (13, 26, 58). The chemiluminescent assay detects predominately the 22-kDa form of GH, with 34% cross-reactivity for 20-kDa GH (methionylated). The median intra-assay coefficient of variation (CV) for the GH assay was 6.0%, and the interassay CV was 9.9%. Total testosterone, free testosterone (analog assay), and estradiol concentrations were measured by solid-phase RIA (Coat-a-Count, Diagnostic Products, Los Angeles, CA). The intra-assay CVs were 6.9, 3.8, and 3.9% for total testosterone, free testosterone and estradiol, respectively, whereas the interassay CVs were 10.3, 4.2, and 9.5%, respectively. Serum total IGF-1 was measured by RIA (Nichols). The intra-assay CV for IGF-1 was 6.7%, and the interassay CV was 13.6%.

Deconvolution analysis. A multiple-parameter deconvolution method was used to estimate pulsatile attributes of GH secretion from the measured serum GH concentrations (56). A pulse of underlying GH secretion was approximated algebraically by a Guassian distribution of secretory rates (54). Basal secretion (time invariant) was estimated concurrently with a subject-specific monoexponential half-life of endogenous GH. The stepwise procedure for deconvolution entails prefitting via an automated waveform-independent technique (PULSE2), in which regions containing significant secretion impulses of undefined waveform are identified successively within a time series when they significantly reduce the total fitted variance by *F* ratio testing (29). Peak locations from PULSE2 were used as estimates in the multiparameter deconvolution analysis, as previously described (20, 58). To avoid overdetermination of peaks (Nyquist concept), putative successive GH peaks separated by <20 min (2 sampling intervals apart) were eliminated and the data were refit. In addition, any presumptive peaks that were outside the sampling window (0–370 min) by more than one sampling interval (10 min) were eliminated.

GH secretory pulses were considered significant if the fitted amplitude (maximal value attained within the computed secretory event) could be distinguished from zero with 95% statistical confidence. The GH secretory pulse half-duration, defined as the duration in minutes of the calculated secretory burst at half-maximal amplitude, GH half-life of elimination, and GH

distribution volume were assumed to be constant throughout any one study period in any individual. The mass of GH secreted per pulse was estimated as the area of the calculated secretory pulse (μg/l distribution volume). The endogenous pulsatile GH production rate was defined as the product of the number of GH secretory pulses and the mean mass of GH secreted per pulse during 6 h. Additionally, the 90-min GH secretory burst mass, defined here as the total mass of GH secreted in 90 min from the end of infusions (0800–0930). This measure limits the effect of spurious spontaneous GH release at later times in the sampling session.

Statistical procedures. Between-group comparisons were expressed in terms of fold change in the value of the geometric mean (GM). The GM is a location parameter, similar to the arithmetic mean and median, and is calculated by simply taking the antilogarithm of the mean response computed from the logarithmically transformed data. We compared GMs instead of arithmetic means, because one of the critical statistical model assumptions for ANOVA states that, to obtain valid statistical tests, residual variation should be approximately equal within all treatment groups. When the magnitude of the variance in the response increases as the mean of the response increases in value, the natural logarithmic transformation is generally used to stabilize the residual variance among two or more treatment groups (18). Therefore, data for serum sex steroids, IGF-1, integrated GH [area under the curve (AUC)], and calculated GH secretion parameters were transformed to the natural logarithm scale. Total testosterone was not logarithmically transformed.

All ANOVA computations were carried out in SAS version 6.12 (SAS/STAT Software Changes and Enhancements, 1996), with the mixed model software of Proc Mixed. Parameter values were estimated by restricted maximum likelihood (REML), and nonexact *F* tests were approximated by using a Satterthwaite approximation (31). A Bonferroni multiplier with a prespecified experimental type I error rate of 0.05 was used to adjust probabilities and confidence limits to maintain a type I error rate of 0.05 for all comparisons of interest. All *P* values presented correspond to statistical tests that were conducted on the log-transformed response data.

Total (integrated) AUC for the serum GH concentration-response curve was computed using the trapezoidal rule (32). These and other GH response estimates were then analyzed by a three-way nested ANOVA model, with gender, condition, and stimulus type considered classification variables.

An analysis of covariance (ANCOVA) model was used to determine whether the values of basal preinjection GH (measured just before each stimulus) predicted subsequent GH release. As a component of the ANCOVA model, the basal GH level was treated as a continuous covariate. Classification variables for gender, condition, and stimulation were also included in the ANCOVA model as well as terms for two-way, three-way, and four-way interactions among the values of basal GH and the classification variables. Parameter values were estimated by REML (see above).

A test of nonadditivity was used to assess the a priori hypothesis that GH release induced by combined G and A infusions is independent or synergistic. In this analysis, we eliminated the baseline intervals of GH release before and after the stimulation period, which is thus defined as 0730–1030. A formal derivation of this statistical test for nonadditivity is included in the . Note

that in this derivation the value of the GH response of each subject is calculated by simply adding his/her baseline-adjusted estimates of serum GH AUC after stimulation by A and stimulation by G and then subtracting this sum from the estimate of baseline-adjusted serum GH AUC after combined AG stimulation. This analysis thus tests the null hypothesis of no synergy (independence) between the GH-stimulatory effects of A and G delivered together (AG). This procedure was repeated for 90-min GH secretory burst mass. Nonadditivity terms were analyzed by a two-way nested ANOVA model, with gender and condition treated as classification variables.

To assess power for the current investigation, values of minimum detectable fold change in the GM were estimated for the primary outcomes [stimulated serum GH integrated area (AUC) and 90-min secretory burst mass] on the basis of the three-way nested ANOVA that was used in the analysis (45). In the computations of minimum detectable fold-change, the power of the statistical test was specified to be 0.80 and the type I error rate was specified to be 0.05. For comparisons of GH AUC and 90-min burst mass that involve two-way interactions the estimates of the minimum detectable fold change were 1.23 and 1.56, respectively, whereas the estimates were 1.47 and 2.26, respectively, for three-way interactions.

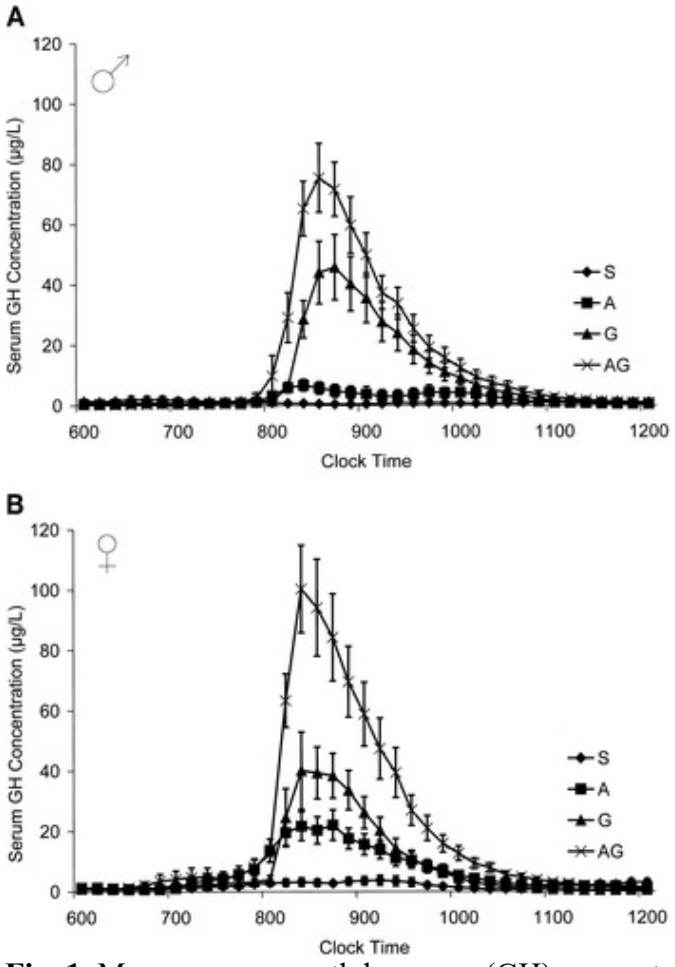

Fig. 1. Mean serum growth hormone (GH) concentration profiles basally and in response to GH-releasing peptide (GHRP)-2 (G) and/or l-arginine (A) infusions in men (*A*) and women (*B*). Data are the means ± SE. Clock time is shown.

