Synergy of L-arginine and GHRP-2 stimulation of growth hormone in men and women: modulation by exercise

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Wideman, Laurie, Judy Y. Weltman, James T. Patrie, Cyril Y. Bowers, Niki Shah, Shannon Story, Johannes D. Veldhuis, and Arthur Weltman. 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.—We investigated the ability of exercise, a multipathway, potent, physiological stimulus for GH release, to alter the synergistic interaction of L-arginine (A) and GH-related peptide (GHRP)-2 (G) observed at rest and the ability of gender to further modulate this putative interaction. Subjects (9 men and 9 early follicular phase women) completed 30 min of constant load aerobic exercise in combination with intravenous infusions of saline (S), A (30 g over 30 min), G (1 µg/kg bolus), or both (AG) in separate study sessions in randomly assigned order. Measures of GH release were logarithmically transformed for statistical analysis. Similar to rest, exercise maintained the rank order (AG > G > A > S) of effective stimulation of GH release for the key response measures in men or women, a gender disparity in the time to reach the maximal serum GH concentration, the calculated endogenous GH half-life, and the observed effect of preinfusion (basal) serum GH concentrations on determining secretagogue responsiveness. Exercise potentiated the individual stimulatory actions of A and G, while blunting the relative magnitude of the synergistic (supra-additive) interaction observed at rest. We infer from the present data that *1*) exercise is likely to induce release of both GHRH and somatostatin, *2*) L-arginine may facilitate the effect of exercise by limiting somatostatin release, *3*) GHRP-2 could further enhance the stimulatory impact of exercise by opposing central actions of somatostatin and/or heightening endogenous GHRH release, and *4*) gender strongly controls the relative but not absolute magnitude of A/G synergy both at rest and after exercise.

male; female; pituitary; somatotropin; regulation; endocrine

EXERCISE IS A POTENT PHYSIOLOGICAL stimulus for growth hormone (GH) release in both sexes (22, 23, 31, 41, 42, 44). Although the neuroendocrine basis underlying exercise-induced GH release remains enigmatic, the mechanism would putatively involve GH-releasing hormone (GHRH) release and/or somatostatin withdrawal and possibly natural GH-releasing peptide (GHRP)-like ligand release or a combination of these mechanisms (18). Application of relevant neurophysiological probes, such as the selective GH secretagogues L-arginine (A) and/or GHRP-2 (G), may help clarify the neuroendocrine pathway of the exercise stimulus. As reviewed further in the DISCUSSION, we reason that if exercise-induced GH release is preferentially mediated by somatostatin withdrawal, then stimulation with A and exercise should evoke equivalent and nonadditive (combined stimuli) responses for GH release. Analogously, if the exercise stimulus depends on release of a putative natural GHRP-like ligand, then the response to exercise and a potent GHRP (e.g., G) should be similar and their combination noninteractive. Conversely, if GHRH is involved as a primary mediator of exercise-induced GH release, then exercise combined with A and/or exercise combined with G should elicit greater GH release then exercise alone. The latter conjecture is based on the synergy observed at rest between GHRP and exogenous GHRH (5, 7, 28, 29), and the interpretation that presumptive release of endogenous GHRH by exercise could also synergize with infused G. Accordingly, here we have investigated the impact of A and/or G infusions on exercise-induced GH release. This strategy thereby assesses indirectly the various mechanistic contributions of endogenous somatostatin withdrawal and GHRH (and/or putative GHRP-like ligand) release to exercise-driven GH secretion in men and women.

Strong gender distinctions in GH neuroregulation are evident in experimental animals and the human (18). Thus we postulated that gender further influences the effects of A and/or G on exercise-induced GH release. Although gender differences in responses to several GH stimulation tests have been reported (1, 18, 21, 25), results of gender comparisons in the limited avail-

able studies with GHRPs have been controversial (2, 4, 27). In corollary, whereas A and GHRP are equally synergistic in both genders studied at rest (companion paper, Ref. 43), the sex-dependency of their interaction with exercise (if any) is not known. Accordingly, here we also explore how gender modulates the interaction between exercise and A or G actions, considered alone and combined. We hypothesized that gender disparities in GH secretory responses to one or both of the secretagogues observed at rest would be effaced by a (potentially multipathway) exercise stimulus.

METHODS

The detailed methodology associated with the resting component (control) of the present study is described fully in the companion paper (43). In the present continuation, we also examined the effects of exercise on GH release. For this paper, the following additional methods were employed.

Subjects completed a peak oxygen consumption ($\dot{V}O_2$)/lactate threshold test on an electronically braked cycle ergometer (Ergo Metrics 800S). Initial power output (PO) was 40 W for women and 60 W for men, and the PO was increased 15 W every 3 min until volitional fatigue. Metabolic measures were collected using standard open-circuit spirometric techniques (Sensormedics metabolic cart 2700Z, Yorba Linda, CA). Heart rate was determined electrocardiographically. An indwelling venous cannula was inserted in a forearm vein, and blood samples were taken at rest and during the last 15 s of each exercise stage for the measurement of blood lactate concentration (YSI Instruments 2700, Yellow Springs, OH). The lactate threshold (LT) was determined from the blood lactate-PO relationship (40). The PO for the 30 min constant load (CL) aerobic exercise sessions (CLPO) was calculated as follows

$$CLPO = PO \text{ at } LT + 0.50(PO \text{ at } \dot{V}O_{2\,peak} - PO \text{ at } LT)$$

Subjects were evaluated in the General Clinical Research Center on four other occasions (exercise). The four stimuli described earlier were applied [saline (S); A alone (30 g iv over 30 min); G alone (1 μg/kg iv bolus); A and G combined] immediately before the 30-min CL exercise bout (0800–0830). Subjects began exercise at the predetermined CLPO, but all subjects were advised that completing 30 min of exercise was more important than remaining at the predetermined CLPO. All decreases in PO were noted, and total work for each admission was calculated as the sum of PO over the 30-min exercise session. Metabolic measures were collected on a minute-by-minute basis, and total number of calories expended during the 30 min CL exercise session was calculated as the sum of the minute values. Heart rate was measured during exercise and was analyzed at 10, 20, and 30 min of exercise. Blood lactate concentrations were measured at 10, 20, and 30 min of exercise. All admissions ($n = 8$; 4 rest, 4 exercise) were randomly ordered and scheduled at least 2 days apart. Women were studied during the early follicular phase (*days 2–8*) of the menstrual cycle, and hence across two or more menstrual cycles to accommodate all eight sessions.

