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14. ABSTRACT

To test the hypothesis that the appearance of disulfide-linked growth hormone (GH) aggregates during and after an acute resistance exercise test (ARET) in men could be influenced by chronic physical training. Fourteen men (28 ± 1 yr) underwent two different 8-wk physical training programs designed to improve military performance. Before and after chronic training, subjects performed an ARET (six sets of 10 repetition-maximum squat) and had venous blood drawn pre-, mid-, and post-ARET (o, 15, and 30 min. post exercise). To determine whether GH molecules were disulfide-linked, serum samples were chemically reduced via glutathione (GSH). Serum immunoreactie GH (IRGH) and immunofunctional GH (IFGH) concentrations were determined using two specific immunoassays, in nonreduced (-GSH) and reduced (+GSH) states. Data were analyzed using repeated-measures ANOVA. Results: No differences were observed in the GH responses of the two training programs; therefore, training group data were combined for analysis.

15. SUBJECT TERMS

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Effects of Acute and Chronic Exercise on Disulfide-Linked Growth Hormone Variants

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ABSTRACT

PIERCE, J. R., A. P. TUCKOW, J. A. ALEMANY, K. R. RARICK, J. S. STAAB, E. A. HARMAN, and B. C. NINDL. Effects of Acute and Chronic Exercise on Disulfide-Linked Growth Hormone Variants. *Med. Sci. Sports Exerc.*, Vol. 41, No. 3, pp. 581–587, 2009. **Purpose**: To test the hypothesis that the appearance of disulfide-linked growth hormone (GH) aggregates during and after an acute resistance exercise test (ARET) in men could be influenced by chronic physical training. **Methods**: Fourteen men (28 ± 1 yr) underwent two different 8-wk physical training programs designed to improve military performance. Before and after chronic training, subjects performed an ARET (six sets of 10 repetition-maximum squat) and had venous blood drawn pre-, mid-, and post-ARET (0, 15, and 30 min postexercise). To determine whether GH molecules were disulfide-linked, serum samples were chemically reduced *via* glutathione (GSH). Serum immunoreactive GH (IRGH) and immunofunctional GH (IFGH) concentrations were determined using two specific immunoassays, in nonreduced (−GSH) and reduced (+GSH) states. Data were analyzed using repeated-measures ANOVA. **Results**: No differences were observed in the GH responses of the two training programs; therefore, training group data were combined for analysis. GSH reduction increased the mean GH signal (−GSH: 1.4 ± 0.3 $\mu g \cdot L^{-1}$ vs +GSH: 1.7 ± 0.3 $\mu g \cdot L^{-1}$; $P < 0.01$) only when quantifying IRGH. *Post hoc* testing indicated that serum contained IRGH disulfide-linked GH aggregates at the mid, 0-, 15-, and 30-min posttime points of the ARET ($P < 0.01$), whereas GSH reduction did not affect IFGH concentrations. Chronic physical training had no effect on the ARET-induced GH response. **Conclusion**: Acute resistance exercise leads to the appearance of disulfide-linked IRGH aggregates, and this response does not appear to be affected by 8 wk of chronic physical training. The physiological significance of increased proportions of disulfide-linked GH aggregates postexercise remains uncertain; however, structural alterations in GH moieties after acute exercise may represent important regulatory steps in mediating GH biological activity at selected target tissues. **Key Words:** SOMATOTROPIN, GLUTATHIONE REDUCTION, RECEPTOR DIMERIZATION, GH DIMERS

Since the early observation of Hunter et al. (11) in 1965, numerous studies have demonstrated that exercise of sufficient intensity and duration elicits increases in circulating growth hormone (GH) concentrations (7,9,15,25). However, the majority of these studies have largely ignored the two most important characteristics of GH physiology: its pulsatile release pattern and its molecular heterogeneity. Utilizing deconvolution analysis, the importance of the circadian rhythm underlying GH release at rest and how it can be modulated by acute exercise have been adequately characterized (14,21,22,27,30,31). Although the pulsatile nature of GH may exert strong influences on eventual cellular adaptations, it is also evident that the structure of the molecule drives its ultimate function at the target tissue (26). For instance, size exclusion chromatography (12) or exclusion assays (28) have been used to demonstrate that exercise leads to the appearance of different GH isoforms into the circulation. After chronic exercise training, these different GH isoforms may offer a partial explanation for the disparate phenotypic outcomes as a result of different modes of chronic exercise training (20).