RESULTS

The group serum GH concentration response patterns over time to each of the stimuli are shown in Fig. 1*A* for men and Fig. 1*B* for women. The maximal serum GH concentration attained was greatest for the AG stimulus and least for S. The rank order of stimulus strength, AG > G > A > S, was the same in men and women. In men, the values of the maximal serum GH concentrations attained after each of the stimuli were 2.4, 7.8, 36, and 73 μg/l (for S, A, G, and AG, respectively). These data show a 30-fold increase in the maximal serum GH concentration in response to AG compared with S in men. In women, the values for the S, A, G, and AG treatments were 6.1, 22, 43, and 93 μg/l and the increase in the maximal serum GH concentration for the AG compared with the S stimulus was 15-fold. Comparing the fold changes between women and men for each of the stimuli revealed a significant 2.8-fold [95% constant load (CL) (1.1,7.2)] greater GH rise in response to A in women compared with men.

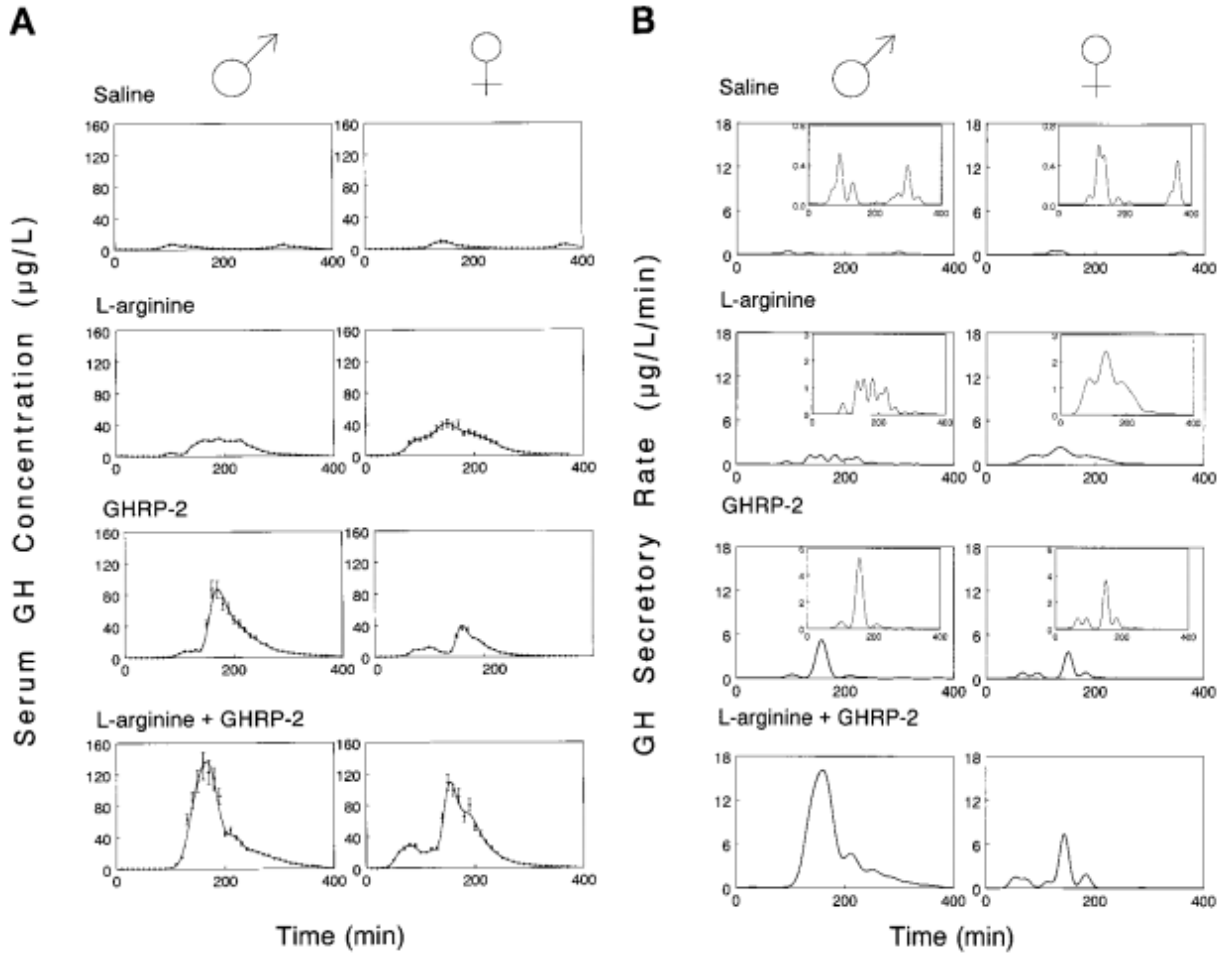

Fig. 2. Representative serum GH concentration profiles (*A*) and the corresponding GH secretion profiles (*B*) in an individual healthy young man and woman basally and in response to G and/or A infusions. *Time zero* corresponds to 0600 clock time in Fig. 1.

In women, the time to reach the maximal serum GH concentration was greatest after the A and AG stimuli (50 and 60 min) compared with G (30 min). The same pattern of responses was observed in men, but the time to reach the maximal GH concentration was greater (delayed) for all three stimuli (60, 70, 40 min for A, AG, G, respectively). This consistent gender difference in median time to reach maximal GH concentration was highly significant ($P < 0.001$).

Representative individual serum GH concentration vs. time curves for a man and a woman are shown in Fig. 2*A*, and the corresponding calculated GH secretion profiles assessed by deconvolution analysis are given in Fig. 2*B* (discussed below).

Figure 3 presents box plots, which summarize the data for total serum integrated GH AUC (logarithmic scale), for men and women after each of the stimuli. Akin to maximal serum GH levels, the order of AUC response magnitudes was AG > G > A > S for both men and women. The increase in serum GH AUC for the AG compared with the S stimulus was 24-fold for men and 11-fold for women. The incremental change in serum GH AUC observed for each stimulus was influenced significantly by gender ($P< 0.001$). After A infusion, women had a 3.3-fold [95% CL(1.2,9.2)] greater median serum GH AUC than men ($P < 0.001$). No gender differences existed for the S, G, and AG stimuli.