A full description of the statistical methodology was described in the companion paper (43). Briefly, data for serum sex steroids, insulin-like growth factor (IGF)-1, integrated GH [area under the curve (AUC)], and calculated secretion parameters were analyzed by three-way nested ANOVA, with gender, condition (rest vs. exercise), and stimulus type considered as classification variables. Nonadditivity data were analyzed by a two-way nested ANOVA, with gender and condition as classification variables. Regression analysis was used to assess the relationship between maximal serum GH concentrations (independent variable) and GH half-life (dependent variable).

RESULTS

Regardless of the stimulus administered before exercise (saline, A, and/or G), there was no difference in end-exercise blood lactate, end-exercise $\dot{V}O_2$, total kilocalories, and total work in response to the 30-min constant load (CL) exercise bout (Table 1). Men had greater absolute $\dot{V}O_2$ values [1.4-fold, 95% CL(1.1,1.65), $P = 0.007$] and total energy expenditure [1.3-fold, 95% CL(1.0–1.55), $P = 0.026$] compared with women. When end-exercise $\dot{V}O_2$ was adjusted for fat-free mass (ml·kg fat free mass^{-1}·min^{-1}), there was no significant gender difference in this measure. Independent of sex, there was a trend ($P = 0.051$) for heart rate to be higher during the S and A admissions compared with the G and AG admissions. There was also a trend for men to have a greater total work output than women during the 30-min CL exercise bout ($P = 0.076$).

The mean GH response patterns after each stimulus 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. As observed in the resting (R) condition (companion paper,

Table 1. *Gender comparisons for 30-min constant load aerobic exercise*

	End-Exercise Blood Lactate, mmol/l		End-Exercise $\dot{V}O_2$, l		Total Calories, kcal		Total Work, kJ	
	M	F	M	F	M	F	M	F
Saline	6.0 ± 0.7 (6.4)	5.5 ± 0.7 (5.5)	2.5 ± 0.2 (2.5)	2.0 ± 0.2 (2.0)	353 ± 24 (349)	296 ± 24 (292)	285 ± 21 (279)	235 ± 20 (243)
Arginine	5.7 ± 0.5 (5.8)	4.5 ± 0.4 (4.5)	2.7 ± 0.2 (2.6)	1.8 ± 0.2 (1.8)	373 ± 23 (352)	282 ± 24 (281)	281 ± 22 (279)	232 ± 19 (243)
GHRP-2	5.9 ± 0.7 (5.4)	5.2 ± 0.7 (5.9)	2.5 ± 0.2 (2.4)	1.8 ± 0.1 (1.9)	353 ± 21 (351)	279 ± 22 (279)	285 ± 21 (279)	223 ± 19 (204)
AG	5.0 ± 0.5 (5.2)	5.1 ± 0.6 (5.1)	2.4 ± 0.1 (2.2)	1.9 ± 0.2 (1.8)	340 ± 24 (321)	274 ± 21 (269)	278 ± 24 (279)	231 ± 21 (209)

Values are means ± SE (geometric mean). M, males; F, females; GHRP, growth hormone-releasing peptide; AG, combined L-arginine and GHRP ($n = 9$ volunteers in each group). Significant gender main effects are end-exercise peak O_2 consumption ($\dot{V}O_2$) and total calories ($P < 0.05$).
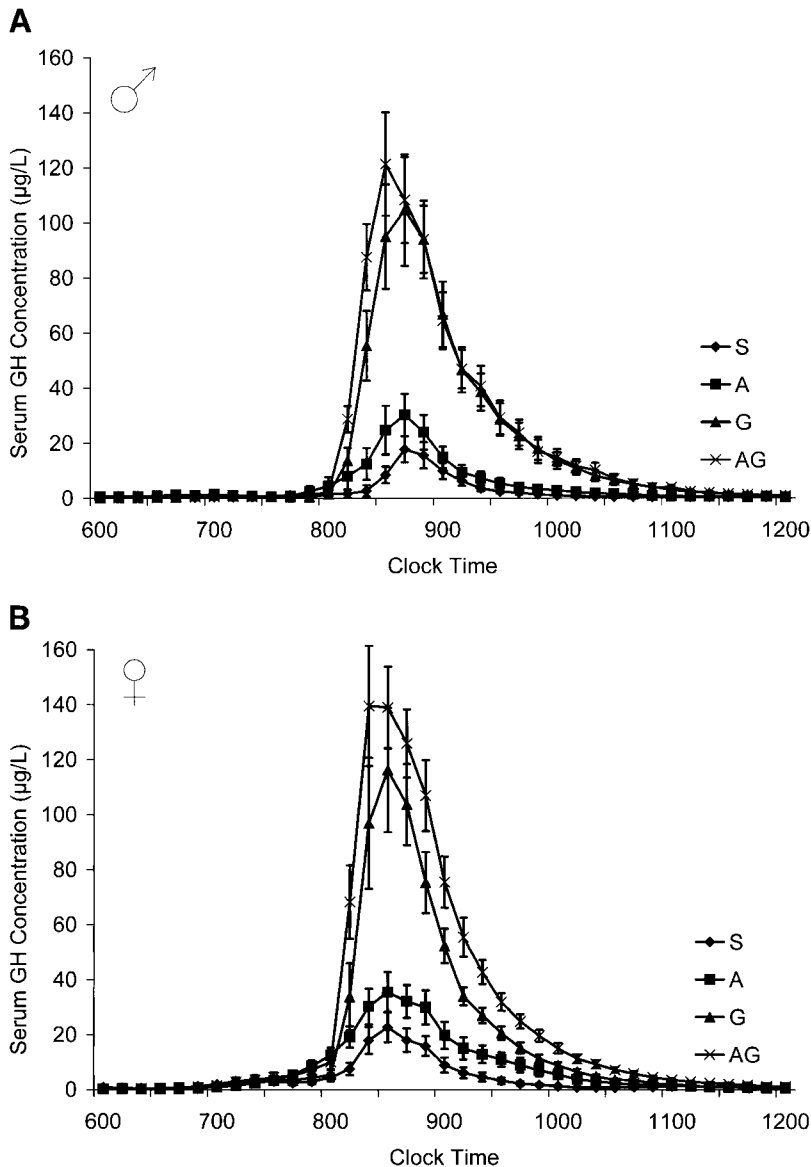
A

