Growth hormone responses during strenuous exercise: the role of GH-releasing hormone and GH-releasing peptide-2

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ABSTRACT

MAAS, H. C. M., W. R. DE VRIES, I. MAITIMU, E. BOL, C. Y. BOWERS, and H. P. F. KOPPESCHAAR. Growth hormone responses during strenuous exercise: the role of GH-releasing hormone and GH-releasing peptide-2. *Med. Sci. Sports Exerc.*, Vol. 32, No. 7, pp. 1226–1232, 2000. **Purpose and Methods:** This study was designed to investigate the role of two effective releasers of growth hormone (GH): GHRH and GHRP-2 during exercise (EX). Eight healthy male subjects (ages: 22 ± 1.2 (mean ± SD) yr, BMI: 22.5 ± 2.2 $kg \cdot m^{-2}$) were exposed to maximally stimulating dose of 100 μg GHRH iv, and 200 μg GHRP-2 iv, during incremental EX on a cycle ergometer to exhaustion. GH responses after EX alone were compared with the responses after the combined administration of the same EX plus GHRH, EX plus GHRP-2, and EX plus GHRH plus GHRP-2. Blood samples were obtained in the fasted state at intervals for 2 h postexercise and the area under the GH response curve (AUC) was calculated by trapezoidal integration. **Results:** Significant differences ($P < 0.003$) were observed between the AUCs after administration of EX alone (mean ± SEM): 2324 ± 312 $\mu g \cdot L^{-1} \cdot 120$ min, after EX plus GHRH: 6952 ± 1083, after EX plus GHRP-2: 14674 ± 2210, and after the combination EX plus GHRH plus GHRP-2: 17673 ± 1670. However, AUCs after each combination did not differ significantly from those after arithmetical addition of each separate stimulus, indicating that the mechanisms of the respective stimuli do not interact. Linear regression analysis on mean GH responses between 20 and 30 min after the start of EX showed that EX alone and GHRH alone explain about 59% (adj. R^2) of the GH response to the combination EX plus GHRH. The ratio of the respective regression coefficients (GHRH vs EX) was about 2:1 (instead of 1:1), indicating that EX seems to potentiate the activity of GHRH. GHRH alone and EX alone also explained about 74% of the response to the combination EX plus GHRP-2. In the latter response, a synergistic action of GHRP-2 on GHRH could be observed. **Conclusions:** The data indicate that under strenuous EX conditions, endogenous GHRH activity causes a further increase of GH release. A GHRP-2 mediated mechanism in the central neuroendocrine regulation acts as a "booster," possibly by stimulating the effects of GHRH and/or an unknown hypothalamic factor, as well as by stimulating the pituitary GH release directly.
Key Words: GROWTH HORMONE SECRETAGOGUES, HEXAPEPTIDES, NEUROENDOCRINE REGULATION, EXERCISE, CYCLE ERGOMETER

The neuroendocrine mechanisms for exercise-induced growth hormone (GH)-release are still incompletely understood. It has already been shown that the GH response to submaximal exercise is mainly mediated by an increased central cholinergic tone (6,11,19,25,32), which reduces hypothalamic somatostatin. Therefore, we assume that in conditions where inhibition of somatostatin is maximal, such as during exercise at an intensity of more than 80% $\dot{V}O_{2max}$ (25), further activation of pituitary GH release must be performed by other mechanisms. We hypothesize that during high-intensity exercise GH-releasing hormone (GHRH) operates as a primary factor for GH release, with the help of a secondary releasing factor as co-agonist.

Recently, we showed that serum GH responses, evoked by the combined administration of exercise plus the synthetic secretagogue GH-releasing peptide-6 (GHRP-6), far exceeded the summated responses after exercise alone and GHRP-6 alone (25). In fact, the levels of these responses resembled those previously reported after the combined administration of GHRP-6 and GH-releasing hormone (GHRH), and the observed increase in frequency and amplitude of GH peaks favors the possibility that exercise-induced GH release is mediated through an increase in endogenous GHRH release (7,25,26).

