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Acute resistance exercise stimulates sex-specific dimeric immunoreactive growth hormone responses

Hui Ying Luk [c], William J. Kraemer [a,*], Tunde K. Szivak [a], Shawn D. Flanagan [a], David R. Hooper [a], Brian R. Kupchak [b], Brett A. Comstock [d], Courtenay Dunn-Lewis [e], Jakob L. Vingren [c], William H. DuPont [a], Wesley C. Hymer [f]

[a] Department of Human Sciences, The Ohio State University, Columbus, OH, USA
[b] Human Performance Laboratory, Department of Kinesiology, University of Connecticut, Storrs, CT, USA
[c] Department of Kinesiology, Health Promotion and Recreation, University of North Texas, Denton, TX, USA
[d] Division of Kinesiology and Sport Science, University of South Dakota, Vermillion, SD, USA
[e] Department of Health Sciences, School of Science and Engineering, Merrimack College, North Andover, MA, USA
[f] Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, USA

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ABSTRACT

Purpose: We sought to determine if an acute heavy resistance exercise test (AHRET) would elicit sex-specific responses in circulating growth hormone (GH), with untreated serum and serum treated with a reducing agent to break disulfide-bindings between GH dimers.
Methods: 19 untrained participants (nine men and ten women) participated in an acute heavy resistance exercise test using the back squat. Blood samples were drawn before exercise (Pre), immediate post (IP), +15 min (+15), and +30 min (+30) afterwards. Serum samples were chemically reduced using glutathione (GSH). ELISAs were then used to compare immunoreactive GH concentrations in reduced (+GSH) and non-reduced (−GSH) samples. Data were analyzed using a three-way (2 sex × 2 treatment × 4 time) mixed methods ANOVA, with significance set at $p \leq 0.05$.
Results: GSH reduction resulted in increased immunoreactive GH concentrations when compared to non-reduced samples at Pre (1.68 ± 0.33 μg/L vs 1.25 ± 0.25 μg/L), IP (7.69 ± 1.08 μg/L vs 5.76 ± 0.80 μg/L), +15 min (4.39 ± 0.58 μg/L vs 3.24 ± 0.43 μg/L), and +30 min (2.35 ± 0.49 μg/L vs 1.45 ± 0.23 μg/L). Also, women demonstrated greater GH responses compared to men, and this was not affected by reduction.
Conclusions: Heavy resistance exercise increases immunoreactive GH dimer concentrations in men and women, with larger increases in women and more sustained response in men. The physiological significance of a sexually dimorphic GH response adds to the growing literature on aggregate GH and may be explained by differences in sex hormones and the structure of the GH cell network.

1. Introduction

Human growth hormone (GH) is a polypeptide family with over one hundred variants [1–3]. Through its tyrosine kinase receptor, GH activates downstream signaling through mTOR, which promotes gene translation and protein synthesis [4,5]. The functional roles of GH have been well documented, and include pleiotropic effects on osmoregulation, tissue anabolism, metabolism, and endocrine signaling [6]. Nevertheless, investigations on recombinant GH supplementation (with or without strength training) in healthy individuals have not reliably demonstrated improvements in muscular strength or protein synthesis [7–10]. Despite the lacking evidence for its efficacy and its status as an illegal performance enhancing substance, recombinant GH (22 kD) has become a popular ergogenic aid among athletes and the aging.

The discrepancy between the known signaling properties of GH and its anabolic effects may be explained by the apparently low biological activity of monomeric 22 kD GH in this respect. In the early days, limited analytical tools and an underappreciated role of GH isoform heterogeneity resulted in the focused examination of the 22 kD isoform. Thus, investigators considered 22 kD the major contributor to observed physiological effects. About two decades ago, different molecular weight GH variants were identified, and found to exist in peripheral circulation. These variants are produced through alternative splicing, as well as post-translational modifications, which may occur to differing degrees

Abbreviations: AHRET, acute heavy resistance exercise test; ANOVA, Analysis of Variance; GH, growth hormone; GHBP, growth hormone binding protein; GHR, growth hormone receptor; GHRH, growth hormone releasing hormone; GSH, glutathione; mTOR, mammalian target of rapamycin; RM, repetition maximum; SST, somatostatin.

* Corresponding author. Tel.: +1 614 688 2354; fax: +1 614 688 3432.
E-mail address: kraemer.44@osu.edu (W.J. Kraemer).

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in cellular compartments [2]. Upon transcription, mRNA splicing is thought to predominately produce two monomeric products (22 kD and 20 kD). Other isoforms are then produced through covalent and non-covalent interactions, resulting in larger GH aggregates [2].

Sex-specific GH releasing patterns [11,12], regulation [13–16], and function have been observed in previous work. This has led to the suggestion that a sexually dimorphic sex hormone environment can affect the organization and the content of anterior pituitary somatotroph populations [14,15]. This contention is supported by observations of sex-specific differences in the types of steroid receptors, mRNA levels in GHRH and somatostatin neurons, and number of GHRH neurons [17–19]. In support of the physiological significance of such differences, GH pulse patterns differ in male and female rats [20], and male rats are more sensitive to the release of somatostatin [21]. Taken together, it is reasonable to theorize that sex-specific differences in the sex hormone environment may affect GH release patterns in humans.