In addition to alternative mRNA splicing of the GH-N gene leading to different isoforms (e.g., 20-kDa GH), other molecular weight variants include dimeric and oligomeric complexes, such as GH bound to GH-binding protein (GHBP) or GH molecules bound to one another *via* either covalent or noncovalent bonds (1). Because binding of GH to its receptor requires free molecules with two intact binding sites, GH bound to GHBP would not be available for receptor binding; however, the potential for receptor dimerization is less evident for GH molecules bound to one another by disulfide bonds. Investigations by Rubin et al. (23) and Hymer et al. (12) have established that when compared with resting serum samples, chemically reducing serum samples with glutathione (GSH) during and after acute exercise leads to a preferential appearance of disulfide-linked GH molecules. More recently, investigations have demonstrated that

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Acute exercise test. Both before and after the 8-wk physical training programs, subjects performed an acute resistance exercise test (ARET). This ARET was comprised of six sets of the individual's 10 repetition maximum (10-RM) squat, separated by 2-min interset rest periods (12), and chosen due to the previous success in subject tolerance and the ability to perturb the hormonal milieu. The initial 10-RM weight was approximated as 75% of the subject's one repetition maximum (1-RM) measured during preexperimental testing sessions. Strong verbal encouragement was provided to the subjects so as to have them complete a full set of 10 squats with good form. If a subject could not successfully perform 10 repetitions, the weight was readjusted to facilitate completion of 10 full range of motion repetitions.

Blood sampling and handling. Before the initiation of each ARET, a venous catheter was inserted in a forearm vein with a saline lock to maintain catheter patency. Subjects had venous blood drawn before (pre), after three sets (mid), and immediately after (post) as well as 15- and 30-min after (+15 and +30, respectively) of the ARET. Blood samples were allowed to clot at room temperature and then centrifuged at 3000 RPM for 20 min at 4°C. After separation, serum aliquots were frozen and stored at −80°C until chemical or water treatment and subsequent assay work were performed.

Chemical reduction of serum samples. At each time point, blood was sampled relative to the ARET (see above); the frozen serum sample was thawed and separated into two 225-μL aliquots. One of the aliquots was combined with 25 μL reconstituted glutathione (GSH) (Sigma-Aldrich, St. Louis, MO) at a final concentration of 10 mM (+GSH), whereas the other aliquot was combined with 25 μL deionized H_2O serving as the nonreduced internal control (−GSH). After the addition of GSH or deionized H_2O, the samples were incubated at room temperature for 18 h (12), and GH concentrations subsequently determined in their respective assays.

Assays. IRGH concentrations were determined on a commercially available two-site immunoradiometric (IRMA) assay from Diagnostic System Laboratories (DSL, Webster, TX). Briefly, this noncompetitive assay used polypropylene tubes coated with an immobilized antibody specific for the GH molecule. After introduction of serum samples in either nonreduced (−GSH) or reduced (+GSH) states to the coated tubes, a second GH-specific antibody radiolabeled with I^{125} was added. According to the manufacturer, only GH molecules that are bound to both antibodies are detected in the assay. Inter- and intraassay coefficients of variation (CV) for the IRGH IRMA measured in our laboratory were below 8.0%.

The work from Strasburger et al. (24) lead to the development of DSL IFGH assay. This assay was an enzymatically amplified "two-step" ELISA sandwich-type assay, which used a specific monoclonal antibody and a biotinylated recombinant GHBP targeted to GHR-binding sites 2 and 1, respectively. Therefore, only GH molecules that are capable of dimerizing the GHR are translated into an assay signal. Inter- and intraassay CV for the IFGH ELISA measured in our laboratory were below 8.5%. Both GH assays were calibrated to the World Health Organization International Reference Reagent for hGH (code 88/624).

Data analysis. Data were analyzed separately with regards to the assay used (e.g., IR vs IF) using a multifactorial [5 (ARET time points) × 2 (chemical reduction) × 2 (chronic training)] ANOVA with repeated measures. All values are expressed as mean ± SE (n = 14), and significance was set at $P \leq 0.05$. When the ANOVA detected a significant F-ratio, *post hoc* analysis (least significant difference) was used to determine statistical differences for within-subject factors. All statistical analyses were performed on SPSS, version 15.0 (SPSS Inc., Chicago, IL).

RESULTS

Because the main outcome of this investigation was to examine the GH response to both acute and chronic physical training and GH concentrations measured before, during, and after the ARET were not statistically different between the two 8-wk exercise training programs (Army Standardized Physical Training and experimental Army training program), data from the two physical training groups were combined for all analyses. Additionally, for both IRGH and IFGH assay data, the original subject number of n = 17 was reduced to n = 14 for data analysis, as three subjects who completed the study consistently fell below the manufacturer's reported sensitivity values in both IRGH (0.01 $\mu g \cdot L^{-1}$) and IFGH (0.06 $\mu g \cdot L^{-1}$) assays.

Body composition, strength, and performance outcomes. As a result of the 8-wk physical training, total body mass did not change significantly (pretraining: 82.8 ± 3.2; posttraining: 82.7 ± 3.2 kg; $P > 0.05$); however, accounting for this nonsignificant change, lean body mass increased (pretraining: 63.8 ± 2.1 kg vs posttraining: 65.6 ± 2.1 kg; $P < 0.01$) and fat mass decreased (pretraining: 19.1 ± 2.4 kg vs posttraining: 17.1 ± 2.5 kg; $P < 0.01$) after the training regimens. As with the GH response to acute exercise, these differences were not dependent on the mode of chronic training (10).

