# The effect of exercise type on immunofunctional and traditional growth hormone

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## Abstract:

The purpose of this study was to compare the growth hormone (GH) response, including the immunfunctional (IF) GH response, between an acute bout of aerobic and resistance exercise in the same subjects. Ten cross-trained males (24.3 ± 1.2 years) performed both 30 min of continuous cycling at 70% of $VO_{2max}$, and intermittent free weight squatting at 70% of 1-RM, in a randomly assigned crossover design, separated by at least 1 week. Blood samples were collected at 10-min intervals for 2 h (30 min rest, 30 min exercise, 60 min recovery) and analyzed for total human and IF GH. After adjusting for the amount of work performed per minute of exercise, integrated GH AUC was significantly greater during the resistance session than the aerobic session as measured by both the total and IF GH assays ($P = 0.008$ and $P = 0.014$, respectively). Peak GH concentrations were significantly greater during the resistance session than the aerobic session ($P = 0.05$). A similar overall GH pattern was observed in response to both types of exercise, with peak values occurring at the end of exercise, regardless of the GH assay used. These data demonstrate that in young, cross-trained males, intermittent resistance exercise elicits a greater response of GH, including IF GH, compared to a continuous aerobic session, when controlling for the work performed per minute, intersubject variability, relative exercise intensity and session duration.

**Keywords** Aerobic exercise - Resistance exercise - Growth hormone isoforms

## Article:

INTRODUCTION

It is widely recognized that exercise is a powerful stimulant for the release of circulating growth hormone (GH) in the human, as measured by traditional immunoassays (Kraemer et al. *2006a*; Nindl et al. *2001*; Rubin et al. *2003*; Tuckow et al. *2006*; Wallace et al. *2001*; Wideman et al. *1999, 2006*). Since GH is known to play an important role in lipid metabolism and protein synthesis, researchers have attempted to link exercise-induced increases in GH to physiological adaptations that traditionally accompany chronic exercise training (i.e., reduced body fat and lean muscle acquisition). While substantial increases in circulating GH occur transiently in response to both acute aerobic and resistance exercise, the physiological effects of these alterations remain unknown. Chronic aerobic and resistance exercise training clearly result in different physiological adaptations at the cellular level and phenotypically produce different body compositions. These differences suggest that the upregulation of protein synthesis and subsequent activation of genes to promote fat utilization and muscle hypertrophy differ between

the two types of exercise. Given the metabolic effects of GH, this hormone may play a role in initiating these adaptations.

Despite this belief, it is still unclear how transient exercise-induced increases in GH regulate post-receptor mechanisms and whether they differ based on the type of exercise completed. A first step in examining the potential effects and differences of exercise-induced GH is to document the pattern of GH release between the two types of exercise in the same individual. To our knowledge, no published data exist examining this in the same subject, which is imperative, based on the large intersubject variability observed with GH release.

Baumann (*1991*) has reported that as many as 100 different molecular variations of GH exist in circulation. Many of these molecular forms of GH have been quantified with traditional immunoassays but may not dimerize the GH receptor (GHR) and thus may produce no downstream physiological effects. Identification of the sequence of amino acids needed for the GH–GHR complex to be formed, allowed Strasburger et al. (*1996*) to develop an immunofunctional (IF) immunoassay that could recognize this sequence, and as a consequence would identify only GH capable of binding and dimerizing the GHR, a step that is critical for cellular transduction of the GH signal (Mellado et al. *1997*). It is suggested that examination of the IF GH profile between the two types of exercise may provide additional information regarding the actions of exercise-induced GH release and provide a framework for future studies to investigate the physiological functions of this hormone in response to exercise.

No known research has examined how the GH response may differ between acute aerobic and resistance exercise in the same individual. Examination of the GH profile, including the IF GH response, may provide new insights into the pattern of GH response to different types of exercise. The purpose of this study was to compare the GH response, between an acute aerobic session and a resistance exercise session, in the same individuals.

## METHODS

### *Subjects*

Ten young, healthy, cross-trained males (Table 1) were recruited to participate in the study. To be defined as cross-trained, subjects were required to be participating in both aerobic (including cycling) and resistance (including squatting) exercise, each for at least 3 days per week for a minimum of 30 min per session, for the past 6 months. The exercise intensity of training sessions was required to be rated a minimum of 15 (i.e., hard) on the Borg rating of perceived exertion (RPE) scale. The majority of aerobic training consisted of continuous, steady state running and cycling, while resistance training consisted of traditional multiple exercise, multiple set, moderate volume bodybuilding type training aimed at whole body hypertrophy/strength development. A more detailed description of the training backgrounds is provided in Table 1. To assure that subjects were adequately cross-trained, inclusion criteria required a maximal oxygen consumption ($VO_{2max}$) of $\geq$40 ml $kg^{-1}$ $min^{-1}$ on the cycle ergometer, and a one-repetition maximum (1-RM) in the bent knee squat exercise of $\geq$1.5-times their body weight, which was verified during the preliminary testing session. The University of North Carolina at Greensboro Institutional Review Board for Human Subjects approved all procedures. All subjects were required to provide written informed consent prior to beginning the study and completed a

medical health questionnaire, which verified that all were non-smokers, were free of endocrine or orthopedic disorders, and were not currently taking anti-inflammatory drugs.