In order to further define underlying neuroendocrine mechanisms of pituitary GH release, this experimental study

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was designed to investigate the role of two effective releasers of GH: GHRH and the hexapeptide GH-releasing peptide-2 (GHRP-2, D-Ala-DβNal-Ala-Trp-D-Phe-Lys-NH_2) during strenuous exercise. GHRP-2 has no structural homology with GHRH and acts both at the level of the pituitary and the hypothalamus via receptors different from that of GHRH or somatostatin (5,30). In general, GHRP-2 acts synergistically with GHRH (3), but its role in the central neuroendocrine regulation during exercise is not known. It is well known that the GH-releasing effects of GHRH and GHRP-2 are dose-related (3,5,14). By giving maximally stimulating doses, tentative conclusions can be drawn along the following line: if, for example, the amount of GH released by the combined administration of exercise plus GHRP-2 is not greater than to GHRP-2 alone, exercise works by a GHRP-2 mediated mechanism, whereas if the amount of GH released by the combined response exercise plus GHRP-2 exceeds the released GH amount by GHRP-2 alone, some other mechanism is likely to be involved.

Subjects were exposed to a maximally stimulating dose of GHRH, GHRP-2, and to a combination of the two at rest, and during incremental exercise to exhaustion. The corresponding GH release was calculated as area under the curve (AUC) for 2 h. Taking into account the different time course of the GH release, we also analyzed the relationship between the exercise-related trials and calculated the relative contribution of each secretagogue to the GH response. Further, we tried to unravel interaction effects of the different mechanisms for GH release. We limited this analysis to three repeated measures, obtained during the time interval, in which peak GH responses were evoked (i.e., 20–30 min after the start of the exercise).

METHODS

Subjects. Eight healthy male subjects, aged 20–25 yr, were studied after approval of the ethics committee of the University Hospital Utrecht and after giving informed consent. Their habitual activity level ranged from sedentary to well-trained, and all subjects were nonsmokers. None had a history of medical illness, and none were taking medication or were obese (body weight: 74.6 ± 6.3 kg, height: 182.4 ± 3.3 cm, body mass index: 22.5 ± 2.2 kg·m^{-2}, body fat percentage: 13.5 ± 4.5%, lean/fat ratio: 6.4 ± 1.9, and waist/hip ratio: 0.87 ± 0.06 (mean ± SD). Assessment of fat mass (FM) and fat free mass (FFM) was performed using a tetrapolar bioelectrical impedance analyzer (BIA-101 analyzer, RJL-systems, Detroit, MI) with the electrodes placed as described by Lukaski et al. (22). FM and FFM were calculated using regression equations, developed by Lukaski and Bolonchuk (23).

Study design. At least 1 wk before the treatment trials, each subject participated in a maximal performance test on the cycle ergometer (Excalibur, Lode, Groningen, The Netherlands) to determine individual levels of peak oxygen uptake (the highest oxygen uptake attained during the test, $\dot{V}O_{2max}$). The test started at a workload of 2 W·kg^{-1} and subsequently every 2 min a load of 0.5 W·kg^{-1} was added until the subjects were exhausted. A breath-to-breath gas sampling method was used to measure oxygen uptake (Oxycon β, Mijnhardt, Bunnik, The Netherlands).

After an overnight fast, each subject started the experiments at 0900 h. Seven trials were performed at least 4 d apart, in order to have enough time for recovery. We started with exercise alone, followed in random order by administration of GHRH or GHRP-2, combined administrations of GHRH plus GHRP-2, exercise plus GHRH, exercise plus GHRP-2, and exercise plus GHRH plus GHRP-2. Thirty minutes before each trial, a catheter was placed in an antecubital vein and was kept patent with a heparin/saline solution. The moment of the start of each experiment was called t = 0. Blood samples were taken immediately before t = 0, and at t = 15, 25, t = max (time point immediately after exhaustion), 30, 35, 45, 60, 90, and 120 min. During the trials without exercise, t = max corresponds to the time point obtained from the trial with exercise alone. Exercise consisted of cycling on an ergometer, according to an incremental protocol: 5 min at 40% $\dot{V}O_{2max}$, 10 min at 60% $\dot{V}O_{2max}$, 10 min at 80% $\dot{V}O_{2max}$, and finally 100% $\dot{V}O_{2max}$ until exhaustion. GHRH (100 μg in 2 mL 0.9% NaCl solution) and GHRP-2 (200 μg in 4 mL 0.9% NaCl solution) were administered separately or combined as a bolus injection at t = 0. In all treatments involving GHRH, one of eight subjects reported transient flushing, whereas no side effects were seen in treatments involving GHRP-2.