In addition, several measures indicative of performance improvement occurred as a result of 8 wk of chronic physical training. Moreover, these measures were not dependent on the mode of exercise in which the subjects participated (10). For instance, as a result of the training, $\dot{V}O_{2max}$ increased 11.8% from baseline (pretraining: 48.4 ± 1.6 $mL \cdot kg^{-1} \cdot min^{-1}$ vs posttraining: 54.1 ± 1.4 $mL \cdot kg^{-1} \cdot min^{-1}$; $P < 0.01$). In addition, measures of both upper and lower body strength increased after 8 wk of chronic training. Specifically, the subjects' 1-RM bench press increased by 11.1% (pretraining: 74.6 ± 3.8 kg vs posttraining: 82.9 ± 3.4 kg; $P < 0.01$), and their 1-RM squat

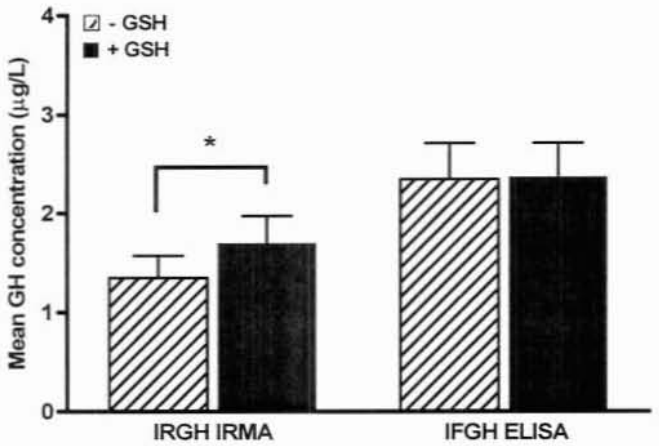

FIGURE 1—Effects of GSH reduction on mean IRGH and IFGH concentrations. Note that the GSH reduction treatment significantly increased the signal only in the IRGH assay. IRGH, immunoreactive GH; IFGH, immunofunctional GH; GSH, glutathione. *$P \leq 0.05$ versus non-reduced concentration. Values are presented as means ± SE; $n = 14$.

strength increased by 18.0% (pretraining: 86.6 ± 4.1 kg vs posttraining: 102.2 ± 4.2 kg; $P < 0.01$).

IRGH. Before the ARET, mean GH concentrations were 0.05 ± 0.03 $\mu g \cdot L^{-1}$ and increased significantly ($P < 0.05$) throughout the ARET to a peak of 2.8 ± 0.6 $\mu g \cdot L^{-1}$ at the post-ARET time point. After the termination of the acute exercise bout, GH concentrations declined but remained above baseline concentrations at least for 30 min into recovery (1.4 ± 0.3 $\mu g \cdot L^{-1}$; $P < 0.05$). After reducing the samples with 10-mM GSH, the mean IRGH concentration increased from 1.3 ± 0.2 to 1.7 ± 0.3 $\mu g \cdot L^{-1}$ (significant main effect of GSH reduction; $P < 0.01$), representing a 26% increase (Fig. 1). Upon further inspection of the ARET response, *post hoc* testing revealed that the +GSH treatment increased mean IRGH concentrations over –GSH treatment ($P < 0.01$) at mid-ARET (1.3 ± 0.4 vs 1.1 ± 0.3 $\mu g \cdot L^{-1}$), post-ARET (3.0 ± 0.6 vs 2.5 ± 0.6 $\mu g \cdot L^{-1}$), +15-min (2.5 ± 0.4 vs 1.9 ± 0.3 $\mu g \cdot L^{-1}$), and +30 min (1.6 ± 0.4 vs 1.2 ± 0.3 $\mu g \cdot L^{-1}$), indicating that disulfide-linked IRGH molecules were present for at least 30 min after acute resistance exercise (Fig. 2). Furthermore, the IRGH AUC analysis revealed that the total output of IRGH during the AET increased as a result of chemical reduction (−GSH: 68.7 ± 11.4 $\mu g \cdot L^{-1} \cdot min^{-1}$ vs +GSH: 86.7 ± 14.0 $\mu g \cdot L^{-1} \cdot min^{-1}$; $P < 0.01$). As a result of 8 wk of chronic physical training, there were no statistically significant effects observed on the mean IRGH response (pretraining: 2.0 ± 0.5 $\mu g \cdot L^{-1}$ vs posttraining: 1.1 ± 0.2 $\mu g \cdot L^{-1}$; $P = 0.21$), the IRGH AUC response (pretraining: 97.3 ± 27.0 $\mu g \cdot L^{-1} \cdot min^{-1}$ vs posttraining: 58.0 ± 12.1 $\mu g \cdot L^{-1} \cdot min^{-1}$; $P = 0.26$), or the IRGH AUC response after chemical reduction ($P = 0.30$) (Fig. 3).