**Table 1** Subject characteristics for cross-trained males ($N$ = 10)

| | **Mean ± SEM** |
|---|---|
| Age (years) | 24.3 ± 1.2 |
| Height (cm) | 180.8 ± 1.8 |
| Body mass (kg) | 89.1 ± 3.5 |
| Percent body fat | 15.1 ± 1.5 |
| Cycle $VO_2$ max (ml $kg^{-1}$ $min^{-1}$) | 44.7 ± 2.4 |
| Aerobic exercise per week (hours) | 2.8 ± 0.7 |
| Cycling exercise per week (h) | 1.2 ± 0.5 |
| Years aerobic exercise training | 4.5 ± 0.7 |
| Dumbbell squat 1-RM (kg) | 152.5 ± 11.3 |
| Resistance exercise per week (h) | 3.8 ± 0.6 |
| Squatting exercise per week (h) | 1.4 ± 0.2 |
| Years resistance exercise training | 5.7 ± 0.9 |

## *Preliminary testing*

### Anthropometric measurements

Skinfold measurements were obtained from seven sites using Harpenden calipers (Creative Health Products, Ann Arbor, MI, USA), as previously described by Jackson and Pollock (*1978*). Body density and percent body fat were then calculated based on the equations described by Jackson and Pollock (*1978*) and Siri (*1961*), respectively.

### Preliminary maximal exercise testing

Maximal oxygen consumption ($VO_{2max}$) was determined for each subject using a graded exercise test on a Lode Excalibur Sport cycle ergometer (Lode B.V. Medical Technology, Groningen, The Netherlands). Oxygen consumption was measured using standard open circuit spirometry (Vmax, Sensormedics, Yorba Linda, CA, USA). Heart rate was determined using a Polar a5 heart rate monitor (Polar Electro Inc, Woodbury, NY, USA). Following a brief warm up period, the workload was initially set at 100 W and increased by 50 W every 2 min until the subject could no longer continue due to fatigue (RPMs dropped below 50), medical concerns, or their heart rate reached the predicted maximum (220-age). The highest mean 1-min $VO_2$ value obtained during testing was used to calculate workload during the submaximal aerobic exercise protocol.

On a separate visit to the Exercise Physiology laboratory, subjects performed a 1-RM test in the bent knee squat exercise using free weights. Subjects were provided instructions on proper performance of this movement, including instruction on the desired depth of the squat, which was standardized for all subjects. Multiple attempts (6–8) were completed with an increasing weight until subjects reached their 1-RM. Three-minute rest intervals were provided between

attempts. The maximum amount of weight that could be lifted at one time, in perfect form (using a 2 s eccentric/concentric tempo), was recorded as the 1-RM, and this was used to calculate workload during the submaximal resistance exercise protocol.

**Submaximal testing sessions**

Within 2 weeks of the maximal assessment, subjects returned to the Exercise Physiology laboratory in the morning (between 7 and 9 a.m.) for submaximal testing following an overnight fast (8 h). They were instructed not to participate in exercise for the 48-h preceding the submaximal protocols. Subjects also completed a food diary for the 3 days prior to each submaximal trial and were instructed to keep their diet similar for both submaximal sessions.

Subjects completed an acute aerobic session (cycling at 70% of $VO_{2max}$ for 30 min on a cycle ergometer) and an acute resistance session (squatting at 70% of 1-RM for 30 min—total time including both work and rest) in random order, separated by 1–2 weeks. While it was not completely possible to control for exercise intensity between these two types of exercise, a similar relative exercise intensity was used for each exercise (i.e., 70% $VO_{2max}$ and 70% 1-RM).

The submaximal cycling protocol consisted of a 6-min warm-up period with a gradual increase in intensity (from 40 to 60% $VO_{2max}$). The workload was then adjusted to an intensity that correlated with 70% $VO_{2max}$, as assessed by the preliminary aerobic assessment, and maintained throughout the 30 min of cycling. Relative intensity was monitored with $VO_2$ values at 5-min intervals and workload was adjusted to maintain 70% $VO_{2max}$.

During the submaximal squatting protocol, subjects were initially requested to perform 5 min of light stationary cycling. After this was completed, the subject performed three warm up squatting sets with free weights (5–6 repetitions, 90–120 second rest, weight equal to 40, 50, and 60% of 1-RM values as assessed during the initial 1-RM test). The weight was then increased to 70% 1-RM, and sets were performed at this weight (or a weight equivalent to 10% less than this weight if subjects could not complete at least 5 repetitions in proper form) for the entire 30-min exercise period. Each repetition was completed to a standardized depth and performed to momentary muscular failure, which allowed for the performance of 5–12 repetitions (mean ± SEM = 7.2 ± 0.5 repetitions per set). Subjects rested for 90 to 120 s between sets and the total time of the session was 30 min, including both work and rest time.

Before each submaximal test, an intravenous catheter was inserted into the antecubital vein. Blood samples (~8 ml) were taken 10 min after initial placement of the catheter and at 10-min intervals throughout the 2-h protocol (30 min pre-exercise, 30 min exercise, 60 min recovery). Due to the pulsatile release of GH, multiple samples were necessary to accurately examine the GH profile. Oxygen consumption, respiratory exchange ratio (RER), heart rate, and RPE were recorded at 5-min intervals throughout the aerobic session. Similarly timed measurements were recorded during the resistance session with the exception of heart rate and RPE, which were recorded at the end of each completed set.

