Growth hormone pulsatility profile characteristics following acute heavy resistance exercise

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Nindl, Bradley C., Wesley C. Hymer, Daniel R. Deaver, and William J. Kraemer. Growth hormone pulsatility profile characteristics following acute heavy resistance exercise. *J Appl Physiol* 91: 163–172, 2001.—This investigation examined the hypothesis that acute heavy resistance exercise (AHRE) would increase overnight concentrations of circulating human growth hormone (hGH). Ten men (22 ± 1 yr, 177 ± 2 cm, 79 ± 3 kg, $11 \pm 1\%$ body fat) underwent two overnight blood draws sampled every 10 min from 1700 to 0600: a control and an AHRE condition. The AHRE was conducted from 1500 to 1700 and was a high-volume, multiset exercise bout. Three different immunoassays measured hGH concentrations: the Nichols immunoradiometric assay (Nichols IRMA), National Institute of Diabetes and Digestive and Kidney Diseases radioimmunoassay (NIDDK RIA), and the Diagnostic Systems Laboratory immunofunctional assay (DSL IFA). The Pulsar peak detection system was used to evaluate the pulsatility profile characteristics of hGH. Maximum hGH was lower in the exercise (10.7 µg/l) vs. the control (15.4 µg/l) condition. Mean pulse amplitude was lower in the exercise vs. control condition when measured by the Nichols IRMA and the DSL IFA. A differential pattern of release was also observed after exercise in which hGH was lower in the first half of sleep but higher in the second half. We conclude that AHRE does influence the temporal pattern of overnight hGH pulsatility. Additionally, because of the unique molecular basis of the DSL IFA, this influence does have biological relevance because functionally intact molecules are affected.

immunofunctional assay; somatotropin; strength training

IT IS WIDELY KNOWN THAT EXERCISE acutely stimulates growth hormone (GH) secretion (13, 24, 33), but the persistence of this stimulation and the effect on GH secretion over a more extended follow-up observation period are still not well characterized. Optimal resolution of the pulsatile release of human GH (hGH) should include both frequent sampling and sensitive assay techniques to define the secretory dynamics of the GH axis (14). It would also seem prudent to sample during the sleeping hours at a time of dynamic hGH release. The few studies that have examined overnight hGH release after exercise have reported conflicting results, which may be attributable to factors such as exercise protocol, sampling scheme, and immunoassay used (1, 16, 22, 23, 29, 43).

The physiological significance of studying the pulsatile profile of hGH lies in the fact that the episodic pattern of release is thought to be integral in the elicitation of optimal agonist function at the cellular level. The pulsatile delivery of intravenous GH has been shown to be more effective than continuous delivery for upregulation of GH-modulated tissue end products (7, 8, 19, 27). The pulsatile release of hGH is also clinically important because it has been shown to be amplified during periods of linear growth (7, 9) and after longitudinal aerobic training (42) and depressed during pathological (18) and aged conditions (11, 18) and under sexually dimorphic regulation in men and women (20). Presently unknown is whether the benefits of resistance training, such as increased strength and muscularity, are accompanied by an increased pulsatile release of hGH.

Few studies have explored the impact of exercise on the pulsatile release of GH in humans. Weltman et al. (42) reported an amplification of the pulsatile release of GH in women undergoing 1 yr of aerobic training above the lactate threshold. Kanaley et al. (22) have published the only study using a frequent sampling scheme (every 10 min for 24 h) and a pulse detection program to examine the impact of acute daytime aerobic exercise on nocturnal pulsatile hGH release. In this study, no impact was observed. Although resistance is the preferred mode of exercise for the development and maintenance of optimal musculoskeletal system and it is documented that the acute program variables influence hGH concentrations immediately postexercise (24), no investigation to date has examined the influence of acute resistance exercise on overnight hGH pulsatility.

A variety of immunoassays are commercially available to detect circulating hGH concentrations. Because these immunoassays employ different monoclonal and polyclonal antibodies directed at specific epitopes on the GH molecule and because the variant molecular forms of GH may or may not have these intact epitopes spatially accessible to these antibodies, the results obtained for GH concentrations from the same sample can vary (10, 15, 25). Recent work evaluating the impact of exercise on in vivo bioassayable vs. immunoassayable hGH have also shown a disparity between the two for hGH concentrations (28). Thus the selection of an appropriate assay method for the detection of hGH becomes an important decision for the scientist/clinician whose interest in measuring hGH may be in the diagnostic evaluation of pituitary function.

Strasburger et al. (38) recently developed a novel enzyme-linked immunosorbant assay (ELISA) based on the molecular interaction between the hormone and its receptor necessary for receptor dimerization and subsequent signal transduction. This assay uses an anti-hGH monoclonal antibody and a biotinylated recombinant hGH binding protein (BP) that bind, respectively, to hGH receptor binding sites 2 and 1, which is the first step in initiating the cascade of intracellular signaling events for hGH (12). Strasburger et al. reported that this assay had a higher correlation than a conventional polyclonal assay compared with the in vitro NB2 bioassay, lending credence to the notion that this assay may be more biologically relevant than other immunoassays. This assay has yet to be evaluated after hGH stimulation tests such as exercise.

We examined the hypothesis that overnight hGH concentrations would be increased after an acute heavy resistance exercise protocol. To examine a more robust response of hGH to resistance exercise than had been performed previously, overnight serial sampling was utilized in a repeated-measures within-group design. Each subject participated in both a control and exercise session/condition. The pulsatile release of hGH was then evaluated and compared. In addition, to gain further insight regarding the impact of assay method, three immunoassays measured hGH concentrations: a conventional immunoradiometric assay, a conventional radioimmunoassay, and a novel "immunofunctional" assay that detects molecules on the basis of the molecular interaction between the molecule and its receptor, which is essential for receptor dimerization and subsequent signal transduction.

