

Human growth hormone response to repeated bouts of aerobic exercise

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Kanaley, J. A., J. Y. Weltman, J. D. Veldhuis, A. D. Rogol, M. L. Hartman, and A. Weltman. Human growth hormone response to repeated bouts of aerobic exercise. *J. Appl. Physiol.* 83(5): 1756–1761, 1997.—We examined whether repeated bouts of exercise could override growth hormone (GH) auto-negative feedback. Seven moderately trained men were studied on three occasions: a control day (C), a sequential exercise day (SEB; at 1000, 1130, and 1300), and a delayed exercise day (DEB; at 1000, 1400, and 1800). The duration of each exercise bout was 30 min at 70% maximal O₂ consumption ($\dot{V}O_{2\max}$) on a cycle ergometer. Standard meals were provided at 0600 and 2200. GH was measured every 5–10 min for 24 h (0800–0800). Daytime (0800–2200) integrated GH concentrations were ~150–160% greater during SEB and DEB than during C: 1,282 ± 345, 3,192 ± 669, and 3,389 ± 991 min·μg·l⁻¹ for C, SEB, and DEB, respectively [SEB > C (*P* < 0.06), DEB > C (*P* < 0.03)]. There were no differences in GH release during sleep (2300–0700). Deconvolution analysis revealed that the increase in 14-h integrated GH concentration on DEB was accounted for by an increase in the mass of GH secreted per pulse (per liter of distribution volume, l_v): 7.0 ± 2.9 and 15.9 ± 2.6 μg/l, for C and DEB, respectively (*P* < 0.01). Comparison of 1.5-h integrated GH concentrations on the SEB and DEB days (30 min exercise + 60 min recovery) revealed that, with each subsequent exercise bout, GH release apparently increased progressively, with a slightly greater increase on the DEB day [SEB vs. DEB: 497 ± 162 vs. 407 ± 166 (*bout* 1), 566 ± 152 vs. 854 ± 184 (*bout* 2), and 633 ± 149 vs. 1,030 ± 352 min·μg·l⁻¹ (*bout* 3), *P* < 0.05]. We conclude that the GH response to acute aerobic exercise is augmented with repeated bouts of exercise.

endocrinology; deconvolution

GROWTH HORMONE (GH) is released from the anterior pituitary gland in an intermittent pulsatile manner (17) and is impacted by age, gender, nutrition, sleep, body composition, fitness, and sex steroid hormones (5–7, 17, 18). In the rodent, a GH secretory episode appears to elicit auto-negative feedback (10, 11). This short-loop feedback has been hypothesized to occur at the level of the hypothalamus by an increase in the release of somatostatin and/or a decrease in the release of GH-releasing hormone (GHRH). Although acute administration of GHRH results in increased serum GH concentrations, repeated administration of GHRH at 2-h intervals resulted in a reduction in GH area under the curve when GHRH was administered on two occasions (4). More recently, a marked attenuation of GH release was observed when high doses of GHRH were administered four times at 2-h intervals (J. A. Aloï

and M. L. Vance, unpublished data). These data suggest that somatotrophs become desensitized or depleted in response to repeated maximal pharmacological stimulation. Alternatively, negative feedback may occur at the level of the hypothalamus.

Exercise is a potent physiological stimulus of GH release (2, 3, 14, 19). Intensity and duration of acute exercise, work output during exercise, muscle mass used during exercise, fitness, and training state influence the GH response to exercise. Intensity of exercise may play a key role, with a threshold of exercise intensity necessary before a significant rise in GH levels is detected (2, 3). Plasma GH concentrations increase within 10–20 min of aerobic exercise, peak within the exercise bout or immediately after exercise, and remain elevated above baseline for ~2 h after exercise (14).

The GH response to repeated bouts of exercise in the human is not known. In this study we examined the following hypotheses: 1) the GH response to repeated bouts of exercise is progressively attenuated, presumably as a result of auto-negative feedback, and 2) the attenuation of the GH response to repeated bouts of exercise is lessened or eliminated when greater recovery time is provided between exercise bouts.

