Immunoreactive and bioactive growth hormone responses to resistance exercise in men who are lean or obese

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Growth hormone (GH) is a family of polypeptide variants, with >100 forms known to exist in circulation that is released from the anterior pituitary in pulsatile manner (41). Heterogeneity of molecules in the GH family results from transcriptional and translational processing in the pituitary somatotroph, which, in turn, may lead to generation of GH isoforms in the circulation. Factors such as age, sex, body composition, sleep, nutritional status, and insulin-like growth factor I (IGF-I) can dramatically affect the pulsatility of GH secretion (6). In healthy, lean individuals, GH is released in three to six pulses throughout the day, with the most pronounced release occurring nocturnally (19, 47). This pulsatile release has multiple effects on peripheral tissue, with one of its key functions being regulation of body fat and lean tissue (30, 47). Recent work in humans has demonstrated that continuous GH administration primarily augments hepatic and muscle IGF-I, while only pulsatile GH administration augments adipose tissue lipolysis (47). One of the most potent stimulators of pulsatile GH release is exercise (48). Exercise has been shown to modify the activity and molecular character of GH variants in circulation (21). However, much remains unknown about the biological action of these GH variants.

Overall GH levels and those stimulated by cardiovascular exercise have been shown to be decreased in obese individuals. It is also well established that increased body fat and body mass index (BMI) suppress spontaneous and stimulated serum GH concentrations but that these pulsatile responses return to normal with reduction to normal levels of body weight (43, 46). Normal GH response to physiological stimuli such as sleep and cardiovascular exercise appears to be impaired in obese individuals (44). Lower cardiovascular exercise-induced GH secretions have been attributed to a reduction in the mass of GH secreted per pulse (26, 50). Alterations in the GH response to exercise may be related to severity of obesity (42) and the progression of obesity status from simple (excess adiposity) to physiological alterations in metabolic homeostasis. In obese individuals, reductions in spontaneous GH secretion (as much as 6% for each unit increase in BMI) and the half-life of circulating GH have been reported (decreases from 15 to 11 min) (46). Additionally, the biological effects of GH in obese humans may not be determined merely by the overall magnitude of GH output but, rather, by complex pulsatile patterns of GH presentation to peripheral tissues (47).

GH has been used as a therapy to reduce total fat mass and abdominal fat mass, with the effect being more pronounced in males than in females (5). The dosages of GH administration have varied, but it seems that low dosages are effective in reducing fat mass and abdominal fat mass (47). GH administration has been successful in reducing abdominal/visceral fat deposits (28) and can prove to be beneficial at reducing obesity-related comorbidities that may be caused by the increased abdominal/visceral adipose tissue (6). A low-dose GH treatment in addition to calorie restriction has been shown to accelerate body fat loss and GH secretion (27). Kim et al. (27) and Johannsson et al. (25) demonstrated that low-dose GH administration and calorie restriction can favorably affect visceral fat levels and may be useful in reducing some of the metabolic complications associated with visceral obesity. GH
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is stimulated by exercise, and exercise may offer an additional avenue for increasing GH in therapeutic approaches for weight loss.

Aerobic exercise and resistance exercise (RE) have demonstrated the ability to increase circulating GH in lean individuals. However, investigations examining responses to both modes of exercise within the same participants have shown that greater GH concentrations occur in response to a RE stimulus (7). In addition, in response to a cardiovascular exercise stimulus, it has been prolifically established that obese individuals’ concentrations are blunted (17). However, only one prior study has examined immunoreactive GH (iGH; i.e., assays that use an antibody-based detection system) response to RE in obese individuals (43). It is important to point out that our understanding of the concentrations of GH variants stimulated by exercise has developed primarily through the use of immunoassays (enzyme immunoassay and RIA), which present an incomplete picture of the GH response to exercise (21). Previous investigations have thus established that RE can increase circulating iGH concentrations in lean individuals (8, 9), but whether bioactive GH (bGH) is also affected is less understood. McCaffrey et al. (37, 38) found an increase in bGH, but not iGH, in men after acute physical activity. Different assays signal different target end points of interaction; thus, divergence can exist for the magnitude and response of human GH to exercise or physiological stimuli (11, 15, 23, 30). Examining the bGH response to RE in obese relative to lean men may clarify the type of altered GH response in obese men.

GH assessment is further complicated by the fact that, in blood, GH is also bound to binding proteins. These GH-specific binding proteins (GHBP) (4) bind in a 1:1 ratio, which is increased in individuals who are GH-deficient, and GHBP concentrations can vary due to nutritional and metabolic status (13). Thus these binding proteins may play an important role in understanding the lower GH values observed in obese individuals (4). This notion is consistent with the observation that obesity is associated with high concentrations of GHBP (20). In normal-weight individuals, GHBP is positively correlated with body fat percentage and intra-abdominal visceral fat (14). While GHBP may help mediate the lower GH concentrations in obese individuals, no prior research has examined whether changes in GHBP concentrations differ in response to exercise in lean vs. obese men.