METHODS

Subjects. Ten young, healthy, fit men 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. From the screening procedure, subjects were determined to be nonsmokers and free of any endocrine, orthopedic, or other medical problems (e.g.,

eating, sleeping, or psychiatric disorders) that would confound the data from this investigation. Subjects who had taken supplements (e.g., creatine or androstenedione, etc.) during the preceding 3 mo were excluded. To eliminate other possible confounding variables known to influence hGH secretion, such as age, fitness, and adiposity (18), subjects were required to meet a stringent set of physical fitness inclusion criteria. These inclusion criteria were: age <25 yr, percent body fat $<20\%$, maximal oxygen uptake >45 ml·kg^{-1}·min^{-1}, and 1 repetition maximum (1 RM) squat strength $>1.5 \times$ body mass. Thus, by meeting these criteria, a homogeneous subject population was recruited for this investigation and could be fairly characterized as having above average degrees of both aerobic and strength fitness. At this physical exam, venous blood was drawn and subsequently assayed for testosterone, triiodothyronine, and insulin-like growth factor-I (IGF-I) to ensure that subjects' concentrations of these hormones were within normal values for young adults. Hydrostatic weighing was performed to determine body density by the methods described by Akers and Buskirk (2). Body density was subsequently used to estimate percent body fat. Maximal volume of oxygen consumption was determined by a graded treadmill protocol. Table 1 gives the subjects' physical and descriptive characteristics.

Strength assessment. 1 RMs were tested on the following exercises: squat, bench press, leg press, and lat pulldown on Universal (Universal Equipment, Omaha, NE) and York Barbell equipment (York Barbell, York, PA). Warm-up consisted of performing 5–10 repetitions at 40–60% perceived maximum, a 3- to 5-min rest and stretching period, and the completion of 3–5 repetitions at 60–80% maximum. Three to

Table 1. *Baseline physical, descriptive data of subjects*

Variables	Mean ± SD	Range
Physical characteristics		
Age, yr	21 ± 2.1	18–25
Height, cm	177.1 ± 7.4	166–189
BM, kg	78.6 ± 6.4	70–90
%BF	11.3 ± 4.1	5–18
FFM, kg	69.8 ± 7.4	60–84
BMI, kg/m^2	25.1 ± 1.9	22–30
FFMI, kg/m^2	22.3 ± 1.6	20–24
Aerobic fitness parameters		
$\dot{V}O_{2\,max}$, l/min	4.4 ± 0.45	3.7–5.1
$\dot{V}O_{2\,max}$, ml·kg·min^{-1}	50.8 ± 2.5	45.4–53.8
HR$_{max}$, beats/min	195.2 ± 6.0	184–203
RER	1.13 ± 0.03	1.07–1.20
Muscular strength measures		
Squat, kg	135.0 ± 37.0	84–206
kg/BM	1.7 ± 0.4	1.0–2.5
kg/FFM	1.9 ± 0.5	1.2–3.0
Bench press, kg	110.2 ± 25.0	70–145
kg/BM	1.4 ± 0.3	0.9–1.8
kg/FFM	1.6 ± 0.3	1.1–2.1
Leg press, kg	196.4 ± 33.3	127–227
kg/BM	2.5 ± 0.4	1.6–3.0
kg/FFM	2.8 ± 0.4	1.9–3.3
Lat pulldown, kg	84.1 ± 9.6	68–95
kg/BM	1.1 ± 0.1	0.8–1.2
kg/FFM	1.2 ± 0.1	1.0–1.5

%BF, percent body fat; BM, body mass; BMI, body mass index; FFM, fat-free mass; FFMI, fat-free mass index; $\dot{V}O_{2\,max}$, maximal oxygen uptake; HR$_{max}$, maximal heart rate; RER, respiratory exchange ratio.

five subsequent lifts were then made to determine the 1 RM with 5-min rests between lifts. An attempt was considered successful when completed through a full range of motion without deviating from proper technique and form.

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. Details of the AHREP are provided in Table 2. This was accomplished by performing multijoint exercises that required the use of major muscle groups in both the lower and upper body. Subjects terminated the exercise set on the achievement of the number of repetitions or muscle failure. In the event that the desired number of repetitions was not achieved for a given set, the load was subsequently reduced before commencement of the next exercise using that same exercise and load. A 90-s rest period was given after each exercise, and alternating exercise groups were utilized as outlined in Table 2. All subjects completed the entire workout. The mean $\pm$ SE time for completion of the AHREP was 125.3 ± 3.4 min.

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. To facilitate familiarization with the facility and to safeguard against a disrupted first night sleeping effect, all subjects slept in the GCRC the night before each overnight serial blood draw. Conditions were mimicked exactly to include the taping of a catheter near the antecubital vein for the night. One of these overnights served as the control 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 by a registered nurse and serial blood was drawn at a rate of one draw every 10 min until 0600 the following morning. Blood sampling followed the same procedures during the exercise condition. During the exercise condition the subject performed the AHREP from ~1500 to 1700 in the university athletic weight room. Blood was ob-

tained as soon as possible after the completion of the last set. The mean $\pm$ SE time after exercise for the first blood draw was 4.3 ± 0.7 min. During each overnight trial, subjects were allowed to move freely on the floor, watch TV, receive phone calls, study quietly, and so on. Bedroom lights were turned off at 2200, and the TV was turned off at 2300. At bedside, a registered nurse and research technicians unobtrusively performed the serial blood draws throughout the night. Blood was collected in glass vacutainers, allowed to clot at room temperature, and centrifuged for 30 min at 800 g at 4°C. After centrifugation, 900 μl of serum was aliquoted into three separate Eppendorf tubes, flash frozen in liquid nitrogen, and stored at −80°C for later analysis.

Dietary control. All subjects completed two 3-day dietary intake diaries (i.e., one 3-day diary before each overnight trial). Subjects were asked to replicate, as much as possible, their first 3-day dietary intake for the second 3-day period on their overnight visits. Dietary analyses (Nutritionist IV, First DataBank, San Bruno, CA) of these records verified that the caloric content and composition were similar for the 3 days before each overnight stay. On each day of their overnight trials, 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. Subjects were allowed to consume water ad libitum.

