Nocturnal Growth Hormone Secretory Dynamics are Altered Following Resistance Exercise:

Deconvolution Analysis of 12 hour Immunofunctional and Immunoreactive Isoforms

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RUNNING HEAD: NOCTURNAL GROWTH HORMONE SECRETION AND EXERCISE
ABSTRACT

To characterize the effects of daytime exercise on subsequent overnight growth hormone (GH) secretion and elimination dynamics, serum was sampled and GH measured every 10 min for 12 h (1800 to 0600 h) in a control (CON) condition and following a 50-set resistance exercise protocol (EX) from 1500 to 1700 h. GH was measured with a conventional immunoreactive (IR) assay and an immunofunctional (IF) assay and values were utilized in multi-parameter deconvolution analysis. EX resulted in a higher overnight secretory burst frequency (CON: 7.6 (SD 2.4) < EX: 9.4 (SD 2.2) bursts per 12 h, \( P = 0.005 \)) but lower mean burst mass (CON: 9.2 (SD 4.7) > EX: 6.0 (SD 2.9) \( \mu \text{g/L} \), \( P = 0.019 \)) and secretory rate (CON: 0.68 (SD 0.29) > EX: 0.48 (0.23) \( \mu \text{g/L/min} \), \( P = 0.015 \); ANOVA main effect means presented). Approximate entropy (ApEn) was greater after EX, indicating a less orderly GH release process than CON. The estimated half-life of IF GH was significantly lower than IR GH (IF: 15.3 (SD 1.1) < IR 19.8 (SD 1.6) min, \( P < 0.001 \)) but similar between the CON and EX conditions (~17 min). Despite the changes in secretory dynamics, 12-h mean and integrated GH concentrations were similar between conditions. The results suggest that although quantitatively similar total amounts of GH are secreted overnight in CON and EX conditions, resistance exercise alters the dynamics of secretion by attenuating burst mass and amplitude yet increasing burst frequency.

KEYWORDS: pulsatile, somatotropin, kinetics, approximate entropy, weight lifting
INTRODUCTION

Acute physical exercise is a well-established model for eliciting the secretion of growth hormone (GH) into circulation. Acute bouts of exercise regardless of mode (i.e., aerobic or resistance) result in a rapid increase in GH concentrations with a return to baseline levels generally within one hour of the cessation of exercise (27). In addition, multiple spontaneous concentration peaks can be observed in a 24-h period due to the pulsatile nature of GH secretion, with the most robust increase typically occurring at night during slow wave sleep (21). While many studies have examined multiple samples for a short period immediately following exercise, few studies have examined the effect of daytime exercise on nocturnal GH concentrations and perhaps less is known about how exercise affects the overnight secretion and elimination dynamics of GH.

The majority of investigations regarding the acute effects of daytime activity on subsequent nocturnal GH concentrations utilized analysis of mean and/or peak GH concentrations compared to a control condition. Previous investigators have demonstrated increases (1) and decreases (5) in peak and/or mean nocturnal GH concentrations following daytime physical activity, while the majority of data contend that mean nocturnal GH concentrations are largely unaltered by earlier exercise (9; 10; 13; 28). However, observations of mean GH concentrations provide limited information due to the highly pulsatile and dynamic nature of GH secretion. An arithmetic mean of concentrations observed over time obscures the secretion events which occur during the period of sampling. Therefore, the analysis of mean GH concentrations from data collected over several hours during sleep, even in combination with peak values, may not present the most accurate picture of the impact that exercise has on hormone secretion per se.
In fact, a single concentration peak (i.e., as assessed by mathematical peak detection programs) is often actually comprised of multiple secretory events that have been completely masked by simultaneous elimination kinetics. As Veldhuis et al. describe: “the plasma GH concentration peak can be dissected into relevant contributions by GH secretory burst amplitude and duration, any concomitant basal secretion, burst frequency, and half-life” (22). In contrast to hormone concentration peak detection alone, multi-parameter deconvolution analysis provides a means to estimate a number of additional hormone secretion and elimination parameters based solely on assayed concentrations over time.

