Characteristics of circulating growth hormone in women after acute heavy resistance exercise

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Characteristics of circulating growth hormone in women after acute heavy resistance exercise. Am J Physiol Endocrinol Metab 281: E878–E887, 2001.—The effects of exercise on the molecular nature of secreted human growth hormone (GH) or its biological activity are not well understood. Plasma from women (average age 23.6 yr, n = 35), drawn before and after an acute heavy resistance exercise test, was fractionated by size exclusion chromatography into three size classes, namely, >60 kDa (fraction A), 30–60 kDa (fraction B), and <30 kDa (fraction C). The concentrations of GH in these fractions, as well as in unfractioned plasma, were measured by the Nichols immunoradiometric assay, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) polyclonal competitive RIA, Diagnostic Systems Laboratory’s immunofunctional assay (measures dimerization-capable species), and the rat tibial bioassay. A chemical reduction of the samples before GH assay. Concentrations of GH in these fractions, as well as in unfractioned and unfractionated plasma samples were used, but they showed no significant change with use of the rat tibial bioassay. Significant increases in circulating GH concentrations of two- to fourfold were observed when immunoassays in unfractionated plasma samples were used, but they showed no significant change with use of the rat tibial bioassay. Significant increases in circulating GH concentrations in fractions B and C, but not in fraction A. Because chemical reduction of the samples before GH immunoassay significantly increased GH concentrations in fractions B and C (Nichols and NIDDK kits) after exercise, it is concluded that exercise may specifically increase release of disulfide-linked hormone molecules and/or fragments. Finally, because most of the GH released after exercise was able to dimerize the GH receptor in vitro, it is also concluded that these forms have the two intact binding sites required to initiate signal transduction in target cells.

growth hormone isoforms; strength training; women; immunofunctional assay

EXERCISE IN HUMANS is a well-known provocative stimulus for growth hormone (GH) release. GH is well known for its anabolic and lipolytic activities. It is believed to be one of the important mediators of training-induced gains in muscle strength and size. Although several studies (9, 14, 16) have defined reasonably well the relationship between intensity and/or type of exercise and the concentrations of GH in the circulation, relatively little is known about the effects of exercise on I) the molecular nature of the secreted GH or 2) its biological activity. Wallace et al. (28) recently reported dynamic changes in certain GH molecular isoforms after acute aerobic endurance exercise in trained adult men, suggesting that the GH response may be complex in its response to exercise stress and supporting the need for further study of the molecular nature of circulating GH after exercise. Currently, no such data exist in women or for more conventional heavy resistance exercise, which is used for producing gains in muscle strength and size.

Antibody-based detection methods are used most frequently to measure concentrations of GH in the blood of humans after exercise. Epitope specificities of different antibodies that are used in different immunoassays help to explain the variability in estimation of hormone concentration sometimes observed (4, 18, 20, 24, 26, 27). In a recent review, Strasburger and Dattani (25) point out that variability can also be explained in part by heterogeneity of GH isoforms as well as the distribution of different isoforms among individuals. The heterogeneity that collectively characterizes the family of GH molecules in the pituitary gland and blood is reasonably well understood (1). In addition to the 22-kDa GH monomer, which has four helix bundles, other forms include the 20-kDa mRNA splice variant, disulfide-linked homodimers and heterodimers of these monomers, glycosylated GH, high molecular weight oligomers, receptor-bound forms of GH, and finally, hormone fragments resulting from proteolysis. The proportion of 22-kDa and non-22-kDa isoforms in human blood is variable and is thought to be due to differences in metabolic clearance of individu-
ual isoforms, the presence of circulating binding proteins, and the generation of fragments in peripheral tissues (3).

Variability in the biological activities of GH isoforms further complicates the problem of understanding the full significance of exercise-induced elevations of GH. Bioassays for GH activities in test animals are often cumbersome and costly. The somatogenic activities of GH are usually measured in hypophysectomized rats by either the weight gain assay or the tibial line bioassay (7, 8, 10, 11). To our knowledge, the recent report by McCaig et al. (21) offers the first information relating to the effect of exercise in men on circulating GH measured by the rat tibial line bioassay. These authors reported that a moderate exercise regimen involving unilateral plantar flexion significantly increased GH bioactivity in plasma but, surprisingly, had no effect on GH concentrations when measured by competitive RIA.