Growth hormone immunoassays. Three separate immunoassays compared circulating serum hGH concentrations: *1)* the Nichols Institute Diagnostics immunoradiometric assay (Nichols IRMA) (San Juan Capistrano, CA); *2)* the National

Table 2. *List of order, exercises, repetitions, and relative loads of acute heavy resistance exercise protocol*

Group 1	1	2	3	4	5	6	7	8	9	10
	Squat	Bench Pr	Squat	Bench Pr	Squat	Bench Pr	Squat	Bench Pr	Squat	Bench Pr
	1×10	1×10	1×5	1×5	1×10	1×10	1×5	1×5	1×10	1×10
Group 2	11	12	13	14	15	16	17	18	19	20
	Leg Pr	Lat Pd	Leg Pr	Lat Pd	Leg Pr	Lat Pd	Leg Pr	Lat Pd	Leg Pr	Lat Pd
	1×10	1×10	1×5	1×5	1×10	1×10	1×5	1×5	1×10	1×10
Group 3	21	22	23	24	25	26	27	28	29	30
	Squat	Bench Pr	Squat	Bench Pr	Squat	Bench Pr	Squat	Bench Pr	Squat	Bench Pr
	1×10	1×10	1×5	1×5	1×10	1×10	1×5	1×5	1×10	1×10
Group 4	31	32	33	34	35	36	37	38	39	40
	Leg Pr	Lat Pd	Leg Pr	Lat Pd	Leg Pr	Lat Pd	Leg Pr	Lat Pd	Leg Pr	Lat Pd
	1×10	1×10	1×5	1×5	1×10	1×10	1×5	1×5	1×10	1×10
Group 5	41	42	43	44	45	46	47	48	49	50
	Squat	Bench Pr	Squat	Bench Pr	Squat	Bench Pr	Squat	Bench Pr	Squat	Bench Pr
	1×10	1×10	1×5	1×5	1×10	1×10	1×5	1×5	1×10	1×10

Multijoint exercises that required the use of major muscle groups in both the lower and upper body were used [e.g., the squat (15 sets), leg press (Leg Pr; 10 sets), bench press (Bench Pr; 15 sets), lateral pulldown (Lat Pd; 10 sets)]. The relative loads for each exercise alternated between 10 and 5 repetition maximum (RM) loads. The 10 and 5 RM loads were calculated as 70% and 85% of the exercise 1 RM, respectively. The 50 total sets were placed into 5 groups of 10 exercises each. *Sets 1–10, 21–30,* and *41–50* were identical with respect to the exercises performed (e.g., squat and bench press) and relative intensity (sets of the same exercises were alternated between 10 RM and 5 RM loads). Likewise, *sets 11–20* and *31–40* were identical with respect to the exercises performed (e.g., leg press and lat pulldown) and relative intensity (see above). The sequential numbers are the order in which the exercises were performed. Start with *Group 1, set 1* and move to right. At *set 10* in *group 1,* the sequence starts with *group 2, set 1* and so forth. Note: *groups 1, 3,* and *5* are composed of identical exercises, order of exercises, and relative loads. *Groups 2* and *4* are also composed of identical exercises, order of exercises, and relative loads. A 90-s rest period was given after each exercise, and a 120-s rest period was given after each group of 10 sets.

Institute of Diabetes and Digestive and Kidney Disease competitive polyclonal radioimmunoassay (NIDDK RIA); and 3) the Diagnostic Systems Laboratory's immunofunctional ELISA (DSL IFA) (Webster, TX). The NIDDK RIA and Nichols IRMA were chosen because they represented a prototype "competitive polyclonal RIA" and a "double monoclonal IRMA," respectively. These two assays are readily accessible, are commonly used in laboratories studying hGH, and were considered representative assays to which the DSL IFA could be compared. The DSL IFA was chosen because of its unique molecular basis and to extend the work of Strasburger et al. (35–38) by comparing this assay after acute resistance exercise to the above-mentioned assays for the first time. The assay was designed to detect only those hGH molecules possessing intact sites 1 and 2, which are a prerequisite for the molecule to induce receptor dimerization. All assays were validated with respect to parallelism, recovery, and linearity. Log-logit, log-log, and linear standard curve-fitting regressions were used for the NIDDK RIA, Nichols IRMA, and DSL IFA, respectively. All standard curve points were run in quadruplicate. To eliminate interassay variance, all assay 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 RIAs and a microplate reader (Bio-Tek Instruments, Winooski, VT) for the ELISA. The sensitivities of the Nichols IRMA, the NIDDK RIA, and the DSL IFA were 0.04, 0.10, and 0.20 μg/l, respectively. For the Nichols IRMA, all values fell above the sensitivity for the assay. For the NIDDK RIA and DSL IFA, values falling below the thresholds were set equal to the sensitivity for that immunoassay. The intraassay variances for low, medium, and high hGH concentrations were as follows: 7.2, 5.2, and 5.4%, respectively, for the Nichols IRMA; <10% for the NIDDK RIA; and 7.1, 7.6, and 8.4%, respectively, for the DSL IFA. Serum IGF-I was measured with a two-site IRMA (Diagnostic Systems Laboratories, Webster, TX) at three times throughout the sampling. The sensitivity and intra-assay variance of the IGF-I assay were 2.06 μg/l and 3.9%, respectively.

Pulsatility analysis. Pulsatile analysis for hGH was performed by using the Merriam and Wachter PC-Pulsar program for the IBM-PC (30), the software for which was kindly provided by J. F. Gitzen and V. D. Ramirez from the Neural and Behavioral Program at the University of Illinois. The G parameters corresponding to a 1% false detection rate were used: G(1): 4.40, G(2): 2.60, G(3): 1.92, G(4): 1.46, G(5): 1.13. The following pulsatility profile characteristics were used for statistical analysis: mean hGH concentration, maximum hGH concentration, number of peaks, mean amplitude of these peaks, mean peak length of the peaks, and mean interpeak interval.

Statistics. All results are reported as means ± SE. An ANOVA with repeated measures was used to determine the effects of exercise vs. control (i.e., the repeated measure) and assay detection method (i.e., Nichols IRMA vs. NIDDK RIA vs. DSL IFA). The raw data from 1800 to 0600 were entered into the pulsar peak detection program to characterize pulse characteristics as described above. The effects of condition and immunoassay on the pulsatility profiles were then tested for statistical significance ($P \leq 0.05$) on the pulsatility profile characteristics (i.e., mean hGH concentration, maximum hGH concentration, number of peaks, mean amplitude of these peaks, mean peak length of the peaks, and mean interpeak interval). The 12-h data were then partitioned into four segments. The mean hGH concentration was then analyzed for the main effects of time period [1800–1900 (I); 1900–2300 (II); 2300–0300 (III); 0300–0600 (IV)], assay

method, and condition. Regression analyses assessed the relationship for hGH concentrations between the Nichols IRMA:NIDDK RIA, Nichols IRMA:DSL IFA, and DSL IFA: NIDDK RIA. All analyses were performed using a commercially available software package (CSS: Statistica, StatSoft, Tulsa, OK).