**Diet analysis**

Subjects were requested to maintain their normal diet throughout the study. Daily food records recorded by subjects for the 3 days prior to each submaximal exercise session verified this.

Records were analyzed for total calories, fat, carbohydrate, and protein intake using Diet Analysis Plus software (ESHA Research, Salem, OR, USA).

**GH analyses**

Serum samples were measured in duplicate using two different Diagnostic Systems Laboratory (DSL) Enzyme-Linked Immunosorbent Assays (ELISAs) (Webster, TX, USA). The DSL Human GH (hGH) ELISA is an enzymatically amplified "two-step" sandwich-type immunoassay. Sensitivity of the DSL hGH ELISA assay is 0.03 ng/ml. The intra- and interassay coefficients of variation (CV) for this assay were 4 and 5%, respectively. The results from this traditional GH assay were referred to as "total GH" as it identifies a number of GH isoforms, including IF GH. The DSL Immunofunctional (IF) GH ELISA is based on an enzymatically amplified "two-step" sandwich-type immunoassay, which identifies GH molecules with both GHR binding sites available (Strasburger et al. *1996*). The minimum detection limit for this assay is 0.06 ng/ml. The intra- and interassay CV were 5 and 10%, respectively. The standards for both assays were calibrated against the World Health Organization standard code 88/624. A microplate reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) with dual wavelength absorbance measurements at 450 and 620 nm was used to quantify hormone concentrations from both assays.

Due to the disparity in the amount of actual exercise time completed between the aerobic and resistance exercise (30.0 ± 0 vs 6.2 ± 0.4 min, respectively), GH analyses were adjusted for the amount of work completed per exercise time. GH area under the curve (AUC) was determined using trapezoidal integration.

### *Statistical analyses*

**Exercise performance measures**

The performance and physiological responses to the cycling and squatting exercise sessions (actual excise time, total energy expenditure, total work completed, total $VO_2$, $VO_2$, % $VO_2$ peak, RER, and heart rate) were analyzed via paired *t* tests.

**Dietary variables**

All dietary data (total calories, % fat, % protein and % carbohydrate), for the 3 days preceding the aerobic and the resistance exercise sessions, were analyzed via paired *t* tests.

**GH measurements**

The data for GH area under the curve (AUC), as well as the data for mean baseline and peak GH were analyzed by way of repeated measures ANCOVA. All of these data were analyzed on the natural logarithmic scale. Logarithmic transformations were carried out so that the normality and equal variance assumptions of the ANCOVA model were not violated.

With regard to the ANCOVA model, the set of predictor variables were comprised of two categorical variables (assay type and exercise type) and one continuous variable. The continuous variable indicated the amount of work that the subject performed per minute of exercise when the work was expressed on the natural logarithmic scale. The rational for including this variable in the model was such that we could standardize all of our statistical comparisons to a common

level of exercise intensity (work/min of exercise). Each of the ANCOVA models also included a set of parameters to estimate assay by exercise interaction.

To compare the distribution of the GH response between two different assays, and between the two forms of exercise we constructed a set of linear contrasts of the least-squares means from the ANCOVA. All of the tests associated with the linear contrasts of the least-squares means were two-sided, and we used Bonferroni type I error rate adjustment to maintain an overall type I error rate of 0.05.

Since the data were analyzed on the logarithmic scale, the comparisons of the GH response between the two different assays, and between the two different forms of exercise were expressed as a ratio of the geometric means. The geometric mean is a location parameter similar to the arithmetic mean and median. The geometric mean is simply the antilogarithm of the arithmetic mean computed from the natural logarithmically transformed data. The ratio of geometric means is commonly referred to as the fold change in the response.

The relationship between the measurements of GH via the DSL human GH ELISA and the DSL IF GH ELISA were assessed by linear regression. The coefficient of determination ($R^2$) was used as the measure of linear association.

All of the statistical computations were carried out with the software of the PROC MIXED Procedure of SAS version 9.1 (SAS Institute Inc. Cary, NC, USA).

## RESULTS

### *Exercise session performance*

Table 2 summarizes the mean physiological responses elicited by the acute aerobic and resistance sessions. Despite similar total session duration (30 min), actual time spent exercising was significantly greater during the aerobic session compared to the resistance session (30.0 ± 0.0 min vs 6.2 ± 0.4 min, $P < 0.001$). The aerobic session resulted in significantly greater caloric expenditure (data not shown) and total work than the resistance session ($P < 0.001$). However, after adjusting for the amount of time spent exercising, the work completed per minute of squatting was significantly greater than per minute of cycling (20.9 ± 1.6 kJ $min^{-1}$ vs 11.2 ± 0.4 kJ $min^{-1}$, $P < 0.001$). A similar pattern was observed when caloric expenditure was expressed per minute of resistance and aerobic exercise ($P < 0.001$). RER and RPE were significantly higher during the resistance session than during the aerobic session ($P < 0.001$ and $P = 0.002$, respectively). Average heart rate did not differ between the two exercise sessions ($P = 0.98$).