The purpose of this study was to examine whether a RE protocol equated on intensity and volume across groups with adequate familiarization would demonstrate differences in GH variables in lean and obese men. Chronic exercise has demonstrated bGH effects in women and in male astronauts (23, 32, 33, 37, 38). Studies have also reported that iGH is blunted in obese individuals, no prior research has examined whether alterations in bGH or iGH in obese vs. lean men in response to RE?

METHODS

Participants and Preliminary Screening Procedures

The study included 18 healthy (9 lean and 9 obese) male volunteers who were free of existing acute or chronic illness (known cardiovascular, endocrine, or metabolic disease), did not take any medications or dietary supplements, and were nonsmokers. A preparticipation health history and a physical activity questionnaire were used to screen all volunteers for inclusion in the study. The study was approved by the University of Connecticut Institutional Review Board; this approval included acceptance of the Pennsylvania State University’s Institutional Animal Care and Use Committee’s approval for use of animals in the study as assay tools. All human participants gave informed written consent after being informed of all the risks and benefits of the study. All participants were untrained (having not participated in a resistance-training protocol for ≥6 mo) and not currently participating in any structured exercise program more than twice per week and >30 min per session. Subject characteristics are presented in Table 1.

Procedures

Body composition analysis. Participants were weighed on an electronic scale, with weight recorded to the nearest 0.1 kg, and height was measured with a standard stadiometer. Fat-free mass, fat mass, and percent body fat were determined using dual-energy X-ray absorptiometry (GE Lunar Prodigy Advance, Madison, WI). Percent body fat was calculated as soft tissue fat mass divided by the sum of soft tissue fat mass, soft tissue lean body mass, and bone mineral content. Additionally, regional analysis of the abdomen was assessed by placing a box between L1 and L4 using commercial software (enCORE version 6.00.270). Quality assurance was assessed by analyzing a phantom spine provided by the software company, and daily calibrations were performed before all scans using a calibration block provided by the software company. Coefficient of variation of percent fat on repeat scans on a group of men ranging from 9.0 to 50.2% in our laboratory was 0.55%.

RE protocol and 10-repetition maximum testing. All participants completed five exercise protocol visits to familiarize them with the protocol, remove any novelty effects, and establish a 10-repetition maximum (RM) prior to the acute RE protocol. The resistance-training protocol consisted of squat, bench press, leg curls, dumbbell rows, dumbbell shoulder press, and dumbbell step-up exercises. These exercises were chosen not only for their ability to produce the desired hormonal response, but also because they represent upper and lower body exercises commonly employed in normal resistance-training routines. Several investigators have noted the importance of including upper and lower body (small and large muscle group) exercises in a previously identified differences in GHBP in obese men. In addition, what role does GHBP play, if any, in understanding alterations in bGH or iGH in obese vs. lean men in response to RE?

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>20.1 ± 2.1</td>
<td>21.0 ± 2.1</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>71.7 ± 5.8</td>
<td>107.1 ± 14.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>177.8 ± 8.7</td>
<td>176.2 ± 3.6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.8 ± 1.36*</td>
<td>35.58 ± 4.45</td>
</tr>
<tr>
<td>Fat body mass, kg</td>
<td>10.1 ± 2.34*</td>
<td>38.7 ± 10.6</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>59.0 ± 6.0</td>
<td>63.71 ± 6.8</td>
</tr>
<tr>
<td>% Body fat</td>
<td>14.7 ± 3.54*</td>
<td>37.4 ± 5.6</td>
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</table>

Values are means ± SD of 9 men in each group. BMI, body mass index.

*Significantly different from obese (P < 0.05).
GH-stimulating acute protocol (29, 34). Therefore, during the acute RE protocol, the participants performed 3 sets of 10 repetitions at 85–95% of previously determined 10 RM for each exercise. Previous research indicates that loads that fall below this level would not adequately stimulate a GH response (31).

**Acute RE protocol.** At the acute RE protocol visit, the participants entered the laboratory after a 12-h overnight fast, and all exercise tests were performed between 0700 and 1100 (24-h clock). They had been instructed to abstain from drinking alcohol or ingesting high doses of caffeine (>2 cups per day) for 24 h prior to the testing protocol. Participants were required to consume 1 liter of water on the night prior to testing and 0.5 liter on the day of visit 6. Adequate hydration (urine specific gravity ≤1.020) was confirmed prior to venous catheterization via urine refractometry (2). Before the acute exercise protocol, an intravenous catheter was inserted into the antecubital vein. Blood samples were taken 15 min after initial placement of the catheter. Participants then engaged in the acute RE protocol. Blood samples were collected at six time points: preexercise, midexercise, immediately postexercise, and 30, 70, 110 min postexercise. Time points were chosen to correspond to differences identified in prior studies (43). The cannula was kept patent with a 10% heparin solution injection. Prior to each blood draw, 3 ml of blood were extracted to avoid inadvertent saline dilution of the blood sample. Whole blood was collected by appropriate tubes with preservatives for processing for determination of the various hormones and metabolites and was centrifuged at 1,500 g for 15 min at 4°C. After centrifugation, plasma and serum were immediately separated into aliquots and added to preservative-treated tubes, flash-frozen in liquid nitrogen, and stored at −80°C until later analysis.