RESULTS

Integrity of AHREP. The exercise volume for the squat/bench press "superset" combination decreased ($P \leq 0.05$) throughout the AHREP [*sets 1–10*: 15,751 ± 1,176 repetitions (reps) × lb; *sets 21–30*: 13,292 ± 988 reps × lb ($\sim$84% of *sets 1–10*); and *sets 41–50*: 11,898 ± 911 reps × lb ($\sim$76% of *sets 1–10*)]. Also, the exercise volume for the leg press/lat pulldown superset combination decreased during *sets 31–40* [16,629 ± 707 reps × lb ($\sim$91% of *sets 11–20*) 18,336 ± 772 reps × lb]. The intensity of the AHREP was further confirmed as the mean peak lactate concentrations increased from pre-AHREP values (2.0 ± 0.4 vs. 17.2 ± 1.2 mmol).

Immediate postexercise growth hormone responses. Figure 1 shows serum hGH concentrations for the first hour after exercise as assessed by the Nichols IRMA (1*A*), the NIDDK RIA (1*B*), and the DSL IFA (1*C*). After exercise, GH concentrations gradually declined during this hour, whereas hGH concentrations increased during this same period in the control condition. hGH concentrations were significantly increased for the first 30 min after exercise. The increase in hGH concentrations during the control period is attributed to a naturally occurring daytime pulse at $\sim$1730. All 10 subjects exhibited a daytime pulse between 1700 and 1830.

Pulsatility profile characteristics of 12-h serial data. Figure 2 illustrates the 12-h pulsatility profiles determined by using either the Nichols IRMA (2*A*), the NIDDK RIA (2*B*), or the DSL IFA (2*C*) assays. The main results were a lower maximum hGH concentration and lower mean peak amplitude after the AHREP. Table 3 lists the main effect means for the pulsatility profile characteristics. Significant assay effects were observed for mean hGH (Nichols IRMA > NIDDK RIA = DSL IFA), maximum hGH (Nichols IRMA > DSL IFA > NIDDK RIA), mean pulse amplitude (Nichols IRMA > NIDDK RIA = DSL IFA), and mean pulse length (Nichols IRMA > DSL IFA; NIDDK equal to both). No assay differences were observed for the number of peaks or the interpeak interval. Significant condition effects were observed only for the maximum hGH concentration [exercise (10.7 μg/l) < control (15.4 μg/l)]. The mean pulse amplitude approached significance [exercise (4.84 μg/l) vs. control (6.65 μg/l); $P =$ 0.08]. The mean hGH concentration ($P = 0.25$), mean pulse length ($P = 0.75$), number of peaks ($P = 0.80$), and interpeak interval ($P = 0.34$) did not statistically differ between the conditions. Figure 3 shows the interaction means for the pulsatility profile characteristics. Figure 3, *A–D*, shows that there were no differences between exercise and control conditions for mean hGH (3*A*), number of peaks (3*B*), mean pulse length (3*C*), or interpeak interval (3*D*). Interaction effects
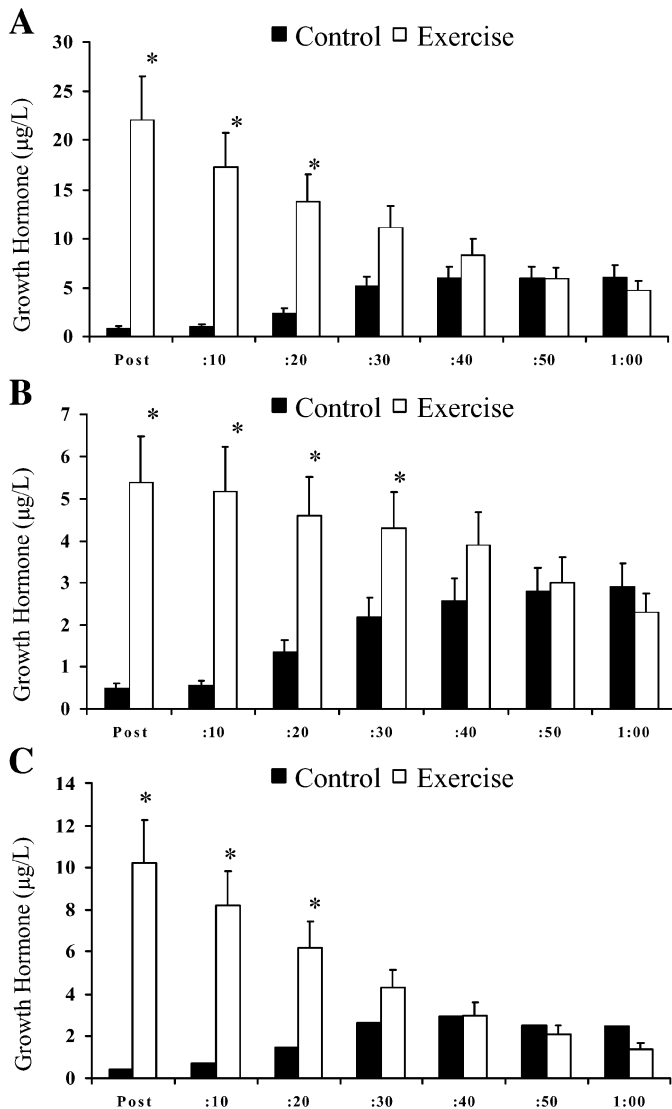
A

B

C

Fig. 1. *A*: Nichols immunoradiometric assay (IRMA)-assessed human growth hormone (hGH) responses for the first hour postexercise between control and exercise conditions. *B*: National Institute of Diabetes and Digestive and Kidney Diseases radioimmunoassay (NIDDK RIA)-assessed hGH responses for the first hour postexercise between control and exercise conditions. *C*: Diagnostic Systems Laboratory immunofunctional assay (DSL IFA)-assessed hGH responses for the first hour postexercise between the control and exercise conditions. *Significant differences ($P \leq 0.05$) between control and exercise conditions.

were evident for the maximum hGH concentration and mean pulse amplitude (Fig. 3, *E* and *F*). These figures show that the Nichols IRMA and DSL IFA detected a lower maximum hGH and mean pulse amplitude for the exercise vs. control condition.