In 1996, a major advance emerged from the report by Strasburger et al. (27), in which the design of a sensitive immunofunctional assay for GH was described. In this assay, only those forms of the hormone capable of inducing receptor dimerization are translated into an assay signal. This assay I uses a monoclonal antibody to target binding site 2 for the GH receptor on GH (a small surface area on the NH2-terminal random coil sequence, the beginning of helix 1, and COOH-terminal parts of helix 3 of the GH molecule) and 2) targets a second, larger site on the hormone involving 31 amino acids on the COOH-terminal portion of the fourth helix bundle, parts of the random coil sequence between helix 1 and helix 4, and parts of helix 1. The functional integrity of the molecule is therefore measured in this assay and represents the initial stages of a cascade leading to signal transduction in target cells. Strasburger and Dattani (25) suggest that the assay “may more closely reflect the biologically active proportion of GH in serum samples than that measured by RIA.”

We have studied the effect(s) of an acute heavy resistance exercise test (AHRET) on circulating GH in 35 young women. Our experimental design was driven by an awareness of circulating GH isoforms and variabilities in estimating their concentrations by bioassays vs. immunoassays. We report here that AHRET significantly increases GH forms contained in two different molecular mass classes (30–60 kDa and <30 kDa), which are active in the Strasburger immunofunctional assay but not in the rat tibial line bioassay.

METHODS

Subjects

Thirty-five women (mean ± SD physical characteristics: 23 ± 4 yr, 166 ± 7 cm, 64 ± 10 kg, 26 ± 6% body fat) were recruited and asked to read and sign an informed consent form before study inclusion. This study had been approved by the Pennsylvania State University Human Use and Review Board, the Institutional Animal Care and Use Committee, and the Human Use Review Office of the Army Surgeon General. All subjects were screened medically for any existing condition (e.g., orthopedic, endocrine) before they were judged as fit to enter the study. None were smokers or recreational drug users. Each subject had received a pregnancy test, and all tests proved negative before enrollment. All subjects were screened for nutritional habits by a registered dietician 2 wk before initial testing to assure normal dietary intakes and food behavior and to eliminate confounding influences of aberrant diets. All subjects were found to have habitually consumed adequate energy, including 30–40% energy from fat and 15–20% energy from protein. All women were menstruating regularly and defined as eumenorrheic (28- to 32-day menstrual cycles during the previous year) (6). All testing was done during the follicular phase of the menstrual cycle with methods described previously (6). Although enrolled subjects were considered healthy and active, none were defined as resistance-trained individuals (i.e., they had not performed structured resistance training for the previous 6 mo).

Exercise Protocol

At least 48 h before the AHRET, each subject had her one repetition maximum strength measured in the squat test by use of the Plyometric Power System (Norsearch, Lismore, Australia) (17).

At the time of testing, each subject rested quietly in the laboratory before the preexercise blood sample was obtained in an effort to reduce anticipatory responses of catecholamines. Whole blood was then obtained by venipuncture 15 min before the AHRET. A light warm-up on a cycle ergometer was given for 2–3 min before the actual AHRET. A second sample of whole blood was obtained immediately after (i.e., within 2 min) completion of the AHRET. All exercise tests were done between 0630 and 1300 after a 6- to 8-h fast.

The AHRET consisted of 6 sets of 10 repetition maximum squats with 2-min rest periods between sets. Initially, the 10 repetition maximum load was calculated as ~75% of the subject’s one repetition maximum squat. If the subject failed to perform the 10 repetitions because of fatigue on any given set, the load was immediately adjusted to permit completion of the remaining repetitions. On average, the time required to complete the entire AHRET was ~12–15 min.

Processing of Whole Blood

Plasma was obtained from each sample by centrifugation (800 g, 10 min) and distributed according to the scheme shown in Fig. 1.

Fractionation

Fifteen milliliters of plasma were fractionated on a 100-cm-long Sephacryl S-100 HR sizing column (26 mm ID), and the resulting 100 tubes were pooled into three larger fractions containing molecules with apparent molecular masses >60 kDa (fraction A), 30–60 kDa (fraction B), and <30 kDa (fraction C). Two Sephacryl columns were used throughout the course of this study, one for processing each of the 60 pre-AHRET plasma samples and the other for the 60 post-AHRET samples. Each of the 2 columns was calibrated using blue dextran [molecular weight (MW) 2,000,000], BSA (MW 66,000), carbonic anhydrase (MW 29,000), cytochrome c (MW 12,400), and aprotinin (MW 6,500), which were provided in a molecular weight standards kit (Pharmacia, Uppsala, Sweden). Columns were washed with 0.05 M NH4HCO3, pH 8.0
(1 column volume) in the intervals between processing of each plasma sample and were recalibrated after 15 plasma samples had been processed. Regression analysis of these calibration curves [log MW vs. the ratio of eluted protein to column void volume \((V/V_o)\)] yielded lines of the form \(y = -1.069 + 6.11444\) and \(y = -1.0196 + 6.0595\). Correlation coefficients for these regressions were 0.997 and 0.996, respectively.