Our laboratory previously reported that following daytime exercise, the nocturnal maximum GH concentration and mean amplitude of GH peaks (as determined by the Pulsar peak detection software) were decreased whereas overall mean GH concentration and the number of peaks were unchanged (14). In the current investigation, we employed a different peak detection program (Cluster; 24) in combination with multi-parameter deconvolution analysis (23; 25) to further analyze our initially published data (14). The deconvolution analysis was utilized with data provided by both a conventional immunoreactive (IR) GH assay as well as an immunofunctional (IF) GH assay which only detects GH molecules that are able to dimerize GH receptors for subsequent signal transduction. The purpose of the current investigation was to characterize the effects of a daytime exercise bout on subsequent overnight GH secretion and elimination dynamics via deconvolution analysis.

METHODS

The experimental design of the resistance exercise protocol and serum sampling described below have been published in a previous report (14).
Subjects. Ten young, healthy, fit men (21 (SD 2.1) yr, 177 (SD 7.4) cm, 78.6 (SD 6.4) kg, 11.3 (SD 4.1) % body fat) participated in this investigation, which was approved by the Pennsylvania State University’s Human Use Institutional Review Board and the General Clinical Research Center Scientific Review Committees. All subjects were briefed on the risks of the investigation and subsequently read and signed the informed consent document. Each subject was medically screened by a physician before inclusion in the study.

Acute heavy resistance exercise protocol. The acute heavy resistance exercise protocol (AHREP) began at 1500 and was designed to be a high-volume workout that included 50 total sets and recruited and activated a large amount of muscle tissue. This was accomplished by performing multijoint exercises that required the use of major muscle groups in both the lower and upper body. Details of the AHREP have been previously published (14). All subjects completed the entire protocol.

Overnight trials. Subjects underwent two randomized counterbalanced overnight trials at the Pennsylvania State University, University Park General Clinical Research Center (GCRC) housed in the Noll Physiological Research Center. One of these overnights served as the control (CON) trial, in which the subject reported to the GCRC at 1430, had a resting blood sample taken via venipuncture, and rested quietly until ~1700, whereupon a catheter was inserted into the antecubital vein and blood was drawn every 10 min until 0600 the following morning. Blood sampling followed the same procedures during the exercise condition.
Dietary control. All meals were provided for the subjects. These meals were prepared by registered dietitians at the GCRC and conformed to the following criteria: no caffeine, aspartame, or snacks; macronutrient distribution was 55% carbohydrate, 15% protein, and 30% fat; and sodium was controlled at 3 g. Calories were based on the Harris Benedict standard formula plus an appropriate activity factor for the subject’s age and physical activity. Meal times were breakfast at 0630, lunch at 1130, and dinner at 1900. Lunch and dinner times were scheduled around the 1500–1700 afternoon workout to ensure that all subjects exercised in the postabsorptive state and also to allow an acute postexercise sampling regimen that was not influenced by caloric consumption.

Growth hormone immunoassays. Two immunoassays compared circulating serum GH concentrations: the Nichols Institute Diagnostics (San Juan Capistrano, CA) immunoradiometric assay (IRMA) and an enzyme-linked immunosorbent assay (ELISA) for IF GH (Diagnostic Systems Laboratories; DSL, Webster, TX). All assays were validated with respect to parallelism, recovery, and linearity. Log-log and linear standard curve-fitting regressions were used for the Nichols IRMA and DSL ELISA, respectively and all standard curve points were run in quadruplicate. To eliminate interassay variance, samples for a particular subject were assayed within the same batch on a gamma counter with curve-fitting algorithms (EG & G Wallac Gamma Counter, Turku, Finland) for the IRMA and a microplate reader (Bio-Tek Instruments, Winooski, VT) for the DSL ELISA. The sensitivities of the Nichols IRMA and the DSL ELISA were 0.04 and 0.20 µg/L, respectively. For the Nichols IRMA, all values fell above the sensitivity for the assay. For the DSL ELISA, values falling below the thresholds were set equal to the assay sensitivity (0.20 µg/L). The intraassay variances for low, medium, and high GH
concentrations were as follows: 7.2, 5.2, and 5.4%, respectively, for the Nichols IRMA and 7.1, 7.6, and 8.4%, respectively, for the DSL IF ELISA.