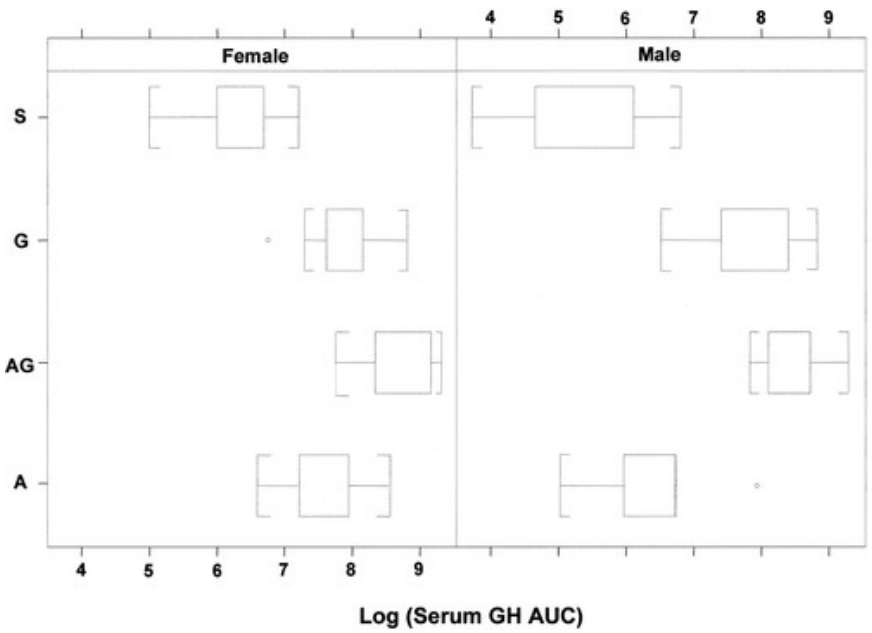

Fig. 3. Box plot representations of log [integrated serum GH area under curve (AUC)] basally and in response to G and/or A infusions in healthy young men and women. ○, Measurements below the 10th or above the 90th percentile.

Table 1 presents data from the deconvolution analysis of GH secretory responses. The basal GH secretion rate was greater in women than men ($P = 0.05$), and this difference was consistent regardless of the stimulus administered. The calculated GH half-life was dependent on the stimulus administered ($P < 0.001$), but independent of gender ($P = 0.9$). The 90-min GH secretory burst mass showed a graded stimulus order, which was the same in men and women (AG > G > A > S). The increase in 90-min GH secretory burst mass after AG infusion compared with S was 242-fold for men and 40-fold for women. The incremental change in 90-min GH

secretory burst mass for each stimuli was significantly influenced by gender (P= 0.005). In the control (S) session, the 90-min secretory burst mass was greater in women than men (4.8 vs. 0.88 μg/l, P = 0.0003). Women tended to exhibit greater 90-min GH mass after A infusion (P = 0.072). For both the G and AG stimuli, men and women had similar 90-min GH mass.

Table 1. Gender comparisons of calculated GH secretion measures

	Basal Secretion Rate, $\mu g \cdot l^{-1} \cdot min^{-1}$		GH Half-Life, min		90-min Mass of GH Secreted, μg/l		Production Rate, $\mu g \cdot l^{-1} \cdot min^{-1}$	
	M	F	M	F	M	F	M	F
Saline	0.006 ± 0.002 (0.004)	0.014 ± 0.004 (0.010)	16.1 ± 1.5 (15.4)	13.9 ± 1.3 (13.3)	2.0 ± 0.8 (0.7)	12.9 ± 5.8 (4.5)	10.5 ± 3.1 (7.2)	34.8 ± 8.3 (28.6)
Arginine	0.004 ± 0.001 (0.003)	0.011 ± 0.002 (0.009)	15.4 ± 0.6 (15.3)	15.8 ± 1.2 (15.4)	22.1 ± 7.8 (15.7)	65.4 ± 18.7 (37.9)	34.0 ± 10.8 (25.2)	101 ± 20.2 (87.3)
GHRP-2	0.005 ± 0.001 (0.004)	0.01 ± 0.002 (0.009)	18.2 ± 1.9 (17.4)	16.4 ± 1.2 (16.1)	115 ± 24.1 (88.2)	117 ± 32.4 (93.1)	128 ± 21.4 (109)	132 ± 36.2 (105)
AG	0.005 ± 0.001 (0.004)	0.011 ± 0.002 (0.010)	19.9 ± 1.6 (19.3)	20.9 ± 1.6 (20.4)	196 ± 45.8 (168)	199 ± 25.0 (182)	212 ± 53.1 (179)	210 ± 29.1 (191)

Values are the means ± SE (geometric mean). M, males (n = 9); F, females (n = 9); GH, growth hormone; GHRP, GH-related peptide; AG, combined L-arginine and GH. Time interval for production rate measurement is 0600–1200.

The order of response magnitude for endogenous GH production rate (AG > G > A > S) was identical to that of 90-min GH mass (above) for both men and women. The increase in the endogenous GH production rate from the S baseline to AG stimulus was 23-fold in men and 7-fold in women. The incremental change in endogenous GH production rate from one stimulus to the next was influenced by gender (P < 0.001). In the S and A treatments, women had greater endogenous GH production rates than men (P < 0.001 and P < 0.001, respectively). Gender differences were not observed for the G and AG stimuli.

There was a main effect of stimulus on mass of GH secreted per burst (P = 0.0001), and the gradation of the effect was AG > G > A > S (data not shown). The mass of GH secreted per burst in the combined groups increased by 3.5-fold in response to A infusion over S [95% CL(2.7,5.4)], by 1.7-fold for G over A stimulation [95% CL(1.1,2.5)], and by 1.8-fold for AG over G stimulation [95% CL(1.2,2.7)] (P < 0.001 for each). The mean GH secretory burst amplitude was greater in women than men for the A and S stimuli (P < 0.001 for both). Women had a 2.9-fold [95% CL(1.2,7.2)] greater mean GH secretory burst amplitude compared with men after the A stimulus and a 3.0-fold [95% CL(1.2,7.5)] greater mean GH secretory burst amplitude in the S (control) setting.

Figure 4 shows a box plot of the results for the test of nonadditivity applied to the 90-min GH secretory burst mass. This analysis revealed a synergistic effect for the combined AG stimulus compared with the individual effects of A and G (P = 0.02). We noted that the combined AG stimulus compared with the summed response for (A + G) for 90-min GH secretory burst mass was 1.3-fold greater in women [95% CL(0.97, 1.8)] and 1.48-fold in men [95% CL(1.1,2.0)].

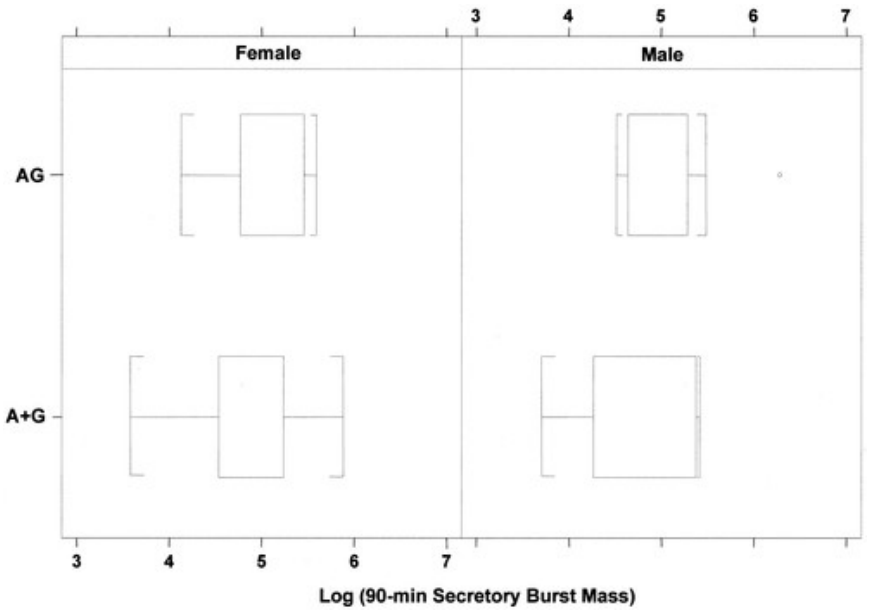

Fig. 4. Box plot representations of the test of nonadditivity of the logarithms of the 90-min GH secretory burst mass after combined A and G infusions (AG) vs. their algebraically summed individual effects (A + G). ○, Measurements below the 10th or above the 90th percentile.