B

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

Ref. 43), the rank order of stimulus strength (AG > G > A > S) was similar in men and women. Regardless of the single or combined stimulus administered, the absolute maximal serum GH concentration attained with exercise was greater than that observed at rest (see Fig. 1 of companion paper, Ref. 43). In men, the maximal serum GH concentrations attained after each stimulus combined with exercise was 14, 25, 101, and 116 μg/l (for S, A, G, and AG, respectively); the corresponding values for women were 20, 32, 105, and 143 μg/l (for S, A, G, and AG, respectively). These data represent a 48 (men)- and 23-fold (women) increase in the maximal serum GH concentration in response to AG combined with exercise compared with resting saline control (i.e., 2.4 and 6.1 μg/l for men and women, respectively). The maximal serum GH concentration attained in response to the secretagogue administered was significantly influenced by gender ($P = 0.016$) and condition (rest vs. exercise, $P < 0.001$). Additionally,

the incremental change associated with the exercise vs. rest condition was significantly influenced by gender ($P = 0.009$).

In women, the time to reach maximal serum GH concentration was longer after the A and AG stimuli (60 min) compared with G (30 min). A significant latency was also observed in men, but was greater than in women for the A and G stimuli (70 and 40 min, respectively), but similar for the combined AG stimulus (60 min). The time to reach the maximal serum GH concentration was dependent on gender ($P < 0.001$) and stimulus administered ($P < 0.001$), but independent of the rest vs. exercise condition ($P = 0.272$).

Representative individual serum GH concentration vs. time curves for a man and woman are illustrated in Fig. 2*A*, and the corresponding (calculated) GH secretion profiles assessed by deconvolution analysis are given in Fig. 2*B* (discussed below).
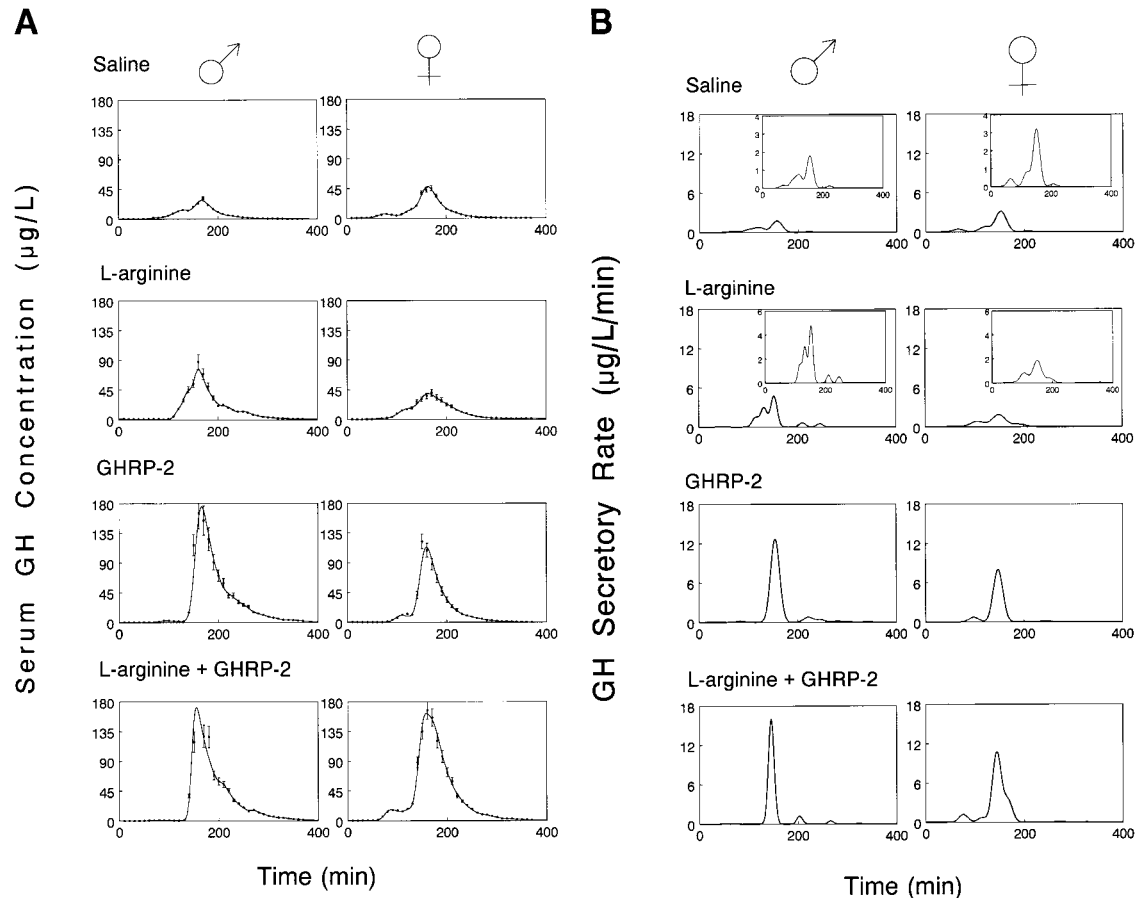
Fig. 2. Representative serum GH concentration profiles (*A*) in an individual healthy young man and woman basally and in response to G and/or A infusions and the corresponding (calculated) GH secretion profiles (*B*). *Time zero* corresponds to 0600 clock time in Fig. 1. GHRP, GH releasing peptide.