Mean hGH concentrations segmented by time periods. Figure 4 illustrates the composite pulsatility profile (i.e., the mean values at each 10-min interval for all three immunoassays). The 12-h profile was partitioned into four segments: 1800–1900 (I), 1900–2300 (II), 2300–0300 (III), and 0300–0600 (IV). The profile was segmented in this manner to determine whether a differential temporal pattern of release existed as sug-

gested after visual observation of the data. The segments were selected on the basis of feeding time, lights out time, and the time for the observed divergence between the exercise and control conditions. Subsequently, the exercise vs. control mean hGH concentrations in each time period were compared for all three immunoassays. This analysis is depicted in Fig. 5. For all three immunoassays, the control hGH was greater than the exercise hGH in *time period I*. No differences between conditions were observed during *time period II*. The control hGH concentrations were greater than the exercise hGH concentrations during *time period III* when assessed by the Nichols IRMA and the DSL IFA but not by the NIDDK RIA. The exercise hGH concentrations were greater than the control hGH concentrations during *time period IV* when assessed by the Nichols IRMA and NIDDK RIA but not by the DSL IFA.
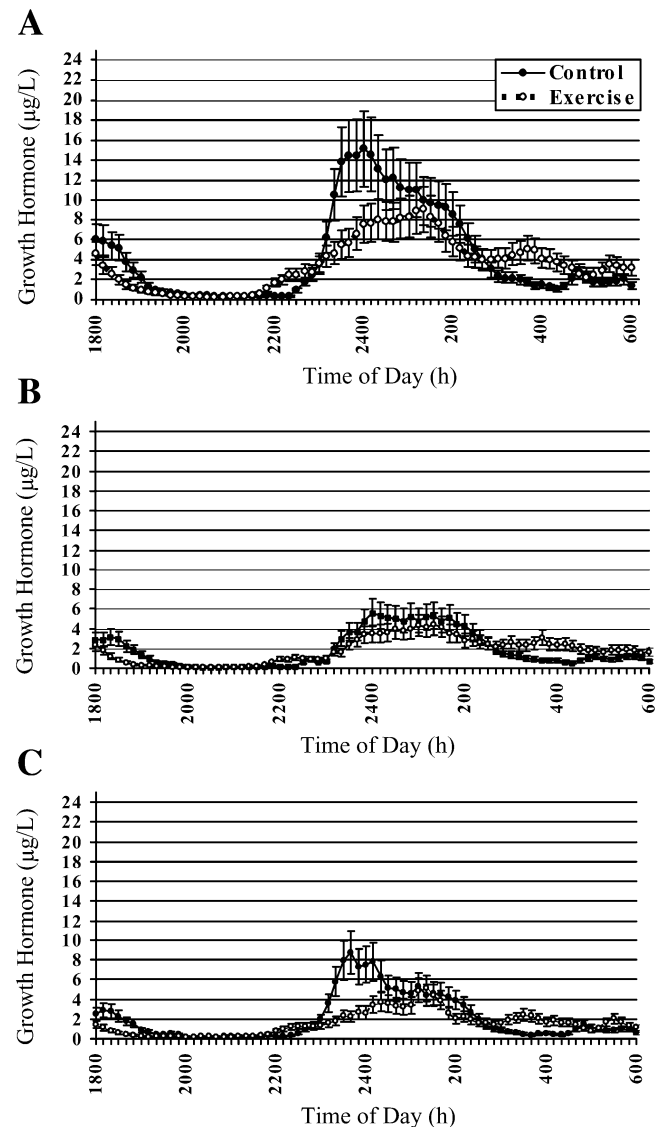
A

B

C

Fig. 2. Comparisons of composite mean pulsatile profiles of hGH between control and exercise conditions assayed by the Nichols IRMA (*A*), the NIDDK RIA (*B*), and the DSL IFA (*C*).

Table 3. *ANOVA marginal main effect means for immunoassays and conditions on 12-h pulsatility profile data*

Parameter	Condition		Assay Method		
	Control	Exercise	Nichols IRMA	NIDDK RIA	DSL IFA
Mean [GH], µg/L	2.28[a]	2.38[a]	3.98[a]	1.89[b]	1.83[b]
Number of peaks	3.9[a]	3.9[a]	4.0[a]	3.3[a]	4.35[a]
Mean pulse length, min	119.7[a]	124.2[a]	140.5[a]	124.9[a,b]	100.0[b]
Interpeak interval, min	223.9[a]	208.0[a]	205.4[a]	235.8[a]	206.7[a]
Maximum [hGH], µg/l	15.4[a]	10.7[b]	19.5[a]	8.0[b]	11.7[c]
Mean pulse amplitude, µg/l	6.65[a]	4.84[a]	8.0[a]	4.65[b]	4.63[b]

IRMA, immunoradiometric assay; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases; RIA, radioimmunoassay; DSL, Diagnostic Systems Laboratory; IFA, immunofunctional assay; [GH], growth hormone concentration; [hGH], human growth hormone concentration. Different letters denote statistical difference within main effect, whereas similar letters denote statistical similarity within main effect ($P = 0.05$). The ANOVA interaction means are shown in Figure 3.

Serum IGF-I responses. There were no differences in IGF-I concentrations between control and AHREP conditions at *hours 1*, *5*, or *9*.

Comparison between immunoassays (via regression analyses). Linear regression analyses revealed significant associations among the three assays (correlations ranged from 0.85 to 0.95; see Fig. 6). The Nichols IRMA and the DSL IFA had the highest correlation, whereas the regression line between the NIDDK RIA and the DSL IFA had a slope of 1.13. From visual inspection of the corresponding scatterplots, it was evident that the data points diverged from the regression lines with increasing hGH concentrations.