METHODS

Subjects. Seven healthy moderately trained men (age 21–29 yr) provided voluntary written informed consent, as approved by the Human Investigation Committee, before entering the study. Each subject underwent a detailed medical history and physical examination, and no subject had a history of pituitary, renal, hepatic, or metabolic disease. The subjects were nonsmokers, were not taking any medication known to affect GH secretion, had not undergone transmeridian travel in the past 8 wk, and were not night-shift workers. Screening laboratory data revealed normal hematologic, renal, hepatic, gonadal, and thyroid function. Subjects refrained from exercise for 24 h before each evaluation.

Experimental design. Each subject completed a resting metabolic rate study (Delta Trac, SensorMedics, Yorba Linda, CA) to estimate 24-h energy expenditure, a treadmill test to assess level of cardiovascular fitness, and underwater weighing for determination of body density at the Exercise Physiology Laboratory of the General Clinical Research Center. Subjects were then evaluated on three separate occasions, each separated by 4 wk, during which serum concentrations of GH were measured for 24 h under the following conditions: 1) no exercise (control day, C), 2) a sequential (SEB) exercise day with three 30-min exercise bouts at 70% maximal O₂ consumption ($\dot{V}O_{2\max}$) at 1000, 1130, and 1300, and 3) a delayed (DEB) exercise day with three 30-min exercise bouts at 70% $\dot{V}O_{2\max}$ at 1000, 1400, and 1800. Each subject served as

his own control. The order of the admissions was randomized and completed within 3 mo (Fig. 1).

Body composition. Body density was assessed by hydrostatic weighing (8). Each subject was weighed in air on an Accu-weigh beam scale accurate to 0.1 kg and subsequently weighed underwater on a Chatillon autopsy scale accurate to 10 g. Residual lung volume was measured using an oxygen-dilution technique (20). The computational procedure of Brozek et al. (1) was used to determine relative fat from body density measurements.

$\dot{V}O_{2max}$. $\dot{V}O_{2max}$ was determined using a continuous cycle ergometer protocol. Subjects started cycling at 100 W for the first 3 min, and the power output was increased by 25 W every 3 min until volitional fatigue. $\dot{V}O_{2max}$ was chosen as the highest O_2 consumption ($\dot{V}O_2$) attained.

Metabolic measures. Metabolic measures were collected using standard open-circuit spirometric techniques (metabolic cart 2700Z, SensorMedics). Heart rate was determined electrocardiographically.

Exercise/control days. Subjects were admitted to the General Clinical Research Center on the evening before the exercise/control studies, with lights out from 2300 to 0600. At 0600 an intravenous cannula was placed in a forearm vein and the subject was fed a standard breakfast (kcal based on the resting metabolic rate data plus an activity factor; 55% carbohydrate, 15% protein, and 30% fat). To avoid the confounding effects of meals on GH secretion, subjects then fasted until 2200, when they consumed a second standardized meal (55% carbohydrate, 15% protein, and 30% fat). Beginning at 0800, blood samples were drawn every 10 min for 24 h for measurement of serum GH concentrations, except during exercise and 1 h of recovery, when blood samples were collected at 5-min intervals. After 2 h of baseline blood sampling, subjects began their first exercise bout (SEB and DEB days) or remained at rest (C day). During the SEB admission, exercise bouts were repeated every 1.5 h; during the DEB admission, exercise bouts were repeated at 4-h intervals. Because sleep stimulates GH secretion (7), subjects were not allowed to sleep until after 2300, when lights were turned off.

GH analysis. Serum GH concentrations were measured in duplicate by immunoradiometric assay using standards diluted in human serum (Nichols Institute, San Juan Capistrano, CA). All samples for a subject were run in the same

assay. The sensitivity of the assay was 0.2 $\mu\text{g/l}$, and the mean intra- and interassay coefficients of variation were 3.9 and 3.5%, respectively. Serum GH concentrations were determined by a new procedure developed at the National Science Foundation Center for Biological Timing at the University of Virginia (13). Briefly, standard curves were evaluated by weighted nonlinear least-squares analysis using three response functions. Uncertainties (SD) associated with each GH concentration were estimated empirically, considering variances associated with assay response and standard curve evaluations. The standard curve parameters and response function to the variably weighted response data were optimized. Confidence limits for the standard curve parameters were then calculated (13). The function yielding the lowest absolute sum of squared residuals was chosen for analyzing the samples.