Hormonal assays. Blood was collected into silicone coated tubes and immediately chilled. After centrifugation serum was stored at −20°C until assayed within 4 wk. Serum GH levels in $\mu g \cdot L^{-1}$ were measured in duplicate by IRMA (Oris Industry Company, Gif-sur-Yvette, France). GH standards were calibrated according to the WHO reference standard 66/217. There was no cross-reactivity with LH, FSH, HCG, TSH, or PRL. The mean interassay coefficients of variation (CV) were 6.1%, 5.0%, and 6.0% at GH concentrations of 6.2, 19.6, and 59.8 $\mu g \cdot L^{-1}$, respectively. The intra-assay CV was 7.7% at a GH level of 8 $\mu g \cdot L^{-1}$.

Statistics. GH responses after administration of the different stimuli were calculated as the area under the response curve (AUC in $\mu g \cdot L^{-1} \cdot 120$ min) by trapezoidal integration. Differences between the response curves were analyzed using nonparametric statistics (Friedman test), followed by a *post hoc* analysis, using Wilcoxon matched-pairs signed-ranks test. Statistical significance was set at P-values <0.05 (two-tailed). Further, for each trial reliability analyses (SPSS for Windows Release 6.1) were performed on three repeated measures, obtained between 20 and 30 min after the start of the trial, to establish whether these data are reproducible, additive, and fit a parallel model. To increase the power of discrimination, these data were summated and averaged. Differences were analyzed with the same nonparametric statistics as used by AUC. Statistical significance (two-tailed) was tested with the method Exact (Monte Carlo significance). Hereafter, a correlation matrix ($N = 8$) between the four (averaged) exercise-related responses was calculated, followed by multiple linear regression analysis.

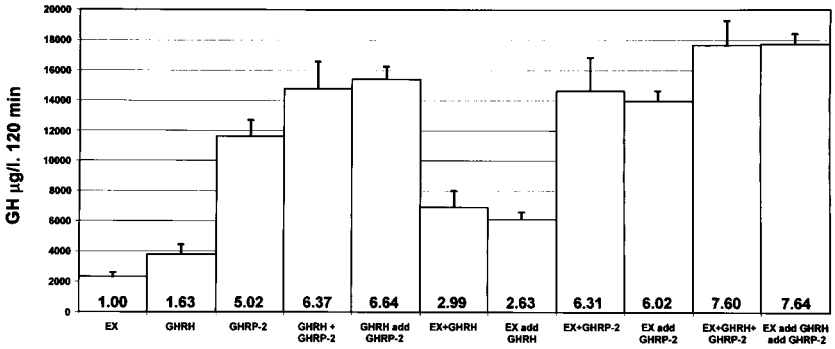

Figure 1—AUC in $\mu g \cdot L^{-1} \cdot 120$ min (mean ± SEM, $N = 8$) after exercise (EX), after GHRH (100 μg, iv), after GHRP-2 (200 μg, iv), and after the combined administrations of GHRH plus GHRP-2, EX plus GHRH, EX plus GHRP-2, and EX plus GHRH plus GHRP-2. The combinations GHRH add GHRP-2, EX add GHRH, EX add GHRP-2, and EX add GHRH add GHRP-2 are the arithmetical additions of the responses to each stimulus separately. The basis of each column depicts the ratio (AUC/AUC EX).

RESULTS

In the maximal exercise test administered before the treatment trials, the highest workload achieved was (mean ± SD) 325 ± 21.7 W with a peak oxygen uptake of 3.9 ± 0.4 $L \cdot min^{-1}$ (52.6 ± 4.3 $mL \cdot kg^{-1} \cdot min^{-1}$).

AUC. Figure 1 shows the AUCs after all trials. No significant difference was found between the AUCs after exercise and GHRH: (mean ± SEM: 2324 ± 312 $\mu g \cdot L^{-1} \cdot 120$ min and 3785 ± 661, respectively), whereas the difference between GHRP-2 and the combination GHRH plus GHRP-2 approached significance (11,655 ± 1083 and 14,803 ± 1,789, $P = 0.06$). Highly significant differences were observed between exercise alone and GHRP-2 alone, and exercise alone versus the combination GHRH plus GHRP-2 (2324 ± 312 and 11,655 ± 1083; 2324 ± 312 and 14,803 ± 1789, respectively, both $P < 0.001$).

