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Recovery using "float" from high intensity stress on growth hormone-like molecules in resistance trained men

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ABSTRACT

Objective: The purpose of this study was to examine the influence of a novel "floatation-restricted environmental stimulation therapy" (floatation-REST) on growth hormone responses to an intense resistance exercise stress.
Design: Nine resistance trained men (age: 23.4 ± 2.5 yrs.; height: 175.3 ± 5.4 cm; body mass: 85.3 ± 7.9 kg) completed a balanced, crossover-controlled study design with two identical exercise trials, differing only in post-exercise recovery intervention (i.e., control or floatation-REST). A two-week washout period was used between experimental conditions. Plasma lactate was measured pre-exercise, immediately post-exercise and after the 1 h. recovery interventions. Plasma iGH was measured pre-exercise, immediately-post exercise, and after the recovery intervention, as well as 24 h and 48 h after the exercise test. The bGH-L was measured only at pre-exercise and following each recovery intervention.
Results: For both experimental conditions, a significant ($P \leq 0.05$) increase in lactate concentrations were observed immediately post-exercise (~14 mmol • L-1) and remained slightly elevated after the recovery condition. The same pattern of responses was observed for iGH with no differences from resting values at 24 and 48 h of recovery. The bGH-L showed no exercise-induced changes following recovery with either treatment condition, however concentration values were dramatically lower than ever reported.
Conclusion: The use of floatation-REST therapy immediately following intense resistance exercise does not appear to influence anterior pituitary function in highly resistance trained men. However, the lower values of bGH suggest dramatically different molecular processing mechanisms at work in this highly trained population.

1. Introduction

First introduced in the 1970s, floatation-REST therapy has only recently become popular with its use in high-performance populations (e.g., elite athletics, military operators) seeking to accelerate the natural recovery process from high intensity physical and stressful demands. Floatation-REST therapy is thought to mediate recovery by inducing a state of deep relaxation through the reduction of external stimuli. With the individual essentially floating in an Epsom salt (magnesium sulfate) liquid filled tank or room maintained at 35 °C, the brain's perceptual boundaries of the body are blurred and also limits the need for thermoregulation. The concentration of salt is maintained at a specific gravity of 1.25–1.28 $g \cdot cm^{-3}$, providing a natural buoyancy that allows for effortless floatation. The full support of the water reduces the compressive forces of gravity and allows for significant muscle relaxation. Additionally, light and sound inputs can be removed, creating an environment of reduced visual, auditory, tactile, and gravitational inputs as used in the current study.

The deep relaxation created by floatation-REST often mimics initial sleep phases and its influence on growth hormone (GH) can even be stronger than the circadian system's influence which caused us to hypothesize that floatation-REST therapy may, in fact, influence both immunoreactive (iGH) and bioactive (bGH-L) isoforms of GH [1]. The endocrine system plays a major role in mediating high levels of force production via sympathetic/adrenergic responses but is also highly involved in the recovery process and anabolic signaling for tissue remodeling processes [2–5]. Interestingly reductions in lactate responses following an isokinetic resistance exercise test have been observed following a one-hour floatation-REST session when compared to passive

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recovery [6]. The known positive associations of lactate and iGH further supported our hypothesis that floatation-REST may influence on iGH and bGH-L recovery patterns [7]. The study of the 22 kD iGH during recovery from exercise, more specifically resistance exercise, has been extensively characterized yet less is known about bGH-L [5,8,9]. Furthermore, data on various types of growth hormone (GH) molecules have not been examined in highly resistance trained men or women. We have previously shown in women that short term resistance training (i.e., 6 months) did result in increases within both resting and acute exercise concentrations of bGH-L [10]. While not gender-specific, such data does open the possibility that with resistance training, highly adapted mechanisms mediating iGH and bGH-L secretions may be more sensitive to different forms of recovery such as floatation-REST therapy [11].

Recovery from exercise stress, usually within hours, is an important factor in many different scenarios; e.g., from health and fitness trainees to athletes. A host of different interventions have been used to enhance recovery from physical stress ranging from nutritional supplements to apparel using cold and/or compression or massage [12]. Increased use of this novel modality, floatation-REST, now called "floatation-restricted environmental stimulation therapy," has been more frequently used in the recovery process, yet our understanding of the modality is still in its early phases in many areas of study [6]. Outside its potential use for improving exercise recovery, it has been used to enhance relaxation and mediate return of physiological systems back to resting homeostasis [13–15]. To our knowledge, only one study has examined iGH responses with floatation-REST. In that study, examining resting responses of iGH over 240 min of floatation-REST was compared to a control rest condition, no significant differences in iGH concentrations were observed [16]. How floatation-REST may mediate exercise-induced recovery patterns of plasma GH responses remains unknown. Thus, due to the paucity of data on GH and floatation-REST, the purpose of the current study was to examine both iGH and bGH-L recovery response patterns to floatation-REST following an intense resistance exercise stress protocol in resistance trained men.

2. Materials and methods

2.1. Experimental approach and design

After the informed consent process was completed, each subject participated in two familiarization visits to get acquainted with the experimental procedures and to determine 1 repetition maximum (1RM) strength in the barbell squat. A balanced cross-over design was used with two identical exercise trials, but different post-exercise recovery interventions (i.e., control or float-REST). A two-week washout period was used between experimental conditions. For each trial, plasma lactate was measured at three timepoints—pre-exercise, immediately post-exercise, and after the 1 h. recovery intervention. Plasma iGH was measured at five timepoints—pre-exercise, immediately-post exercise, after the 1 h. recovery intervention, as well as 24 h and 48 h after the exercise test. Due to cost constraints the bGH-L was measured at two timepoints—pre-exercise and following recovery.