Figure 5 shows a box plot of the results for the test of nonadditivity for stimulated GH AUC. After adjustment for baseline (treatment effect at rest), the test for nonadditivity also revealed that the individual effects of A and G in determining GH release are not simply additive, but rather significantly synergistic (supra-additive) when administered in combination (joint stimulus, AG) ($P = 0.003$). The combined AG stimulus compared with summed response for (A + G) was 1.6-fold greater in women [95% CL(1.2,2.0)] and 1.6-fold in men [95% CL(1.2,2.0)].

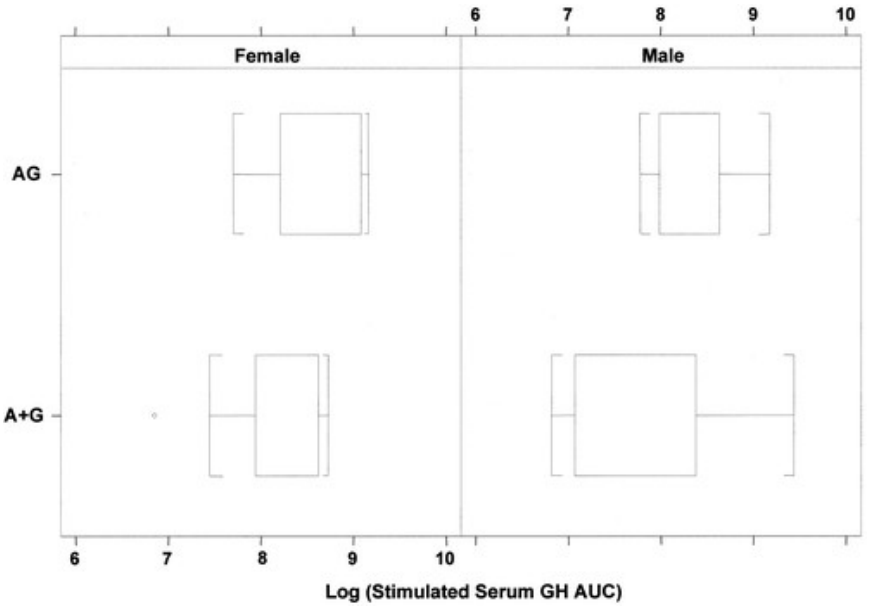

Fig. 5. Box plot representations of the test for nonadditivity of the logarithms of the stimulated serum GH AUC after combined AG infusions vs. their algebraically summed individual effects (A + G). ○, Measurements below the 10th or above the 90th percentile.

There were no significant gender or stimulus differences in serum estradiol concentrations or serum IGF-1 concentrations. Men had significantly greater total and free testosterone concentrations ($P < 0.001$, for both), independent of stimulus.

ANCOVA revealed that the linear relationship between log (basal serum GH AUC) and log (stimulated GH AUC) varied with gender ($P = 0.02$) and stimulus type ($P < 0.001$). The relationship between these two variables had a positive slope for the S, A, and AG stimuli, but not for G (results not shown). When considering all possible interactions between the log (basal serum GH AUC) and the indicator variables for gender and stimulus type, ANCOVA showed that including log (basal serum GH AUC) explains substantial residual variance otherwise unaccounted for by gender and stimulus ($P < 0.001$). However, the additional covariate [log (basal serum GH AUC)] failed to change the composition of the terms that were judged to be important in the original ANOVA model.

DISCUSSION

A major observation in the current study is that the effect on GH release of A and G administered together is not additive but rather is synergistic and that such synergism is expressed equivalently in men and women. Second, a gender-independent rank order of maximal GH secretory responses is demonstrated, i.e., AG > G > A > S. The synergistic interaction between G and A evoked a massive outpouring of GH over the succeeding 90 min; namely, a 242-fold (men) and a 40-fold (women) augmentation of GH secretory burst mass over the saline control. Third, mean integrated and maximal serum GH concentrations, the (90 min) mass of GH secreted, GH secretory burst amplitude, and the GH production rate after A infusion are all higher in women than men. Last, a further gender difference was identified in that men had a consistent delay in attaining maximal serum GH concentrations compared with women. In contrast, the GH half-life, burst duration, burst frequency, and GH secretory responses to G infusions are sex independent.

The GH secretory response patterns to each of the distinct secretagogues were similar qualitatively in men and women (Fig. 1,*A* and *B*). Although the absolute value of the maximal serum GH concentration attained during the combined AG stimulus was greater in women than in men (93 vs. 73 μg/l), this difference was not statistically significant. However, given the gender disparity in basal (prestimulus) serum GH concentrations, we also calculated the fractional (fold) GH increase over basal after combined AG infusions compared with the unstimulated admission (S). This fold effect of AG was greater in men (30-fold) than women (15-fold). The augmented fractional secretory response (despite similar maximal serum GH concentrations) in men reflected the lower mean serum GH concentrations in the unstimulated state (S), as recognized in other gender comparisons (17, 52). Thus, by the way of simple integrated serum GH responses, men evinced a 24-fold and women an 11-fold rise above control (S) under joint AG drive ($P< 0.001$). The possible physiological role that this difference may play in mediating gender differences in target organs of GH action (i.e., body mass and structure) is unknown.

The time to reach the maximal serum GH concentration was greater after the A and AG stimuli compared with G in both genders. For each secretagogue, men attained a maximal serum GH concentration ~10 min later than women (in A, G, and AG). This temporal disparity is similar to that observed in a recent study using exercise as an alternative stimulus (63). Deconvolution analysis showed that the delay was not due to postponed GH secretion, a more prolonged GH half-life, or an extended GH secretory burst in men. There are several possible mechanistic explanations for this gender distinction. First, men's lower basal serum GH levels may have extended their time to reach an equivalent maximal GH peak. Second, procedural factors such as the introduction of intravenous fluids (S, A, or G) may have elicited unequal stress responses in the two sexes, given that corticotropin-releasing hormone (CRH) release (at least in the rat) evokes somatostatin secretion (38). In addition, the sexually dimorphic patterns of GH secretion are likely controlled at hypothalamic loci, as inferred from laboratory studies in rats (47, 48, 49). In the human, Jaffe et al. (27) also reported a gender-dependent pattern of IGF-1 feedback control of GH secretion, in which women exhibit reduced inhibitory responsiveness to IGF-1 infusions, possibly reflecting a lesser effect of (daytime) somatostatin in women. Earlier, Carlsson et al. (12) described sexually dimorphic GH autofeedback control of GHRH and somatostatin actions in the adult rodent. Thus we reason that if women have diminished GH and/or IGF-1 (auto) negative feedback compared with men, then peak GH release may occur more rapidly in women than men. In this regard, application of the approximate entropy statistic, taken as an indirect barometer of within-axis feedback signaling, at the hypothalamic-pituitary level, strongly distinguished the orderliness of GH release in the female and male in both human and rat (40). The latter strongly suggests (but does not prove) unequal GH-IGF-1 feedback strength in the two sexes (60).