Highly significant differences in AUCs after exercise alone, exercise plus GHRH, exercise plus GHRP-2, and exercise plus GHRH plus GHRP-2 were observed (2324 ± 312, 6952 ± 1083, 14764 ± 2210, and 17673 ± 1670, respectively, all $P < 0.003$). No significant differences were observed between the AUCs of all combined administrations and the arithmetical sum of the respective secretagogue-mediated GH release.

Peak values and repeated measures. Figure 2 illustrates the difference in time course of the relative GH responses after exercise alone, GHRH alone, and GHRP-2 alone. The peak value of each stimulus was set at 100%. At the moment that exercise reached its 100% level, those of GHRH or GHRP-2 were about 80% of their maximal response.

Table 1 and Figure 3 show the mean GH responses from baseline to all stimuli as a function of time. Peak serum value after administration of GHRH was reached at t = 60 min, after GHRP-2 at t = 35, and after the combination GHRH plus GHRP-2 at t = 30. For all exercise-related trials we observed peak values at t = max, i.e., immediately after exhaustion. The incremental protocol showed no significant difference in time to exhaustion: 25 min 24 s ± 2 min 4 s for exercise alone, 26 min 12 s ± 2 min 10 s for exercise plus GHRH, 23 min 48 s ± 1 min 55 s for exercise plus GHRP-2, and 24 min±2 min 13 s for exercise plus GHRH plus GHRP-2.

Reliability analyses on the responses between 20 and 30 min after the start of each of the seven trials showed that the unbiased estimate of reliability ranged between 0.98 and 0.99, and that there were no significant differences from nonadditivity or from fitting a parallel model. No significant differences were observed between the averaged responses after exercise versus GHRH (56.2 ± 8.3 $\mu g \cdot L^{-1}$ and 39.9 ±

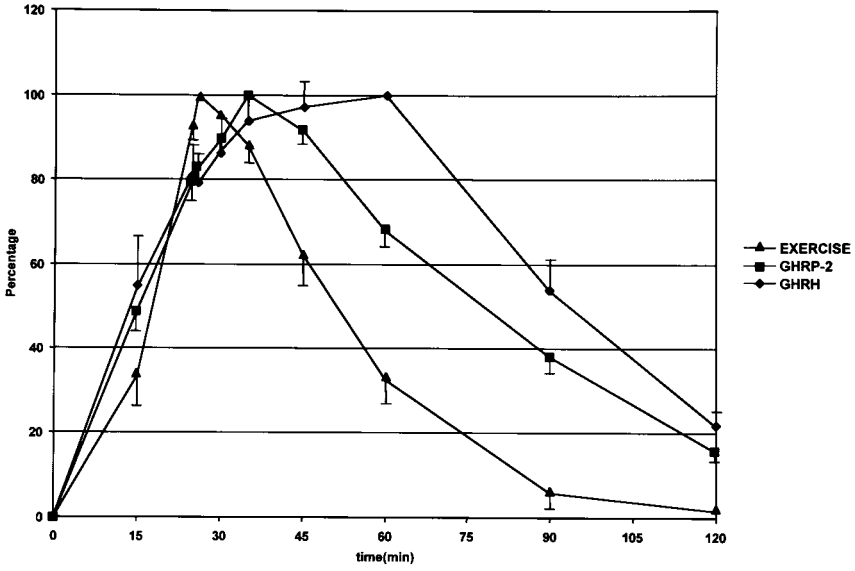

Figure 2—Relative GH responses in % (mean ± SEM, $N = 8$) as a function of time, to exercise, GHRH and GHRP-2. Peak values were set at 100%.

TABLE 1. Mean (±SEM) growth hormone (GH) responses ($\mu g \cdot L^{-1}$, $N = 8$) from baseline to all stimuli as a function of time.

	t (min)								
	15	25	Max	30	35	45	60	90	120
GHRH	26.4	39.3	38.3	42.2	45.5	47.2	48.4	25.9	10.4
	4.8	4.9	5.1	6.0	7.3	8.9	10.7	6.7	2.6
GHRP-2	88.2	143.5	149.2	161.4	179.9	165.1	121.9	68.1	27.9
	9.7	15.0	15.2	16.1	18.7	16.3	14.1	7.9	4.0
GHRH + GHRP-2	132.7	167.6	175.9	176.3	168.5	170.8	154.6	106.4	58.4
	14.3	25.0	25.8	18.9	26.6	23.4	17.8	13.3	12.7
EX	19.4	54.6	58.4	55.6	51.5	36.2	18.9	3.4	0.9
	5.4	8.8	9.3	7.6	7.2	4.8	3.0	1.2	0.4
EX + GHRH	72.6	125.8	134.7	122.0	108.1	95.6	68.3	26.2	8.6
	14.6	17.3	15.2	16.0	15.8	18.2	13.4	5.0	2.1
EX + GHRP-2	149.0	239.9	261.2	255.4	228.5	195.1	133.1	66.9	27.6
	13.2	30.6	37.1	40.8	35.3	33.0	21.1	14.0	5.8
EX + GHRH + GHRP-2	209.6	262.4	267.3	242.5	245.1	204.0	157.8	106.9	48.7
	14.0	26.1	29.0	32.8	18.5	26.6	18.9	13.7	6.1