In addition to the three pooled Sephacryl fractions, an aliquot of unfractionated plasma was also lyophilized before reconstitution in 10 ml of 0.001 M \(\text{Na}_4\text{HCO}_3\), pH 8.0, and subsequent GH assay. To determine what effect lyophilization might have on GH measurements, an aliquot of unfractionated plasma was not lyophilized and was subsequently assayed by the Nichols immunoradiometric assay (Fig. 1).

**GH Assays**

Circulating plasma human GH was determined by use of three different immunoassays: 1) The Nichols Institute Diagnostics immunoradiometric assay (Nichols IRMA; San Juan Capistrano, CA), 2) The National Institute of Diabetes and Digestive Kidney Diseases competitive radioimmunoassay (NIDDK RIA), and 3) The Diagnostic Systems Laboratory's (DSL) immunofunctional ELISA (IFA; Webster, TX). All assays were validated with respect to linearity, parallelism, and recovery. Log-log, log-log, and linear standard curve-fitting regression methods were used for the NIDDK RIA, Nichols IRMA, and DSL IFA, respectively. To eliminate interassay variance, all samples from a subject were assayed within the same batch by use of a gamma counter with curve-fitting algorithms (EG & G Wallac Gamma Counter, Turku, Finland) for the RIAs or a microplate reader (Bio-Tek Instruments, Winskosi, VT) for the ELISA.

In each case, recoveries of immunoreactive (i)GH after chromatography were 1.5- to 3.0-fold higher than GH concentrations measured in unfractionated plasma. High recoveries of GH after column chromatography have been reported by other investigators (7); the reason(s) is unknown but may reflect removal of inhibitory substance(s) in the starting material.

**Nichols IRMA.** This commercially available assay used two monoclonal antibodies of high affinity and specificity for GH; each detects a different epitope on the GH molecule. One of the antibodies was labeled for detection; the other was coupled to biotin. The sensitivity for this assay by use of the \(B_0 \pm 2\) SD method was 0.04 ng/ml. Intra-assay variances for low, medium, and high GH concentrations were 7.2, 5.2, and 5.4%, respectively.

**NIDDK RIA.** The NIDDK RIA reagents were obtained from Dr. A. F. Parlow and the National Hormone and Pituitary Program of the NIDDK. The GH antigen (NIDDK-GH-I-3; AFP-11019B) was iodinated on site. The primary polyclonal antibody was rabbit NIDDK-anti-GH-2. The biological potency of the GH reference preparation (NIDDK-GH-RP-1; AFP-4793B) was 2.2 IU/mg, as determined in the hypophysectomized female rat body weight gain bioassay. This reference preparation was serially diluted from 25 to 0.195 ng/ml (8 total points) and subsequently used for the standard curve in all three immunoassays. The secondary antibody was sheep anti-rabbit globulin and was used in conjunction with polyethylene glycol. After centrifugation, tubes were aspirated, and the pellet was counted in a gamma counter for 120 s (EG & G Wallac Gamma Counter). The sensitivity for this assay by use of the \(B_0 \pm 2\) SD method was 0.10 ng/ml. Intra-assay variances for low, medium, and high GH concentrations were all 10.0%.

**DSL IFA.** This commercially available assay uses an enzymatically amplified "two-step" sandwich-type assay requiring an anti-GH monoclonal antibody and biotinylated recombinant GH binding protein (GHBP) that bind, respectively, to GH receptor-binding site 2 and site 1 present on all "biologically active" GH molecules. Plasma samples were incubated sequentially (with intervening wash steps) with 1) an immobilized anti-GH antibody, 2) biotinylated GHBP followed by streptavidin labeled with horseradish peroxidase, and finally 3) tetramethylbenzidine substrate. After addition of an acidic stop solution, enzyme turnover was determined by dual wavelength absorbency measurements at 450 and 630 nm. The absorbancy measured is directly proportional to the concentration of biologically active "intact" GH that possesses both GH receptor-binding sites. The sensitivity for this assay, when the \(B_0 \pm 2\) SD method was used, was 0.20 ng/ml. Intra-assay variances for low, medium, and high GH concentrations were 7.1, 7.6, and 8.4%, respectively.