Cluster Analysis. The cluster analysis program (24) was used to analyze the overall mean GH concentration (µg/L), 12-h integrated AUC via trapezoidal rule, and characteristics of GH concentration “peaks”. The cluster program was configured in a 1 x 2 fashion, defining significant peaks with two samples and nadirs with one sample. Cluster analysis was utilized to provide descriptive information regarding observed GH concentrations as a function of time, such as the number of peaks in 12 h, the mean interval between peaks, the mean peak width (i.e., duration), the mean peak height (i.e., amplitude), and the mean area under the peaks.

Deconvolution Analysis. Multi-parameter deconvolution analysis (23; 25) was employed for estimates of the hormone secretory and elimination dynamics which accounted for the observed 12-h serum GH concentration profiles. The outcome variables estimated by deconvolution analysis for the present study included the following burst parameters: the number of secretory bursts per 12 h, mean interval between bursts, mean burst mass, and mean burst amplitude (i.e., maximal secretory rate). Additional calculations included the basal GH secretion rate, 12-h basal GH secretion (basal secretion rate × 720 min), half-life, 12-h pulsatile GH secretion (mean burst mass × number of secretory bursts), 12-h total GH secretion (i.e., the sum of basal and pulsatile secretion), and the ratio of pulsatile to total GH secretion. Initially the PULSE2 program was used to estimate the number and position of secretory bursts that might comprise the final GH concentration profile. The file generated by PULSE2 was then utilized with the deconvolution
analysis program, where iterative nonlinear least squares parameter estimation at 95% statistical confidence intervals was utilized to quantify all aforementioned parameters of secretion.

*Approximate Entropy (ApEn).* The ApEn statistic was used as an estimate of the regularity of the GH release process in each condition and as measured by each assay. ApEn is a single value calculated for a hormone time-series where a higher ApEn denotes greater process irregularity or greater disorderliness of hormone release (16-18). The ApEn analysis was applied with parameters of m=1 (i.e., run length), r =0.20 (i.e., tolerance window), and 1000 Monte Carlo simulations per series.

*Statistical Analyses.* A 2 × 2 repeated measures ANOVA with condition (i.e., CON vs. EX) and assay detection method (i.e., Nichols IRMA vs. DSL IF ELISA) as repeated-measures factors was used on each of the GH parameters from the cluster, deconvolution, and ApEn analyses. A Tukey’s *post hoc* test was utilized when appropriate. Paired two-tailed *t* tests were utilized to compare IR/IF GH ratios in the two conditions for several parameters. All statistical analyses were performed with SigmaStat software package version 3.1 (SYSTAT Software, Point Richmond, CA). An alpha level of 0.05 was used to determine statistically significant findings for all analyses. All data are presented as mean (SD) unless noted otherwise. Values reported for CON or EX conditions represent the main effect means for the condition (i.e., combined IR and IF values for the respective condition) unless otherwise noted. Values reported for IR or IF assay represent the main effect means for the type of assay (i.e., combined CON and EX values for the respective assay) unless otherwise noted.
RESULTS