**Table 2** Performance and physiological responses to cycling and squatting exercise sessions ($N = 10$)

| | Cycling mean ± SEM | Squatting mean ± SEM |
|---|---|---|
| Actual exercise time (min) | 30.0 ± 0.0** | 6.2 ± 0.4 |
| Total work completed (kJ) | 335 ± 11** | 128 ± 10 |
| Total $VO_2$ (l) | 87.5 ± 3.4** | 53.3 ± 2.6 |
| $VO_2$ (ml $kg^{-1}$ $min^{-1}$) | 32.6 ± 1.7** | 20.2 ± 1.3 |

| | Cycling mean ± SEM | Squatting mean ± SEM |
|---|---|---|
| Percentage of $VO_{2max}$ | 73.1 ± 0.5** | 45.2 ± 1.2 |
| Percentage of 1-RM | | 61.8 ± 1.6 |
| Number of repetitions per set | | 7.2 ± 0.5 |
| Number of sets | | 13.1 ± 0.3 |
| Respiratory exchange ratio (RER) | 0.94 ± 0.01** | 1.03 ± 0.01 |
| Heart rate (beats $min^{-1}$) | 160.1 ± 4.8 | 160.2 ± 4.2 |
| RPE | 14.9 ± 0.4* | 17.0 ± 0.4 |

* Significantly different from response during squatting session ($P = 0.002$)
** Significantly different from response during squatting session ($P < 0.001$)

**Diet**

No statistical difference was observed between the mean total calories consumed during the 3 days prior to the resistance and aerobic session (2,802 ± 249 kcal vs 2,887 ± 204 kcal, $P = 0.79$). In addition, % fat, % protein, and % carbohydrate did not differ during the 3 days prior to the resistance and aerobic sessions ($P = 0.34$).

**GH measurements**

The profiles for the exercise-induced total human GH and IF GH response to acute aerobic and resistance exercise are shown in Fig. 1. Similar GH patterns were observed, with GH concentrations peaking at the end of exercise and declining immediately after exercise, regardless of the type of exercise or assay used. Baseline GH concentrations (mean of the 0, 10, and 20 min time points) did not differ between the two exercise sessions.

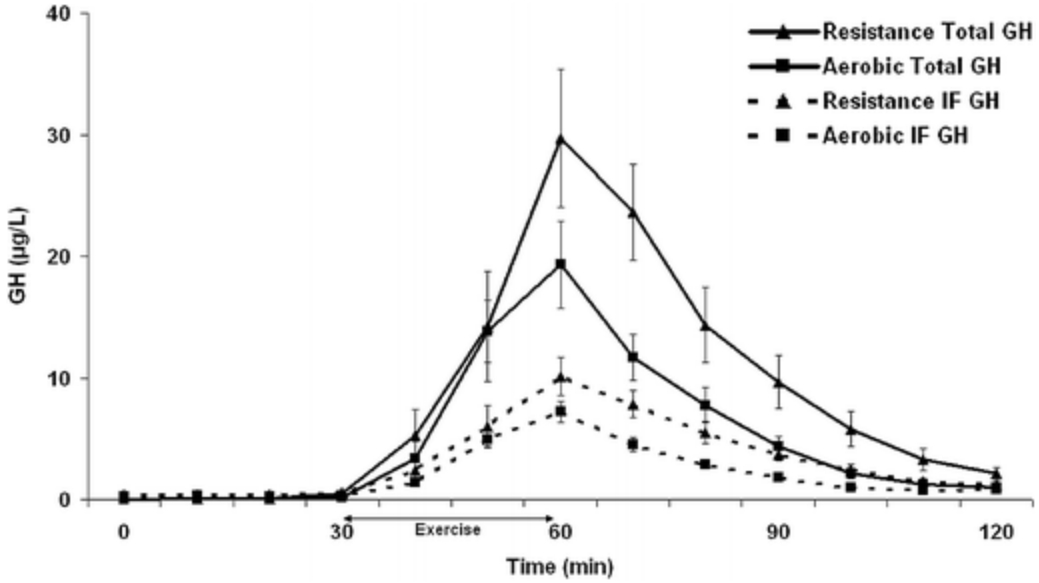


**Fig. 1** Serum human total GH and IF GH measurements during the aerobic and resistance exercise sessions. Values are mean ± SEM

There were obvious differences in the amount of work completed per minute between the resistance and aerobic sessions (20.9 ± 1.6 kJ $min^{-1}$ vs 11.2 ± 0.4 kJ $min^{-1}$, respectively), therefore, exercise-induced GH responses (AUC and peak GH) were adjusted for the amount of work completed per minute for each session. After adjusting for the amount of work completed per minute, significant main effects for assay type and exercise condition were present for integrated GH AUC and peak GH values. As expected, total GH, as measured by the traditional human GH ELISA, was significantly greater than IF GH for both integrated GH AUC ($P = 0.002$) and peak GH measurements ($P < 0.001$). As illustrated in Fig. 2, resistance exercise produced an approximate twofold greater integrated GH AUC response compared to the response during the aerobic session when measured by both the total GH and IF GH assays ($P = 0.008$ and $P = 0.014$, respectively). When analyzing peak GH concentrations, a significant main effect for exercise type was observed with peak GH levels significantly greater during the resistance session than the aerobic session ($P = 0.05$). Despite assay comparisons demonstrating total and IF peak GH levels nearly twofold greater during the resistance exercise session, significance was not reached ($P = 0.065$ and $P = 0.115$, respectively; Fig. 3).