As first described by Merimee et al. (34) over 30 years ago, we also observed that women attained a significantly (2.8-fold) greater maximal serum GH concentration and (3.3-fold) greater AUC during l-arginine infusion than men. By deconvolution analysis, we could corroborate this sex discrepancy and further explicate mechanistically for the first time that higher maximal serum GH concentrations during A infusion in women do not reflect any gender-dependent differences in GH half-life or GH secretory burst duration or women's higher interpulse serum GH concentrations, but rather is due to a specific amplification of GH secretory burst mass in the female. The gender difference in women's heightened GH response to A is likely estrogen dependent, because men also show elevated A-stimulated GH secretion after short-term estrogen exposure (34). However, the early follicular phase women studied here maintained serum estradiol concentrations that were no different from those in the normal men and still manifested accentuated GH release. Thus an inhibitory effect of testosterone on the GH secretory response to l-arginine is a possible explanation, as suggested indirectly by other earlier studies of spontaneous (basal) 24-h GH release in men treated with nonaromatizable androgen (59) or an androgen-receptor antagonist (35). This putative inhibitory effect of androgen could be mediated via augmented somatostatin release, as suggested in the rat (3), and/or increased responsiveness of the pituitary gland to somatostatin. Although clinical data relevant to the latter conjecture are not available to our knowledge. Recent clinical experiments indicate that estrogen does modulate somatostatin action in women (10). Gender differences after A are distinct and evidently do not extend to the putative endogenous GHRP-ligand activated pathway, because at least at the G dose of 1 μg/kg used here and in earlier studies, men and women exhibit similar GHRP-stimulated GH release (present data and Refs. 6, 37).

A recent clinical investigation of gender differences in GH release noted 68-fold higher morning ambulatory serum GH concentrations determined by immunofluorometry (IFA) in young women compared with young men (17). As observed earlier by immunoradiometric assay and IFA or 24-h GH profiles (52), daily GH secretion rates are greater in young women than men. We show here that this gender difference is also present as assessed by ultrasensitive chemiluminescence-based assay. Furthermore, by deconvolution analysis we could observe that the sex difference is accounted for solely by augmented GH secretory burst mass, but not half-life, in women. Although the calculated (endogenous) GH half-life rose after combined AG infusions (compared with the individual secretagogues), this effect was unrelated to gender. Sex invariance of GH half-life also was evident in experiments using recombinant human GH infusions in octreotide-suppressed young women and men (44). The increase in estimated GH half-life observed here after joint AG administration thus is more likely due to the higher serum GH concentration attained in this context, because several investigations have reported a concentration dependence of metabolic clearance rate of GH in humans (25, 43, 44).

In the current analysis, we calculated the mass of GH secreted over the entire 6-h study and also evaluated the mass of GH secreted over the 90-min poststimulus interval to obviate the effects of spurious spontaneous GH release at later times in the sampling session. The rank order of stimulus effects (AG > G > A > S) was the same for both measures. In both analyses, women maintained greater values in both the control (S) and A sessions compared with men, with no gender difference after G or combined AG infusions.

Regulation of GH secretory burst amplitude explained unstimulated gender differences as well as the stimulatory effects of A, G, and AG infusions in this study. Thus the median GH secretory burst amplitude was greater in women compared with men both under control conditions and in response to A stimulation, but not after the G and AG stimuli. On the basis of analytic considerations (57), GH secretory burst amplitude thus also explicates the gradation of secretagogue effects (AG > G > A > S). Secretagogue drive of GH secretory burst amplitude is here shown to be highly specific, given the failure of any single stimulus to alter GH secretory burst duration, frequency, or half-life, which are the other (analytic) determinants of the serum GH concentration (54, 56, 57).

There were no gender differences in serum IGF-1 or estradiol concentrations in this study, given that women were evaluated during the early follicular phase of the menstrual cycle. As expected, men maintained significantly greater total and free serum testosterone concentrations. Of interest, no sex steroid measures correlated with GH secretory responses to any of the secretagogue stimulus combinations used here.

Available clinical studies report considerable variability in GH secretory responses to virtually all stimuli, e.g., exercise, GHRH challenge, etc. (42, 50, 61). This biological variability in the GH response has led investigators to examine the statistical power of studies with small subject numbers (such as the current study with 18 subjects), particularly when two- or three-way interactions are investigated. To this end, we performed power analysis for the primary GH outcome variables (stimulated GH AUC and 90-min GH burst mass) to estimate the minimal detectable difference required for two- and three-way interactions. For these primary outcome

variables, the fold changes observed in the present investigation (range 3.3- to 242-fold) were much larger than the minimum detectable fold change values (range 1.23- to 2.26-fold), thus ensuring our ability to detect differences in the GH response despite the large biological variability. Devesa et al. (16) suggested further that the magnitude of GH release varies depending on the timing of GHRH infusion, i.e., whether GHRH is given during a trough or a peak of GH release, reflecting presumptively high or low somatostatinergic tone, respectively. Given this predicate, we also evaluated how the prestimulus serum GH concentration influenced the calculated GH secretory response in relation to gender and secretagogue type. This analysis revealed positive relationships between log(basal serum GH integrated AUC concentrations) and log(stimulated AUC serum GH concentrations) at baseline and in response to the A and AG stimuli, but not the G stimulus. This new observation for these secretagogues has several implications. First, because the linear relationship between these two variables depends on the secretagogue administered, it would become inappropriate to use an ANCOVA with log(basal GH) as a covariate for the G stimulus if the intent is to adjust treatment means (46). Second, the evident lack of any significant relationship between basal serum GH level and G-stimulated GH release warrants further mechanistic evaluation, especially because no (or a negative) correlation existed in both men and women. As such, the G stimulus would appear to differ mechanistically from A and GHRH actions (16). Anatomic evidence for interconnections between GHRH and somatostatin neurons in the hypothalamus (5, 33) might provide one mechanism for this observation. For example, an elevated basal serum GH concentration before G administration might impose greater GH autofeedback on endogenous GHRH (and elevated feedforward on somatostatin) release and thereby result in reduced (stimulated) GH secretion. This scenario would not prevail equally in the face of A or AG infusions, because A is believed to restrain somatostatin release and somatostatin is thought to mediate GH autofeedback (24). Last, our exploration of an ANCOVA model demonstrates that significant residual variance in selective secretagogue-stimulated GH secretion is indeed explicable by basal GH levels, even beyond the influence of gender.

The mechanism underlying A-induced GH release is currently believed to include somatostatin withdrawal (2, 21, 22). Although this mechanism has not been confirmed directly in humans, A has no known direct effects on GHRH release or the GHRP effector pathway. Current evidence supports the view that G acts via novel, non-GHRH and non-somatostatin receptor mechanisms. Therefore, we predicted that the impact of combined AG stimulation on GH release would exceed that of either A or G alone, or their algebraically additive effects; i.e., represent a supra-additive (or synergistic) interaction. We here support this important postulate in both men and women. To this end, we statistically evaluated the null hypothesis that GH release produced by combined AG stimulation exceeds that of the summed effects of A and G given on different days. Several previous clinical studies have reported synergistic (supra-additive) interactions between GHRP and GHRH (but A was not evaluated) on GH release in humans (6, 8, 9,39, 41), although another study did not (51). A synergistic interaction between GHRH and GHRP cannot be accounted for readily via stimulation of endogenous GHRH secretion or by inhibition of somatostatin release (6, 41). Alternative hypothetical mechanisms thus include inhibition of somatostatin actions at the pituitary level or release of another unknown hypothalamic factor (“U factor”). In other studies, pyridostigmine [which also presumptively acts in part via hypothalamic somatostatin withdrawal and in part via GHRH release (24)] augmented the GH secretory response to GHRP-6 (37), but did not enhance the amount of GH secreted in response to GHRH

given with GHRP-6 (15). The authors concluded that somatotrope secretory responsiveness to the combined administration of GHRH and GHRP-6 is largely independent of somatostatinergic tone (15). However, one must also consider the possibility that maximal GH release was attained after combined GHRH and GHRP-6 infusions and that the addition of pyridostigmine (or any other stimulus) could not enhance GH secretion further. The present study indicates clearly that combined AG stimulation is synergistic in both men and women, i.e., greater than the summed effects of individual infusions of A and G ($P < 0.001$). To our knowledge, this is the first study to show a synergistic impact of A and G on GH release. In addition, we demonstrate that the effect of combined AG is gender independent ($P = 0.28$).