Cluster analysis. The least square means for the main effects of condition (i.e., CON vs. EX, combined IR and IF values) and assay (i.e., IR vs. IF, combined CON and EX values) on cluster analysis parameters are presented in Table 1. No significant \( P > 0.05 \) interaction effects were observed for any of the parameters. A significant exercise effect was found for the mean amplitude of GH peaks (CON: 7.8 (SD 4.2) > EX: 6.1 (SD 3.7) µg/L, \( P = 0.041 \)). Overall 12-h mean GH and 12-h AUC did not differ significantly between CON and EX conditions. No significant differences existed in the number of GH peaks identified by cluster analysis or the mean interval between peaks by assay or condition. No other significant differences existed with regard to the remaining cluster parameters in the CON vs. EX conditions. Significant assay main effects (i.e., IR vs. IF) existed for 12-h mean GH, total GH AUC, the mean duration of peaks, mean amplitude, and mean area under GH peaks (Table 1) with all parameters being higher when utilizing the IR assay values. Mean nadir GH concentrations were similar for all conditions and assays. Representative graphic plots of the concentration profiles of one subject as determined by cluster analysis of CON and EX data via IR and IF assay are presented in Figure 1.

Deconvolution analysis. The least square means for the main effects of condition (i.e., CON vs. EX values, combined IR and IF values) and assay (i.e., IR vs. IF values, combined from CON and EX conditions) on deconvolution analysis parameters are presented in Table 2. A number of significant condition effects were demonstrated. The number of GH secretory bursts in the 12-hour sampling period was significantly increased in the EX compared to the CON condition (CON: 7.6 (SD 2.4) < EX: 9.4 (SD 2.2) bursts, \( P = 0.005 \)). The mean interval between bursts (CON: 79.4 (SD 37.1) > EX: 44.7 (SD 12.9) min, \( P = 0.005 \)), mean burst mass (CON: 9.2 (SD
4.7) > EX: 6.0 (SD 2.9) µg/L, \( P = 0.019 \), and GH secretion rate (CON: 0.68 (SD 0.29) > EX: 0.48 (SD 0.23) µg/L/min, \( P = 0.015 \)) were each significantly lower in the exercise condition. The 12-h pulsatile and total GH secretion values were not significantly different between the CON and EX conditions. Estimated GH half-lives were similar in the CON and EX conditions (CON: 17.8 (SD 2.6) ≈ EX: 17.3 (SD 2.6) min, \( P = 0.251 \)); however, the half-life for IR GH was significantly greater than that of IF GH (IR: 19.8 (SD 1.6) > IF: 15.3 (SD 1.1) min, \( P \leq 0.001 \)).

With regard to assay differences, the number of secretory bursts, mass secreted per burst, burst rate, 12-h pulsatile secretion, 12-h total secretion, and ratio of pulsatile to total secretion were all significantly greater for the IR compared to the IF assay (\( P \leq 0.005 \) for all). A significant interaction existed for basal secretion rate and 12-h basal secretion (\( P = 0.042 \) for both variables). A Tukey’s post hoc revealed that for both basal secretion rate and 12-h basal secretion, IR GH values in the CON condition were significantly lower than IR GH values in the EX condition, whereas the remainder of comparisons between assay and condition were not significantly different. Representative graphic plots of the secretion events determined by deconvolution analyses in the CON and EX conditions via IR and IF assay are presented beneath their respective concentration profile plots in Figure 1.

\textit{ApEn}. ApEn values were significantly (\( P = 0.032 \)) greater the evening following EX compared to the CON condition. ApEn values were not affected by the method of assay (i.e., IR vs. IF, \( P = 0.808 \)). ApEn least square means for the ANOVA main effects of condition and time are presented along with the deconvolution outcomes in Table 2.
IR/IF Ratios. The ratios of immunoreactive to immunofunctional GH were calculated for the following variables in the CON and EX conditions: mean GH concentration, 12-h AUC, mean amplitude of peaks, mean AUC of peaks, mean burst mass, mean secretory rate, 12-h pulsatile secretion, and 12-h total secretion. The IR/IF ratios for CON and EX conditions were compared via paired two-tailed t tests. No significant differences existed in IR/IF ratios for any of the variables between conditions (ratios and P values presented in Table 3).
DISCUSSION