2.2. Participants

Nine resistance trained men who had used the squat exercise in their training were recruited to participate in this investigation. All participants were healthy and cleared of any injuries or medical complications that might confound the study findings. The subject characteristics ($n = 9$) were: age: 23.4 ± 2.5 yrs.; height: 175.3 ± 5.4 cm; body mass: 85.3 ± 7.9 kg, back squat 1RM: 150.2 ± 21.0 kg; squat-strength to body mass ratio: 1.76 ± 0.2. Each participant had been involved with a progressive heavy resistance training program, including the squat exercise, as represented by the high squat to body mass indicator. Each participant signed a consent form to participate after having the benefits and risks of the study explained to them. The study was approved by The Ohio State University's Institutional Review Board for the use of human subjects in research.

2.3. Procedures

2.3.1. Familiarization and preliminary exercise testing

Participants were carefully familiarized with all of the testing protocols prior to the initial testing. Following anthropometric measurements, a standardized dynamic warmup was completed followed by 3 to 5 single lifts to determine the one repetition maximum (1-RM) squat using a standard protocol previously described in detail [17]. The 1 RM squat was performed using an Olympic barbell and power rack (EliteFTS, London, OH, USA). A full range of motion was required, with the endpoint of the squat defined by a 90-degree relationship between the femur and the lower leg (i.e., knee at 90°). The depth requirement was confirmed through the use of a plum line that the bottom of the thigh had to reach in order for the repetition to be counted as correct technique.

2.3.2. Testing and controls

Testing started at 6 AM, with participants reporting to the laboratory after an 8 h overnight fast. Other restrictions included 12 h without caffeine, 24 h without alcohol/medications, and 72 h without exercise. Hydration status was assessed at each session via urine specific gravity (USG) using a handheld refractometer (Reichert, New York, NY, USA) with a minimum hydration requirement of USG ≤ 1.020 prior to testing. Participants remained fasted throughout the entire testing laboratory visit but were encouraged to consume water ad libitum. A 3-day diet log approach was used to assure the same dietary intakes prior to and during the experimental time frame. Prior to the first laboratory visit, participants were carefully instructed on how to record their food intakes and record the time of each meal or snack. Therefore, they started this 3-day diet record the day before or, in other words, 24 h before the start of the first laboratory testing visit. They then continued their recording for the next two days. Each participant then used that detailed 3-day dietary record and was asked to replicate it for their next experimental testing sequence. No other form of recovery interventions were allowed during each 48-h trial as typical with our work in muscle damage studies (e.g., no hot tubs, baths, showers > 10 min, ice, compression, heat pads, foam rollers, massage, medications, or other devices that may artificially enhance the speed of recovery) [18].

2.3.3. Exercise testing protocols

Prior to the acute heavy resistance exercise test protocol (AHRET), a standardized dynamic warmup was performed. Using the data from preliminary 1 RM testing, the acute AHRET consisted of 6 sets of 10 repetitions at 80% of the 1 RM in the squat exercise with two minutes rest between sets [2]. Using the same equipment as in the 1 RM testing, the protocol started at the prescribed loading, and then if the participant could not lift it through a full repetition spotters helped to complete the set and a 10% reduction in the following set was used.

2.3.4. Floatation recovery intervention

The floatation-REST sessions took place in our laboratory float room, which contains a deluxe Quest Float Suite (Superior Float Tanks, Norfolk, VA). The fiberglass tank contained approximately 300 gal of water, creating a depth of about 10 in. The water was saturated with greater than 1200 pounds of USP grade Epsom salt (MgSO4) and maintained at a specific gravity of ~1.26 g•cm^3. In-tank heaters provided a steady thermal environment (94 °F), approximating that of skin temperature. Each participant showered and inserted earplugs before entering the float tank in the nude to reduce any further sensory cues. The session was conducted in the absence of light and sound. At the completion of the hour session, a dim light came on within the tank, and a gentle voice alerted the participant that the session was over. The participant then exited the tank and showered before returning to the lab for a post-recovery blood draw.

2.3.5. Control recovery intervention

A relaxing yet sensory-stimulating environment matching the timing of the floatation-REST exposure was used for the control recovery intervention. The recovery intervention started 20 min after the

AHRET protocol. Participants sat in a reclining chair in a well-lit room and in order to provide a continuous auditory, and visual stimulation watched episodes of Planet Earth (BBC Earth) on a tablet computer. These carefully selected episodes of geographic landscapes and pleasant wildlife scenes were standardized across participants and chosen to minimize emotional arousal, excluding content that may have impeded relaxation [15]. At the end of the hour session, participants showered prior to returning to the laboratory for the post-recovery blood draw.

2.3.6. Blood sampling and storage

At each time point, participants sat in a partially reclined phlebotomy chair while blood was collected by a trained phlebotomist from an antecubital vein with a 21-gauge needle (BD Vacutainer Safety-Lok Blood Collection Set, Becton Dickinson and Company, Franklin Lakes, NJ). Plasma was immediately centrifuged at 2000 x *g* for 15 min (4 °C). Resulting plasma was aliquoted and stored at −80 °C. Samples underwent a single freeze-thaw cycle prior to the assay with duplicate analyses for all assays.

2.3.7. Biochemical analysis

Plasma lactate was analyzed using a *ChemWell* automated chemistry analyzer (Awareness Technology, Inc., Palm City, FL) using a lactate reagent kit (Pointe Scientific, Inc. is in Canton, MI, USA) with an intra-assay CV of 4.7%. Growth hormone immunoreactivity (iGH) was analyzed by ELISA using a sandwich enzyme immunoassay technique using a monoclonal antibody specific for human GH with a sensitivity of 2.10 pg•mL^{-1} (USA R&D Systems, Inc., Minneapolis, MN, USA). Samples were analyzed in duplicate at the appropriate absorbance using a Biotek Synergy H1 monochromatic multi-mode plate reader (Biotek, Winooski, VT, USA). The intra-assay variance was 4.3%.