Because the combined AG stimulus response exceeded GH release driven by the summed A plus G responses in both men and women, we speculate that A and G might interact in some excitatory manner so as to increase net GH secretion. Several hypotheses could explain the foregoing observation. Although considered a minor action of GHRP (7), partial attenuation of somatostatin action at the pituitary level by GHRP and/or release of another unknown hypothalamic factor by GHRP may be responsible for the synergistic action of A and G. Additionally, inferred bidirectional neuronal connections between GHRH, somatostatin, and/or endogenous GHRP-like neurons within the hypothalamus may increase net GH release during the combined infusion of A and G, i.e., GHRH neurons, which are known to be activated by GHRP in experimental animals (24), may also stimulate somatostatin withdrawal. Thereby, the joint AG effect could be greater than the summed effect of A and G given on separate days. Accordingly, further analysis of the mechanisms underlying the distinctly synergistic effects of A and G will likely be fruitful in future clinical and experimental investigations in both sexes.

Perspectives

The basis for the sexually dimorphic patterns of GH secretion in humans is a complex issue. Indeed, isolation of factors contributing to gender difference is made challenging by the inability to appraise directly in vivo each of the neuroregulatory pathways involved. The present study uses two selective neuroregulatory probes to explore the mechanisms underlying the sexually dimorphic pattern of GH secretion. Thereby, we observe both common and sex-specific features of GH control. First, a common effect of secretagogues on GH release is to amplify GH secretory burst mass in both men and women. This mechanism may be important, because data in experimental animals indicate that increased GH secretory burst mass and elevated basal GH secretion impact intermediary metabolism, hepatic gene expression, body fat deposition, and lean muscle mass differentially. Second, these analyses unveil several gender-specific regulatory features, including basal GH secretion and individual vs. synergistic secretagogue actions (see abstract). Finally, the prestimulus basal GH secretion rate influences the response to secretagogues beyond that explicated by gender alone. The predictive power of presecretagogue GH levels on responsiveness to A but not G thus is both gender specific and secretagogue dependent. The latter may be due to the unequal impact of the concurrent level of somatostatinergic activity on responsiveness to different specific secretagogue types. According to this reasoning, the response to G in either men or women could be largely independent of somatostatin tone. These elements of GH neuroregulation highlight the need for further investigations of gender differences in the multisecretagogue control of the GH-IGF-1 axis.

Test of Additivity

Model statement.

$$y_{j(i)kl} = \mu + \tau_i + \rho_{j(i)} + \lambda_k + \alpha_l + (\tau\lambda)_{ik} + (\tau\alpha)_{il} + (\lambda\alpha)_{kl} + (\tau\lambda\alpha)_{ikl} + \varepsilon_{j(i)kl}$$

$i = 1, 2 \quad j = 1, 2, \ldots, 9 \quad k = 1, 2, 3 \quad l = 1, 2$

where μ is the grand mean; τ_i, the *i*th gender effect, assumed to be fixed; $\rho_{j(i)}$ is the *j*th subject effect nested within the *i*th gender, assumed to be random; λ_k is the *k*th treatment effect, assumed to be fixed; α_l is the *l*th condition effect, assumed to be fixed; $(\tau\lambda)_{ik}$ is the effect of the *i*th gender by *k*th treatment interaction; $(\tau\alpha)_{il}$ is the effect of the *i*th gender by *l*th condition interaction; $(\lambda\alpha)_{kl}$ is the effect of the *k*th treatment by *l*th condition interaction; $(\tau\lambda\alpha)_{ikl}$ is the effect of the *i*th gender by *k*th treatment by *l*th condition interaction; and $\varepsilon_{j(i)kl}$ is the random error.

Model constraints.

$$\sum \tau_i = 0, \sum \lambda_k = 0, \sum \alpha_l = 0,$$

$$\sum\sum (\tau\lambda)_{ik} = 0, \sum\sum (\tau\alpha)_{il} = 0,$$

$$\sum\sum (\lambda\alpha)_{kl} = 0, \sum\sum\sum (\tau\lambda\alpha)_{ikl} = 0$$

$$\rho_{j(i)} \sim N(0, \sigma_s^2) \quad \varepsilon_{j(i)kl} \sim N(0, \sigma^2)$$

Test of additivity. Let λ^*_1 be the baseline adjusted treatment effect of A, λ^*_2 be the baseline adjusted treatment effect of G, and λ^*_3 be the baseline adjusted treatment effect of combined A and G (AG).

Note that

$$\begin{aligned}
y_{(\mathrm{A+G})j(i)l} &= 2\mu + 2\tau_i + 2\rho_{j(i)} + (\lambda_1^* + \lambda_2^*) + 2\alpha_l + \tau_i(\lambda_1^* + \lambda_2^*) + 2(\tau\alpha)_{il} + \alpha_l(\lambda_1^* + \lambda_2^*) \\
&\quad + \tau\alpha_{il}(\lambda_1^* + \lambda_2^*) + \varepsilon_{(\mathrm{A+G})j(i)l} \\
y_{(\mathrm{A+G})j(i)l} &= \mu + \tau_i + \rho_{j(i)} + \lambda_3^* + \alpha_l + \tau_i\lambda_3^* + (\tau\alpha)_{il} + \alpha_l(\lambda_3^*) + \tau\alpha_{il}(\lambda_3^*) + \varepsilon_{(\mathrm{AG})j(i)l} \\
y_{(\mathrm{AG-A-G})j(i)l} &= \mu + \tau_i + \rho_{j(i)} + (\lambda_3^* - \lambda_1^* - \lambda_2^*) + \alpha_l + \tau_i(\lambda_3^* - \lambda_1^* - \lambda_2^*) + (\tau\alpha)_{il} \\
&\quad + \alpha_l(\lambda_3^* - \lambda_1^* - \lambda_2^*) + \tau\alpha_{il}(\lambda_3^* - \lambda_1^* - \lambda_2^*) + \varepsilon^*_{(\mathrm{AG-A-G})j(i)l}.
\end{aligned}$$

Thus for the new response $y^*_{j(i)l} = y_{(\mathrm{AG-A-G})j(i)l}$, the model can be expressed as

$$y^*_{j(i)l} = \mu^* + \tau_i + \rho_{j(i)} + \alpha_l + \tau\alpha_{il} + \varepsilon^*_{j(i)l}$$

where

$\mu^* = \mu + (\lambda_3^* - \lambda_1^* - \lambda_2^*) + \tau_i(\lambda_3^* - \lambda_1^* - \lambda_2^*) + \alpha_l(\lambda_3^* - \lambda_1^* - \lambda_2^*) + \tau\alpha_{il}(\lambda_3^* - \lambda_1^* - \lambda_2^*).$
$\varepsilon_{j(i)l}^* = \varepsilon_{(\mathrm{AG-A-G})j(i)l}^*$

Hence

$$\mathrm{E}\left[y_{j(i)l}^*\right] = \mathrm{E}\left[\mu^* + \tau_i + \rho_{j(i)} + \alpha_l + (\tau\alpha)_{il} + \varepsilon_{j(i)l}^*\right] = \mu^*$$

and the null hypothesis for a global test of additivity is that μ*= 0.