IFGH. In addition to IRGH, an acute bout of resistance exercise also lead to the appearance of molecules capable of GHR dimerization (molecules containing both receptor sites). In a pattern similar to IRGH, *post hoc* analysis revealed that the mean IFGH concentration significantly increased ($P < 0.01$) throughout the ARET to a peak at immediately postexercise (pre-ARET: 0.2 ± 0.1 $\mu g \cdot L^{-1}$; mid-ARET: 1.9 ± 0.5 $\mu g \cdot L^{-1}$; and post-ARET: 3.8 ± 0.7 $\mu g \cdot L^{-1}$). In the postexercise recovery period, IFGH concentrations began to return toward preexercise values but remained above baseline values ($P < 0.01$) 30 min into recovery.

Unlike the IRGH concentrations, chemical reduction with GSH did not increase IFGH concentrations as measured in the IF ELISA. Figure 1 illustrates that when GSH was added to the serum samples, no significant differences were noted in the mean IFGH concentration (−GSH: 2.3 ± 0.4 vs +GSH: 2.4 ± 0.3 $\mu g \cdot L^{-1}$; $P = 0.62$). However, similar to IRGH concentrations, no significant effects were observed in the IFGH response after chronic physical training (pretraining: 2.7 ± 0.6 $\mu g \cdot L^{-1}$ vs posttraining: 2.0 ± 0.4 $\mu g \cdot L^{-1}$; $P = 0.30$). Furthermore, 8 wk of chronic physical training had no significant effect on the mean IFGH AUC (pretraining: 128.2 ± 28.0 $\mu g \cdot L^{-1} \cdot min^{-1}$ vs posttraining: 102.5 ± 22.4 $\mu g \cdot L^{-1} \cdot min^{-1}$; $P = 0.49$) or the percent increase in IFGH AUC after chemical reduction ($P = 0.96$) (Fig. 3).

DISCUSSION

This study evaluated the effects of 8 wk of chronic physical training on concentrations of IR and IF disulfide-linked GH aggregates during and after an ARET. As GH exhibits a great deal of molecular heterogeneity, which is thought to possibly influence biological activity at the target tissue (26,28), it is important to study whether exercise can induce alterations in the structural composition of the GH molecule, thereby potentially modulating GH action. The current investigation used two novel methodologies in an attempt to glean more information on the impact that acute exercise and chronic physical training exert on GH physiology: 1) chemical reduction *via* GSH to examine

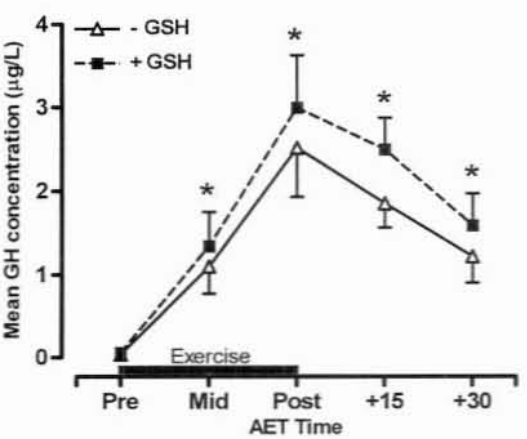

FIGURE 2—After chemical reduction of serum samples with GSH, IRGH concentrations were augmented at Mid, Post, +15, and +30 min of the ARET, suggesting that disulfide-linked IRGH aggregates were present at these time points. *$P \leq 0.05$ versus –GSH condition. Values are presented as means ± SE; $n = 14$.

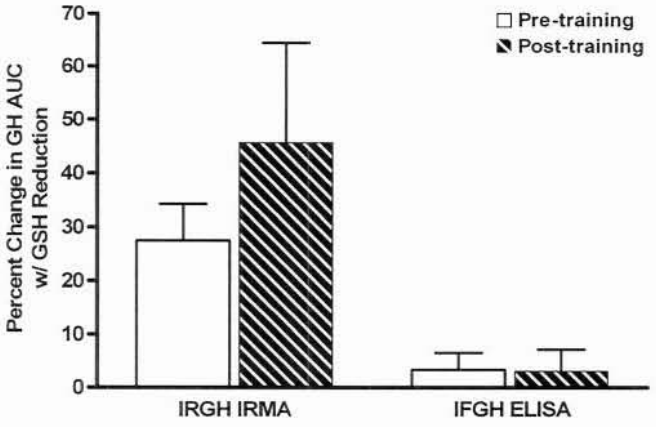

FIGURE 3—Eight weeks of chronic physical training had no significant effect on the disulfide-linked GH AUC response (percent increase in GH AUC with GSH reduction) to acute resistance exercise for either IRGH ($P = 0.30$) or IFGH ($P = 0.96$). Values are presented as means ± SE; $n = 14$.

disulfide-linked GH aggregates and 2) comparison of a conventional IR assay to an IF assay that detects GH molecules based on the interaction between the molecule and its receptor that is required before cell signal transduction. Our findings demonstrate 1) that disulfide-linked aggregates of IRGH are present during and after acute resistance exercise and persist for at least 30 min into the recovery period, 2) that this response pattern is not altered by chronic physical training, and 3) that chemical reduction did not increase the concentration of IFGH, suggesting that these molecules are not linked *via* disulfide bonds.