**Rat Tibial Line Bioassay for GH**

Concentrations of biologically active GH in unfractionated and fractionated plasma samples were determined according to the method of Greenspan et al. (11). Briefly, female Sprague-Dawley rats (Hilltop Labs, Scottsdale, PA), hypophysectomized at 26–28 days of age, were used 2 wk after surgery. Animals that weighed <80 g or >100 g at the time of sample injection were excluded. The following criteria were taken as evidence for completeness of hypophysectomy: failure to gain >7 g in the 10 days after the operation, deterioration of body tonus, maintenance of infantile ("smooth") hair, and absence of pituitary remnants in the sella turcica at autopsy. Animals were injected subcutaneously once daily for 4 days with 1) experimental plasma samples, 2) a standard GH preparation (USDA bovine GH B-1 AFP 5200, 1.4 IU/mg at total doses of 10, 30, or 90 μg), or 3) physiological saline (control). Twenty-five hours after the last injection, the animals were killed, tibial epiphyseal plates were stained, and plate widths were measured in...
double-blind fashion using an ocular micrometer (10 readings averaged across the plate width for each sample). GH responses were expressed in terms of a purified human pituitary preparation (3.0 IU/mg). Assay variance was 7.2%. Average (± SD) tibial widths of animals injected with bovine GH standard were for saline, 147 ± 11 µm (SD); for 10 µg, 168 ± 14 µm; for 30 µg, 190 ± 24 µm; and for 90 µg, 233 ± 22 (y = 1.0768x − 166.04; r² = 0.96).

Statistical Analysis

Analysis of variance (ANOVA) or ANOVA with repeated measures was used to compare GH concentrations before and after exercise for the assays (CSS:Statistica, StatSoft, Tulsa, OK). Where appropriate, α-level corrections were made to maintain the 0.05 level of significance. Tests for normality of distribution (Kolmogorov-Smirnov χ²-test) and homogeneity of variance (Levene’s test) were used on all data sets before ANOVAs. Post hoc comparisons were accomplished via a Fisher’s least significant difference test. Linear regression analysis was used to evaluate the relationship between the NIDDK and Nichols assays and the DSL IFA. Statistical power was determined to be >0.80 for all measures for the sample size used at the 0.05 α-level (nQuery Advisor software, Statistical Solutions, Saugus, MA). All statistical significance in this study was set at P ≤ 0.05. For all figures, identical letters represent no significant differences.

RESULTS

The chromatographic profiles of circulating GH obtained from the plasma of a single subject before and immediately after AHRET are shown in Fig. 2, A and B, respectively. These results were obtained using the Nichols IRMA kit on nonlyophilized samples. A majority (71% preexercise; 75% postexercise) of the recovered hormone was associated with molecules having apparent molecular masses between 14 and 29 kDa. A small percentage of the recovered hormone was associated with molecules of apparent sizes between 70 and 120 kDa (10% preexercise; 9% postexercise). A third region, encompassing 30–60 kDa, made up the remainder (16–19%) of the hormone recovered from this sample. These profiles correspond to the “big-big,” “big,” and “little” iGH variants reported by Stolar et al. (23). They defined the regions of the 70 plasma samples that were pooled and concentrated (Fig. 1) to generate fractions A, B, and C either before or immediately after AHRET. Also shown in Fig. 2, C and D, are the A₂₈₀ profiles of the fractionated plasmas from the same single test subject before and after exercise. These profiles reflect the distributions of the plasma proteins

![Fig. 2. Gel chromatography profiles of human (h) growth hormone (GH) (Nichols assay) and A₂₈₀-nm absorbances in individual fractions of plasma from a single test subject obtained before the acute resistance exercise test (AHRET, A and C) and immediately after AHRET (B and D). Elution positions of molecular mass standards are marked by arrows: BD, blue dextran; BSA, bovine serum albumin; CA, carbonic anhydrase; CC, cytochrome c; A, aprotinin. Regions pooled to generate fractions A (>60 kDa), B (30–60 kDa), and C (<30 kDa) are indicated. This strategy for combining fractions was identical for all 70 plasma samples represented by the data in Figs. 3–5. Vₑ/Vₒ, ratio of eluted protein (Vₑ) to column void volume (Vₒ).](image-url)
after chromatography; except for minor changes in region B, the profiles are generally similar.

**Nichols GH assay**

Average concentrations of circulating iGH before and after AHRET in plasmas of the 35 participants, measured using the Nichols reagents, are shown in Fig. 3. Significant increases after exercise were found in the unfractionated as well as in the B and C fractions. In both the pre- and postexercise samples, GH concentrations were highest in the B and C fractions combined, accounting for 89% of the total hormone recovered at both time points. Largest exercise-induced increases were in the unfractionated and B samples (4.1- and 4.2-fold, respectively).