We again used the same established bGH-L bioassay procedure previously reported and used many times over the past 19 years [10,19–21], which was originally described by Greenspan and Li [22]. Our definition of bGH-L is defined and restricted to in vivo growth responses of the test organism upon injection [11]. Briefly, female Sprague-Dawley rats (Hilltop Labs, Scottsdale, PA) were hypophysectomized at 26–28 days of age and were used 2 weeks after surgery. Two animals per cage were housed at the animal care facility at The Pennsylvania State University following handling guidelines for animal care in accordance with the approval of The Pennsylvania State University Institutional Animal Care and Use Committee. A 12:12-h light-dark cycle was employed with food and water ad libitum. Animals that had a body mass of < 80 g or > 100 g at the time of sample injection were not used in the bioassay. Criteria used as evidence for completeness of hypophysectomy were a 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 as determined by visual inspection under magnification. Animals were injected intraperitoneally once daily for 4 days with either 1) experimental plasma samples, 2) a standard GH preparation (United States Department of Agriculture bovine GH B-1 AFP 5200, 1.4 IU/mg at total doses of 5,15,45,90 μg), or 3) physiological saline (control). Duplicate samples (i.e., 2 animals) were used for the values determined for the standard curves and for each time point for each participant. At twenty-four hours after the last injection, the animals were killed, tibias removed and immersed in phosphate-buffered saline for further processing, tibial epiphyseal plates were stained with silver nitrate, and plate widths were measured in double-blind fashion using a computerized ocular micrometer (10 readings averaged across the plate width for each sample). Additionally, two separate independent readings for determination of the bGH-L values were accomplished in a blinded fashion by two different investigators. GH responses were expressed in terms of a purified human pituitary preparation (3.0 IU/mg). Assay variance was 7.1% and minimal detection limit of 5 μg·L^{-1}. Average tibial widths of animals injected with bovine GH standard were as follows (means ± SD): saline, 141.1 ± 6 μm, 5 μg,152.7 ± 8 μm, 15 μg 182.0 ± 11 μm,45 μg, 221.8 ± 12 μm, 90 μg 251.4 ± 9 μm, with the line of best fit for the standard curve was $r^2 = 0.95$.

$$\hat{y} = 1.00158 \times +169.39102$$

2.4. Statistical analyses

Means and standard deviations were calculated for each variable. A 2 × 2 (Treatment × Time) analysis of variance (ANOVA) was used to assess differences in bGH-L, a 2 × 3 (Treatment × Time) ANOVA was used to assess differences in lactate while a 2 × 5 ANOVA was used for iGH. In the event of a significant F test, pairwise comparisons were further evaluated using Fisher's LSD. Statistical significance for all analyses was set a priori at $p \leq 0.05$. All data met the assumptions of linear statistics. Data were analyzed using SPSS version 25 (IBM, Armonk, NY).

3. Results

The intense demands of the exercise test are displayed in Fig. 1, as evidenced by the significant increase in plasma lactate pre to post-exercise.

The changes in plasma iGH can be observed in Fig. 2. One can see in Fig. 2 panel A the full profile of plasma iGH over the experimental timeline. With variations in iGH observed in the prior resting study on floatation-REST, we wanted to show the individual responses for the two conditions which are presented in the remaining panels B to F [16]. Significant increases occurred as expected immediately post-exercise and then remained elevated at the post-recovery time point but returned to pre-exercise resting concentrations at 24 and 48 h after the exercise test. No significant differences were observed between the recovery conditions. Fig. 3 presents the responses of bGH-L to the post-exercise recovery interventions. Panel A shows the mean values for each treatment condition pre-exercise and after the 1 h. recovery. Panel B displays the individual responses. No significant differences were noted for treatment or time for bGH-L.

4. Discussion

In this study, we examined the exercise recovery process using a novel intervention of an acute floatation-REST recovery protocol. Our findings showed no recovery treatment differences in plasma GH concentrations as viewed from the perspective of two different assays after intense exercise that elevated blood lactate beyond 12 mmol• L^{-1}. Our iGH data followed the expected increases reflecting the relationship between iGH and blood lactate [7,23,24]. We had hypothesized that floatation-REST would impact GH responses based on a prior study by Morgan et al. [6] that showed floatation-REST reduced blood lactate concentrations after a repetitive isokinetic knee extension-flexion test (50 repetitions at 60o • s^{-1}).

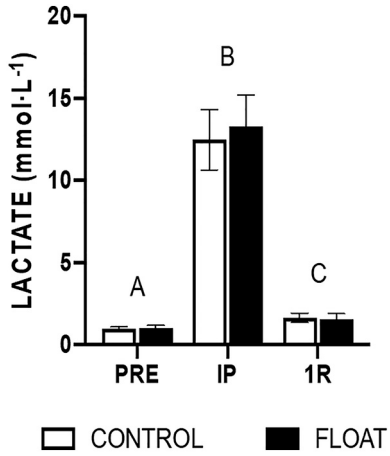

Fig. 1. Lactate (mmol·L^{-1}) response to exercise and recovery. Data presented as means ± SD. Values not sharing a common letter are significantly different ($P \leq 0.05$).