Figure 3 presents a box plot summarizing values of total serum (6 h) GH AUC for men and women in response to each stimulus during exercise. The gradation of the responses of serum GH AUC (AG > G > A >

S) was similar to that obtained at rest and the same for men and women. The increase in serum GH AUC for the AG stimulus combined with exercise compared with S (resting control) was 31-fold for men and 15-fold

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. ○, Single measurements below the 10th or above the 90th percentile.
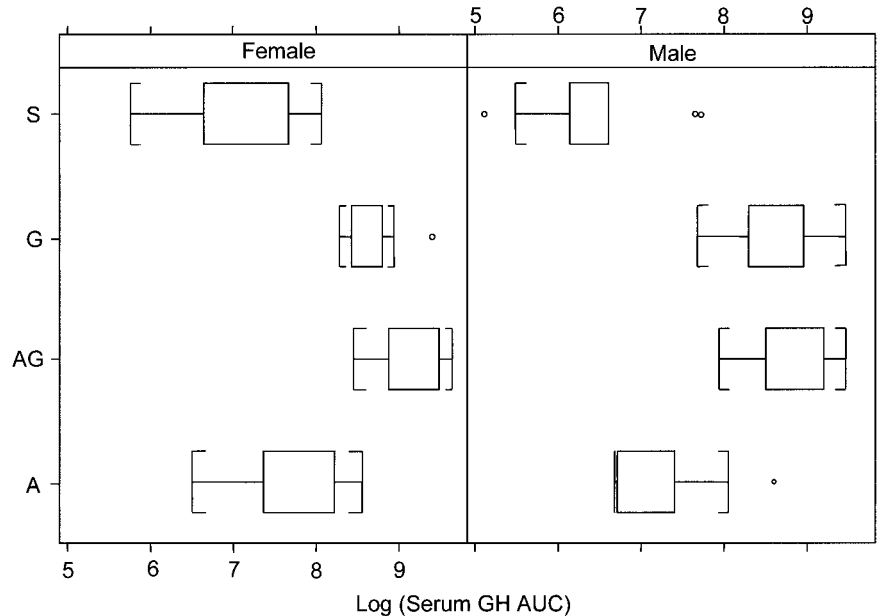
for women. The incremental change in serum GH AUC observed after stimulus administration was influenced significantly by gender ($P < 0.001$) and condition (rest vs. exercise) ($P = 0.007$). In both men and women, exercise elevated significantly the serum GH AUC compared with rest for the S and G stimuli ($P < 0.001$ for both), but not for AG. With the A infusion, exercise resulted in significantly greater serum GH AUC compared with rest in men ($P < 0.001$), but not women. In both women and men, the fold increase observed in GH AUC for the AG stimulus compared with control was greater during exercise than at rest (31-fold vs. 24-fold in men; 15-fold vs. 11-fold for women). When the results for serum GH AUC were combined for men and women during exercise, responses to the A or G stimulus given alone were significantly greater than the S stimulus ($P < 0.001$ for both), but AG compared with G was not significantly different. Exercise alone (saline infusion with exercise) and A infusion alone without exercise exerted equivalent effects on GH AUC.

Figure 4 presents a box plot summarizing values of 90-min GH secretory burst mass. This measure reflects stimulus-driven secretion after correction for half-life. A graded stimulus order was observed for 90-min GH secretory burst mass (AG > G > A > S) in both genders. The increase in 90-min GH secretory burst mass for the AG stimulus combined with exercise relative to the S stimulus at rest was 362-fold for men and 55-fold for women. Men and women had similar absolute values of 90-min GH secretory burst mass after the combined exercise/AG stimulus (252 vs. 248 µg/l). Although 90-min GH secretory burst mass tended to be greater in women than men after exercise combined with S infusion (36.4 vs. 15.7 µg/l), A infusion (73.4 vs. 41.5 µg/l), or G infusion (198 vs. 161 µg/l), none of the differences was statistically significant. The incremental changes in 90-min GH secretory burst mass after secretagogue infusions were significantly influenced by gender ($P = 0.005$) and condition (rest vs. exercise)

($P < 0.001$). In men and women, the fold change in 90-min GH secretory burst mass for the AG compared with the S stimulus was greater with exercise (363-fold for men and 55-fold for women) than at rest (242-fold for men and 40-fold for women). When absolute 90-min secretory burst mass values were combined in men and women, neither comparison of A infusion vs. control (S) nor AG vs. G stimulus showed significant differences during exercise. As was observed for GH AUC, GH pulse mass after exercise alone (saline infusion) and A infusion alone (no exercise) were not significantly different.

The order of magnitude of endogenous GH production rates (over 6 h) was the same for men and women (AG > G > A > S) and identical to that observed at rest (companion paper, Ref. 43). Women tended to have a greater endogenous GH production rate than men for each stimulus, but these differences were nonsignificant (Table 2). The fold change in endogenous GH production rate for the AG stimulus combined with exercise compared with control (S at rest) was 38-fold for men and 10-fold for women. The increase stimulated by AG was greater during exercise than rest in men (38- and 25-fold), but similar in women (10- and 7-fold). The fold change in endogenous GH production rate associated with secretagogue administered was significantly influenced by gender ($P < 0.001$) and condition ($P = 0.017$). Additionally, the incremental change associated with the exercise vs. rest condition was significantly influenced by gender ($P = 0.044$).

Although the number of GH peaks detected per 6 h by deconvolution analysis tended to be greater in women than men in response to the S, A, and G stimuli, none of the differences was significant (data not shown, range = 4–6). Men and women had a similar number of GH peaks after the AG stimulus. However, stimulus type interacted with gender in influencing the number of GH peaks ($P = 0.011$).
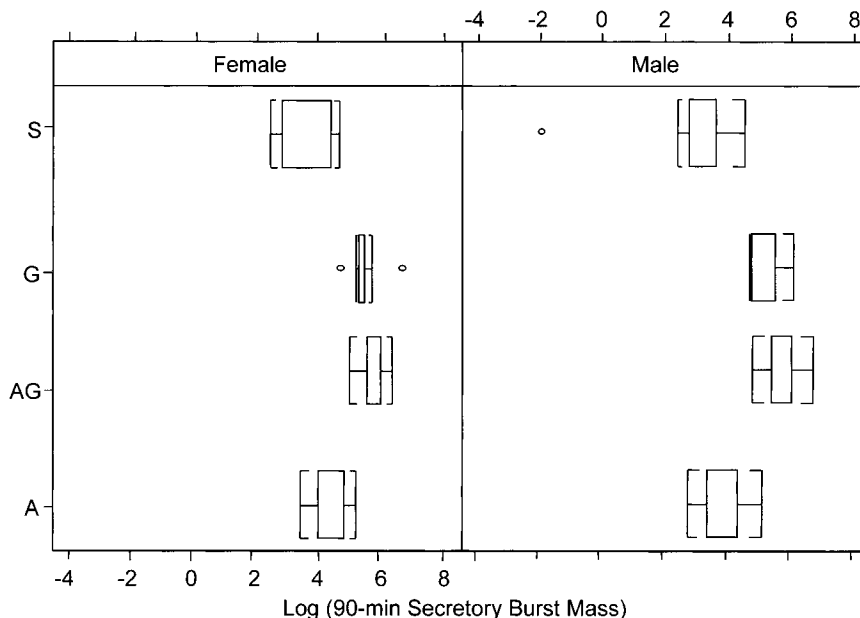

Fig. 4. Box plot representations of log(90-min GH secretory burst mass) during saline infusion and in response to G and/or A infusions in healthy young men and women. ○, Occasional measurements below the 10th or above the 90th percentile.