GHRH, GH-releasing hormone; GHRP-2, GH-releasing peptide-2; EX, exercise; EX + GHRH, exercise plus GHRH; EX + GHRP-2, exercise plus GHRP-2; and EX + GHRH + GHRP-2, exercise plus GHRH plus GHRP-2.

5.2, respectively) and between GHRP-2 versus GHRH plus GHRP-2 (151.4 ± 15.1 and 173.2 ± 22.8). In the exercise-related trials, the responses showed almost the same trend as observed for the AUCs. Highly significant differences ($P < 0.001$) were found between exercise (56.2 ± 8.3 $\mu g \cdot L^{-1}$), exercise plus GHRH (127.5 ± 16.0), and exercise plus GHRP-2 (252.2 ± 35.7). Latter value, however, did not differ significantly from that after exercise plus GHRH plus GHRP-2 (257.4 ± 28.7). No significant differences were observed between the arithmetical addition of responses and the combined responses, with the exception for the difference between the combination exercise plus GHRH versus addition (127.5 ± 16.0 and 96.2 ± 8.5, respectively, $P = 0.02$).

The correlation matrix (Table 2) showed a significant relationship between the response after exercise plus GHRH and exercise plus GHRH plus GHRP-2, whereas the relationship between exercise plus GHRH and exercise plus GHRP-2 approached significance ($P = 0.06$). Multiple regression analysis showed that about 59% (adj. R^2) of the variance in the GH response to exercise plus GHRH could be explained by the contributions of exercise and GHRH alone. The ratio of the regression coefficients GHRH versus exercise is about 2 (Table 3A). Exercise and GHRH also explained about 74% of the variance in GH response to exercise plus GHRP-2. The ratio of the regression coefficients GHRH versus exercise increased to about 3 (Table 3B). Finally, about 57% of the variance in GH response to exercise plus GHRH plus GHRP-2 could be explained by the combined administration of exercise plus GHRH, without a significant contribution of GHRP-2 (Table 3C).

TABLE 2. Correlation matrix of the (averaged) growth hormone (GH) responses after four exercise (EX)-related conditions; significance was accepted at $P < 0.05$.

EX	EX + GHRH	EX + GHRP-2	EX + GHRH + GHRP-2
EX	0.42	0.22	0.01
	$P = 0.30$	$P = 0.61$	$P = 0.98$
EX + GHRH		0.68	0.80
		$P = 0.06$	$P = 0.02$
EX + GHRP-2			0.38
			$P = 0.36$

DISCUSSION

As the exact neuroendocrine mechanisms by which exercise stimulates GH release are poorly understood, we postulate the following model. At relatively low work loads,