Integrated GH concentrations (area under the curve) for several time periods were calculated as previously described (16). A multiple-parameter deconvolution method was employed to derive quantitative estimates of attributes of GH secretory events from the measured serum GH concentrations. This was done while the subject-specific monoexponential half-life of endogenous GH was estimated. It was assumed that each pulse of GH secretion is approximated by a Gaussian distribution of secretory rates (15). Basal secretion was estimated as previously described (17) for the control day and was assumed not to change during the exercise days. GH secretory pulses were considered significant if the fitted amplitude could be distinguished from zero (i.e., pure noise) with 95% statistical certainty. The GH secretory pulse half-duration (duration at half-maximal amplitude), GH half-life of elimination, and GH distribution volume were assumed to be constant throughout the study period for each individual. The mass of GH secreted per pulse was estimated as the area of the calculated secretory pulse ($\mu\text{g/l}$ distribution volume, l) (15). The endogenous pulsatile GH production rate was estimated as the product of the number of secretory pulses and the mean GH mass secreted per pulse.

Statistical analysis. Analysis of variance with repeated measures was employed to determine mean differences. Where mean differences were observed, preplanned mean comparisons were examined. Values are means \pm SE. $P < 0.05$ was chosen a priori.

RESULTS

Subject characteristics. The mean age of the subjects was 24.9 ± 0.9 yr, mean $\dot{V}O_{2max}$ was 53.4 ± 2.9 $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, and mean percent body fat was $13.4 \pm 2.0\%$.

$\dot{V}O_2$ during repeated bouts of exercise. During SEB exercise bouts, the mean $\dot{V}O_2$ ranged from 2.62 ± 0.05 (bout 1) to 2.36 ± 0.09 l/min (bout 3). During DEB exercise bouts, the mean $\dot{V}O_2$ ranged from 2.65 ± 0.05 (bout 1) to 2.61 ± 0.07 l/min (bout 2).

GH release. Figure 2 shows the mean serum GH concentrations during blood sampling at 10-min intervals over 24 h during C, SEB, and DEB conditions. In SEB and DEB conditions, each exercise bout resulted in a distinct GH pulse. The 24-h integrated serum GH concentrations are shown in Fig. 3. Twenty-four-hour integrated GH concentrations were $\sim 60\%$ greater during the SEB and DEB days than on the C day: SEB $>$ C ($P < 0.16$) and DEB $>$ C ($P < 0.14$).

To examine the impact of repeated exercise on GH release more closely, 14-h (0800–2200) integrated GH

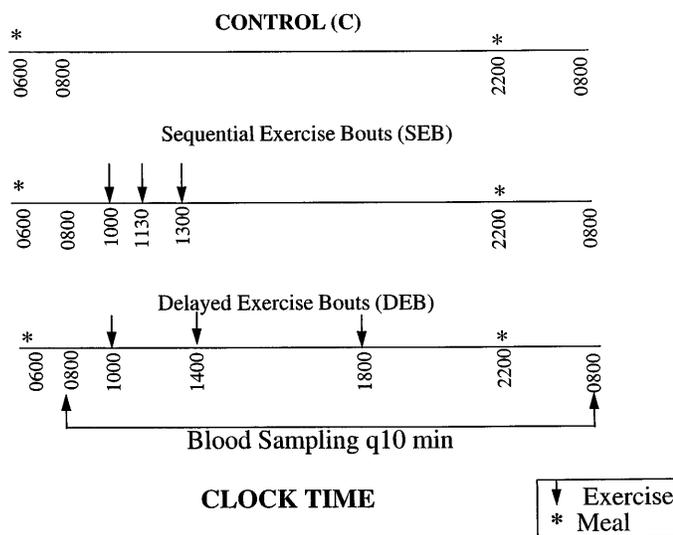


Fig. 1. Study design for control, sequential exercise bouts (SEB), and delayed exercise bouts (DEB) days.