Table 2. *Gender comparisons for calculated GH secretion measures*

	Basal GH Secretion Rate,[a] $\mu g \cdot l^{-1} \cdot min^{-1}$		GH Half-Life,[b] min		Production Rate,[c,d,e] $\mu g \cdot l^{-1} \cdot 6 \ h^{-1}$	
	M	F	M	F	M	F
Saline	0.007 ± 0.001 (0.006)	0.011 ± 0.002 (0.010)	14.1 ± 0.8 (13.9)	14.4 ± 0.8 (14.3)	36.9 ± 9.2 (28.2)	63.2 ± 12.7 (52.3)
Arginine	0.006 ± 0.002 (0.004)	0.01 ± 0.001 (0.009)	16.4 ± 0.7 (16.3)	16.0 ± 1.5 (15.4)	63.7 ± 16.6 (51.4)	111 ± 18.5 (98.2)
GHRP-2	0.006 ± 0.001 (0.005)	0.01 ± 0.001 (0.009)	20.4 ± 1.4 (20.0)	18.7 ± 1.2 (18.3)	206 ± 38.9 (180)	245 ± 58.2 (214)
AG	0.007 ± 0.001 (0.006)	0.007 ± 0.002 (0.006)	17.4 ± 1.8 (16.3)	22.2 ± 0.9 (22.0)	319 ± 66.6 (270)	298 ± 39.9 (276)

Values are means ± SE (geometric mean). M, males ($n = 9$); F, females ($n = 9$). Time interval for production rate measurement is 0600–1200. All basal GH secretion rate data were obtained during an exercise bout. See (Ref. 43) for analogous results obtained in these subjects at rest. [a]Significant gender main effect, [b]significant treatment main effect, [c]significant treatment × gender interaction effect, [d]significant treatment × condition interaction effect, [e]significant condition × gender interaction effect.

There were no significant gender, stimulus, or condition (rest vs. exercise) differences in fasting serum concentrations of estradiol or IGF-1. Men had significantly higher total and free testosterone concentrations ($P < 0.001$, for both), independent of condition ($P = 0.570$ and $P = 0.539$, respectively) and stimulus type ($P = 0.512$ and $P = 0.560$, respectively).

When the test of nonadditivity was applied to stimulated GH AUC during exercise, the joint AG infusion was synergistic (i.e., supra-additive), compared with the summed individual effects of A and G (A + G) ($P = 0.003$). The difference between AG and the summed individual effects (A + G) in the both genders was small, 1.1-fold for women [95% CL(0.9,1.4)] and 1.0-fold for men [95% CL(0.8,1.3)] (a fold change of 1.0 indicates no difference between the 2 measures) (data not shown). At rest, the fold difference between these responses was 1.6-fold for both women [95% CL(1.2,2.0)] and men [95% CL(1.2,1.9)]. Thus the fold change observed in the synergistic response for stimulated GH AUC was dependent on condition (rest > exercise) ($P < 0.001$), but independent of gender ($P = 0.7$). Figure 5 (box plot) summarizes the difference data

for the test of nonadditivity for stimulated GH AUC during rest and exercise. The incremental change in the synergistic (AG) response at rest compared with exercise was 1.4-fold [95% CL(1.1,1.8)] for women and 1.6-fold [95% CL(1.2,2.0)] for men. These differences were highly significant ($P = 0.007$ and $P = 0.001$ in women and men, respectively).

Analogously, during exercise the test for nonadditivity for 90-min GH secretory burst mass revealed that the combined stimulus (AG) was synergistic compared with the summed individual effects of A and G (A + G) ($P = 0.02$). The response to AG was 0.9-fold greater than the summed effects of (A + G) in exercising women [95% CL(0.67,1.2)] and 1.2-fold for men [95% CL(0.9,1.65)] (data not shown). At rest, the fold difference between these responses was 1.3-fold for women [95% CL(1.0,1.7)] and 1.5-fold for men [95% CL(1.1,2.0)] (companion paper, Ref. 43). Thus there was a trend for the fold change in synergy for 90-min GH secretory burst mass to depend on condition (rest > exercise) ($P = 0.06$), but not on gender ($P = 0.14$). The incremental changes in the synergistic response at rest compared with exercise were 1.5-fold [95% CL(1.0,2.2)]
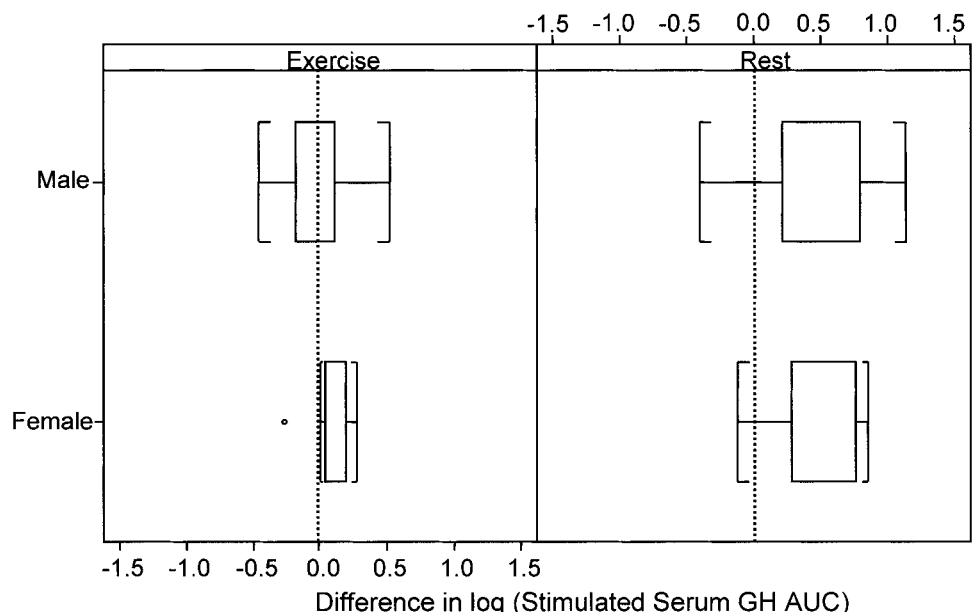