**NIDDK GH assay**

The patterns of exercise-induced increase in circulating iGH with this kit were generally similar to those found using the Nichols reagents. Thus fractions B and C both contained significantly more GH after AHRET than before (Fig. 4). On average, GH concentrations in fraction C were significantly greater than in all other fractions. Frequently, concentrations of hormone detected by the NIDDK reagents tended to be about one-half of those measured with the Nichols reagents. However, an important exception was in fraction C; in this case, the average concentrations of iGH were not significantly different (7.6 vs. 8.1 ng/ml; \( P > 0.05 \); Nichols vs. NIDDK).

**IFA**

Results obtained from this kit yielded patterns that were similar to those obtained with the other two kits (compare Fig. 5 with Figs. 3 and 4). Thus the unfractionated, fraction B, and fraction C samples all increased post-AHRET. Three additional interesting findings were that 1) on average, fraction C contained 16.5 ng/ml post-AHRET, the highest concentration of hormone in any of the groups represented by the data in Figs. 2–5; 2) the average exercise-induced increase in hormone concentration was higher in fraction B than in fraction C (5.2- vs. 3.0-fold, respectively); and 3) GH molecules in these samples contain both binding sites necessary for receptor dimerization and can therefore be presumed to be “biologically” active (26).

**GSH Treatment**

Results of a preliminary trial on pooled plasma samples from five randomly selected subjects in which three concentrations of GSH were added (both pre- and post-AHRET) to the samples at different concentrations (1, 3, or 10 mM for 2, 5, 10, or 18 h at room temperature) indicated that maximum increases in GH concentrations were found when 10 mM GSH was used for 18 h (data not shown). This GSH treatment condition was then used to determine the effects of chemical reduction in each of the samples from the 35 subjects before and after AHRET.
As shown in Table 1, GSH treatment significantly increased apparent GH concentrations in only 2 of the 12 groups of plasma samples obtained pre-AHRET. However, in the post-AHRET group, 6 of the 12 groups treated with GSH contained significantly more GH. These effects were seen in a majority (5 of 6) of the unfractionated plasma sample groups; they were of greater magnitude after exercise. These GSH-induced increases in GH concentrations were not found in high molecular mass material (fraction A). However, they tended to be found in fraction B samples post-AHRET. These results suggest that disulfide-linked hormone aggregates/fragments are released into the circulation after the exercise regimen used in this study.

Rat Tibial Line Bioassay

Although GH was readily detected in all samples, AHRET was without effect on bioassayable GH (Fig. 6). In this case, the distribution pattern of recovered hormone was quite different from patterns shown in Figs. 2–5, in that each of the three fractions contained about one-third of the recovered hormone. As before, hormone recoveries from the column were >100% (151% pre-AHRET and 143% post-AHRET).

Lyophilization

The experimental design (Fig. 1) required lyophilization of diluted fractions collected from the long chromatography column to make measurements of GH by four different assays feasible. In an effort to determine what effect, if any, lyophilization and subsequent rehydration might have on GH concentrations, an aliquot from each fraction was not lyophilized before immunoassay (Nichols). Sample volumes were insufficient to do this test for all four assays. As shown in Table 2, the average GH concentrations (n = 35) in all Sephacryl fractions, both pre- and post-AHRET, were not significantly affected by the lyophilization/reconstitution procedures. However, this was not true for the unfractionated plasma samples. In this case, GH concentrations in both the pre- and post-AHRET samples were twofold lower after lyophilization than before. This result may partly explain why hormone recoveries from the column were considerably higher than their corresponding input levels. Although the reason for this result is unknown, the data in Table 2 indicate that preferential loss of a GH isoform(s) during chromatography is unlikely.