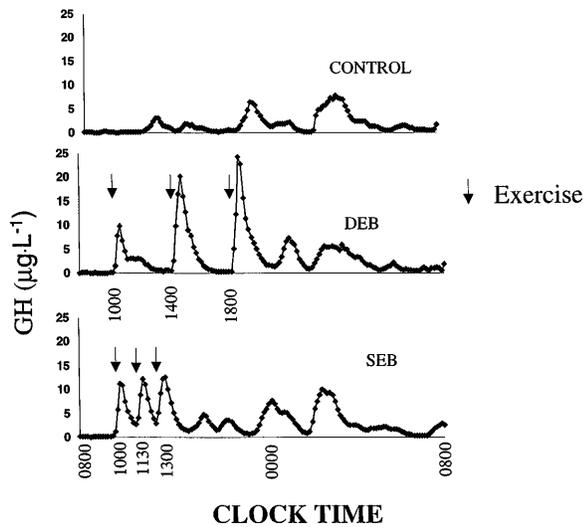


Fig. 2. Mean serum growth hormone (GH) concentrations during blood sampling at 10-min intervals over 24 h on C, SEB, and DEB days.

concentrations were also examined. This time frame was chosen to eliminate the confounding effects of the second standardized meal (2200) and sleep. These data are presented in Fig. 4. Fourteen-hour integrated GH concentrations were ~150–160% greater during SEB and DEB days than on the C day: SEB > C ($P < 0.06$) and DEB > C ($P < 0.03$). Examination of the sleep portion of our sampling period (2300–0700) revealed no significant differences in 8-h integrated GH concentrations: $1,227 \pm 368$, 889.2 ± 107.5 , and $1,055.1 \pm 108.3$ $\text{min} \cdot \mu\text{g} \cdot \text{L}^{-1}$ for C, SEB, and DEB, respectively.

To examine the impact of each exercise bout on GH release, integrated GH concentrations for the 30 min of exercise and 1 h of recovery after each exercise bout were analyzed with a 2×3 analysis of variance (SEB vs. DEB; exercise bouts 1, 2, and 3) with repeated measures (Fig. 5). A significant main effect was observed, with integrated GH concentrations increasing with each subsequent exercise bout ($P < 0.05$). The apparent progressive increase in GH concentrations with repeated exercise tended to be greater on the DEB day than on the SEB day.

Table 1 shows the results of the multiparameter deconvolution analysis of serum GH concentrations

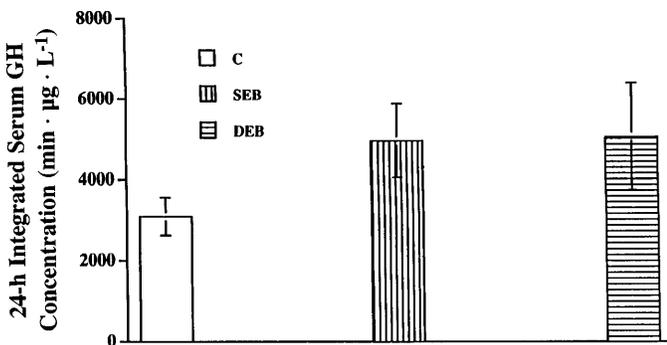


Fig. 3. Twenty-four-hour integrated serum GH concentrations on C, SEB, and DEB days. Values are means \pm SE.