In contrast to the study by Rubin et al. (23), which demonstrated that disulfide-linked GH aggregates were present during but not after acute aerobic exercise in men, we report that disulfide-linked GH molecules remain present during and after acute resistance exercise into the recovery period in men. Hymer et al. (12) also reported that disulfide-linked GH aggregates are present immediately after acute resistance exercise in women; however, plasma GH concentrations were only measured pre- and immediately post-exercise in their investigation. An explanation for this disparate response could be the modes of exercise used: incremental aerobic exercise used by Rubin et al. (23) versus resistance exercise with constant relative workload used by Hymer et al. (12) and the current investigation. Although the reason for a disproportionate GH response is not apparent, it may be that aerobic and resistance exercise exert a disparate release pattern for GH variants of altered biological half-lives (28) that may modify downstream GH function (lipolytic vs somatogenic actions) (18).

As a consequence of the 8 wk of chronic training, measures indicative of positive changes in performance and body composition were observed; however, there were no differences observed between the two training groups (10). In accordance with GH's metabolic properties, as well as its potential for altering body composition due to exercise training, we also observed no differences in the GH responses to acute exercise between the two exercise training programs. A possible explanation for this finding is that the two different exercise programs both involved whole-body exercise, gradually progressed throughout the training, and also shared in some overlap in terms of running and sprinting exercises (10). Therefore, given the time constraint of 8 wk, we were not able to distinguish any statistical differences between the two exercise training groups. Perhaps, if the study were of longer duration, we may have observed more discernable changes in performance and body composition, both of which might be explained by differences in the hormonal milieu.

In contrast to our hypothesis, we did not observe a significant decrease in the exercise-induced GH response (using the same relative workload) after 8 wk of training. However, a previous investigation by Weltman et al. (29) demonstrated that the exercise-induced GH response is diminished after 6 wk of chronic aerobic training when exercising at the same absolute workload. In agreement with our study, Kraemer et al. (16) showed no difference in GH immunoreactivity after 24 wk of chronic resistance training in women. More importantly, they demonstrated that GH bioactivity increased among all the fractions examined. Therefore, despite the current apparent decrease or the previous significant decrease (29) in the exercise-induced GH response after chronic exercise, the GH released into the circulation may be of higher bioactivity after chronic training. If the GH response to acute exercise is reduced after chronic exercise training, then altering the binding potential of the molecules released could be an important regulatory step in mediating GH biological activity at selected target tissues.

The importance of disulfide-linked GH molecules has not yet been fully elucidated; however, there are some interesting speculations about this phenomenon. One potential reason for the formation could be to extend the biological half-life of the GH molecule (2,28), whereas another plausible reason for linking molecules together may be to create functional molecules from potentially nonfunctional molecules (e.g., antagonists) (17,32). As demonstrated by Langerheim et al. (17), substituting the Gly residue for an Arg on the GH molecule (hGH-G120R) results in a mutated molecule with only binding site 1 intact (site 2 is rendered nonfunctional). The mutated GH molecules were then linked to form homodimeric complexes and had their functionality evaluated *via* the Nb2 cell bioassay. Interestingly, the dimeric complexes with each constituent of the complex having only an intact site 1 due to the mutation were capable of inducing *in vitro* cell proliferation (17). Subsequent investigations also demonstrated that G120R dimeric complexes also lead to JAK2 phosphorylation and downstream STAT5 activation in a human fibrosarcoma cell line (C14), albeit with less activity than intact GH molecules containing both binding sites (32). Relating this to the current study, if the IRGH molecules detected only had binding site 1, it is possible that they could possibly

interact with the IRMA immobilized antibodies but not interact and translate into an assay signal in the IFGH ELISA (requiring both binding sites). Thus, IRGH may not be considered bioactive by standards of the IFGH ELISA, but if the IRGH molecules were disulfide-linked in response to acute exercise as indicated presently, dimeric GH may still induce signal transduction with the GHR by having only two site 1 available for binding (17,32). Although not statistically significant, the percent change in IRGH AUC with GSH reduction during acute exercise appeared higher after chronic exercise training, suggesting that a higher percentage of IRGH was disulfide-linked after 8 wk of exercise. Bioactive molecules as measured by the IFGH assay already have two intact binding sites required for receptor dimerization and would not have to be disulfide-linked to induce signal transduction at the target cell (demonstrated by our inability to observe increased IFGH concentrations with chemical reduction in response to acute and chronic exercise). Therefore, it is plausible that aggregation of GH molecules, due to acute and chronic exercise, is one mechanism to enhance biological activity of IRGH molecules, which only contain one binding site and otherwise might not be able to initiate cellular signal transduction.