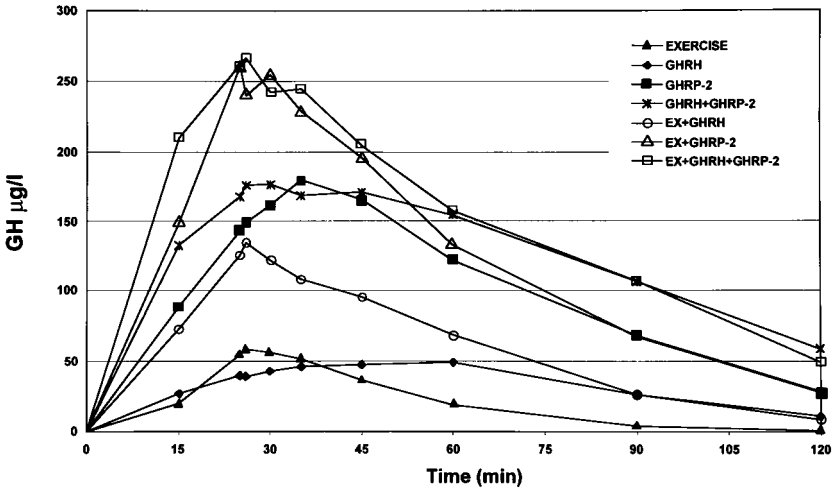

Figure 3—Mean responses of serum GH ($\mu g \cdot L^{-1}$, $N = 8$) as a function of time to exercise (EX), to GHRH (100 μg), to GHRP-2 (200 μg), and to the combined administrations of GHRH plus GHRP-2, EX plus GHRH, EX plus GHRP-2, and EX plus GHRH plus GHRP-2. Intravenously administration of secretagogues was done at t = 0. For reasons of clarity, no SEM data are depicted.

TABLE 3A. Regression analysis of the averaged growth hormone (GH) response (in $\mu g \cdot L^{-1}$) to the combined administration of exercise (EX) plus GHRH dependent on the averaged response to EX alone and to GHRH alone.

Variables	Coefficient	Standard Error	t	P
EX	1.22	0.48	2.53	0.05
GHRH	2.30	0.76	3.03	0.03
Constant	−33.09	47.15	−0.70	0.51

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Regression	2	10165.69	5082.84	6.12	0.04
Residual	5	4152.95	830.59		

the moderate GH responses are mainly due to activation of the central cholinergic system, which results in inhibition of the hypothalamic somatostatinergic tone, which on its own is a weak stimulus for GH release (6,11,19,25,32). Inhibition of somatostatinergic tone is a saturated process. At high-intensity exercise, assuming complete suppression of hypothalamic somatostatinergic tone, a further increase of GH release must be due to other mechanisms, such as a GHRH-dependent mechanism (25). We hypothesize that GHRH will operate as a primary factor with the help of a secondary releasing factor as co-agonist. Although the mechanism of action of GHRP-2 has not been fully established, the evidence that this secretagogue has its own specific receptor (5,30) suggests the existence of an endogenous GHRP-2-like ligand. By giving maximally stimulating doses of GHRH and GHRP-2 at rest and during incremental exercise, GH responses were compared in an effort to add clarity to our understanding of the underlying neuroendocrine mechanisms for exercise-induced GH release.

Based on the fact that no significant differences could be observed between the AUCs, obtained from arithmetical addition and from the combined administration of the secretagogues, the tentative conclusion might be drawn that the mechanisms of each secretagogue (exercise, GHRH, or GHRP-2) do not interact. However, analysis of AUCs does not take into account differences in time course of the responses, which are related to interactions of the stimuli given. A significant relationship between the averaged values, obtained 20–30 min after the start of the exercise, was observed for the response to exercise plus GHRH versus exercise plus GHRH plus GHRP-2, whereas the relationship between exercise plus GHRH versus exercise plus GHRP-2 approached significance (Table 2). It cannot be ruled out that the latter relationship may have physiological significance, because a small sample size may fail to detect as significant a difference that is real. Therefore, this must be investigated further. During the time period between 20 and 30 min, the responses to exercise were maximal, whereas the responses to GHRH alone and GHRP-2 alone were submaximal, but at least 80% of their respective peak levels (Fig. 2). In spite of this asynchronicity, interactions between stimuli could be unraveled: in the response to exercise plus GHRH, the regression equation showed that the addition of exercise to GHRH in a ratio 1:1 would result in an underestimation of the combined response. In fact, the ratio of the regression coefficients GHRH versus exercise was about 2:1 (Table 3A), indicating that in the combined response GHRH activity seems to potentiated by exercise. The responses to exercise plus GHRH (127.5 ± 16.0), however, remained lower than to GHRP-2 alone (151.4 ± 15.1). When GHRP-2 was added to the combined administration of exercise plus GHRH, the averaged GH response of 257.4 ± 28.7 $\mu g \cdot L^{-1}$ exceeded that of exercise plus GHRH, suggesting that GHRP-2 acts either by increasing the activity of GHRH and/or by disinhibition of pituitary GH release, whereas activation of an unknown hypothalamic factor is also possible (3). The significant differences between the GH responses to exercise and GHRP-2 alone (peak values, averaged values, AUCs), indicate that during exercise the involvement of a potent GHRP-2-mediated mechanism is limited, and our analysis points to a potentiating effect on the GHRH activity, as the ratio of the regression coefficients GHRH versus exercise increased from about 2 to about 3 in the response to exercise plus GHRP-2 (Table 3B). Moreover, as the combined administration of exercise plus GHRP-2 acts additive on the GH response, a substantial interaction between the mechanisms of these two secretagogues is unlikely, in contrast to the interaction between exercise and the GHRH-mediated mechanism.