DISCUSSION

It has been known for 35 years that exercise is a potent stimulus for the release of immunoreactive GH from the human pituitary gland (12). However, many details of the exercise-induced responses are lacking. This situation can be attributed in part to the complexities involved in measuring circulating GH. These include 1) the epitope specificities of the different antibodies used in different immunoassays, 2) the wide spectrum of different molecular GH isoforms circulating in human plasma, 3) the fact that some GH circu-

Table 1. Effects of GSH treatment on GH concentrations in unfractionated and fractionated plasmas of 35 young women pre- or post-AHRET

<table>
<thead>
<tr>
<th></th>
<th>Pre-AHRET, µg/l</th>
<th>Post-AHRET, µg/l</th>
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<tbody>
<tr>
<td></td>
<td>−GSH</td>
<td>+GSH</td>
</tr>
<tr>
<td>NIDDK RIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated</td>
<td>1.3±0.2</td>
<td>1.8±0.3*</td>
</tr>
<tr>
<td>Fraction A</td>
<td>0.5±0.1</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>Fraction B</td>
<td>1.3±0.2</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>Fraction C</td>
<td>2.6±0.5</td>
<td>3.2±0.7</td>
</tr>
<tr>
<td>Nichols IRMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated</td>
<td>2.1±0.5</td>
<td>2.8±0.6*</td>
</tr>
<tr>
<td>Fraction A</td>
<td>0.6±0.1</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>Fraction B</td>
<td>1.9±0.3</td>
<td>2.1±0.4</td>
</tr>
<tr>
<td>Fraction C</td>
<td>2.6±0.6</td>
<td>3.1±0.8</td>
</tr>
<tr>
<td>DSL IFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated</td>
<td>1.5±0.3</td>
<td>1.8±0.5</td>
</tr>
<tr>
<td>Fraction A</td>
<td>0.5±0.1</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>Fraction B</td>
<td>1.7±0.3</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>Fraction C</td>
<td>5.6±1.4</td>
<td>3.0±0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE of growth hormone (GH) concentrations determined by 3 different immunoassays: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) RIA, the Nichols immunoradiometric assay (IRMA), and the Diagnostic Systems Laboratory (DSL) immunofunctional ELISA assay (IFA). GH treatment conditions were 10 mM GSH for 18 h at room temperature immediately before assay, AHRET, acute heavy resistance exercise test. *Significant difference (P ≤ 0.05) between −GSH and +GSH treatments.

Table 2. Effects of lyophilization on GH concentrations (assessed via Nichols IRMA) in unfractionated and fractionated plasma samples of 35 young women either pre- or post-AHRET

<table>
<thead>
<tr>
<th></th>
<th>Pre-AHRET, µg/l</th>
<th>Post-AHRET, µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not lyophilized</td>
<td>Lyophilized</td>
</tr>
<tr>
<td>Unfractionated</td>
<td>4.0±1.5</td>
<td>2.1±0.5</td>
</tr>
<tr>
<td>Fraction A</td>
<td>0.4±0.7</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>Fraction B</td>
<td>1.0±1.3</td>
<td>1.9±0.3</td>
</tr>
<tr>
<td>Fraction C</td>
<td>1.6±1.2</td>
<td>2.6±0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE.
lates bound to the GH receptor, and 4) the well-described pulsatile nature of GH release. The heterogeneity of the hormone’s action on different targets further contributes to that complexity. Thus there is some evidence that the effect of the hormone on lipid, carbohydrate, and protein metabolism, in addition to its effect in mediating longitudinal bone growth, may also be controlled by different GH isoforms (22). Many of these considerations had direct bearing on our experimental design. Two key elements of that design were 1) assessments of acute heavy resistance exercise on both the biological and immunological activities of the circulating hormone and 2) assessments of these activities in different size classes of circulating hormone variants separated by size exclusion chromatography.

The results of several previous studies show that exercise of the type and duration used in this study result in significant increases in the amounts of circulating immunoreactive GH (14–16). Our study confirms and extends this general finding in the sense that significant increases in circulating GH were found in the unfractionated plasma samples after acute heavy resistance exercise.

To our knowledge, this study is one of the first to begin characterization of the underlying details of the exercise-induced GH response as related to the size ranges of the GH molecules that are released into the circulation. Wallace et al. (28) recently reported dynamic changes in certain GH molecular isoforms after acute exercise in trained adult men. Their results showed that 1) all circulating isoforms studied increased after exercise, 2) 73% of the total exercise-induced GH peak was accounted for by the 22-kDa form, 3) the IFA detected ~50% of the 22-kDa peaks, and 4) not all forms had the same disappearance rate from the circulation. Wallace et al. concluded that the biological consequences of GH isoform alterations after exercise “. . . remain to be determined.” It is difficult to compare our study with that of Wallace et al. in any more than a general way, because the experimental design and test parameters were very different (e.g., mode of exercise, assay approaches, duration of exercise, and the like). However, it seems clear that the results of both studies reinforce the need for further study of GH isoforms after exercise.