Our findings indicate that in addition to an IRGH molecule only, acute exercise also leads to the release of IFGH, which is in agreement with previous investigations (4,12,19,23,27). However, in contrast to the existing studies that have examined IFGH responses to acute exercise and have consistently observed that the IF concentrations were about 50% of the IRGH concentrations (4,19,27), we observed that the GH concentrations measured by the IF assay were higher than the IR concentrations. It is important to note that we used a different assay for measurement of IRGH (DSL IRMA) than the previous studies (DSL hGH ELISA (4) or Nichols IRMA (19,27)). Although our observation that IFGH concentrations were higher than IRGH concentrations cannot be fully explained and differs from previous reports, this finding was confirmed by independent laboratory analysis (unpublished observations). Consitt et al. (4) also reported that in resting conditions, IFGH concentrations were higher than IRGH at rest, lending the to the notion that the IFGH assay may detect segments and fragments of GH that are not detected in the IRGH assay. Importantly, the documented disparity for GH measurement across immunoassays can be attributed to antibody specificity, matrix effects, reference standard preparation, and tracer used (6,13).

Although the potential modulation of GH bioactivity through aggregation is interesting, some additional limitations of the currently used IFGH assay must be considered. In comparison to available bioassays (8,33), the IFGH ELISA assay only allows information to be gleaned at the extracellular level and the membrane (e.g., GHR), whereas intracellular signaling cascades remain largely uncharacterized. However, a recent study by Consitt et al. (5) reported that in response to acute aerobic exercise, the absolute change in IFGH as well as the peak IFGH concentrations were related to the fold change in STAT5 phosphorylation, demonstrating that molecules detected in the IFGH assay most likely are capable of initiating postreceptor intracellular signaling. As a caveat, molecules detected in the IFGH assay are deemed bioactive by containing both sites necessary for GHR dimerization; however, there also has to be an adequate number of receptors available at the target cell (dependent on up- or down-regulation of receptors, saturation kinetics between GH and its receptor, necessary conformational receptor changes, etc.), all of which occur before the downstream intracellular signaling. How the possible alterations in the bioactivity of GH and differential outcomes resulting from long-term aerobic and resistance exercise training (decreased body fat and increased lean mass) remains to be explored.

In conclusion, we demonstrated that both IRGH and IFGH molecules are present in the circulation during and after an acute resistance exercise bout. However, after treating the serum samples with a reducing agent, GSH, we observed that only IRGH concentrations increased during acute exercise and remained elevated until 30 min postexercise, suggesting that only molecules detected in the IRGH assay appear to be disulfide-linked. Moreover, this GH response to acute exercise, although diminished after 8 wk of exercise training, was not significantly different from pretraining values. With specific regard for the current study, although the physiological significance of acute exercise-induced disulfide-linked GH aggregates is still unknown and concentrations appear reduced after exercise training, it is possible that aggregation of GH monomers may contribute to enhancing biological action postacute exercise. Collectively, alterations in the structure of GH moieties after acute exercise may represent important regulatory steps in mediating GH biological activity at selected target tissues. Future investigations may wish to explore the use of bioassays and examine effector proteins within the GH signaling cascade (5) in an attempt to clarify the role of acute and chronic exercise-induced GH aggregation.

REFERENCES

1. Baumann G. Growth hormone heterogeneity: genes, isohormones, variants, and binding proteins. *Endocr Rev.* 1991;12(4):424–49.

2. Baumann G, Stolar MW, Buchanan TA. The metabolic clearance, distribution, and degradation of dimeric and monomeric growth