Fig. 5. Box plot representations of the nonadditivity (synergism) of the joint vs. single A and G stimuli. Data are logarithms of the differences between AG (combined A and G infusions) and the summed effects of A and G individually (A + G) at rest and during exercise for 3-h stimulated GH AUC. ○, Measurements below the 10th or above the 90th percentile.
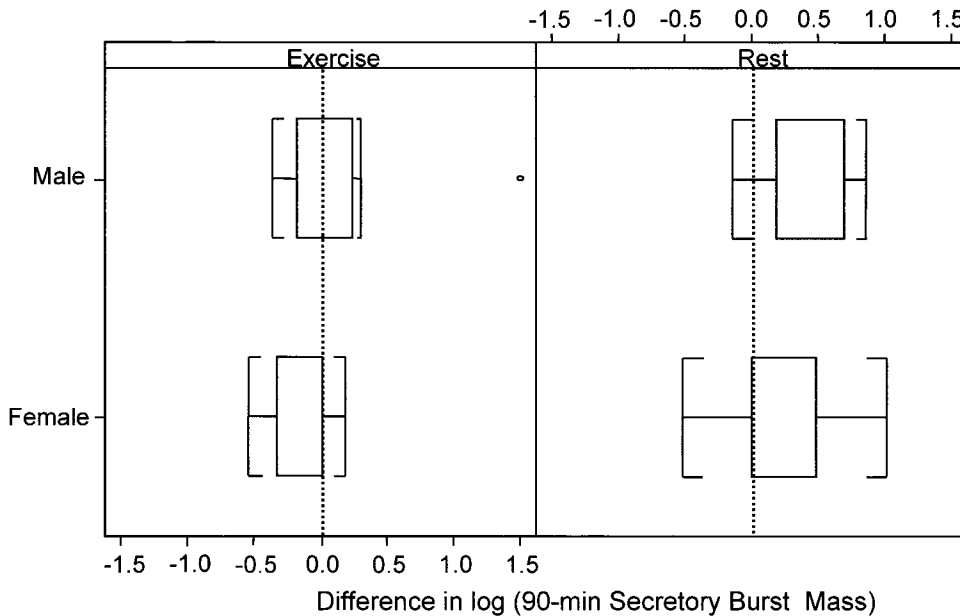
Fig. 6. Analog nonadditivity test for A/G synergism applied to 90-min GH secretory burst mass (see Fig. 5). ○, Measurements below the 10th or above the 90th percentile.

in women and 1.2-fold [95% CL(0.8,1.8)] in men (Figure 6). This comparison was nonsignificant for men and women.

ANCOVA revealed that during exercise (as at rest; companion paper, Ref. 43), the linear relationship between log (basal serum GH AUC) and log stimulated (GH AUC) varied depending on stimulus administered. The relationship between these two variables had a positive slope for the S, A, and AG stimuli, but not for G (results not shown). Whereas substantial residual variance was explained by log(basal serum GH AUC), it failed to change the composition of the terms judged to be important in the original ANOVA model.

With exercise, the calculated GH half-life was higher after the G and AG stimuli compared with the control (S) or A stimuli. The estimated half-life of GH was maximal in women for exercise combined with the AG stimulus (21 min) and in men for exercise and the G stimulus (20 min) (Table 2). The apparent GH half-life was dependent on the stimulus administered ($P < 0.001$) and serum GH concentration attained, but independent of gender ($P = 0.956$) and condition (rest vs. exercise, $P = 0.537$). Power function ($y = ax^b$) regression analysis of maximal serum GH concentrations (independent variable) on GH half-life (dependent variable) in men and women revealed significant curvilinear relationships ($P < 0.001$) (Figure 7). There was no gender difference in these relationships.

DISCUSSION

A major finding of the current investigation in men and women is that A and G interact synergistically in driving GH secretion even during exercise. Indeed, the fold change observed for the combined AG stimulus over control (S) was greater during exercise than at rest. In addition, during exercise the rank order of the GH secretory responses remains AG > G > A > S as recognized earlier in humans studied at rest (compan-

ion paper, Ref. 43). Moreover, exercise potentiated all measures of GH secretion compared with rest. Last, the timing of the maximal serum GH concentration attained during exercise showed a greater delay in men than women, as observed earlier at rest.

Exercise of appropriate intensity and duration serves as a powerful physiological stimulus to GH release (22, 31, 35, 41, 42, 44). Akin to the response nonuniformity inherent in other recognized GH secretagogues (18), the exact magnitude of the exercise response is variable among individuals (22, 23, 42). Although some studies have reported gender differences in exercise-induced GH release (8, 14), other studies have reported exercise-driven GH release to be independent of gender (23, 44). In this regard, we achieve statistically indistinguishable total energy expenditure and total work during constant-load exercise among all the stimuli studied here, which thus eliminates unequal exertion as a source of variability. The
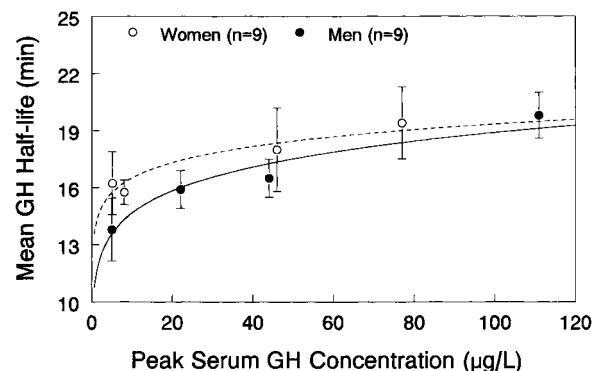