Deconvolution analysis revealed that the number of nocturnal GH secretory events is increased by a resistance exercise bout performed in the late afternoon. An increased burst frequency would suggest a greater amount of hormone secreted, however overall amounts of GH secreted in the CON and EX conditions were not significantly different. Interestingly, the increase in the number of secretory bursts in the EX condition was countered by the concomitant attenuation of mean burst mass and mean burst amplitude (i.e., GH secretion rate), which explains the unchanged total and pulsatile secretion. In the present study, the first hour of immediate post-exercise data (1700-1800) was deliberately omitted to allow GH to return to baseline values. Our intent in omitting these data was to reduce the probability of a false peak detection by catching only the latter portion of a GH peak and to prevent falsely elevated means in the exercise condition. The goal of the current investigation was to examine and compare only the normal nocturnal (i.e., non-exercise induced) GH secretion.

ApEn of overnight GH release was greater the night following EX compared to the CON condition, signifying a more disorderly GH release process the night following the resistance exercise task. The computed mono-exponential half-life of GH was similar between conditions (CON: 17.8 (SD 2.6) vs. EX: 17.3 (SD 2.6) min). Our findings regarding deconvolved hormone half-lives are in accordance with the findings of Pritzlaff et al. (19), who also found GH half-life to be unaltered by exercise. This suggests that elimination kinetics don’t appear to be largely affected by exercise. The results of the present study suggest that exercise does not affect either the total amount of GH secreted overnight or the hormone elimination, yet the dynamics of secretion that result in observed GH concentrations are altered. Based on the current results, the nocturnal GH secretion pattern after daytime resistance exercise is characterized by more
frequent yet quantitatively smaller bursts along with a lower hormone secretion rate and greater irregularity compared to an overnight control condition.

Few reports have been published utilizing frequent serial sampling for several hours following acute exercise bouts. The consistent finding among previously published studies is that exercise, although a potent stimulus for GH release immediately during and after the bout, does not appear to affect the number of GH peaks in the several hours following the exercise (9; 14; 19; 26). Consistent with the results of the Pulsar analysis previously published (14), cluster analysis indicated that the number, duration, and interval between GH concentration peaks in addition to the 12-h mean and integrated AUC GH concentrations were similar between CON and EX conditions. However, the current deconvolution analysis revealed a number of differences in hormone secretory dynamics during the night following resistance exercise that were not apparent in our previous publication which used only peak analysis (i.e., Pulsar).

The increased frequency of secretory events with reduced mean burst mass and secretory rates found in the present study differ with the findings of Pritzlaff et al. (19). Pritzlaff et al. measured GH for 6 h during the day (0900 to 1300) following a 30 min aerobic exercise bout from 0900 to 0930 and found no change in burst frequency but increases in burst mass and amplitude. In contrast, our study involved 50 sets of resistance exercise of approximately 2 h duration performed later in the day (1500 to 1700) along with 12 h of sampling overnight (1800-0600). The differences in exercise mode, intensity, and duration alone may markedly affect the outcome of the exercise on GH secretion. Clearly, the interaction of exercise and sleep (i.e., a time of dynamic GH secretion) also represents a different model when compared to morning exercise with daytime sampling. However, while Kanaley et al. (9) demonstrated that multiple bouts of aerobic exercise progressively increased daytime integrated GH concentrations via
increased burst mass, they also reported that nocturnal GH secretion was not altered. Based on the current finding that our exercise model perturbed overnight GH secretion, the volume of the 50-set resistance exercise protocol (~2 h in duration) in combination with the relatively high intensity resistance exercise likely represents an extreme physiological stress whereas the exercise tasks in the aforementioned studies may not have been of sufficient intensity and/or volume to induce changes in hormone secretion. Thus, with regard to the exercise task, it appears that the type, volume, and intensity of exercise employed can result in dramatically different effects on GH secretory dynamics. In the context of the current investigation, it appears that resistance exercise of ~2 h duration adversely affected the overnight GH pulse profile.