hormone (GH): implications for the pattern of circulating GH forms. *Endocrinology*. 1986;119(4):1497–501.
3. Celniker AC, Chen AB, Wert RM Jr, Sherman BM. Variability in the quantitation of circulating growth hormone using commercial immunoassays. *J Clin Endocrinol Metab*. 1989;68(2):469–76.
4. Consitt LA, Bloomer RJ, Wideman L. The effect of exercise type on immunofunctional and traditional growth hormone. *Eur J Appl Physiol*. 2007;100(3):321–30.
5. Consitt LA, Wideman L, Hickey MS, Morrison RF. Phosphorylation of the JAK2-STAT5 pathway in response to acute aerobic exercise. *Med Sci Sports Exerc*. 2008;40(6):1031–8.
6. Felder RA, Holl RW, Martha P Jr, et al. Influence of matrix on concentrations of somatotropin measured in serum with commercial immunoradiometric assays. *Clin Chem*. 1989;35(7):1423–6.
7. Felsing NE, Brasel JA, Cooper DM. Effect of low and high intensity exercise on circulating growth hormone in men. *J Clin Endocrinol Metab*. 1992;75(1):157–62.
8. Greenspan FS, Li CH, Simpson ME, Evans HM. Bioassay of hypophyseal growth hormone; the tibia test. *Endocrinology*. 1949; 45(5):455–63, illust.
9. Hakkinen K, Pakarinen A. Acute hormonal responses to two different fatiguing heavy-resistance protocols in male athletes. *J Appl Physiol*. 1993;74(2):882–7.
10. Harman EA, Gutekunst DJ, Frykman PN, Nindl BC, Alemany JA, Mello RP. Effects of two different eight-week training programs on military physical performance. *J Strength Cond Res*. 2008; 22(2):1–11.
11. Hunter WM, Fonseka CC, Passmore R. Growth hormone: important role in muscular exercise in adults. *Science*. 1965;150(699): 1051–3.
12. Hymer WC, Kraemer WJ, Nindl BC, et al. Characteristics of circulating growth hormone in women after acute heavy resistance exercise. *Am J Physiol Endocrinol Metab*. 2001;281(4):E878–87.
13. Jansson C, Boguszewski C, Rosberg S, Carlsson L, Albertsson-Wikland K. Growth hormone (GH) assays: influence of standard preparations, GH isoforms, assay characteristics, and GH-binding protein. *Clin Chem*. 1997;43(6 Pt 1):950–6.
14. Kanaley JA, Weltman JY, Veldhuis JD, Rogol AD, Hartman ML, Weltman A. Human growth hormone response to repeated bouts of aerobic exercise. *J Appl Physiol*. 1997;83(5):1756–61.
15. Kraemer WJ, Marchitelli L, Gordon SE, et al. Hormonal and growth factor responses to heavy resistance exercise protocols. *J Appl Physiol*. 1990;69(4):1442–50.
16. Kraemer WJ, Nindl BC, Marx JO, et al. Chronic resistance training in women potentiates growth hormone in vivo bioactivity: characterization of molecular mass variants. *Am J Physiol Endocrinol Metab*. 2006;291(6):E1177–87.
17. Langenheim JF, Tan D, Walker AM, Chen WY. Two wrongs can make a right: dimers of prolactin and growth hormone receptor antagonists behave as agonists. *Mol Endocrinol*. 2006; 20(3):661–74.
18. Nindl BC. Exercise modulation of growth hormone isoforms: current knowledge and future directions for the exercise endocrinologist. *Br J Sports Med*. 2007;41(6):346–8; discussion 8.
19. Nindl BC, Kraemer WJ, Hymer WC. Immunofunctional vs immunoreactive growth hormone responses after resistance exercise in men and women. *Growth Horm IGF Res*. 2000;10(2): 99–103.
20. Nindl BC, Kraemer WJ, Marx JO, Tuckow AP, Hymer WC. Growth hormone molecular heterogeneity and exercise. *Exerc Sport Sci Rev*. 2003;31(4):161–6.
21. Pritzlaff-Roy CJ, Widemen L, Weltman JY, et al. Gender governs the relationship between exercise intensity and growth hormone release in young adults. *J Appl Physiol*. 2002;92(5):2053–60.
22. Pritzlaff CJ, Wideman L, Weltman JY, et al. Impact of acute exercise intensity on pulsatile growth hormone release in men. *J Appl Physiol*. 1999;87(2):498–504.
23. Rubin MR, Kraemer WJ, Kraemer RR, et al. Responses of growth hormone aggregates to different intermittent exercise intensities. *Eur J Appl Physiol*. 2003;89(2):166–70.
24. Strasburger CJ, Wu Z, Pflaum CD, Dressendorfer RA. Immunofunctional assay of human growth hormone (hGH) in serum: a possible consensus for quantitative hGH measurement. *J Clin Endocrinol Metab*. 1996;81(7):2613–20.
25. Sutton J, Lazarus L. Growth hormone in exercise: comparison of physiological and pharmacological stimuli. *J Appl Physiol*. 1976; 41(4):523–7.
26. Takahashi S, Satozawa N. The 20-kD human growth hormone reduces body fat by increasing lipolysis and decreasing lipoprotein lipase activity. *Horm Res*. 2002;58(4):157–64.
27. Tuckow AP, Rarick KR, Kraemer WJ, Marx JO, Hymer WC, Nindl BC. Nocturnal growth hormone secretory dynamics are altered after resistance exercise: deconvolution analysis of 12-hour immunofunctional and immunoreactive isoforms. *Am J Physiol Regul Integr Comp Physiol*. 2006;291(6):R1749–55.
28. Wallace JD, Cuneo RC, Bidlingmaier M, et al. The response of molecular isoforms of growth hormone to acute exercise in trained adult males. *J Clin Endocrinol Metab*. 2001;86(1):200–6.
29. Weltman A, Weltman JY, Womack CJ, et al. Exercise training decreases the growth hormone (GH) response to acute constant-load exercise. *Med Sci Sports Exerc*. 1997;29(5):669–76.
30. Wideman L, Consitt L, Patrie J, et al. The impact of sex and exercise duration on growth hormone secretion. *J Appl Physiol*. 2006;101(6):1641–7.
31. Wideman L, Weltman JY, Shah N, Story S, Veldhuis JD, Weltman A. Effects of gender on exercise-induced growth hormone release. *J Appl Physiol*. 1999;87(3):1154–62.
32. Yang N, Langenheim JF, Wang X, Jiang J, Chen WY, Frank SJ. Activation of growth hormone receptors by growth hormone and growth hormone antagonist dimers: insights into receptor triggering. *Mol Endocrinol*. 2007;22(4):978–88.
33. Ziros PG, Georgakopoulos T, Habeos I, Basdra EK, Papavassiliou AG. Growth hormone attenuates the transcriptional activity of Runx2 by facilitating its physical association with Stat3beta. *J Bone Miner Res*. 2004;19(11):1892–904.