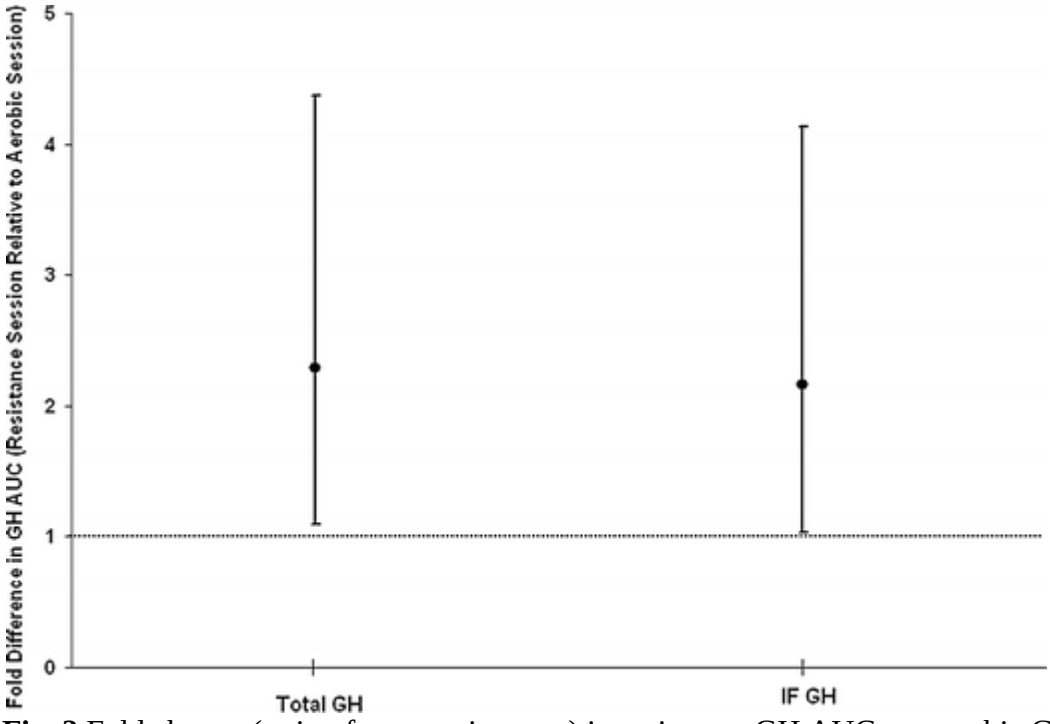


**Fig. 2** Fold change (ratio of geometric mean) in resistance GH AUC vs. aerobic GH AUC for total and IF GH. The values represent fold change (*solid dot*), the Bonferroni-adjusted 95% CIs (*solid line*), and the aerobic session change (onefold) change (*dotted line*)

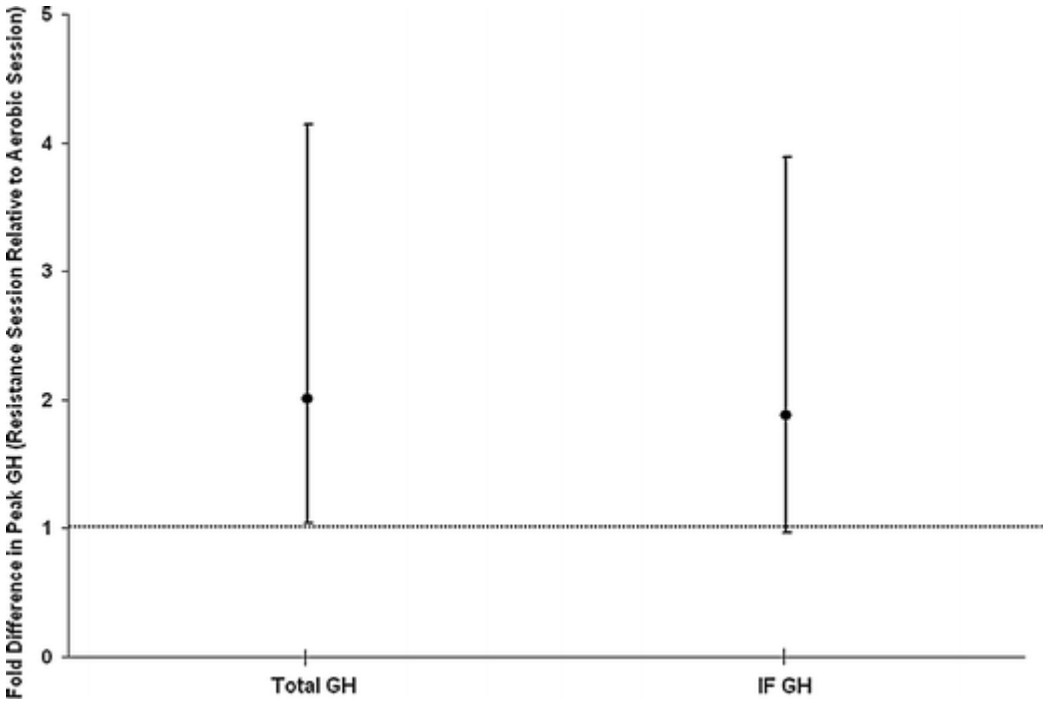


**Fig. 3** Fold change (ratio of geometric mean) in resistance peak GH versus aerobic peak GH for total and IF GH. The values represent fold change (*solid dot*), the Bonferroni-adjusted 95% CIs (*solid line*), and the aerobic session change (onefold) change (*dotted line*)