DISCUSSION

This study provides additional insight into hGH secretory dynamics by using a rigorous sampling scheme to characterize, for the first time, the impact of acute, heavy resistance exercise on hGH pulsatility and uses a newly established immunofunctional assay (i.e., the DSL IFA), which detects hGH molecules on the basis of
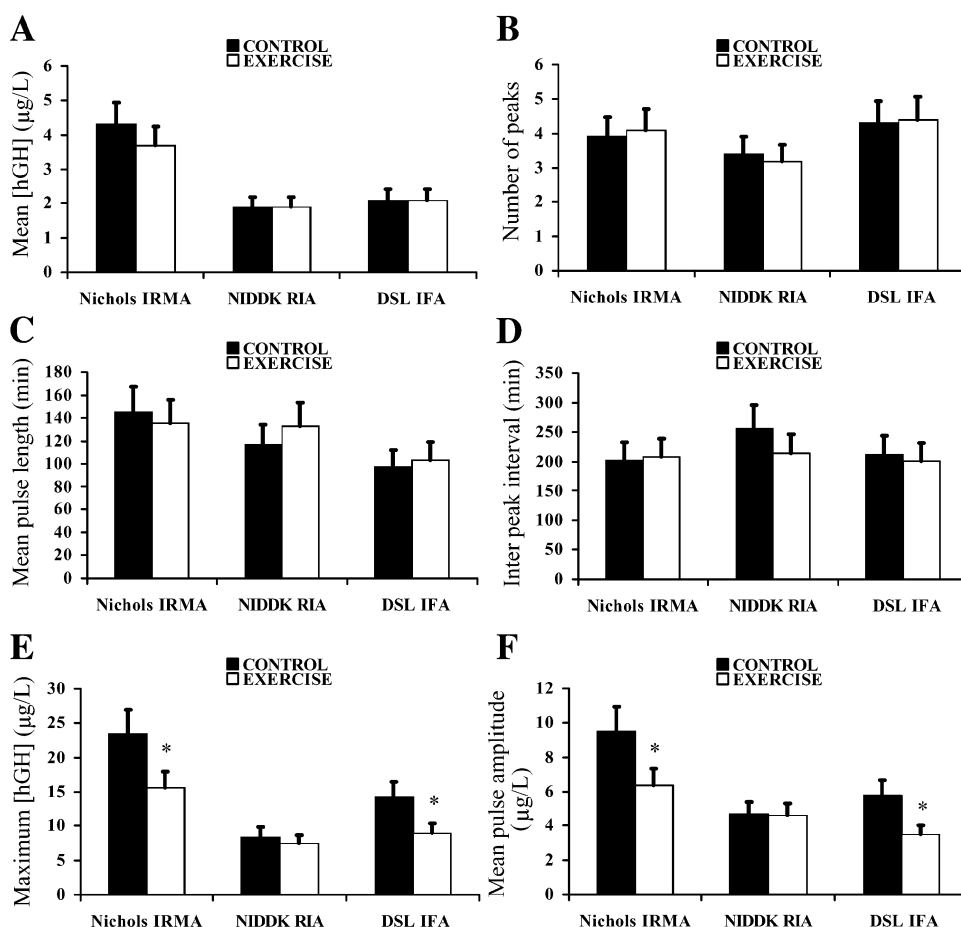
Fig. 3. Comparison between exercise and control among the immunoassays for the pulsatility profile characteristics displaying ANOVA interaction means: mean hGH concentration ([hGH]; *A*), number of peaks (*B*), mean pulse amplitude (*C*), interpeak interval (*D*), maximum [hGH] (*E*), and mean pulse amplitude (*F*). *Significant difference ($P \leq 0.05$) between control and exercise conditions.
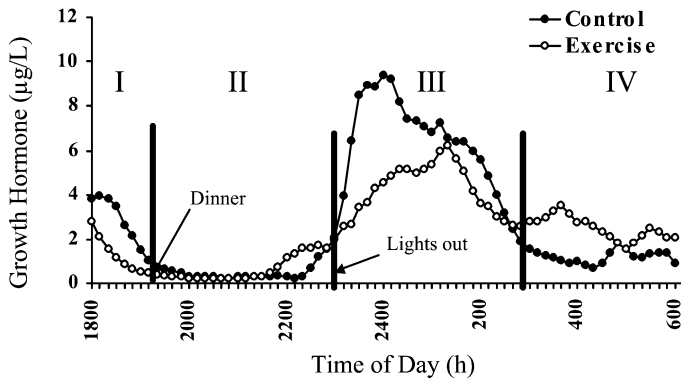
Fig. 4. Composite (mean of all 3 immunoassays) pulsatility hGH profiles between the control and exercise conditions illustrating the segmentation of the serial data. The profile was partitioned in this manner into 4 segments (labeled I–IV) to determine whether a differential temporal pattern of release existed as suggested after visual observation of the data. The segments were selected on the basis of feeding time, lights out time, and the time for the observed divergence between the exercise and control conditions. Mean [hGH] for the segments are given in Fig. 5.

the molecular interaction between the molecule and its receptor (12, 38). Our data demonstrate that heavy resistance exercise in the late afternoon decreased overnight maximum hGH concentrations and hGH pulse amplitudes, although overall mean hGH concentrations were not significantly reduced. Acute heavy resistance exercise differentially influenced the temporal pattern of the overnight release because hGH was lower during the first half of sleep but greater during the second half for the exercise vs. control conditions. Another finding of particular interest is that the DSL IFA yielded less than one-half the mean hGH concentration that the widely used Nichols IRMA did.

There were no differences in the number of hGH peaks between the exercise and control conditions. This finding is consistent with the studies of Pritzlaff et al. (31) and Kanaley et al. (22) who reported that aerobic exercise bouts did not alter the number of hGH episodes in a 6-h or 24-h period, respectively. Weltman et al. (42) also reported that the number of peaks was a stable phenomenon even in the presence of a significant amplification of hGH release after 1 yr of aerobic training in premenopausal women. Considering the fact that the overall mean hGH concentration over the entire 12-h sampling period was also not affected by exercise, the data suggest that the overall amount of hGH released may be tightly regulated, even when the data in Fig. 4 show that the intensity of the exercise regimen was clearly affecting other pulse parameters.