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References

1. Akman MS, Girard M, O'Brien LF, Ho AK, and Chik CL. Mechanisms of action of a second generation growth hormone-releasing peptide (Ala-His-D-BNal-Ala-Trp-D-Phe-Lys-NH2) in rat anterior pituitary cells. Endocrinology 132: 1286–1291, 1993.

2. Alba-Roth J, Muller OA, Schopohl J, and Von Werder K. Arginine stimulates growth hormone secretion by suppressing endogenous somatostatin secretion. J Clin Endocrinol Metab 67: 1186–1189, 1988.

3. Argente J, Chowen BJ, Steiner RA, and Clifton DK. Somatostatin messenger RNA in hypothalamic neurons is increased by testosterone through activation of androgen receptors and not by aromatization to estradiol. Neuroendocrinology 52: 342–349, 1990.

4. Argente J, Chowen JA, Zeitler P, Clifton DK, and Steiner RA. Sexual dimorphism of growth hormone-releasing hormone and somatostatin gene expression in the hypothalamus of the rat during development. Endocrinology 128: 2369–2375, 1991.

5. Bertherat J, Dournaud P, Berod A, Normand E, Bloch B, Rostene W, Kordon C, and Epelbaum J. Growth hormone-releasing hormone-synthesizing neurons are a subpopulation of somatostatin receptor-labeled cells in the rat arcuate nucleus: a combined in situ hybridization and receptor light-microscope radioautographic study. Neuroendocrinology 56: 25–31, 1992.

6. Bowers CY. GH releasing peptides—structure and kinetics. J Pediatr Endocrinol Metab 6: 21–31, 1993.

7. Bowers CY and Granda-Ayala R. Stimulated release of GH in normal younger and older men and women. In: Sex-Steroid Interactions with Growth Hormone, edited by Veldhuis JD and Giustina A. Norwell, MA: Serono Symposia USA, 1999, p. 277–289.

8. Bowers CY, Maumenee FA, Reynolds GA, and Hong A. On the in vitro and in vivo activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. Endocrinology 114: 1537–1545, 1984.

9. Bowers CY, Veeraragavan K, and Sethumadhavan K. Atypical growth hormone releasing peptides. In: Growth Hormone. II. Basic Clinical Aspects, edited by Bercu BB and Walker RF. New York: Springer-Verlag, 1994, p. 203–222.

10. Bray M, Shah N, and Veldhuis JD. Impact of estrogen on the dose-dependent inhibition by somatostatin of somatostatin of GH secretion in postmenopausal women. Abstracts of the 81st Annual Endocrine Society Meeting, San Diego, CA, 1999.

11. Brozek J, Grande F, Anderson JT, and Keys A. Densitometric analysis of body composition of men from girth measurements: revision of some quantitative assumptions. Ann NY Acad Sci 110: 113–140, 1963.

12. Carlsson LMS, Clark RG, and Robinson ICAF. Sex difference in growth hormone feedback in the rat. J Endocrinol 126: 27–35, 1990.

13. Chapman IM, Hartman ML, Straume M, Johnson ML, Veldhuis JD, and Thorner MO. Enhanced sensitivity growth hormone chemiluminescence assay reveals lower post-glucose nadir GH concentrations in men than women. J Clin Endocrinol Metab 78: 1312–1319, 1994.

14. Cheng K, Chan WWS, Barreto A, Convey EM, and Smith RG. Evidence for a role of protein kinase-C in His-D-Trp-AlaTrp-D-Phe-Lys-NH2-induced growth hormone release from rat primary pituitary cells. Endocrinology 129: 3337–3342, 1991.

15. Cordido F, Penalva A, Peino R, Casanueva FF, and Dieguez C. Effect of combined administration of growth hormone (GH)-releasing hormone, GH-releasing peptide-6, and pyridostigmine in normal and obese subjects. Metabolism 44: 745–748, 1995.

16. Devesa J, Lima L, Lois N, Fraga C, Lechuga MJ, Arce V, and Tresguerres JAF. Reasons for the variability in growth hormone (GH) responses to GHRH challenge: the endogenous hypothalamic-somatotroph rhythm (HSR). Clin Endocrinol (Oxf) 30: 367–377, 1989.

17. Engstrom BE, Karlsson FA, and Wide L. Marked gender differences in ambulatory morning growth hormone values in young adults. Clin Chem 44: 1289–1295, 1998.

18. Fisher LD and van Belle G. Biostatistics: A Methodology for the Health Sciences. New York: Wiley, 1993, p. 462–466.

19. Food and Nutrition Board. National Academy of Science-National Research Council. Recommended Dietary Allowances. Washington, DC: National Academy Press, 1989.

20. Friend K, Iranmanesh A, and Veldhuis JD. The orderliness of the growth hormone (GH) release process and the mean mass of GH secreted per burst are highly conserved in individual men on successive days. J Clin Endocrinol Metab 81: 3746–3753, 1996.

21. Ghigo E, Arvat E, Gianotti L, Ramunni J, Maccario M, and Camanni F. Interaction of salbutamol with pyridostigmine and arginine on both basal and GHRH-stimulated GH secretion in humans. Clin Endocrinol (Oxf) 40: 799–802, 1994.

22. Ghigo E, Arvat E, and Valente F. Arginine reinstates the somatotrope responsiveness to intermittent growth hormone-releasing hormone administration in normal adults. Neuroendocrinology 54: 291–294, 1991.

23. Ghigo E, Bellone J, Mazza E, Imperiale E, Procopio M, and Valente F. Arginine potentiates the GHRH- but not the pyridostigmine-induced GH secretion in normal short children. Further evidence for a somatostatin-suppressing effect of arginine. Clin Endocrinol (Oxf) 32: 763–777, 1990.

24. Giustina A and Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 19: 717–797, 1998.

25. Haffner D, Schaefer F, Girard J, Ritz E, and Mehls O. Metabolic clearance of recombinant human growth hormone in health and chronic renal failure. J Clin Invest 93: 1163–1171, 1994.

26. Iranmanesh A, Grisso B, and Veldhuis JD. Low basal and persistent pulsatile growth hormone secretion are revealed in normal and hyposomatotropic men studied with a new ultrasensitive chemiluminescence assay. J Clin Endocrinol Metab 78: 526–535, 1994.

27. Jaffe CA, Ocampo-Lim B, Guo W, Krueger K, Sugahara I, DeMott-Friberg R, Bermann M, and Barkan AL. Regulatory mechanisms of growth hormone secretion are sexually dimorphic. J Clin Invest 102: 153–164, 1998.

28. Jansson JO, Eden S, and Isaksson O. Sexual dimorphism in the control of growth hormone secretion. Endocr Rev 6: 128–150, 1985.

29. Johnson ML and Veldhuis JD. Evolution of deconvolution analysis as a hormone pulse detection method. Methods Neurosci 28: 1–24, 1995.

30. Katch FI, Micheal ED, and Horvath SM. Estimation of body volume by underwater weighing: description of a single method. J Appl Physiol 23: 811–816, 1967.

31. Kuel RO. Statistical Principles of Research Design and Analysis. Belmont, CA: Duxbury, 1994.

32. Mathews JNS, Altman DG, Campell MJ, and Royston P. Analysis of serial measurements in medical research. Br Med J 300: 230–235, 1990.

33. McCarthy GF, Beaudet A, and Tannenbaum GS. Co-localization of somatostatin receptors and growth hormone-releasing factor immunoreactivity in neurons of the rat arcuate nucleus. Neuroendocrinology 56: 18–24, 1992.

34. Merimee TJ, Rabinowitz D, and Fineberg SE. Arginine-initiated release of human growth hormone: factors modifying the response in normal man. N Engl J Med 280: 1434–1438, 1969.