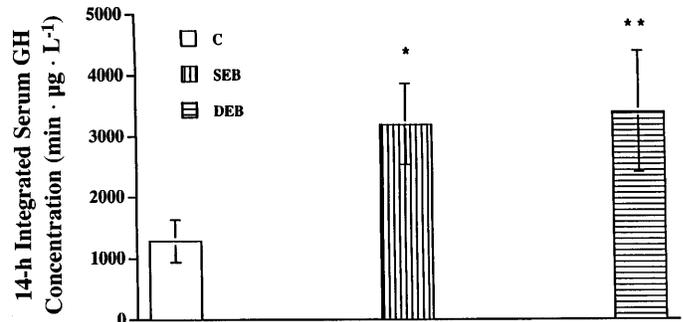


Fig. 4. Daytime (14-h, 0800–2200) integrated serum GH concentrations on C, SEB, and DEB days. Values are means \pm SE. * $P < 0.06$; ** $P < 0.03$ vs. C.

between 0800 and 2200 ($n = 6$, because 1 subject did not complete the SEB condition). The total amount of GH secreted during the 14-h period (production rate) increased 1.7-fold on the SEB day and 2.1-fold on the DEB day compared with the C day: DEB > C ($P < 0.01$). This was accounted for by a 1.5-fold (SEB) to 2.3-fold (DEB) increase in the mass of GH secreted per pulse (DEB > C, $P < 0.01$), with no change in the number of GH secretory pulses or the GH half-life of elimination. The amplitude of GH secretory pulses increased 2-fold (SEB) to 4.3-fold (DEB; DEB > C, $P < 0.01$), but the secretory pulse half-duration decreased to 59% (DEB) and 71% (SEB) of that observed on the C day ($P < 0.01$). Thus, with repeated bouts of exercise, GH secretory pulses were of shorter duration but much greater amplitude.

To determine the effects of repeated bouts of exercise on specific attributes of GH secretion, serum GH concentrations during the 30 min of exercise and the initial 1.5 h of recovery of each exercise bout in the DEB condition were examined using deconvolution analysis (Table 2). The apparent progressive increase in GH integrated concentrations with repeated exercise was related to significant increases in the mass of GH secreted per pulse and greater GH secretory pulse

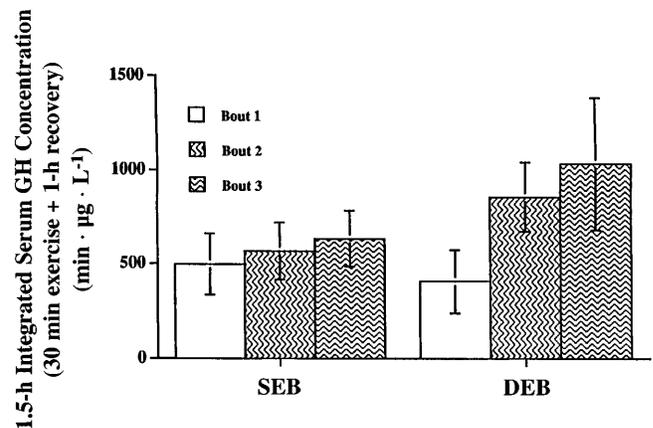


Fig. 5. Effects of repeated bouts of exercise on integrated serum GH concentrations during 30 min of exercise and 1 h of recovery during SEB and DEB days. A significant main effect was observed with a progressive increase in integrated serum GH concentrations with repeated bouts of exercise ($P < 0.05$).

Table 1. Effects of repeated bouts of exercise during SEB and DEB days on specific measures of GH secretion and half-life during daytime (0800–2200) as determined by multiparameter deconvolution analysis

| Variable | C Day | SEB Day | DEB Day |
|---|-------------|-------------|---------------|
| GH secretory pulses per 14 h | 10.0 ± 1.3 | 9.7 ± 0.4 | 7.5 ± 1.3 |
| GH secretory pulse half-duration, min | 23.9 ± 1.6 | 17.0 ± 1.1* | 14.2 ± 1.6* |
| GH half-life, min | 16.9 ± 1.4 | 18.3 ± 1.8 | 19.0 ± 2.3 |
| Mass of GH/pulse, µg/l _v | 7.0 ± 2.9 | 10.4 ± 2.5 | 15.9 ± 2.6* |
| GH pulse amplitude, µg · l _v ⁻¹ · min ⁻¹ | 0.3 ± 0.1 | 0.6 ± 0.1 | 1.3 ± 0.3*† |
| 14-h GH production rate, µg/l _v | 59.3 ± 15.7 | 99.5 ± 12.6 | 126.4 ± 34.6* |