The mean maximum hGH concentration and mean pulse amplitude were lower in the exercise vs. the control condition. There are several possible interpretations for this finding, one of which could be an increased somatostatin tone after exercise because GH releasing hormone is required for the initiation of a GH pulse, whereas somatostatin modulates the amplitude of the GH pulse (17, 39). Although somatostatin is known to inhibit GH release, it does not negatively affect GH biosynthesis. This is an important concept

because this may explain a "rebound" in GH secretion after somatostatin priming/withdrawal (14). The nocturnal peaks were lower for the exercise vs. control conditions during 2300–0300 h, but higher during 0300–0600. Thus, even though the mean hGH concentration was similar between the control and exercise conditions, the temporal pattern of hGH release was clearly influenced by daytime exercise. From a mechanistic perspective, we suggest that the acute heavy resistance exercise bout resulted in an elevated somatostatin tone during 2300–0300. During this time, hGH release was inhibited to some degree. Concurrently, hGH biosynthesis was not inhibited. At ~0300, this somatostatin tone was withdrawn, and hGH molecules biosynthesized and stored during the time when hGH release was inhibited were then available and readily released. Our data also demonstrated that IGF-I inhibitory feedback on pituitary hGH release was unlikely because serum IGF-I concentrations did not differ between the control and exercise conditions. An array of other metabolic and hormonal signals, such as changes in GHRH, hexapeptides, or Ghrelin release, could also have mediated the observed hGH response.
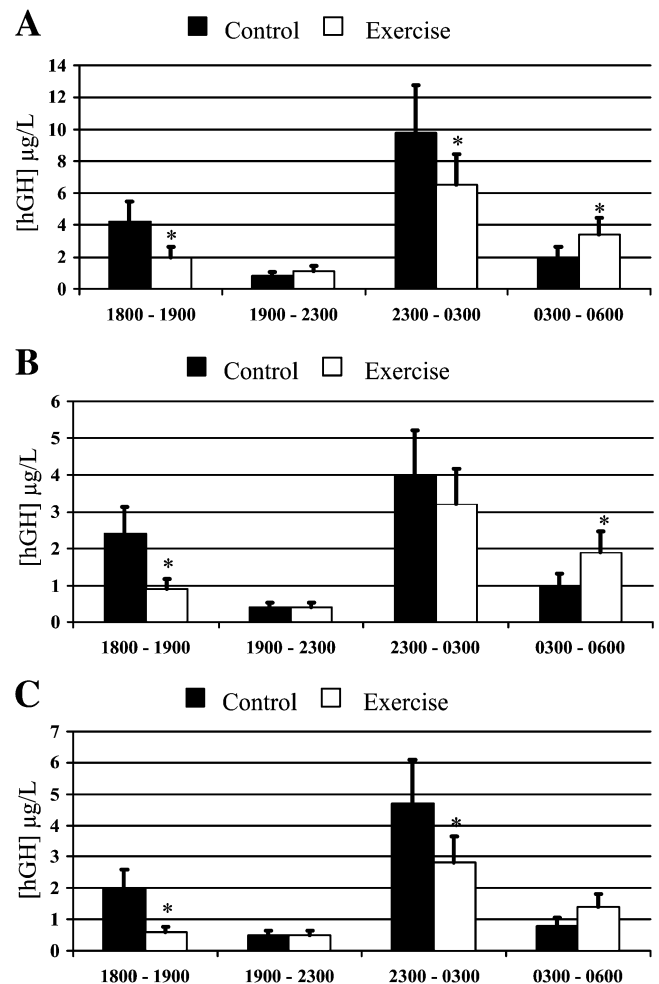

Fig. 5. Mean [hGH] for *time periods I* through *IV* for the Nichols IRMA (*A*), the NIDDK RIA (*B*), and the DSL IFA (*C*). *Significant difference between control and exercise conditions ($P \leq 0.05$).
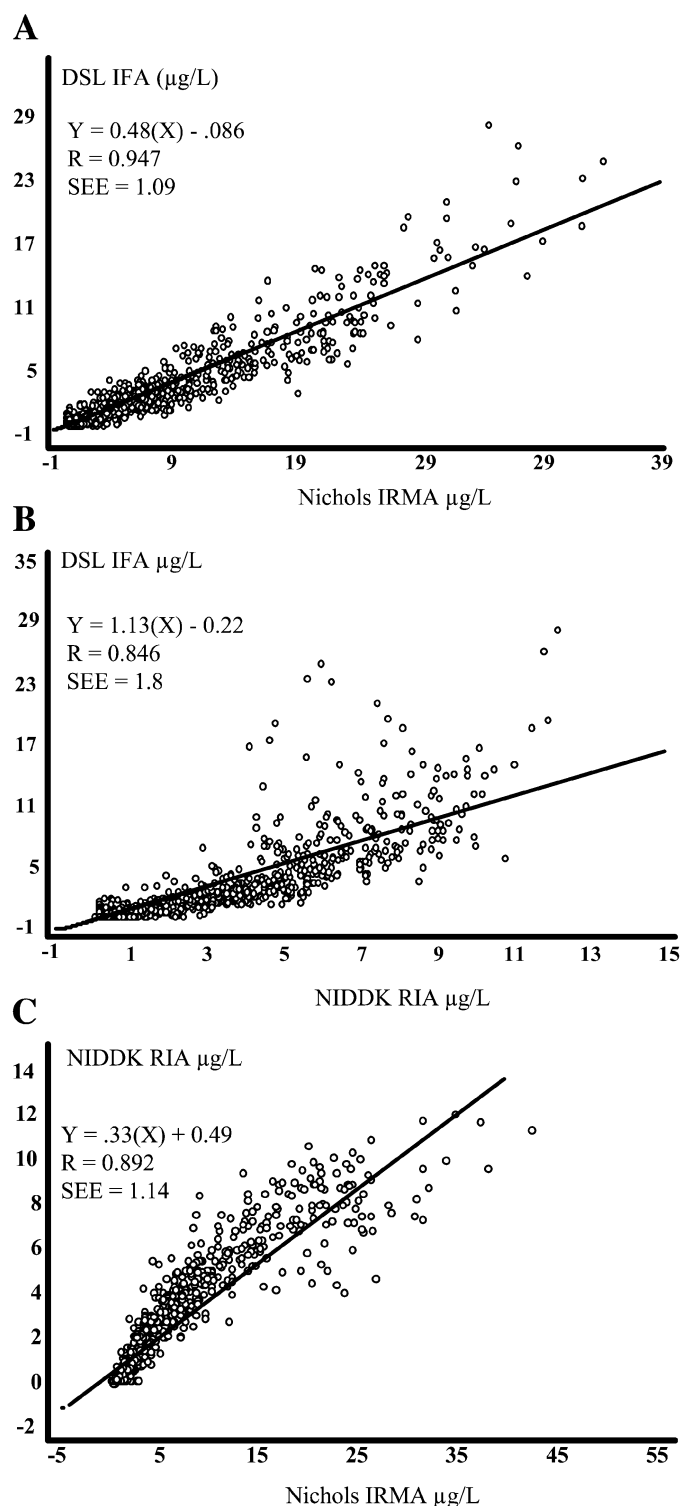
A