35. Metzger DL and Kerrigan JR. Androgen receptor blockade with flutamide enhances growth hormone secretion in late pubertal males: evidence for independent actions of estrogen and androgen. J Clin Endocrinol Metab 76: 1147–1152, 1993.

36. Painson JC and Tannenbaum GS. Sexual dimorphism of somatostatin and growth hormone-releasing factor signaling in the control of pulsatile growth hormone secretion in the rat. Endocrinology 128: 2858–2866, 1991.

37. Penelva A, Pombo M, Carballo A, Barreiro J, Casanueva FF, and Dieguez C. Influence of sex, age and adrenergic pathways on the growth hormone response to GHRP-6. Clin Endocrinol (Oxf) 38: 87–91, 1993.

38. Peterfreund RA and Vale WW. Ovine corticotropin-releasing factor stimulates somatostatin secretion from cultured brain cells. Endocrinology 112: 1275–1278, 1983.

39. Pihoker C, Middleton R, Reynolds GA, Bowers CY, and Badger TM. Diagnostic studies with intravenous and intranasal growth hormone-releasing peptide-2 in children of short stature. J Clin Endocrinol Metab 80: 2987–2992, 1995.

40. Pincus SM, Gevers E, Robinson ICAF, Berg GVD, Roelfsema F, Hartman ML, and Veldhuis JD. Females secrete growth hormone with more process irregularity than males in both human and rat. Am J Physiol Endocrinol Metab 270: E107–E115, 1996.

41. Popovic V, Damjanovic S, Micic D, Djurovic M, Dieguez C, and Casanueva FF. Blocked growth hormone-releasing peptide (GHRP-6)-induced GH secretion and absence of the synergic action of GHRP-6 plus GH-releasing hormone in patients with hypothalamopituitary disconnection: evidence that GHRP-6 main action is exerted at the hypothalamic level. J Clin Endocrinol Metab 80: 942–947, 1995.

42. Rasmussen MH, Juul A, Kjems LL, Skakkebaek NE, and Hilsted J. Lack of stimulation of 24-hour growth hormone release by hypocaloric diet in obesity. J Clin Endocrinol Metab 80: 796–801, 1995.

43. Schaefer F, Baumann G, Faunt LM, Haffner D, Johnson ML, Mercado M, Ritz E, Mehls O, and Veldhuis JD. Multifactorial control of the elimination kinetics of unbound (free) GH in the

human: regulation by age, adiposity, renal function, and steady-state concentrations of GH in plasma. J Clin Endocrinol Metab 81: 22–31, 1996.

44. Shah N, Aloi J, Evans WS, and Veldhuis JD. Time-mode of growth hormone (GH) entry into the bloodstream and steady-state plasma GH concentrations rather than sex, estradiol, or menstrual-cycle stage primarily determine the GH elimination rate in healthy young women and men. J Clin Endocrinol Metab 84: 2862–2869, 1999.

45. Sokal RR and Rohlf JF. Biometry. New York: Freedman, 1995.

46. Steel RG and Torrie JH. Principles and Procedures of Statistics: A Biometrical Approach. New York: McGraw-Hill, 1980.

47. Tannenbaum GS. Genesis of episodic growth hormone secretion. J Pediatr Endocrinol Metab 6: 273–282, 1993.

48. Tannenbaum GS. Multiple levels of cross-talk between somatostatin (SRIF) and growth hormone (GH)-releasing factor in genesis of pulsatile GH secretion. Clin Ped Endocrinol 3: 97–110, 1994.

49. Tannenbaum GS and Ling N. The interrelationship of growth hormone (GH)-releasing factor and somatostatin in generation of the ultradian rhythm of GH secretion. Endocrinology 115: 1952–1957, 1984.

50. Thorner MO, Rivier J, Spiess J, Borges JLC, Vance ML, Bloom SR, Rogol AD, Cronin MJ, Kaiser DL, Evans WS, Webster JD, Macleod RM, and Vale W. Human pancreatic growth hormone-releasing factor selectively stimulates growth hormone secretion in man. Lancet 1: 24–28, 1983.

51. Tiulpakov AN, Brook CGD, Pringle PJ, Pererkova VA, Volevodz NN, and Bowers CY. GH responses to intravenous bolus infusions of GH releasing hormone and GH releasing peptide-2 separately and in combination in adult volunteers. Clin Endocrinol (Oxf) 43: 347–350, 1995.

52. Van den Berg G, Veldhuis JD, Frolich M, and Roelfsema F. An amplitude-specific divergence in the pulsatile mode of GH secretion underlies the gender difference in mean GH concentrations in men and premenopausal women. J Clin Endocrinol Metab 81: 2460–2466, 1996.

53. Veldhuis JD. Gender differences in secretory activity of the human somatotropic (growth hormone) axis. Eur J Endocrinol 134: 287–295, 1996.

54. Veldhuis JD, Carlson ML, and Johnson ML. The pituitary gland secretes in bursts: appraising the nature of glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. Proc Natl Acad Sci USA 84: 7686–7690, 1987.

55. Veldhuis JD, Evans WS, Shah N, Story S, Bray MJ, and Anderson SM. Proposed mechanisms of sex-steroid hormone neuromodulation of the human GH-IGF-I axis. In: Sex-Steroid Interactions with Growth Hormone, edited by Veldhuis JD and Giustina A. Norwell, MA: Serono Symposia USA, 1999, p. 93–121.

56. Veldhuis JD and Johnson ML. Specific methodological approaches to selected contemporary issues in deconvolution analysis of pulsatile neuroendocrine data. Methods Neurosci 28: 25–92, 1995.

57. Veldhuis JD, Lassiter AB, and Johnson ML. Operating behavior of dual or multiple endocrine pulse generators. Am J Physiol Endocrinol Metab 259: E351–E361, 1990.

58. Veldhuis JD, Liem AY, South S, Weltman A, Weltman J, Clemmons DA, Abbott R, Mulligan T, Johnson ML, Pincus S, Straume M, and Iranmanesh A. Differential impact of age, sex steroid hormones, and obesity on basal versus pulsatile growth hormone secretion in men as assessed in an ultrasensitive chemiluminescence assay. J Clin Endocrinol Metab 80: 3209–3222, 1995.

59. Veldhuis JD, Metzger DL, Martha PM Jr, Mauras N, Kerrigan JR, Keenan B, Rogol AD, and Pincus SM. Estrogen and testosterone, but not a non-aromatizable androgen, direct network integration of the hypothalamo-somatotrope (growth hormone)-insulin-like growth factor I axis in the human: evidence from pubertal pathophysiology and sex-steroid hormone replacement. J Clin Endocrinol Metab 82: 3414–3420, 1997.

60. Veldhuis JD and Pincus SM. Orderliness of hormone release patterns: a complementary measure to conventional pulsatile and circadian analyses. Eur J Endocrinol 138: 358–362, 1998.

61. Weltman A, Weltman JY, Schurrer R, Evans WS, Veldhuis JD, and Rogol AD. Endurance training amplifies the pulsatile release of growth hormone: effects of training intensity. J Appl Physiol 72: 2188–2196, 1992.

62. Wideman L, Weltman JY, Patrie JT, Bowers CY, Shah N, Story S, Veldhuis JD, and Weltman A. Synergy of L-arginine and GHRP-2 stimulation of growth hormone in men and women: modulation by exercise. Am J Physiol Regulatory Integrative Comp Physiol 279: R1467–R1477, 2000.

63. Wideman L, Weltman JY, Shah N, Story S, Veldhuis JD, and Weltman A. The effects of gender on exercise-induced growth hormone release. J Appl Physiol 87: 1154–1162, 1999.

64. Wilmore JH. A simplified method for the determination of residual lung volume. J Appl Physiol 27: 96–100, 1969.