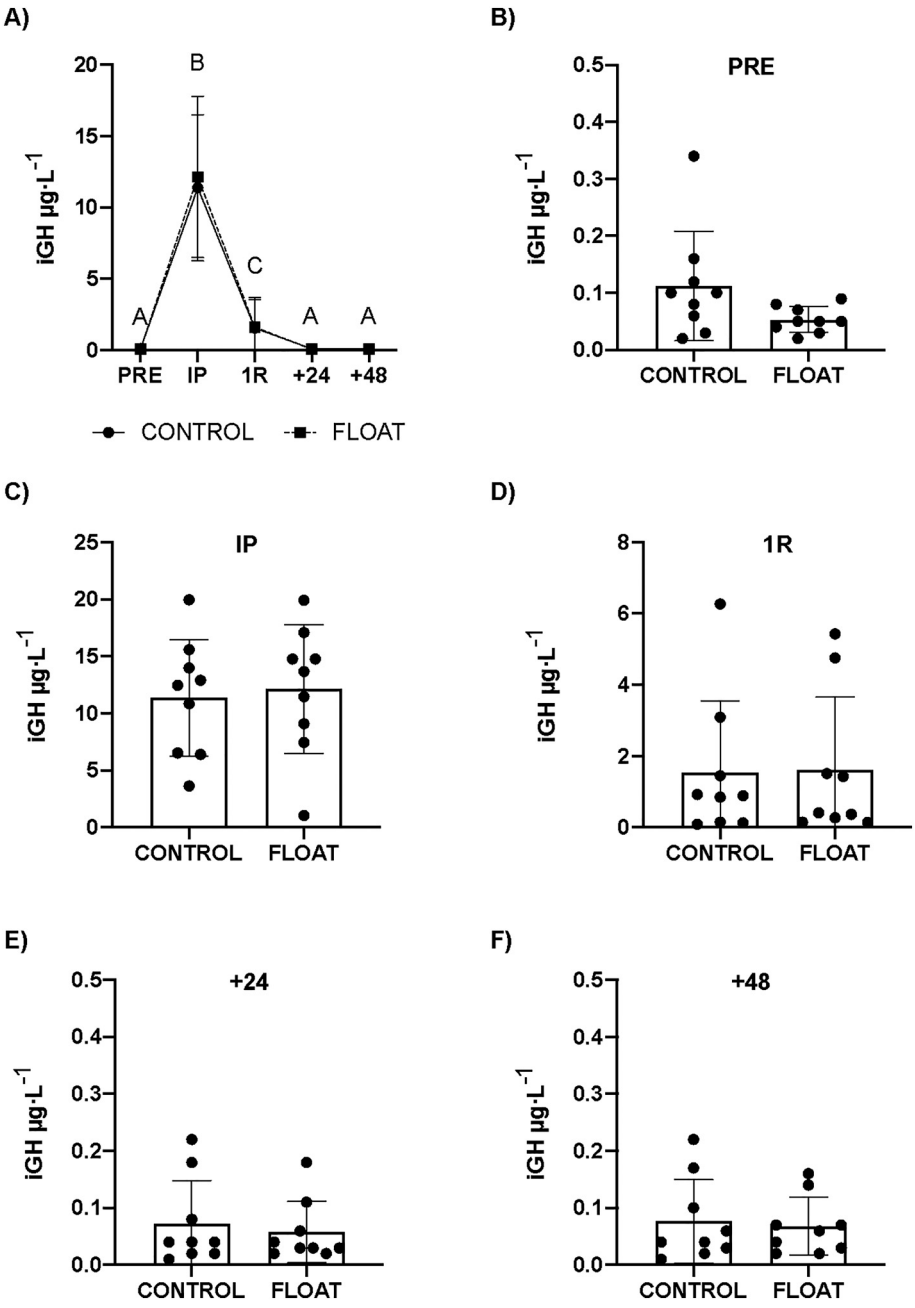

Fig. 2. iGH ($\mu g \cdot L^{-1}$) response to post exercise recovery. Data presented as means ± SD. Values not sharing a common letter are significantly different ($P \leq 0.05$). (A) represents the main effect for significant differences over time while the remaining letters display the difference between recovery conditions at each specific assessment timepoint (B) PRE (C) IP (D) IR (E) +24 (F) +48.

However, we saw no such differences. This may be due to the dissimilarities in the exercise testing protocols used, local isolation exercise with lactate levels less than 4 mmol• L^{-1} versus our whole body AHRET protocol with resulting dramatically higher blood lactate concentrations. However, from the perspective of resting iGH concentrations, our data did reflect those data reported by Schulz and Kaspar [25] that also found no treatment effects for floatation-REST compared to control conditions on resting concentrations of iGH over 240 min.

Interestingly, we also demonstrated a lack of a treatment effect for floatation-REST therapy for bGH-L. It is possible that the inability for us to be able to measure more time points in our design for bGH-L concentrations presented only part of the recovery pattern, especially for extended recovery time points. Thus, further study of the recovery process is needed to fully understand bGH-L recovery responses to intense exercise. However, this study does add to a series of studies done previously by our group investigating bGH-L in humans after aerobic or resistance exercise [10,19,20,26]. In one of our prior studies, untrained women, tested in the early follicular phase of their menstrual cycle, performed that same relative AHRET protocol and showed no acute exercise increases in bGH-L. [10] However, with short term resistance training program of 6 months, these same women demonstrated that significant increases in resting and acute AHRET exercise responses were observed for bGH-L. [10] In two prior studies, McCall et al. [27,28] had also reported increases after performing a small muscle group exercise protocols (unilateral isometric plantar flexor contractions) in male astronauts. Surprisingly in this study, we did not observe similar responses with highly resistance trained men. In fact, no increases in bGH-L concentrations were observed, and interestingly, the concentrations were dramatically lower even in comparison to untrained or moderately trained men or women [10,19,20,26–28]. This may reflect chronic