A series of investigations have established characteristic GH responses to resistance and endurance exercise. Heavy resistance and endurance exercise can alter the distribution of circulating GH isoforms in women [22,23] and men [24–26]. Previously, we used GSH to reduce unfractionated and fractionated (based on molecular size) GH before and after heavy resistance exercise [23]. In addition to increasing detectable immunoreactive 22 kD GH concentrations in unfractionated samples, GSH predominately increased the detectable 22 kD GH in <30 kD and 30–60 kD fractions. These observations indicated that disulfide-bound GH dimers were present, and that reduction allowed for the detection of these aggregates using conventional 22 kD immunoassays. Furthermore, GH dimers represented a large proportion of the total GH recovered immediately after exercise. It is particularly important to consider that compared to monomeric GH isoforms, dimeric isoforms demonstrate stronger reactivity in immunofunctional assays [27]. This finding suggests that dimeric GH may play an important bioactive role with respect to resistance exercise.

Comparing the GH responses of men and women will help us begin to understand the physiological significance of sex-specific differences in GH with respect to resistance exercise. In this study, we sought to extend our knowledge on the response profiles of different GH isoforms, as determined by GSH reduction, in the context of an acute heavy resistance exercise test (AHRET) in men and women. GSH treatment was hypothesized to change detectable GH concentrations, revealing the presence of disulfide-bound GH dimers. Further, based on previous work, it was reasonable to anticipate different GH secretion patterns in men and women.

2. Materials & methods

2.1. Participants

Nineteen untrained participants (nine men, ten women), who had not participated in a systematic resistance program within one year of participation, were recruited for this study, which was part of a larger study [28]. Untrained subjects were recruited to optimize the gains in lean body mass with resistance training and thus provided the independent variable context for this study and its findings. Participants characteristics are presented in Table 1 with expected differences in size and body composition and were matched for activity using the previously described technique [28]. Body composition was determined by DEXA as previously described in detail [28]. Women who participated had normal menstrual cycles (28–32 days). To minimize the influence of variable estrogen concentrations and resting between-group GH concentrations, AHRETs were performed between days two and four of the follicular phase [29]. Prior to participation, all participants provided written informed consent and were medically screened by a physician. All procedures were approved by the local Institutional Review Board.

Table 1
Characteristics of study participants (mean ± SE).

	Men	Women
N	9	10
Age, yr	22.3 ± 1.8	21.7 ± 2.4
Body Mass, kg	76.2 ± 4.3	60.9 ± 7.0*
Height, cm	176.8 ± 16	163.4 ± 1.5*
Fat body mass, kg	15.0 ± 3.3	20.8 ± 6.4*
Fat free mass, kg	50.96 ± 2.1	37.6 ± 3.9*
% body fat	19.1 ± 3.3	33.6 ± 7.5*

* $p \leq 0.05$ from corresponding value for men.

2.2. Procedures

2.2.1. Familiarization and preliminary exercise testing

Participants were familiarized with the AHRET exercise one week prior to the testing day. Following anthropometric measurements, a standardized warm up and three to five subsequent single lifts were performed to determine the one repetition maximum (1-RM) squat strength. The guidelines of Fleck and Kraemer [30] were utilized during this procedure. This involved the following procedures. The 1 RM squat was performed on a Smith Machine using proper technique and the same starting positions each time. A full range of motion was required for each repetition including the maximal repetition resistance lifted (i.e., 1 RM). The end point of the squat was defined by a parallel depth defined as the 90-degree relationship between the femur and the lower leg (i.e., knee at 90°) for each subject and was set with a plum line that the bottom of the thigh had to reach with each repetition in order to be counted as correct technique. Subjects performed a dynamic warm-up consisting of 5 min of cycle ergometer exercise followed by a series of dynamic stretches. Two warm-up sets were completed at 50% estimated 1 RM (8–10 repetitions) and 80% estimated 1 RM (2–5 repetitions) with 2–3 min of rest between sets. The testing protocol consisted of 3–5 attempts with 3–5 min between attempts with the highest mass lifted with proper form recorded as the 1 RM.

2.2.2. Acute heavy resistance exercise test (AHRET)

On the AHRET visit, participants reported to the laboratory after a 12-h overnight fast, including caffeine and alcohol. No exercise was allowed 48-h prior to testing, and participants were asked to consume 16–24 oz of water prior to the test. The AHRET is a standard protocol used in our laboratory [22,23,31] consisting of 6 sets of 10 repetitions in the Smith-squat exercise with 2 min rest periods between sets. With untrained subjects we used an initial load of 75% of the previously determined 1-RM for the first set of 10 repetitions and this load was adjusted on subsequent sets to accommodate fatigue and ensure completion of 10 repetitions per set with the target being a 10 RM for each set.

2.2.3. Blood sampling and storage

Before the AHRET, a catheter was inserted in an antecubital forearm vein. Venous blood was obtained from the subject at rest before the AHRET (Pre), and then immediately after (IP), 15 min (+15), and 30 min (+30) into recovery. Blood samples clotted at room temperature and were then centrifuged at 3000 RPM for 15 min at 4 °C, and aliquots were stored at −80 °C.

2.3. Biochemical analysis

2.3.1. Growth hormone

Growth hormone was analyzed by ELISA (CALBiotech, Spring Valley, CA), with sensitivity of 0.08 μg/L, intra-assay coefficient of variation (CV) of 6.3% and inter-assay CV of 4.9%. The assay wavelength was read at 450 nm on a Molecular Devices VERSAmax tunable microplate reader.