One explanation for the observed changes may lie in the quality of the sleep following the earlier exercise bout. Normal nocturnal GH secretion is associated with slow wave sleep (i.e., Stages III and IV) (21) and nocturnal awakenings have been shown to inhibit GH secretion (20). Therefore, impaired sleep and particularly the disruption of slow wave sleep as a result of intense exercise may disrupt the normal nocturnal pattern of GH secretion. A meta-analysis of studies investigating the effects of exercise on sleep indicated that high intensity exercise >2 h in duration can disrupt rather than improve sleep, and that power-trained athletes have shorter sleep durations and lower levels of slow wave sleep compared to endurance athletes (3).

Differences in the response of various organs and tissues to the administration of GH in a pulsatile versus continuous fashion have been well demonstrated. A pulsatile pattern has been shown to be more potent and effective for upregulation of several GH-modulated target cell end products compared to a continuous (i.e., prolonged duration of exposure) signaling despite similar mean GH concentrations. The existing literature suggests that the most potent biological signal with regard to local muscle and bone IGF-I mRNA and protein expression promoting
growth and anabolism is elicited from intermittent narrow, high amplitude peaks of circulating GH (6; 7; 12). Therefore, it has been suggested that intermittent GH pulses are primarily responsible for muscular and skeletal growth via the induction of the expression of certain genes (e.g., local tissue IGF-I as well as markers of bone formation and resorption) (4; 7; 22). In contrast, continuous stimulation influences many of GH’s metabolic actions including increased GH and LDL receptor expression as well as hepatic/circulating IGF-I and IGFBP-3 synthesis (7; 8; 11; 12; 22). The current results suggest that while 12-h mean and integrated GH concentrations were similar, the more prolonged “broad and flat” GH peaks in the exercise condition may approximate that of a more continuous GH stimulation as compared to the CON condition in which distinct higher amplitude bursts and concentration peaks are apparent. Such changes may be quite physiologically relevant, particularly with respect to chronic habitual exercise (e.g., partaking in exercise several times per week). On the other hand, the contribution of the exercise-induced GH secretion (i.e., during and immediately post-exercise) must also be considered and may be more important than the changes occurring to nocturnal GH secretion.

The deconvolved GH elimination half-life was significantly shorter when calculated for IF assayable GH compared to IR assayable GH (IR: 19.8 (SD 1.6) > IF: 15.3 (SD 1.1) min). The range for the final deconvolved IF GH half-lives was 13.4 to 17.0 min while the range for deconvolved IR GH half-lives was 17.0 to 23.8 min. The fact that the ranges of the deconvolved half-lives do not overlap to a large degree indicates that IF GH does indeed appear to eliminate more rapidly from the circulation in almost all 10 subjects. Furthermore, exercise does not appear to affect this observed difference between IR and IF GH half-lives. The fact that GH aggregates, fragments, and bound GH may be detected by the IR assay would explain the longer
half-life of IR GH. GH oligomers may be present in circulation longer and would therefore still be detectable in serum after being reduced to monomeric GH or proteolyzed into fragments with intact epitopes for binding in the IRMA. Indeed, GH oligomers persist in circulation longer than monomeric GH due to slower clearance (2). Some portion of the additional GH forms being detected by the IR but not IF assay may have prolonged biological activity, which would explain the apparent longer half-life of IR GH (2; 15).