Exercise alone evoked peak GH values of 58.4 ± 9.3 $\mu g \cdot L^{-1}$, which were in general observed at the time point of exhaustion. Such a response is in line with data obtained from

TABLE 3B. Regression analysis of the averaged GH response (in $\mu g \cdot L^{-1}$) to the combined administration of exercise (EX) plus GHRP-2 dependent on the averaged response to EX alone and to GHRH alone.

Variables	Coefficient	Standard Error	t	P
EX	2.02	0.86	2.35	0.06
GHRH	6.20	1.36	4.57	0.01
Constant	−108.82	83.92	−1.30	0.25

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Regression	2	58266.47	29133.24	11.07	0.01
Residual	5	13155.27	2631.05		

TABLE 3C. Regression analysis of the averaged GH response (in $\mu g \cdot L^{-1}$) to the combined administration of exercise (EX) plus GHRH plus GHRP-2 dependent on the averaged response to the combined administration of EX plus GHRH (EX + GHRH).

Variables	Coefficient	Standard Error	*t*	*P*
EX + GHRH	1.43	0.44	3.24	0.02
Constant	75.16	59.35	1.27	0.25

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F*	*P*
Regression	1	29251.13	29251.13	10.47	0.02
Residual	6	16767.15	2794.52		

sedentary and trained subjects (4,8,10,13,16,20,21,29,31). Therefore, the incremental protocol used in this study could be considered to be maximally stimulating.

Based on the dose-relationship for GHRH in young adults, the maximal effective dose of GHRH is at least 1 $\mu \cdot kg^{-1}$ iv (14). GHRH (100 μg) evoked peak GH values of 48.4 $\pm$ 10.7 $\mu g \cdot L^{-1}$ at a time point, which was about 45–60 min after iv administration. The relatively wide range and different times to reach peak values are in line with earlier studies and are assumed to be due to variations in hypothalamic somatostatin activity (9,12,17,24,26). The observed levels in this study are relatively high (and not significantly different from that of exercise), and this may partially due to the fact that our subjects had a relatively low body fat percentage (13.5 $\pm$ 4.5%), which may positively contribute to their responsiveness (15,18,28). A further indication that maximally stimulating doses were used is the observation that the AUC after GHRH (3785 $\pm$ 661 $\mu g \cdot L^{-1} \cdot 120$ min) was much higher than that reported by a study of Peñalva et al. (26), where a GHRH dosage of 1 $\mu g \cdot kg^{-1}$ was used.

GHRP-2 (200 μg) evoked peak GH levels of 179.9 $\pm$ 18.7 $\mu g \cdot L^{-1}$ at about 35 min after iv administration. In most studies (1,2,25,26), hexapeptides were used in lower dosages, resulting in GH values ranging from 18–70 $\mu g \cdot L^{-1}$. Relatively high doses of GHRP-2 are rarely used. In a study of Popovic et al. (27) using GHRP-6 in a dosage of 2 $\mu g \cdot kg^{-1}$, peak responses to 83.2 $\mu g \cdot L^{-1}$ were observed. Compared with these observations, a dose of 200 μg GHRP-2 can be considered to be maximally stimulating. This is corroborated by the observation that the AUC after GHRP-2 (11655 $\pm$ 1083) was higher than reported in studies of Peñalva et al. (26) and of Arvat et al. (1), where doses of 1 or 2 $\mu g \cdot kg^{-1}$ were used.

The AUC after exercise plus GHRH plus GHRP-2 (17673 $\pm$ 1670) exceeded the values of all other combinations and was 7.6 times higher than that of exercise alone. As combined administration of exercise plus GHRH plus GHRP-2 does not show a time delay to peak values in comparison to exercise alone, this combination of secretagogues may be considered to be a highly powerful provocative test for GH release.

In conclusion, the data indicate that under strenuous exercise conditions, endogenous GHRH activity causes a further increase of GH release. A GHRP-2-mediated mechanism acts as a "booster," possibly by stimulating the effects of GHRH and/or an unknown hypothalamic factor, as well as by stimulating the pituitary GH release directly.

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