Time to reach peak GH did not differ between the aerobic and resistance exercise sessions (2 min before exercise cessation and 1 min into recovery, respectively), regardless of the type of assay used. Linear regression analyses revealed a significant association between the two assays during the aerobic session ($r = 0.93$, $P < 0.001$) and resistance session ($r = 0.94$, $P < 0.001$) (Fig. 4a, b, respectively).

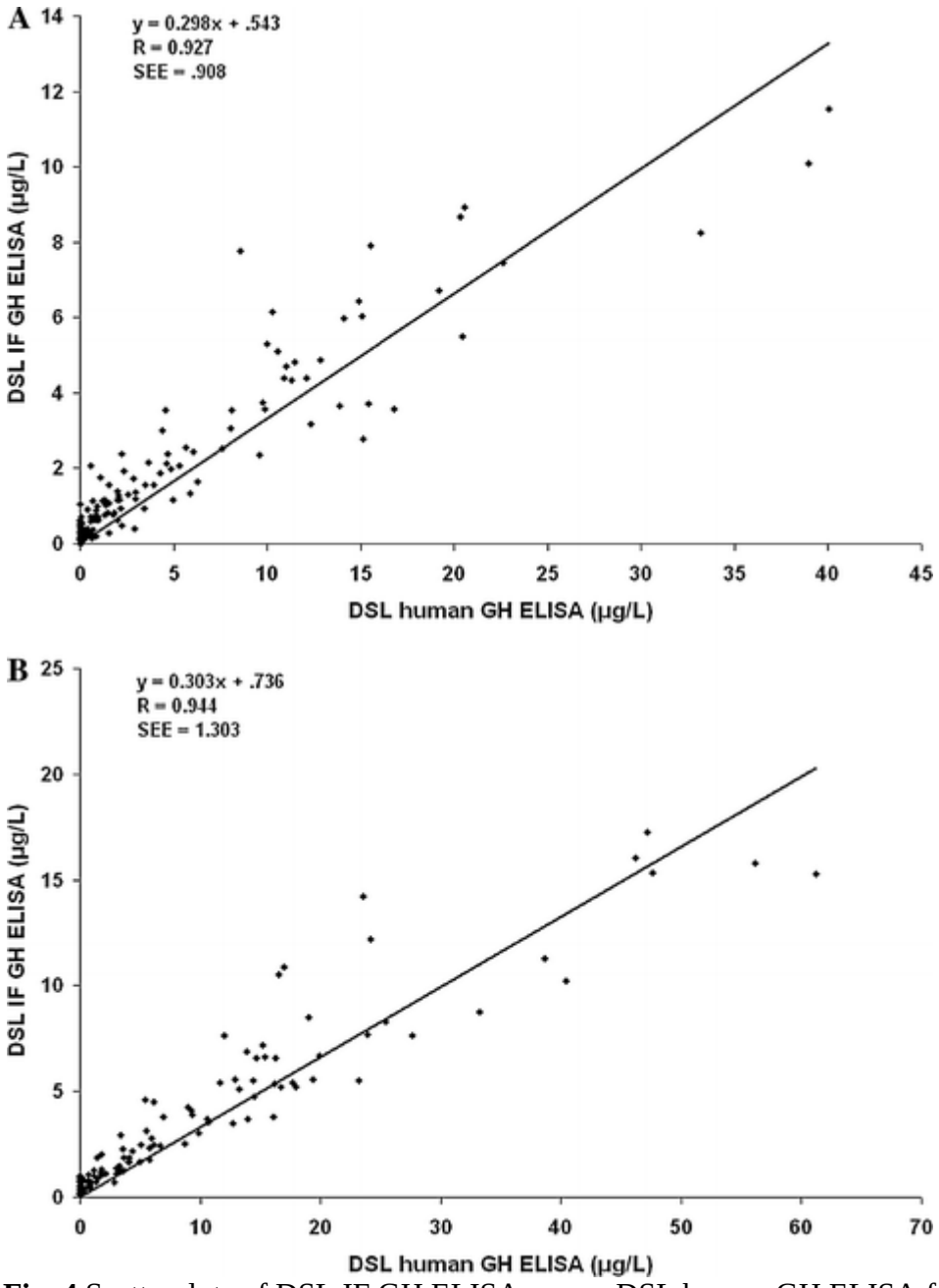


**Fig. 4** Scatterplots of DSL IF GH ELISA versus DSL human GH ELISA for the aerobic session (**a**), and the resistance session (**b**)

## DISCUSSION

The major findings from this study revealed that when adjusting for the amount of work completed per minute within a single individual; (1) acute intermittent resistance exercise demonstrated an approximate twofold greater GH AUC and peak GH response than acute continuous aerobic exercise; and (2) the exercise-induced pattern of circulating GH was similar for both types of exercise, and was independent of the assay used.