In summary, overall mean nocturnal GH concentrations were relatively unchanged by earlier resistance exercise yet the shape of the GH peaks and the secretory events that generated them were altered substantially. A resistance exercise bout performed late in the afternoon resulted in less orderly release of GH characterized by a greater frequency of secretory bursts yet with a smaller mass of GH secreted per burst the night following the exercise. The physiological significance of the altered secretion profile following is unresolved, but previous studies demonstrate that the pattern of GH secretion can dramatically alter gene expression differentially in various tissues. Clinical implications of these results may reside in the ability of chronic resistance training to modulate GH secretion favoring a somatotrophic profile most beneficial to maintaining muscle, bone and metabolic health. While the abundance of literature demonstrates that resistance exercise increases GH immediately following exercise, an extended period of sampling and deconvolution analysis reveal that the pattern of overnight GH secretion is also affected by exercise and may even be reduced. Future investigations are necessary to examine the relationship between alterations in the endogenous GH secretion patterns in response to resistance exercise of differing volumes and intensities and the associated biological outcomes, with particular emphasis on chronic alterations in GH secretion due to repetitive bouts of resistance exercise and whether these alterations persist with training.
DISCLAIMER

The views, opinions, and/or findings contained in this publication are those of the authors and should not be construed as an official Department of Army position, policy, or decision unless so designated by official documentation.

GRANTS

This study was supported, in part, by NIH Grant M01-RR-10732 (to The Pennsylvania State University) and grants received by the American College of Sports Medicine and the National Strength and Conditioning Association (to B.C. Nindl).
FIGURE LEGENDS

Figure 1. Representative cluster-analyzed growth hormone (GH) concentration peak profiles (top figures of panels A and B) and their associated deconvolved secretion events (bottom figures of panels A and B). Cluster and deconvolution analyses were performed on data measured every 10 min overnight from 1800 to 0600 hours by immunoreactive (IR; panel A) and immunofunctional (IF; panel B) assays in control (left columns of Panel A and B) and exercise (right columns of Panel A and B) conditions. Exercise occurred from 1500 to 1700 h. Exercise attenuated the mean amplitude of concentration peaks and the mean amplitude of the secretory bursts that comprise the peaks. The number of secretory bursts was increased following exercise, but the mean burst mass and rates of hormone secretion were lower (ANOVA main effects of condition), resulting in similar overall 12-h mean and integrated GH concentrations between conditions. Figure 1 also visually demonstrates that the IF assay (Panel B) resulted in GH concentrations approximately one-half that of the IR assay (Panel A) for the same serum samples and that this ratio was unaltered by exercise.
Table 1. ANOVA least square means for main effects of condition and assay on cluster analysis of 12-h GH data ($n = 10$; values presented as mean (SD)).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
<th>Assay</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Exercise</td>
</tr>
<tr>
<td>12-h mean GH ($\mu$g/L)</td>
<td>3.3 (2.4)</td>
<td>2.6 (1.9)</td>
</tr>
<tr>
<td>Total GH AUC ($\mu$g/L)</td>
<td>2321.4 (1681.0)</td>
<td>1837.4 (1334.6)</td>
</tr>
<tr>
<td># GH concentration peaks</td>
<td>3.9 (1.6)</td>
<td>3.3 (1.2)</td>
</tr>
<tr>
<td>Mean interval between peaks (min)</td>
<td>148.1 (65.0)</td>
<td>117.7 (61.4)</td>
</tr>
<tr>
<td>Mean duration of peaks (min)</td>
<td>108.5 (29.2)</td>
<td>112.9 (44.8)</td>
</tr>
<tr>
<td>Mean amplitude of peaks ($\mu$g/L)</td>
<td>7.8 (4.2)</td>
<td>6.1 (3.7)$^A$</td>
</tr>
<tr>
<td>Mean AUC of peaks ($\mu$g/L)</td>
<td>478.6 (382.2)</td>
<td>371.4 (408.0)</td>
</tr>
<tr>
<td>Mean nadir GH concentration ($\mu$g/L)</td>
<td>1.1 (1.2)</td>
<td>1.1 (1.0)</td>
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</table>

$^A P = 0.041$ control versus exercise (condition main effect)