Values are means ± SE; *n* = 6. C, control; SEB, sequential exercise; DEB, delayed exercise; GH, growth hormone; l_v, liter of distribution volume. **P* < 0.01 vs. C; †*P* < 0.05, SEB vs. DEB.

amplitudes during exercise bouts 2 and 3 than during bout 1 (*P* < 0.05).

It should be realized that the number of subjects used in the present analyses (*n* = 6–7) is associated with an increased risk of a type II error in several of the comparisons of means. Therefore, nonsignificant mean differences should be viewed as indeterminate findings.

DISCUSSION

Although it is well known that a single bout of aerobic exercise of appropriate intensity will result in a dramatic increase in serum GH concentrations (2, 3, 14), little information is available regarding the effects of repeated bouts of exercise on GH release. We hypothesized, on the basis of studies of repeated administration of pharmacological stimuli on GH secretion, that the GH response to each subsequent exercise bout would be progressively attenuated. We also postulated that the attenuation of GH release to repeated bouts of exercise would be lessened, or eliminated, when greater recovery time was provided between exercise bouts. The exercise intensity of 70% $\dot{V}O_{2\max}$ was chosen on the basis of published data, which indicate that this intensity of exercise will elevate serum GH levels independently of training state (12).

The present data support the hypothesis that repeated bouts of aerobic exercise increase daytime serum GH concentrations without a significant change in nocturnal GH release. Integrated GH concentrations

Table 2. Effects of repeated bouts of exercise on 2-h (30 min exercise + 1.5 h recovery) measures of GH secretion and half-life during daytime (0800–2200) on DEB day

| Variable | Bout 1 | Bout 2 | Bout 3 |
|---|------------|-------------|-------------|
| GH secretory pulses per 2 h | 2.7 ± 0.5 | 2.0 ± 0.3 | 2.7 ± 0.4 |
| GH secretory pulse half-duration, min | 13.8 ± 0.7 | 16.2 ± 3.5 | 13.8 ± 1.6 |
| GH half-life, min | 14.7 ± 2.2 | 17.6 ± 1.2 | 18.9 ± 1.8 |
| Mass of GH/pulse, µg/l _v | 6.1 ± 1.4 | 21.6 ± 2.5* | 16.9 ± 2.3* |
| GH pulse amplitude, µg · l _v ⁻¹ · min ⁻¹ | 0.5 ± 0.2 | 1.3 ± 0.2* | 1.3 ± 0.3* |
| 2-h GH production rate, µg/l _v | 21.8 ± 6.6 | 45.5 ± 10.0 | 48.0 ± 10.4 |

Values are means ± SE; *n* = 7. **P* < 0.01 vs. bout 1.

between 0800 and 2200 were increased ~150–160% (Fig. 4) on the SEB and DEB days compared with the C day. This enhanced daytime GH secretion was related to a 1.7- to 2.1-fold increase in the mass of GH secreted per pulse and a 2- to 4.3-fold increase in secretory pulse amplitude and occurred despite a decrease in secretory pulse half-duration (Table 1). There was no major effect of repeated bouts of exercise on the number of secretory GH episodes or in GH half-life during the 14 h of daytime. In contrast to the marked elevation in GH release during the daytime, no significant differences were observed between exercise and control conditions during sleep (2300–0700). These data are consistent with recent findings of Kern et al. (9), who reported that neither long-duration exercise of low intensity nor long-duration exercise of moderate intensity affected the mean amount of GH released during the night (although the temporal pattern of GH release at night may be affected by higher-intensity daytime exercise). The net result was that 24-h integrated GH concentrations were ~60% higher during the exercise days than on the control day, although this trend did not achieve statistical significance (Fig. 3, *P* < 0.16).