Results shown in Figs. 3–5 indicate that the patterns of immunoreactive GH recovered in the three different fractions were somewhat consistent among the three different immunoassays. Thus, as shown in Table 3, only 4–11% of the recovered immunoreactive hormone was associated with molecules in fraction A, 22–45% was associated with molecules in fraction B, and the majority (44–72%) was recovered in fraction C. The extensive work by Baumann and coworkers (1, 2, 3, 23), which also used size exclusion chromatography to characterize proportions of GH isoforms in plasma, suggested the following distributions: monomeric 22-kDa form, 50%; monomeric 20-kDa form, 10%; homodimeric and heterodimeric forms, 20% (noncovalent); and disulfide-linked and oligomeric forms, 8 and 10%, respectively. Recent work from the Lewis and Sinha groups (Lewis et al., Ref. 19) suggests the possibility that the 22-kDa form of the hormone may not be the major form in human serum; their evidence indicates that high concentrations of 22-kDa hormone fragments (representing amino acids 1–43 and 44–191) predominate. In either case, it seems probable that fraction C contains a mixture of GH molecules, namely, 22-, 20-, 16-, 12-, and 5-kDa forms. Fraction B would be expected to contain both homodimers and heterodimers, whereas fraction A should contain oligomers and/or monomeric GH bound to the receptor. Several investigators have reported that the immunoreactivity of oligomeric forms is poor relative to the monomeric form. This may be true in our study as well, because a significant exercise-induced rise in GH in fraction A was not found in any of the three immunoassays. Celniker et al. (4) previously compared serum GH concentrations measured by four different RIAs and two different immunoradiometric assays. In that study, effects of differences in standards, assay diluents, and antibody specificity were evaluated. Although these variables had effects on the final result, these authors found that high correlations between the different assay methods (r ≥ 0.8) “indicated that these immunoassays perform similarly in a relative sense.” The primary emphasis of our study was an evaluation of the effects of an acute heavy resistance exercise test on circulating GH isoforms and not on a comparison of results obtained among the three different immunoassays we used. Clearly, the collective data (Figs. 3–5) show, in a relative sense, that the primary exercise effects were in fractions B and C regardless of assay method used.

Our experiment used two very different bioassays to test for effects of acute resistance exercise on circulating GH. One relied on the effects of the hormone on tibial epiphyseal cartilage plate chondrocytes of the hypophysectomized rat. Even though the rat tibial line assay is considered by some to be one of the only “true” biological assays for evaluating the somatogenic activity of the hormone, it suffers from 1) high cost and 2) relative insensitivity (limit of detectability of GH is ~5 μg). Nevertheless, it is well known that human plasma contains forms of GH that are detectable in this bioassay. Studies that compare the biological vs. immunological activities of GH released after exercise are rare.

| Table 3. Summary of percentage distributions of plasma GH recovered pre- and post-AHRET in different molecular mass fractions (A, B, and C) obtained by size exclusion chromatography |
|-----------------------------------|------------------|------------------|------------------|------------------|
|                                   | Pre-AHRET         | Post-AHRET        |                  |                  |
|                                   | Total GH recovered, μg/l | A | B | C | A | B | C |
| NIDDK RIA                         | 4.37              | 11 | 29 | 60 | 13.39 | 6.5 | 33 | 60.5 |
| Nichols IRMA                      | 5.02              | 11 | 37 | 52 | 17.30 | 11 | 45 | 44  |
| DSL IFA                          | 7.74              | 6  | 22 | 72 | 26.34 | 4  | 33 | 63  |
| Tibial Assay                      | 3.927             | 34 | 36 | 30 | 3.787  | 35 | 22 | 27  |

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In fact, McCall et al. (21) were the first to report a relationship between exercise and bioactive GH (tibial line assay) by demonstrating elevated levels of circulating GH in men after a moderate exercise regimen involving unilateral isometric plantar flexions. However, our study clearly shows that acute heavy resistance exercise has no effect on circulating GH as measured by the tibial line bioassay. One of the explanations for this negative result could be that our exercise regimen generated a form(s) of the hormone molecule(s) that is (are) not recognized by rat GH receptors on in vivo tissue targets. A simpler explanation would be that the type of exercise may be the important factor in determining pituitary responsiveness. In this regard, it is interesting that the moderate exercise protocol used in the McCall study had no effect on circulating levels of immunoreactive GH. Our results are exactly the opposite (Figs. 3–5). Because the same bioassay was used in both studies, and because the concentrations of bioactive GH in plasma samples of control subjects were of the same order in both studies [2.2–2.7 mg/l plasma (21) vs. 2.3 mg/l plasma of control subjects were of the same order in both studies], the negative results in Fig. 6 would not appear to be explained by differences in bioassay techniques. We believe that the most obvious explanation(s) for the different results between the study of McCall et al. and ours lies either in 1) the type of exercise used or 2) the gender of the test subjects. Evidence for the latter possibility was reported recently by Jaffe et al. (13), who showed that the regulatory mechanisms involved in controlling the GH axis were found to be sexually dimorphic.