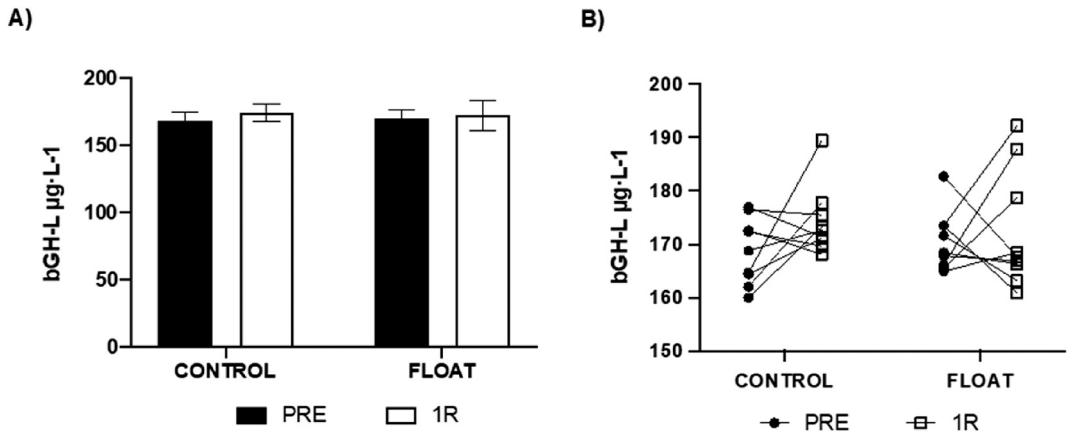

Fig. 3. bGH response ($\mu g \cdot L^{-1}$) to exercise recovery. Panel A presents bGH-L values for both the control and float treatment conditions, pre black bars, 1 h R post treatment, open bars. Panel B presents line graphs to display individual effects for each of the recovery conditions. Dark circles represent the pre-exercise (PRE) values while open squares represent values after the one hour recovery (1R).

training adaptations of the hypo-pituitary axis and somatotroph processing to intense high-level training or be an actual sexual dimorphism. An obvious difference among all studies has been the dramatically different concentrations of GH in the blood using the bGH-L assay. The concentrations are often 100 fold or higher than those using iGH assays [5]. This suggested that the different types of somatotrophs subpopulations may not be functioning in a similar manner [11,29]. How exercise stress modulates anterior pituitary function arising from the stimulation of the different somatotrophs remains speculative [5]. Obviously, more research will be needed to address these speculations, but as Moller and Jorgensen [30] have stated, "…there is a need for study of more direct effects of GH during exhaustive exercise".

However, in spite of these results, novel insights are presented in this study as the different concentrations of iGH and bGH-L may reflect differential activity from two different anterior pituitary somatotroph subpopulations [11,29]. While the resting and exercise response patterns of iGH were similar to what we and others have observed, we speculate that iGH released from lightly granulated [band I] somatotrophs may be very sensitive to the metabolic demands of the exercise protocol, more specifically, its glycolytic demands [7,23]. Our findings confirm the fact that dramatic increases in lactate, an indirect marker of acidotic changes in pH and H+ ion concentrations follow the same pattern of iGH responses with an elevation post-exercise and a return to resting values over an hour [8]. Moreover, even while no changes in the bGH-L concentrations were observed between floatation-REST treatment and control, we measured bGH-L concentrations that were orders of magnitudes lower than has ever been reported in the literature. We believe this finding deserves further attention simply because of the limited reports on plasma bGH-L in the literature [5,11].

The two major findings of our current investigation were totally unexpected. These were that: a) in a small group of highly trained men, plasma concentrations of bGH-L are not affected by heavy resistance exercise, and b) that bGH-L concentrations were anywhere from ~15 to 50 times less than those measured in other cohorts studied previously (see later). In spite of these unexpected results, plasma concentrations of iGH are similar to those reported repeatedly in the literature of other human exercise trials and are therefore unsurprising and expected [8,31].

Further studies in this area could benefit from our speculation here on how these findings might be possible. Thus, future investigations in this study of bGH-L need to carefully understand these underlying mechanisms that may well be operationally and differentially responsive to resistance exercise, training status, and sex of the participants. Thus the low plasma concentrations of bGH-L measured in this study might be attributed to exercise-induced resistance exercise stress on the endoplasmic reticulum (ER) of a subpopulation of pituitary somatotrophs. Intracellular degradation of misfolded bGH-L forms within the ER lumen would ultimately result in producing the low concentrations we observed in this study in our highly resistance trained men.

Protein concentrations in the ER lumen are generally thought to be high [~100 mg/ml]. In professional secretory cells, such as pancreatic endocrine and exocrine cells, as well as somatotrophs, the secretory protein rate is estimated to range in the millions of molecules/min [32]. A large amount of energy is required to maintain homeostasis in this crowded environment and is thought to operate "near the limits of its secretory capacity". During stress, the secretory capacity of a cell will be challenged. To meet physiological demands, dynamic, complex, and interconnected intracellular signaling pathways are utilized to regulate protein folding (e.g., chaperones, glucose regulatory protein, BiP, etc) and ultimately maintain homeostasis in that subcellular compartment. Somewhat surprisingly, the success rate of this unfolded protein response (UPR) program is often quite low, and ER-associated degradation pathways ensue [33,34]. Thus, our studies that show these high values compared to the current study may reflect this low success rate in UPR degradation due to metabolism and UPR sub pathways in specialized (secretory) cells that can proceed from regulation of differentiation to induction of apoptosis [32,35].

Studies investigating exercise-induced responses on ER stress indicate that the UPR programs in skeletal muscle may lead to atrophy, adaptation, and/or insulin resistance [36]. Others indicate that exercise may set a new balance between intracellular pathways leading to cell survival (e.g. apoptosis, autophagy) [37]. The study by Bugliani et al. [37] is particularly relevant from another viewpoint in that a chemical ER stressor, palmitic acid, was found to lead to decreased B cell granule volume density and obvious physiological consequences. However, this response could be reversed using autophagy- activating agents. Collectively, these observations invoke initial involvement of UPR mechanisms in the ER that ultimately lead to reversible effects on populations of cytoplasmic hormone-containing secretion granules. Because bGH-L is also contained in 300 nm diameter secretion granules, as well as being produced and secreted from subpopulations of somatotrophs, the exercise stressor in our current study would result in lower plasma concentrations of bGH-L.