Fig. 7. Power function ($y = ax^b$) regression analysis of the data from all 9 men and 9 women. Regressions relate the mean ($\pm$SE) maximal serum GH concentrations attained after various stimuli (x-axis) to the mean ($\pm$SE) calculated half-lives of endogenous GH (min, y-axis). The power-function fits were significant ($P < 0.001$) in both sexes, with no evident gender differences.

last consideration is important experimentally, because we recently demonstrated that the mass of GH secreted in response to a 30-min exercise stimulus is linearly proportional to exercise intensity (30). Although men attained greater absolute maximal $\dot{V}_{O_2}$ rates at the end of exercise (as expected), it is unlikely that the gender differences observed in GH secretion are related to this distinction, because men and women achieved a similar relative $\dot{V}_{O_2}$ ($\sim$73.5%) and end exercise $\dot{V}_{O_2}$ expressed per kilogram fat-free mass was not different between men and women.

Exercise potentiated the maximal serum GH concentration attained after each secretagogue, compared with responses to the same secretagogue given at rest. The GH secretory response patterns after each stimulus were similar in men and women both during exercise (Figure 1, A and B) and at rest (companion paper, Ref. 43). Similar to previous reports (11, 37), women exhibited higher mean morning fasting baseline serum GH concentrations before the administration of any stimulus compared with men. Therefore, the fold increase in the peak serum GH concentration in response to exercise combined with A and G in men was approximately twice that in women; e.g., at rest 30- vs. 15-fold for men and women, respectively, and during exercise 48- vs. 23-fold for men and women, respectively, reflecting the relatively lower baseline serum GH concentrations in men. Thus, whereas men maintain lower mean baseline serum GH concentrations (and lower basal GH secretion rates) than women, the maximal absolute serum GH concentration attained in response to the exercise stimulus combined with a secretagogue (G and/or A) is similar in men and women, indicating a greater capacity for stimulated GH release in men. These results are similar to Bunt et al. (8), who reported that despite similar absolute maximal GH values for men and women, men had an approximately twofold greater increase in GH concentration compared with women in response to an exercise stimulus of 60% maximal $\dot{V}_{O_2}$. This finding was maintained regardless of the training status of the individual (8).

The integrated serum GH concentration (AUC) over 6 h was used as a complementary and statistically robust measure of total GH release. Similar to the maximal serum GH concentration, GH AUC rose significantly further in response to exercise combined with A, G, or AG compared with the response to each (corresponding) stimulus alone. This observation points to the novel nature of the exercise stimulus in the human (22, 31, 35, 41, 42, 44). Remarkably, exercise potentiated even the absolute GH response observed when A and G were coadministered. We reported earlier that at rest, the coadministration of A and G resulted in a 24- and 11-fold rise in GH AUC above basal (saline) in men and women, respectively. Exercise potentiated this effect in both men and women. In view of the twofold lower basal GH secretion rates in men, the fractional (fold) increase in GH AUC in AG-treated exercising men (31-fold, expressed relative to basal) was also approximately two times larger than that in women (15-fold). However, absolute measures of GH release driven by the threefold stimuli of A, G, and exercise in combination were equivalent in the two sexes, suggesting gender-independent maximal pituitary GH secretory capacity.

As a more direct measure of the immediate hypothalamopituitary secretory response to the foregoing agonists, we calculated 90-min GH secretory burst mass. This value encapsulates all GH release immediately after any given stimulus while obviating the effects of unequal GH half-lives, spurious spontaneous GH release at later times in the sampling session, and variable GH concentrations in the blood before secretagogue infusion (38). According to this reasoning, the 90-min GH secretory burst mass may best represent the actual GH secretory response independent of differences in recovery of spontaneous GH pulsatility, GH half-life, and/or prestimulus GH levels. With this metric, we tested the hypothesis that the predominant mechanism of exercise-induced GH release involved withdrawal of hypothalamic somatostatin. To this end, we assessed the impact of the A stimulus alone, exercise alone, and their combination on the 90-min GH secretory burst mass. This strategy reflects current inference that A (via muscarinic, cholinergic, or unknown mechanisms) restrains hypothalamic somatostatin release (17). If the sole mediator of exercise-induced GH release were somatostatin withdrawal, then A combined with exercise would result in no further increase in 90-min GH secretory burst mass above that of each stimulus alone. Conversely, a contributory role for GHRH (or other secretagogues) would be implied by significant interactive amplification of GH release by A and exercise (18). Under these assumptions, data from the current investigation indicate that the mechanism of exercise-induced GH release mimics at least in part, the action of A, because the 90-min GH secretory burst mass was similar in response to A infusion and exercise alone; moreover, combined A infusion and exercise did not significantly potentiate GH release. Several mechanistic considerations could explicate these findings. First, in several studies, β-adrenergic stimulation of somatostatin release was able to overcome the ability of A (or pyridostigmine) to increase GH secretion (15, 16). Exercise likely activates several central nervous system neurotransmitter pathways, including the adrenergic, cholinergic, and opioid systems (26, 35, 36). Given that exercise stimulates adrenergic outflow (34, 36, 39), we speculate that this inhibitory neurotransmitter response may oppose in part the stimulatory impact otherwise achieved by exercise or A. The approximate doubling of absolute 90-min GH secretory burst mass by A plus exercise vs. exercise alone in each sex (from 30 to 70 for women and 15 to 41 for men) (Fig. 4) would point to this possible explanation; i.e., A infusion partially overcomes the β-adrenergic (or other) inhibition otherwise induced by exercise itself.

Measures of 6-h GH AUC suggest additional mechanistic insights. A alone and exercise alone elicited similar GH release over 6 h, but A combined with exercise evoked a significantly greater GH AUC than

exercise alone. This also suggests that A and exercise do not release GH exclusively via the same mechanism. We note that 6-h GH AUC and 90-min GH secretory burst mass provide complementary measures of GH axis activity. For example, the calculated GH AUC is influenced by GH half-life, whereas the 90-min GH secretory burst mass is corrected for GH half-life variations. More importantly perhaps, the calculated GH AUC encapsulates the entire 6 h of data collection and, therefore, could be modulated by differences in GH autofeedback emerging >90 min after the secretagogue/exercise stimulus. This consideration is of interest, because A demonstrably blunts GH-induced inhibition of GH release (18). We speculate that the positive interaction between A and exercise on 6-h GH AUC may thus reflect A-mediated suppression of GH autofeedback.