The patterns of tibial line GH bioactivity seen after column chromatography (Fig. 6) are reminiscent of the earlier reports of Ellis and Grindeland (7) and Ellis et al. (8), in which significant concentrations of bioactive hormone were found in both the 22-kDa molecular mass region and in higher molecular mass regions. These higher molecular mass forms were attributed to disulfide-linked aggregates of the 22-kDa form. In the case of molecules recovered from fraction B, our data (Table 1) offer some support for that hypothesis, because GSH treatment significantly increased concentrations of immunoreactive hormone when the Nichols or NIDDK antisera were used. However, that explanation cannot be used for those hormone molecules in fraction A, because chemical reduction was without effect. Although we have no supporting data, the 22-kDa form bound to its receptor could explain the fact that 4–11% of the GH was recovered in fraction A.

In the 1990s, two major advances in GH research opened new avenues for the study of GH physiology. These were 1) elucidation of the details involving underlying molecular interactions between a GH molecule and its receptor on a target cell (5) and 2) the development and availability of a sensitive immunofunctional assay for GH (27) that was based on those interactions. A positive signal in the immunofunctional assay implies that the test sample contains GH molecules that possess the two receptor-binding sites required for receptor dimerization. As suggested by Strasburger et al. (27), only those GH forms having two intact binding sites are capable of initiating the signal transduction process on target cells. In this context, the GH immunofunctional assay is a sensitive bioassay that offers an estimate of the biological activity of GH contained in a test sample. In addressing the specific issue of identification of exercise-induced release of GH forms from the pituitary that are biologically active, we view the results obtained from the immunofunctional assay as particularly relevant. Because previous studies have used RIAs or commercial assay kits to evaluate GH quantities in serum after exercise, we compare in Table 4 correlations of data obtained between the immunofunctional assay and the Nichols/NIDDK kits.

Note that 14 of the 16 subgroups showed good correlations between the immunofunctional assay and the Nichols or NIDDK kits ($P \leq 0.05$). However, the relationship between the values obtained in the three different immunoassays (as reflected by differences in

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Fraction</th>
<th>Time point</th>
<th>Slope</th>
<th>Intercept</th>
<th>$R$</th>
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<tr>
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<td>0.83*</td>
</tr>
<tr>
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* $P \leq 0.05$ for regression relationship.
values of slopes and y-intercepts) varied considerably. This latter point is similar to that made earlier for the data set described by Celniker et al. (4). Nevertheless, these comparisons showed interesting trends. These included the findings that the exercise regimen resulted in 1) decreased quality of correlation in GH concentrations in the unfractionated and fraction C plasma samples but actually increased the quality of correlation for GH amounts in fraction A while 2) having little effect on GH forms recovered from fraction B.

The data in Figs. 3–5 and Table 1 support the idea that AHRET stimulates the release of dimeric forms of GH from the pituitary gland. Clearly, these forms are active in the IFA. Strasburger et al. (27) report that GH dimers, on a molar basis, have slightly higher reactivity in the immunofunctional assay (110%) relative to GH monomeric forms; however, to our knowledge no information is available concerning activities of higher molecular mass forms (>60 kDa) in this assay. Because 22–45% of the total GH recovered within 2 min after AHRET is dimeric hormone, we view the most important effect of AHRET to be on circulating hormone molecules of this size class.

It is interesting to speculate on the functional significance of the observation that biological activities of dimeric GH forms are increased after AHRET. Because complexes of GH and binding proteins have longer half-lives than free GH, it is possible that a dimeric GH molecule might also have a longer half-life as well (1–3). Because evidence for the presence of GH dimers is offered in Table 1, it seems reasonable to conclude that the increase in the B fraction after AHRET might have the net effect of prolonging the biological activities of these forms after exercise. Of course, these interpretations must be tempered by the fact that gel exclusion chromatography used in the manner required by our study is a relatively crude way to dissect the complexities of circulating GH isoforms after AHRET.

Given the availability of new, sensitive GH assays for “biological activity,” it will be possible to refine the sampling strategies to further purify active GH isoforms contained in fractions B and C. In this way, a more precise definition and identification of those molecules affected by the exercise protocol can be realized.

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