Yet another study by Eizirik's group offers provocative evidence to show that exercise training may protect humans against ER stress [38]. Their underlying hypothesis proposes that exercise imposes "a mild stress," which, in fact, leads to protection on skeletal muscle, a hormetic response. Their data invoke increased expression of plasma IL-6 [a myokine] that, in turn, leads to STAT-3 activation pathways and downstream signaling. We suggest that the balance between "excessive" vs. "mild" exercise stress will have very different physiological outcomes in terms of bGH-L processing/concentrations. In conclusion,

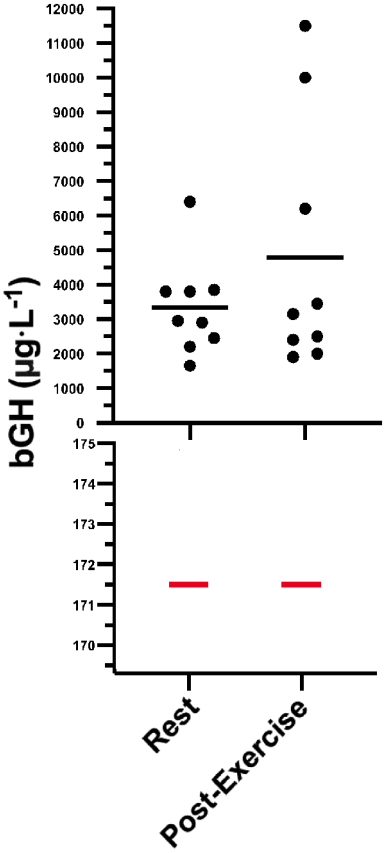

Fig. 4. A composite view of the different studies that have examined bGH-L with the current study mean values marked as a solid line showing the dramatically lower mean values observed in this study of highly resistance trained men at rest and in response to intense exercise stress.

although ER stress mechanisms are well understood (discussed above), appreciation of relationships between ER stress and exercise must still be considered in its infancy.

During exercise recovery, rapid changes are made in anabolic signaling, and glandular activity is dramatically accelerated [8]. The most frequent and important studies on this subject utilize models that involve mis-folded GH molecules that are intended to mimic iGHDII disease in humans. The 22 kDa GH isoform comprises all 5 exons spliced together to encode the major bioactive form of GH [39]. Splicing of exon 3 generates a 17.5 kDa isoform (lacking amino acids 32–71), and the human disease is caused by mutations in and around exon 3. This mutation leads to lower concentrations of the 22 kDa monomer. The 17.5 variant may be retained in the ER, and via UPR mechanisms, disrupts Golgi and impairs secretory protein trafficking. In severe mutations, GH storage is impaired, and ultimately somatotrophs are damaged to varying degrees [40–42]. These events eventually lead to loss of negative feedback control and up-regulation of GHRH expression; Petkovic et al. [40] characterize this disease as one of "autodestruction of somatotrophs".

When the misfolded GH is transfected into COS 7 cells, traffic from the ER to the Golgi is disrupted, and GH containing secretory granules are defective [42–44]. Electron microscopic images reveal severe damage to organelles; vacuolation is estimated to increase 2-3X [45]. Importantly, in vivo trials reveal that the growth curves of transgenic mice bearing these mutant forms of GH are significantly smaller. Thus, the flow of molecular events, from gene mutation - UPR – GH subcellular processing- secretion of defective hormone ↓ decreased growth seems established. Our collective studies, focused on a) details of GH organization/activity in rat pituitary samples coupled with b) study of effects of physical exercise in humans on circulating bGH-L [5,11] begin to offer initial glimpses as to how such molecular flows within the human GH pituitary system may be occurring.

A few rodent studies have addressed the potential role of hypothalamic releasing peptides on pituitary cell events associated with the UPR. Do et al. [46] reported that GnRH would induce UPR in a pituitary gonadotroph cell line by activating the ER stress sensor EIF2AK3. GH cell studies in rodents employing models involving either continuous water stress for 5 days or restraint/immobilization stress both definitively show significant changes in somatotroph populations. For example, GH cells from water-stressed animals are smaller, have increased numbers of secretion granules, decreased concentrations of serum GH, and up-regulated GHRH receptor responsiveness to added GHRH [47]. Another stress model, viz. animal restraint (both acute and repeated immobilization), show somewhat similar changes in the somatotroph population; viz. a 20% reduction in pituitary gland volume, a 20% decrease in volume density of somatotrophs and a 2 fold increase in the concentration of pituitary GH [48]. Although not specifically addressed in either of these animal stress studies, we speculate that the changes in a specific, highly granulated somatotroph subpopulation reported recently by our group are likely to utilize analogous ER stress mechanisms in response to severe stress [29].

Membrane-less organelles, formed by liquid-liquid phase separation (LLPS), are currently recognized as critical in a myriad of regulatory processes used by cells in health and disease. In 2019 we offered circumstantial evidence to support the idea that a 3.4 kDa peptide (shown to have bGH-L activity after purification from human anterior pituitary glands), might also function as a membrane-less organelle. Two recent studies offer additional roles that may well be related to the general issue of ER stress and cellular "noise". In one of these, the ER (a membrane-bound organelle) contacts stress granules (a membrane-less organelle) to influence cell behavior. The stress granule may store messenger RNAs that, although not actively translated, may do so when stress represses translation [49]. The other study is based on the knowledge that the expression of proteins inside cells is noisy, causing variability in protein concentration among identical cells [50]. Questioning the hypothesis that linked such noise to LLPS, the investigators used physical modeling to show that "… as long as phase separation is much faster than protein synthesis and degradation, droplets can reduce noise in protein concentration down to the Poisson limit".

A striking feature of our previous studies was the large variation in the range of circulating concentrations of plasma bGH-L [Fig. 4]; a range that makes it impossible to compare effects of physical exercise between experiments on this variable. In contrast, the current trial is noteworthy not only for the low concentrations of bGH-L but also for their lack of extreme variability [Fig. 4]. Obviously, more experimental trials testing exhaustive exercise regimens in highly trained individuals will be required to address and test the mechanistic ideas discussed herein (ER stress, membrane-less organelles, quality of released bGH-L molecules, and recovery from muscle injury) caused by these exercise protocols.