Despite extensive research investigating the GH response to aerobic and resistance exercise, the findings of the present study are significant because to our knowledge they are the first to compare these two types of exercise in the same individual. In a recent review by Wideman et al. (*2002*) it was stated that although the magnitude of GH release appeared to be similar between these two types of exercise, a direct comparison could not be made unless studied in the same individual since a large interindividual variability occurs in GH release. Moreover, while it is acknowledged that controlling all exercise variables is impossible when comparing an aerobic and resistance exercise session, this study is also the first to our knowledge that has examined the two types of exercise after adjusting for the amount of work completed per exercise time. In addition, it should be noted that typical exercise sessions were utilized for both types of exercise and that relative exercise intensity and session duration were matched between the two types of exercise.

The results from the present study suggest that within a single, cross-trained male, the amount of absolute work completed during an exercise session may not be a key predictor of exercise-induced GH release. Early results by VanHelder et al. (*1984*) reported a greater GH response during intermittent anaerobic exercise compared to continuous aerobic exercise, despite similar total work completed. Unfortunately, only a single post-exercise blood sample was used to measure GH; therefore, interpretation of the pattern of GH release was not possible. The results from the present study are significant in that the GH response was greater during the intermittent resistance exercise session, despite the fact that more than twice as much total work was completed during the aerobic session (128 ± 10 kJ vs 335 ± 11 kJ, respectively). Despite a lack of statistical significance, a trend was evident for the amount of work completed per exercise time to predict GH AUC. While both exercise intensity (Pritzlaff-Roy et al. *2002*) and duration (Wideman et al. *2006*) have been shown to influence GH release, data from the present study indicate that total work completed may not be a factor in predicting the GH response to exercise when comparing different modes of exercise in a given individual. In this case, exercise intensity or the amount of work completed per unit time (i.e., rate of energy produced) may be a better indicator of the exercise-induced GH response.

A number of metabolic factors may have contributed to the greater GH response during the intermittent resistance exercise. Earlier research has suggested that the increase in catecholamines observed during moderate exercise may be involved in the exercise-induced release of GH (Weltman et al. *2000*). Though catecholamines were not measured in the present study, previous research suggests that a greater catecholamine response may have occurred during our resistance protocol, given the anaerobic nature of this activity (Kraemer et al. *1999*). High intensity anaerobic exercise, including resistance exercise, is known to create an environment of oxygen deficit, as well as stimulate lactate production (Rubin et al. *2005*) and hydrogen ion accumulation (Gordon et al. *1994*). While controversial, each of these factors has been suggested to play a possible role in stimulating GH release during exercise (Gordon et al. *1994*; Lassarre et al. *1974*; Rubin et al. *2005*). Although these factors were not measured in the current study, the greater RER value, and the intermittent, anaerobic nature of our resistance exercise suggest that these metabolic factors were likely greater during this session, and therefore, may have influenced the magnitude of the GH response.

The role of greater neuromuscular recruitment during resistance exercise cannot be overlooked as a factor influencing GH release. In addition to the use of leg muscles for squatting, arm muscles were recruited to hold the free weights and trunk muscles were recruited for postural stabilization. Neuromuscular activity has long been suggested as a means of augmenting GH release and has been effective in increasing bioassayable GH (McCall et al. *1997*). Recruitment of small muscle groups (i.e., arm muscles) has been particularly successful in increasing GH release (Kozlowski et al. *1983*). It is possible that during the resistance exercise session "metabolic receptors" in the smaller arm muscles were more sensitive to changes in the aforementioned local metabolic factors and produced a greater peripheral neural afferent signal, and either increased GH directly, or indirectly through the activation of the sympathetic-adrenergic system as discussed previously. It should also be noted that increased neuromuscular recruitment would have occurred due to any mechanical inefficiency in the lifting procedure. In an attempt to limit this factor, all subjects were regular resistance exercisers and had a history of performing squatting exercises. The fact that mechanical efficiency may play a role, suggests that generalization of the results from the present study to other populations, should be made with caution.

Based on the force production necessary to complete resistance exercise, it is probable that a greater recruitment of type II motor units occurred during this type of exercise. Thus, it is conceivable that this produced a greater stimulatory feedback to the pituitary, contributing to a greater GH release during resistance exercise. Unfortunately, due to the difficulties in measuring motor unit recruitment in this setting, a direct relationship between type II motor unit recruitment and GH release is only speculative at this time. Previous research examining concentric and eccentric contractions has supported the role of motor unit recruitment in the release of GH (Durand et al. *2003*). Using the same absolute load, concentric contractions (which require greater motor unit recruitment), produced a greater GH response than eccentric contractions (Durand et al. *2003*). What remains to be elucidated is, whether increased motor unit recruitment is directly involved in GH release or whether it occurs indirectly through the metabolic stressors produced during their recruitment (i.e., lactate, hydrogen ions).

In an attempt to ascertain the events leading to GH post-receptor signaling, and determine if these initial events differ between exercise types, the present study also used an IF GH assay designed to identify only GH capable of binding to the GHR. It is clear from the present study, that the exercise-induced increase in GH included biologically active GH, as measured by the IF assay, regardless of the type of exercise completed. The finding that IF GH increases in response to exercise provides support for the idea that exercise-induced GH may have a biological impact and supports continued research in this area.