B

C

Fig. 6. Scatterplots of [hGH] for Nichols IRMA vs. DSL IFA (*A*), NIDDK RIA vs. DSL IFA (*B*), and Nichols IRMA vs. NIDDK RIA (*C*). All correlations were significant ($P < 0.01$). The data represent all serial data points for all subjects ($n = 1,580$).

The temporal pattern of hGH release in the present study is strikingly similar to the data reported by Kern et al. (23) after low- and moderate-intensity aerobic exercise. The hGH concentrations in Kern's study were higher for the control condition compared with both exercise conditions for the first half of sleep, whereas the control hGH concentrations were lower than both exercise conditions during the second half of sleep. In contrast to the findings of the present study and Kern's study, two other studies, one using an aerobic (22) and one a resistance exercise protocol (29), did not find any impact of daytime exercise on overnight hGH release. It is important to note that our protocol and Kern's protocol were longer in duration and therefore resulted in a higher exercise volume than the studies by Kanaley et al. (22) and McMurray et al. (29). Collectively, the data currently available on the impact of daytime exercise on overnight hGH release support the following conclusions: *1*) daytime exercise of sufficient duration and intensity can influence the temporal release of overnight hGH release, and *2*) this influence is similar for aerobic and resistance modes of exercise.

A naive interpretation of the lowered hGH pulse amplitude during the first half of sleep, albeit somewhat surprising, would be that the resistance exercise had a negative effect on the somatotrophic influences of hGH. Peripheral serum concentrations yield little information concerning uptake by various biocompartments or target tissue responsiveness. It is unlikely that the immunoassays used in the study were able to detect all molecular forms of hGH. Higher molecular weight forms have been shown to have greater bioactive/immunoreactive ratios as measured by the in vivo tibial rat bioassay (28). Thus forms of hGH demonstrating superior biological activity may be released that are not detected by conventional immunoassays. Regardless of the underlying factors responsible for the altered overnight hGH concentrations after resistance exercise, this influence likely reflects compensatory responses to energy deficits and localized tissue damage caused by the episodes of physical activity. We can only speculate on the ultimate effect that exercise exerts on the temporal shift in facilitating the hormone's metabolic and somatogenic activities. Additional work is required to determine whether the high-volume exercise-induced serum hGH concentrations in the hours before daybreak continue into the later morning hours. Although it has been demonstrated that chronic aerobic training amplifies the pulsatile release of hGH (42), no data are available to indicate whether those engaged in regular resistance training also exhibit an increased amplification of hGH release.

Another finding in the present study was the disparity among immunoassays in detecting hGH concentrations. Such variability has been attributed to several factors including antibody specificity, reference preparation purity, and the molecular heterogeneity of the hormone being measured (5, 6, 10, 15, 25, 32). The common reference preparation used in all three immunoassays in our study was of pituitary origin and, in theory, better represents the mixture of hGH forms in the circulation than a single, highly purified form. Even though the results from all three immunoassays were highly correlated, the Nichols IRMA gave higher values for hGH concentrations as well as higher pulse

amplitudes. Because the DSL IFA only purports to measure "biologically active" forms of hGH, it remains unknown precisely what additional molecules the Nichols IRMA could be measuring. The Nichols IRMA has been shown to have the capability of detecting high-molecular-weight variant forms of hGH (21, 26). It seems reasonable to suggest that, on average, 50% of the GH released into the circulation of our exercising subjects overnight was bound to protein and detected by the Nichols IRMA but not the antisera in the DSL IFA and NIDDK RIA. It is possible that different hGH isoforms are coreleased and are detected by one assay but not another. This could have contributed to the curvilinear relationship among the assays for the data at the higher concentrations.

It is important to consider the impact of BPs in the IFA. The IFA uses a recombinant hGHBP to bind to site 1. Thus one could infer that an hGH molecule already complexed to a GH BP would not be detected in this assay system, because site 1 would not be freely accessible. In addition, a hGH-BP complex might be configured such that site 2 is not exposed to the monoclonal antibody (MAb7B11). This could be an important distinction with the DSL IFA, given that Strasburger reported that endogenous GH BP levels in physiological concentrations did not interfere with the hGH results derived from the DSL IFA (38). It has been reported that the high-affinity GH BP inhibits GH binding to receptors and in vitro bioactivity via competition for ligand (4). If it is true that GH complexed to BP is too large a molecule to traverse the capillary endothelium to bind to cellular receptors, the lack of detection of the hGH complexed in the IFA provides further support for the "functional selectivity" of the IFA. The assay does not detect GH already complexed to BP, neither does GH complexed to BP dimerize receptors.

Another possible explanation for the difference between the IRMA and the IFA could be that the IRMA could detect fragments or cleaved forms not detected by the IFA. It has recently been reported that the GH fragment 44–191 is detectable in substantial levels in human serum (34). Because this form lacks part of the NH_2 terminus, it is unlikely to be detected by the IFA, but, depending on the targeted epitopes, it could be detected by the IRMA.

Molecular heterogeneity in circulating hGH forms has been known about and studied for many years. How this molecular heterogeneity fits into the secretory patterns of GH release over extended time periods is largely unknown. Nevertheless, our findings conclusively show that at least some of the molecules released during secretory bursts are able to dimerize hGH receptors. In that sense, these molecules are "biologically active." On the basis of the high correlations among the immunoassays and the similar detection of the number of peaks and interpeak intervals, it appears that the immunoassays report comparable qualitative pictures of the GH response. The quantitative differences among the immunoassays have yet to be fully explained but are likely due to the existence of various molecular isoforms. Future studies aimed at separating different forms of hGH variants during different stages of the GH pulse after exercise would seem productive.

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