5. Conclusions

The response of both iGH and bGH-L to floatation-REST intervention showed no differences between treatments. The responses of iGH followed similar patterns to those previously observed with intense exercise stress. The lack of changes between treatment conditions reflected what had previously been shown with just resting conditions. The lack of changes in bGH-L to the exercise was surprising in these highly trained men; however, the dramatically lower concentrations has never been observed before. This suggested the potential for different types of molecular processing in the band 2 anterior pituitary somatotrophs in such highly trained men. As such, this study offers opportunities for a new line of research in humans studying adaptive mechanisms to intense exercise and training.

Declaration of Competing Interest

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References

[1] C.J. Morris, D. Aeschbach, F.A. Scheer, Circadian system, sleep and endocrinology, Mol. Cell. Endocrinol. 349 (2012) 91–104.
[2] D.N. French, W.J. Kraemer, J.S. Volek, et al., Anticipatory responses of catecholamines on muscle force production, J. Appl. Physiol. 102 (2007) (1985) 94–102.
[3] A. Pratesi, F. Tarantini, M. Di Bari, Skeletal muscle: an endocrine organ, Clin. Cases Miner. Bone Metab. 10 (2013) 11–14.
[4] M.R. Deschenes, W.J. Kraemer, C.M. Maresh, J.F. Crivello, Exercise-induced hormonal changes and their effects upon skeletal muscle tissue, Sports Med. 12 (1991) 80–93.
[5] W.J. Kraemer, N.A. Ratamess, W.C. Hymer, B.C. Nindl, M.S. Fragala, Growth hormone(s), testosterone, insulin-like growth factors, and cortisol: roles and integration for cellular development and growth with exercise, Front. Endocrinol. (Lausanne) 11 (2020) 33.
[6] P.M. Morgan, A.J. Salacinski, M.A. Stults-Kolehmainen, The acute effects of flotation restricted environmental stimulation technique on recovery from maximal eccentric exercise, J. Strength Cond. Res. 27 (2013) 3467–3474.
[7] S.E. Gordon, W.J. Kraemer, N.H. Vos, J.M. Lynch, H.G. Knuttgen, Effect of acid-base balance on the growth hormone response to acute high-intensity cycle exercise, J. Appl. Physiol. 76 (1994) (1985) 821–829.
[8] W.J. Kraemer, N.A. Ratamess, B.C. Nindl, Recovery responses of testosterone, growth hormone, and IGF-1 after resistance exercise, J. Appl. Physiol. 122 (2017) (1985) 549–558.
[9] U.J. Lewis, Growth hormone what is it and what does it do? Trends Endocrinol. Metab. 3 (1992) 117–121.
[10] W.J. Kraemer, B.C. Nindl, J.O. Marx, et al., Chronic resistance training in women potentiates growth hormone in vivo bioactivity: characterization of molecular mass variants, Am. J. Physiol. Endocrinol. Metab. 291 (2006) E1177–E1187.
[11] W.C. Hymer, M.J. Kennett, S.K. Maji, et al., Bioactive growth hormone in humans: controversies, complexities and concepts, Growth Hormon. IGF Res. 50 (2020) 9–22.
[12] F. Brown, C. Gissane, G. Howatson, K. van Someren, C. Pedlar, J. Hill, Compression garments and recovery from exercise: a meta-analysis, Sports Med. 47 (2017) 2245–2267.
[13] A. Kjellgren, J. Westman, Beneficial effects of treatment with sensory isolation in flotation-tank as a preventive health-care intervention - a randomized controlled pilot trial, BMC Complement. Altern. Med. 14 (2014) 417.
[14] J.S. Feinstein, S.S. Khalsa, H.W. Yeh, et al., Examining the short-term anxiolytic and antidepressant effect of Floatation-REST, PLoS One 13 (2018) e0190292.
[15] J.S. Feinstein, S.S. Khalsa, H. Yeh, et al., The elicitation of relaxation and interoceptive awareness using floatation therapy in individuals with high anxiety sensitivity, Biol. Psychiatry Cogn. Neurosci. Neuroimag. 3 (2018) 555–562.
[16] P. Schulz, C.H. Kaspar, Neuroendocrine and psychological effects of restricted environmental stimulation technique in a flotation tank, Biol. Psychol. 37 (1994) 161–175.
[17] W.J. Kraemer, N.A. Ratamess, A.C. Fry, D.N. French, Strength training: development and evaluation of methology, in: P.J. Maud, C. Foster (Eds.), Physiological Assessment of Human Fitness Human Kinetics Pub, Champaign, IL, 2006, pp. 119–150.
[18] W.J. Kraemer, M.F. Joseph, J.S. Volek, et al., Endogenous opioid peptide responses to opioid and anti-inflammatory medications following eccentric exercise-induced muscle damage, Peptides 31 (2010) 88–93.
[19] W.C. Hymer, W.J. Kraemer, B.C. Nindl, et al., Characteristics of circulating growth hormone in women after acute heavy resistance exercise, Am. J. Physiol. Endocrinol. Metab. 281 (2001) E878–E887.
[20] G.A. Thomas, W.J. Kraemer, M.J. Kennett, et al., Immunoreactive and bioactive growth hormone responses to resistance exercise in men who are lean or obese, J. Appl. Physiol. 111 (2011) (1985) 465–472.
[21] W.J. Kraemer, M.J. Kennett, A.M. Mastro, et al., Bioactive growth hormone in older men and women: It's relationship to immune markers and healthspan, Growth Hormon. IGF Res. 34 (2017) 45–54.
[22] F.S. Greenspan, C.H. Li, et al., Bioassay of hypophyseal growth hormone; the tibia test, Endocrinology 45 (1949) 455–463 (illust).