In contrast to our hypothesis, we observed an augmentation of the GH response to subsequent exercise bouts. This was true for SEB and DEB conditions (i.e., independent of recovery time between exercise bouts; Fig. 5). These data suggest that exercise of appropriate intensity is a powerful stimulus of GH release that is able to override GH auto-negative feedback. The mechanisms for the stimulatory effects of exercise on GH secretion are not known but may involve stimulation of hypothalamic GHRH secretion and/or suppression of hypothalamic somatostatin release. Because GH auto-negative feedback is thought to involve exactly the opposite mechanisms, it is reasonable to hypothesize that the exercise stimulus is more potent than the inhibitory feedback of GH on these hypothalamic neurons. It is also evident that pituitary stores of GH do not limit the GH response to repeated bouts of exercise and that desensitization to this stimulus of GH secretion does not occur.

Although there was a trend for a greater increase in serum GH levels with subsequent exercise bouts in the DEB than in the SEB condition, this did not reach statistical significance. Perhaps the longer recovery period between exercise bouts on the DEB day than on the SEB day may have allowed for greater responsiveness of the hypothalamopituitary unit to subsequent exercise stimuli. It is also possible that the apparent progressive increase in serum GH concentrations with subsequent exercise bouts (in both conditions) may be related to fasting. Fasting has been shown to augment GH secretion without affecting the GH half-life (5). In the present study, subjects were fasted from 0600 (the first standardized meal) until 2200 (the second standardized meal) on each of the study days to avoid the possibility that ingestion of meals might blunt the GH response to later exercise bouts. Therefore, the augmentation in the GH response to subsequent exercise bouts might be related to an interaction between fasting and

exercise. Because subjects were fasting for a longer interval before exercise *bouts 2* and *3* on the DEB day than on the SEB day, this might account for the slightly higher integrated GH concentrations in response to exercise *bouts 2* and *3* on the DEB day (Figs. 1 and 5). However, although an interaction between exercise and fasting may have been present, it is unlikely that fasting alone had a major impact on GH levels, inasmuch as we did not observe an augmentation in serum GH concentrations during the daytime in the control condition (Fig. 2).

Separation of the data on the DEB day into three discrete exercise and recovery periods allowed us to examine the impact of repeated bouts of exercise on measures of GH secretion and clearance. The mass of GH secreted per pulse increased by 3.5- and 2.8-fold during exercise *bouts 2* and *3* compared with the initial exercise bout, and the GH pulse amplitude was ~2.5 times greater with subsequent exercise. In contrast, there was no change in the number of pulses or the pulse width with subsequent exercise (Table 2). Although the GH half-life appeared to increase with subsequent exercise bouts (from 14.7 to 18.9 min, Table 2), this trend did not reach statistical significance. In addition, the GH half-life under exercise conditions did not differ from that observed under control conditions. It is possible that GH clearance rates may be altered during acute exercise, but this could not be discerned with the design employed in the present study. The multiple-parameter deconvolution method requires a period of time corresponding to about four to five hormone half-lives to estimate the hormone half-life. Because the exercise bouts were only 30 min in duration, GH clearance rates during exercise could not be distinguished from those during recovery. Thus the half-lives of GH shown reflect an average of exercise and recovery conditions.

In summary, results of the present study indicate that the GH response to repeated bouts of aerobic exercise corresponding to 70% $\dot{V}O_{2\max}$ is not attenuated but, rather, may be augmented, at least under semifasting conditions. The increase in GH secretion observed with repeated bouts of exercise was related to an increase in GH pulse amplitude and the mass of GH secreted per pulse. Three 30-min bouts of aerobic exercise (independent of recovery periods) significantly increased daytime integrated GH concentrations without a significant change in nocturnal GH concentrations compared with control conditions. We conclude that high-intensity aerobic exercise is a potent stimulus of GH secretion that is able to overcome GH auto-negative feedback. Thus repeated bouts of exercise on the same day are able to consistently stimulate GH secretion without attenuation of the GH response.

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