Based on the multiplicity of GH molecules in circulation, it is difficult to directly link GH identified by the IF GH assay to intracellular events at the post-receptor level. More than 100 different GH variants have been identified in circulation, including numerous monomeric and oligomeric moieties, as well as, fragmented forms of GH (Baumann *1991*). To date, the physiological implications of these different GH isoforms remain elusive, but it is conceivable that some isoforms may interfere with GH-GHR binding and as a result inhibit intracellular signaling. GH circulates both free and bound to one of two binding proteins (GHBPs). The circulating high affinity GHBP corresponds to the extracellular portion of the GHR and inhibits

the binding of biologically active GH to the GHR by competing with the receptor for the ligand (Baumann *2002*). Therefore, there are a number of factors that may impede the binding of biological active GH to its receptor which is not accounted for by simply measuring the availability of IF GH. Nevertheless, it is felt that examination of the IF GH response to exercise provides evidence that bioactive forms of GH are increased in circulation and would provide the critical first step in signal transduction.

Previous research demonstrated increased biologically active GH, as measured with the rat tibial line assay, in response to acute isometric exercise (McCall et al. *1997*) but not with an acute heavy resistance protocol (Hymer et al. *2001*; Kraemer et al. *2006b*). These preliminary findings supported the hypothesis that different types of exercise might stimulate the release of different GH variants.

The IF GH ELISA has been found to be highly correlated with the results obtained from the Nb2 cell bioassay (Strasburger et al. *1996*), and is more sensitive, less expensive, and more practical for the use of repeated GH measurements. Preliminary research demonstrated that acute exercise was responsible for an increase in IF GH (Hymer et al. *2001*; Nindl et al. *2000*; Rubin et al. *2003*; Wallace et al. *2001*). However, prior to the research reported here, it was unclear whether the IF GH response differed in magnitude or profile between acute aerobic and resistance exercise.

In the present study, the human GH ELISA and the IF GH assay produced the same response pattern for both exercise sessions. In addition, both assays resulted in a greater GH AUC and peak GH values during the resistance exercise session. The significance of a greater IF GH response during resistance exercise at the level of the tissue remains unknown. For example, it is not clear, whether a linear relationship exists between the magnitude of the GH concentration measured in the blood and the degree of physiological response at the levels of the tissue. Furthermore, while the present study suggests that the pattern of IF GH is similar between the two types of exercise, the current study was not designed to determine whether the IF GH-GHR binding and GH signal transduction were similar between the two types of exercise.

Similar to other studies (Nindl et al. *2000*, *2001*), our study found a strong correlation between IF GH and standard total GH in response to exercise. As has been previously reported, the IF GH ELISA mirrored the standard GH ELISA results but was approximately half that of the GH response identified by the conventional assay (Nindl et al. *2000*). The results from the conventional GH assay were referred to as "total GH" based on the multiple GH moieties that the assay likely identifies; however, it is probable that this assay does not identify all GH isoforms. Therefore, it is important to mention that the results from the present study are limited to the epitope specificity of the antibodies used.

Exactly which GH isoforms are being identified by conventional GH assays such as the human GH ELISA, and not by the IF GH assay, remains unclear. One theory is that antibodies used in conventional assays may identify GH bound to GHBP and this interferes with the quantification of GH (Chapman et al. *1994*). In contrast, it has been previously demonstrated that endogenous human GHBP (within a physiological range of $\leq$2,000 pmol/l) had no apparent interference with the IF GH assay (Strasburger et al. *1996*). On average, 50% of circulating GH is complexed to

endogenous GHBP (Baumann et al. *1988*), which is consistent with the percentage of GH to IF GH observed in this and other studies (Nindl et al. *2000*, *2001*).

Also of interest in the present study was the surprising finding that under resting conditions, many of our samples showed higher GH values with the IF GH assay than the human GH ELISA. Previously, Baumann et al. (*1985*) demonstrated that under basal conditions many fragmented forms of GH exist. Specifically, Wallace et al. (*2001*) reported that under resting conditions, individuals demonstrated a high variability of GH fragments (i.e., 30-, 16-, and 12 kDa). It is possible that based on the physical composition of these fragments and the epitope specificity of the antibodies used in the assay, these fragments are identified by the IF GH ELISA but not the human GH ELISA. Although purely speculative at this time, it is possible that these GH fragments, as well as GH molecules not recognized by any GH assay, could affect GH-related events at the site of the receptor, either by enhancing or inhibiting GH binding (Rowlinson et al. *1996*). Clearly, more research is needed to identify the role of these GH fragments.

In summary, the results from the present study support previous research showing an increase in GH with acute aerobic and resistance exercise. However, this is the first known study to use a repeated sampling regimen in the same subjects to compare the two types of exercise. The results from this study indicate that intermittent resistance exercise is capable of eliciting a greater response of GH, including IF GH, in young, cross-trained men, compared to a continuous aerobic session, when adjusting for the amount of work completed per minute and controlling for relative exercise intensity and session duration. What remains to be determined is the physiological importance of the difference in the magnitude of the response to aerobic versus resistance exercise and whether or not it translates to different cell signals and biological end points.

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