[23] W.J. Kraemer, L. Marchitelli, S.E. Gordon, et al., Hormonal and growth factor responses to heavy resistance exercise protocols, J. Appl. Physiol. 69 (1990) (1985) 1442–1450.
[24] W.J. Kraemer, S.E. Gordon, S.J. Fleck, et al., Endogenous anabolic hormonal and growth factor responses to heavy resistance exercise in males and females, Int. J. Sports Med. 12 (1991) 228–235.
[25] P. Schulz, S. Lustenberger, R. Degli Agosti, R.W. Rivest, Plasma concentration of nine hormones and neurotransmitters during usual activities or constant bed rest for 34 H, Chronobiol. Int. 11 (1994) 367–380.
[26] S.E. Gordon, W.J. Kraemer, D.P. Looney, S.D. Flanagan, B.A. Comstock, W.C. Hymer, The influence of age and exercise modality on growth hormone bioactivity in women, Growth Hormon. IGF Res. 24 (2014) 95–103.
[27] G.E. McCall, C. Goulet, R.E. Grindeland, J.A. Hodgson, A.J. Bigbee, V.R. Edgerton, Bed rest suppresses bioassayable growth hormone release in response to muscle activity, J. Appl. Physiol. 83 (1997) (1985) 2086–2090.
[28] G.E. McCall, C. Goulet, R.R. Roy, et al., Spaceflight suppresses exercise-induced release of bioassayable growth hormone, J. Appl. Physiol. 87 (1999) (1985) 1207–1212.
[29] R.E. Grindeland, W.J. Kraemer, W.C. Hymer, Two types of rat pituitary somatotrophs secrete growth hormone with different biological and immunological profiles, Growth Hormon. IGF Res. 36 (2017) 52–56.
[30] N. Moller, J.O. Jorgensen, Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects, Endocr. Rev. 30 (2009) 152–177.
[31] A.C. Hackney, H.C. Davis, A.R. Lane, Growth hormone-insulin-like growth factor axis, thyroid axis, prolactin, and exercise, Front. Horm. Res. 47 (2016) 1–11.
[32] J. Wu, R.J. Kaufman, From acute ER stress to physiological roles of the unfolded protein response, Cell Death Differ. 13 (2006) 374–384.
[33] C. Hetz, F.R. Papa, The unfolded protein response and cell fate control, Mol. Cell 69 (2018) 169–181.
[34] O. Chakrabarti, N.S. Rane, R.S. Hegde, Cytosolic aggregates perturb the degradation of nontranslocated secretory and membrane proteins, Mol. Biol. Cell 22 (2011) 1625–1637.
[35] D. Ariyasu, H. Yoshida, Y. Hasegawa, Endoplasmic reticulum (ER) stress and endocrine disorders, Int. J. Mol. Sci. 18 (2017).
[36] K.R. Bohnert, J.D. McMillan, A. Kumar, Emerging roles of ER stress and unfolded protein response pathways in skeletal muscle health and disease, J. Cell. Physiol. 233 (2018) 67–78.
[37] M. Bugliani, S. Mossuto, F. Grano, et al., Modulation of autophagy influences the function and survival of human pancreatic Beta cells under endoplasmic reticulum stress conditions and in type 2 diabetes, Front. Endocrinol. (Lausanne) 10 (2019) 52.
[38] F.M.M. Paula, N.C. Leite, P.C. Borck, et al., Exercise training protects human and rodent beta cells against endoplasmic reticulum stress and apoptosis, FASEB J. 32 (2018) 1524–1536.
[39] G.P. Baumann, Growth hormone isoforms, Growth Hormon. IGF Res. 19 (2009) 333–340.
[40] V. Petkovic, M. Godi, D. Lochmatter, et al., Growth hormone (GH)-releasing hormone increases the expression of the dominant-negative GH isoform in cases of isolated GH deficiency due to GH splice-site mutations, Endocrinology 151 (2010) 2650–2658.
[41] W.C. Hymer, R. Grindeland, I. Krasnov, et al., Effects of spaceflight on rat pituitary cell function, J. Appl. Physiol. 73 (1992) (1985) 151S–157S.
[42] T.K. Graves, S. Patel, P.S. Dannies, P.M. Hinkle, Misfolded growth hormone causes fragmentation of the Golgi apparatus and disrupts endoplasmic reticulum-to-Golgi traffic, J. Cell Sci. 114 (2001) 3685–3694.
[43] K. Kannenberg, N.E. Wittekindt, S. Tippmann, H. Wolburg, M.B. Ranke, G. Binder, Mutant and misfolded human growth hormone is rapidly degraded through the proteasomal degradation pathway in a cellular model for isolated growth hormone deficiency type II, J. Neuroendocrinol. 19 (2007) 882–890.
[44] L. McGuinness, C. Magoulas, A.K. Sesay, et al., Autosomal dominant growth hormone deficiency disrupts secretory vesicles in vitro and in vivo in transgenic mice, Endocrinology 144 (2003) 720–731.
[45] D. Ariyasu, H. Yoshida, M. Yamada, Y. Hasegawa, Endoplasmic reticulum stress and apoptosis contribute to the pathogenesis of dominantly inherited isolated GH deficiency due to GH1 gene splice site mutations, Endocrinology 154 (2013) 3228–3239.
[46] M.H. Do, S.J. Santos, M.A. Lawson, GNRH induces the unfolded protein response in the LbetaT2 pituitary gonadotrope cell line, Mol. Endocrinol. 23 (2009) 100–112.
[47] T. Ogawa, H. Sei, H. Konishi, N. Shishioh-Ikejima, H. Kiyama, The absence of somatotroph proliferation during continuous stress is a result of the lack of extracellular signal-regulated kinase 1/2 activation, J. Neuroendocrinol. 24 (2012) 1335–1345.
[48] S. Trifunovic, I. Lakic, P. Vujovic, et al., Morphofunctional parameters of rat somatotrophes after acute and repeated immobilization or restraint stress, Acta Histochem. 121 (2019) 29–34.
[49] B. Kornmann, K. Weis, Liquid but not contactless, Science 367 (2020) 507–508.
[50] A. Klosin, F. Oltsch, T. Harmon, et al., Phase separation provides a mechanism to reduce noise in cells, Science 367 (2020) 464–468.