$^B P \leq 0.05$ immunoreactive versus immunofunctional GH (assay main effect)
Table 2. ANOVA least square means for main effects of condition and assay on deconvolution and ApEn analysis 12-h GH parameters (n = 10; values presented as mean (SD)).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Exercise</td>
</tr>
<tr>
<td># of GH secretory bursts per 12 h</td>
<td>7.6 (2.4)</td>
<td>9.4 (2.2)&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean interval between secretory bursts (min)</td>
<td>79.4 (37.1)</td>
<td>44.7 (12.9)&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean burst mass (AUC; µg/L)</td>
<td>9.2 (4.7)</td>
<td>6.0 (2.9)&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean secretion rate (amplitude; µg/L/min)</td>
<td>0.68 (0.29)</td>
<td>0.48 (0.23)&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal secretion rate (µg/L/min)</td>
<td>0.009 (0.004)</td>
<td>0.010 (0.007)&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>12-h basal secretion (µg/L·12h)</td>
<td>6.3 (3.2)</td>
<td>7.3 (5.0)&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>12-h pulsatile secretion (µg/L·12h)</td>
<td>75.3 (53.3)</td>
<td>59.7 (38.8)</td>
</tr>
<tr>
<td>12-h total secretion (µg/L·12h)</td>
<td>81.6 (53.5)</td>
<td>67.0 (41.3)</td>
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<td>Ratio of pulsatile/total secretion</td>
<td>0.89 (0.09)</td>
<td>0.87 (0.07)</td>
</tr>
<tr>
<td>GH half-life (min)</td>
<td>17.8 (2.6)</td>
<td>17.3 (2.6)</td>
</tr>
<tr>
<td>Approximate Entropy (ApEn)</td>
<td>0.41 (0.11)</td>
<td>0.51 (0.10)&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>A</sup> $P \leq 0.05$ control versus exercise (condition main effect)

<sup>B</sup> $P \leq 0.005$ immunoreactive versus immunofunctional GH (assay main effect)

<sup>C</sup> $P = 0.042$ significant interaction
Table 3. Calculated ratios and paired two-tailed $t$ test results of immunoreactive to immunofunctional growth hormone values for select parameters in control and exercise conditions ($n = 10$; values presented as mean (SD)).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Immunoreactive/Immunofunctional GH Ratio</th>
<th>Control</th>
<th>Exercise</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean GH concentration</td>
<td></td>
<td>2.1 (0.4)</td>
<td>2.2 (0.5)</td>
<td>0.243</td>
</tr>
<tr>
<td>12-h AUC</td>
<td></td>
<td>2.1 (0.4)</td>
<td>2.2 (0.5)</td>
<td>0.282</td>
</tr>
<tr>
<td>Mean amplitude of peaks</td>
<td></td>
<td>1.6 (0.7)</td>
<td>2.0 (1.0)</td>
<td>0.242</td>
</tr>
<tr>
<td>Mean AUC of peaks</td>
<td></td>
<td>2.0 (0.7)</td>
<td>3.5 (2.8)</td>
<td>0.136</td>
</tr>
<tr>
<td>Mean burst mass</td>
<td></td>
<td>1.4 (0.3)</td>
<td>1.5 (0.4)</td>
<td>0.781</td>
</tr>
<tr>
<td>Mean secretory rate</td>
<td></td>
<td>1.3 (0.3)</td>
<td>1.5 (0.4)</td>
<td>0.252</td>
</tr>
<tr>
<td>12-h pulsatile secretion</td>
<td></td>
<td>1.8 (0.4)</td>
<td>1.8 (0.5)</td>
<td>0.989</td>
</tr>
<tr>
<td>12-h total secretion</td>
<td></td>
<td>1.6 (0.3)</td>
<td>1.7 (0.4)</td>
<td>0.583</td>
</tr>
</tbody>
</table>
A

CONTROL

EXERCISE

IMMUNOREACTIVE GROWTH HORMONE

11 secretory bursts
Mean Amplitude = 0.96

14 secretory bursts
Mean Amplitude = 0.43

IMMUNOFUNCTIONAL GROWTH HORMONE

11 secretory bursts
Mean Amplitude = 0.77

10 secretory bursts
Mean Amplitude = 0.34
Reference List