Because we did not infuse GHRH in the present investigation, we cannot comment directly on the role of GHRH in exercise-induced GH release. However, several studies have shown that GHRP and GHRH typically act synergistically to stimulate GH release in humans (4, 6, 7, 28, 29). In addition, GHRP infusion promotes GHRH secretion in the sheep (13, 19). Thus enhancement of G action by exercise could reflect exercise and/or G's potentiation of endogenous GHRH release.

Addition of A did not further amplify the 90-min GH secretory burst mass stimulated by G combined with exercise. This new observation could signify that *1*) somatostatin release is minimal during the G plus exercise stimulus and/or *2*) near-maximal stimulation of pituitary GH secretion is achieved by G alone, with or without corelease of endogenous GHRH. In addition, GHRPs can partially oppose the actions of somatostatin (33), in which circumstance the addition of A might exert little further effect. Alternatively, these collective issues could be harmonized by the thesis that A might act via nonsomatostatinergic pathways. Whereas exercise alone stimulates some somatostatin restraint of GH release, coadministration of G during exercise opposes this effect, thus *1*) achieving a synergy between exercise and G and *2*) limiting yet further potentiation by A during triple-secretagogue drive.

In the experimental animal, hypothalamic GHRH can stimulate somatostatin release, whereas somatostatin inhibits GHRH secretion (3, 12, 20, 24, 45). Thus A might heighten the effect of exercise alone by reducing somatostatin's suppression of GHRH release (18). Analogously, GHRP's reported antagonism of somatostatin's action (above) could potentiate the exercise effect. Both of these models would predict that the threefold stimulation (AG and exercise) would not interact further, as indeed observed here.

In men, A augmented the joint stimulatory effects of exercise and G on endogenous (6 h) GH production rate (summed pulse mass) over S control. This gender difference could indicate that GH secretion in women is less susceptible to GH (auto) feedback, which might be expressed over the 6-h observation interval (but not

necessarily within the first 90 min). This perspective would be consistent with gender differences in GH autofeedback in the rat (10) and with the ability of A to limit GH autofeedback in the human presumptively via somatostatin withdrawal (18).

Conversely, somatostatin infusions in the human strongly suppress GH pulse mass (9). Thus a gender difference in somatostatin withdrawal by A is a relevant consideration in explicating the more marked GH secretory output of men in this unique triple-secretagogue setting.

Combined (joint) AG stimulation remained synergistic during exercise compared with the summed effects of separate A and G infusions (A + G) during exercise ($P = 0.02$). Possible mechanisms for this synergistic effect, as initially observed at rest, are outlined in the companion paper (43). In addition, we now show that the magnitude of this synergism tends to decrease with exercise (Fig. 6) ($P = 0.06$); i.e., the foregoing joint response was 1.2- to 1.5-fold greater during rest than exercise in men and women, respectively. The lesser synergy between A and G during exercise than at rest could indicate that exercise drives elements in the GH response pathway(s) that are already activated by A and/or G. Alternatively, exercise might evoke near-maximal GH secretion and/or unrecognized secondary neuroregulatory effects that partially antagonize the joint effects of A or G.

The tendency of the synergy between A and G to be blunted by exercise was especially conspicuous in men and women in relation to the 3-h GH AUC (vs. 90-min GH secretory burst mass). This finding could indicate first that additional GH secretion occurs after 90 min especially in women, e.g., because of purported sex differences in GH autofeedback (see companion paper, Ref. 43). Second, the combined AG stimulus and exercise may have altered basal GH release in opposite directions. Present methods of secretory pulse analysis cannot readily address the latter speculations, because underlying basal (nonpulsatile) GH secretion is difficult to quantitate during massive GH outpouring. Last, because AUC calculations are also influenced by GH half-life (32), we compared the latter in men and women in response to these joint stimuli. A power function regression of GH half-life against GH concentration was statistically identical in men and women (Fig. 7), thus excluding this notion.

In summary, exercise potentiates the individual and joint stimulatory actions of A and G in both men and women. We postulate that exercise stimulates GHRH secretion and partially restrains further GH release (e.g., by activating endogenous adrenergic and somatostatinergic outflow); A facilitates the effects of exercise by limiting somatostatin release, and G enhances the stimulatory effect of exercise by heightening endogenous GHRH release and/or by opposing central actions of somatostatin. Last, compared with rest, exercise blunts the relative (but not absolute) magnitude of the synergy between A and G, suggesting a novel threefold neuroendocrine interaction among these distinct stimuli.

Perspectives

Several gender differences emerged under the physiological stimulus of exercise. Men have a greater fold change in GH secretion compared with women in response to exercise. Women attain a maximal GH concentration more rapidly than men under exercise drive. The precise biological significance of these response differences to the target tissue is still unknown. However, the greater reliance of women on fat metabolism during exercise might relate to the foregoing contrasts. In absolute terms, near-maximal secretion of GH achieved by triple stimulation with A, G, and exercise was evidently gender independent. These distinctions might arise if exercise evokes multiple and complementary neuroregulatory responses by the GH axis. Such an inference is consistent with the unique ability of exercise to enhance GH secretion more than either secretagogue alone or combined. This threefold interaction allows for the conjecture that exercise recruits nonsomatostatinergic, non-GHRH, and non-GHRP-dependent pathways of cosecretagogue outflow as well. The nature of such ancillary stimulatory pathways is not known. On the other hand, relief of coinhibitory factors would also explicate the present data, because exercise facilitated the synergistic action of G and A. The unexpected ability of exercise to blunt the relative magnitude of the synergistic action of A and G achieved at rest also supports a putative multifold impact of exercise on GH neuroregulation. For example, other recent exercise studies have suggested that exercise may be a unique stimulus in overriding GH auto-negative feedback, possibly independent of gender. Accordingly, the novel and manifold GH-stimulating nature of exercise makes this intervention both an investigative challenge and an excellent clinical tool for evaluating the GH hypothalamopituitary axis. Additionally, exercise might be meritoriously combined with other secretagogues in enhancing GH output, because this stimulus appears to effect GH secretion at multiple levels.

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