GH dimers may also induce GH receptor (GHR) dimerization and signal transduction (17,32). The role that circulating disulfide-linked GH molecules have during exercise remains to be investigated, particularly in terms of chronic exercise response patterns.

Immunoassays used in the measurement of GH can vary considerably in antibody specificity and reference preparation (3) and may also lack some precision in assessing GH bioactivity. Bioassays measuring *in vitro* cell proliferation (33) or *in vivo* tibial growth (8) may provide a better understanding of signal transduction induced by GH molecules, yet they can be time consuming and costly. Alternatively, Strasburger et al. (24) developed an ELISA for the detection of GH molecules possessing both binding sites intact for signal transduction using an anti-GH monoclonal antibody and a biotin-labeled recombinant GHBP. Because this immunofunctional (IF) assay only detects GH molecules capable of dimerizing GHR, it has been proposed that this assay become the gold standard for diagnosing GH bioactivity (24). The few studies that have used this assay indicate that IFGH (GH molecules that have the capability to initiate signal transduction at the target cell) only represents approximately 50% of the total GH concentration (4,19,27). Although the IFGH assay does not directly assess a physiological outcome, it does provide insight on GHR binding and could provide further evidence on the bioactivity of the GH dimer.

Exercise of sufficient intensity can lead to a robust response of GH and its molecular variants (12,16,28), but the mechanisms leading to their preferential release and what role these variants play during and after exercise remain unanswered. One of these variants, disulfide-linked GH, is released in response to acute exercise (12,23), and its functional effect may be 1) to sustain the biological half-life of GH or 2) to establish function in nonfunctional molecules. Currently, the only studies examining this particular exercise-induced response have only been designed around acute bouts of aerobic (23) and resistance exercise (12), whereas no studies have examined the chronic effects of physical training on this response. Therefore, the purpose of this study was to test the hypothesis that the acute exercise-induced appearance of the disulfide-linked GH aggregates would be altered by 8 wk of chronic physical training. A secondary purpose of this investigation was to determine how acute and chronic exercise possibly alters the GHR-binding potential of disulfide-linked GH aggregates as compared between the immunoreactive GH (IRGH) and the immunofunctional GH (IFGH) assays.

METHODS

Subjects. Seventeen healthy, untrained men (28 ± 1 yr) were recruited for this study, had all experimental methods explained to them, and only participated in these studies after giving their free and voluntary written informed consent. Their physical characteristics (mean ± SE) were as follows: height = 177.6 ± 2.0 cm; weight = 82.9 ± 3.2 kg; body mass index = 26.3 ± 1.0 $kg \cdot m^{-2}$. All were prescreened *via* a health history examination, a physical examination by a physician, and were excluded if they had any conditions known to affect hormonal responses. Before implementation, all methods were reviewed and approved by the Human Use Review Committee of the US Army Research Institute of Environmental Medicine. The investigators adhered to the policies for protection of human subjects as prescribed in Army Regulation 70-25, and the research was conducted in adherence with the provisions of 32 CFR Part 219.

Experimental design. The study was designed to examine the effects of chronic (8 wk) physical exercise training on acute resistance exercise-induced hormonal responses. Toward this end, subjects were randomly assigned to one of two training regimens aimed at improving performance on simulated battlefield physical activities (e.g., load carriage and physical training) and performed an acute resistance exercise test (ARET) before and after the chronic training. The two 8-wk training exercise programs were 1) the recently implemented Army Standardized Physical Training that consisted mainly of calisthenics, body weight exercises, and running and 2) an experimental Army training program that emphasized free weight and machine exercises, agility drills, and interval running. Both groups trained for 1–1.5 $h \cdot d^{-1}$, 5 $d \cdot wk^{-1}$, for the 8 wk of training (10). Table 1 provides a detailed description of the two training regimens designed to improve performance on the military tasks.

TABLE 1. Typical training week for standardized and experimental Army physical training groups.

	Monday	Tuesday	Wednesday	Thursday	Friday
Army Standardized Physical Training	Conditioning drills, military movement drills, body weight exercises, and progressive distance running based on run time performance	Conditioning drills, military movement drills, stretching drills, and interval sprinting	Conditioning drills, military movement drills, body weight exercises, and 300-yd shuttle run	Conditioning drills, military movement drills, and progressive distance running based on run time performance	Conditioning drills, military movement drills, stretching drills, and body weight exercises
Experimental Army Physical Training	Warm-up, stretching, free weights, machine exercises, and 3.2-km run	Warm-up, stretching, agility drills	Warm-up, stretching, 8-km road march with additional load carriage that progressed (0–33 kg) throughout the training	Warm-up, stretching, free weights, machine exercises, and 3.2-km run	Warm-up, stretching, and interval sprinting

Description of two different exercise training regimens designed to improve performance on military tasks. The Army Standardized Physical Training emphasized calisthenics and body weight exercises, whereas the experimental Army training program emphasized free weights and machine exercises. For further clarification of training regimens, see Harman et al. (10).