**Biochemical analyses.** Serum samples were analyzed in duplicate for iGH and high-affinity GHBP (Diagnostic Systems Laboratory, Webster, TX) via commercially available ELISAs. Coefficients of variation for these assays were 6.9% and 4.5%, respectively. The human GHBP ELISA has a minimum detection limit of 1.69 pmol/l, and the sensitivity for the iGH assay was 0.03 ng/ml. Serum and plasma samples were thawed only once before analysis.

**bGH.** Concentrations of bGH in plasma samples were determined by the method of Greenspan and Li (18) exactly as reported by our laboratory. Plasma samples were thawed only once before analysis. Concentrations of bGH were determined by the method of Greenspan and Li (18) exactly as reported by our laboratory.

**Immunoreactive GH**

A mixed ANOVA (group × time) was performed to examine changes in iGH. The interaction effect for group × time was not significant ($F = 0.386$, degrees of freedom (df) $= 4,64$, $P = 0.818$, $\eta^2 = 0.024$; Fig. 1A). The main effect of time was significant ($F = 29.00$, df $= 4,64$, $P < 0.001$, $\eta^2 = 0.610$). The main effect of group was not significant ($F = 0.414$, df $= 1,16$, $P = 0.529$, $\eta^2 = 0.025$). Serum iGH concentrations were not different at rest between lean and obese participants (Fig. 1A). iGH was significantly lower at preexercise than at midexercise and immediately postexercise. iGH was significantly lower at midexercise than immediately postexercise and greater than at 110 min postexercise. iGH immediately postexercise was significantly different from all other time points. Additionally, iGH at 50 min postexercise was different from 110 min postexercise. A one-way ANOVA examined differences between groups in iGH area under the curve (AUC). No significant differences were found between groups ($F = 0.645$, df $= 1,17$, $P = 0.434$, $\eta^2 = 0.042$; Fig. 2A).

**Bioactive GH**

A mixed ANOVA (group × time) was performed to examine changes in bGH. The interaction effect for group × time was not significant ($F = 1.95$, df $= 4,64$, $P = 0.113$, $\eta^2 = 0.109$). The main effect of time was not significant ($F = 0.527$, df $= 4,64$, $P = 0.716$, $\eta^2 = 0.032$). However, the main effect of group was significant ($F = 27.24$, df $= 1,16$, $P < 0.001$, $\eta^2 = 0.630$). Lean participants had significantly greater bGH than obese participants at midexercise, immediately postexercise, and 50 min postexercise (Fig. 1B). A one-way ANOVA was used to test differences in bGH AUC. The between-groups effects indicate significant differences ($F = 11.03$, df $= 1,16$, $P = 0.004$, $\eta^2 = 0.408$), consistent with the main effect from the mixed ANOVA. Lean participants had significantly greater bGH than obese participants. Mean differences are depicted in Fig. 2B.
GH-Binding Protein

A mixed ANOVA (group × time) was performed to examine changes in GHBP. The interaction effect for group × time was not significant \( (F = 1.89, \text{df} = 4.64, P = 0.123, \eta^2 = 0.106) \). The main effect of time was not significant \( (F = 2.18, \text{df} = 4.64, P = 0.081, \eta^2 = 0.120) \). However, the main effect of group was significant \( (F = 46.11, \text{df} = 1.16, P < 0.001, \eta^2 = 0.742) \). GHBP was significantly greater in obese than lean participants at all time points (Fig. 1C). A one-way ANOVA was used to test differences in GHBP AUC. The between-groups effects indicate significant differences \( (F = 36.5, \text{df} = 1.17, P < 0.001) \), consistent with the main effect from the mixed ANOVA. Obese participants had significantly greater GHBP AUC than lean participants. Mean differences are depicted in Fig. 2C.

Correlations between iGH, bGH, and GHBP AUCs were examined in lean and obese men. In lean men, iGH was significantly positively correlated with bGH \( (r = 0.70) \). Additionally, GHBP was significantly positively correlated with bGH \( (r = 0.78) \) and iGH \( (r = 0.79) \). However, in obese men GHBP was significantly negatively correlated with iGH \( (r = -0.72) \). Additional correlations examined GH variables across the acute exercise time points (preexercise, at midexercise, and immediately postexercise) for each group. Correlations between these variables for lean and obese men are presented in Table 2. bGH at preexercise is negatively associated with GHBP at midexercise for lean and obese men \( (r = -0.63 \text{ and } -0.70, \text{respectively}) \). In lean men, GHBP at preexercise is positively associated with iGH at midexercise and immediately postexercise \( (r = 0.71 \text{ and } 0.67, \text{respectively}) \); in the obese group, GHBP at preexercise is negatively associated with iGH at midexercise and immediately postexercise \( (r = -0.65 \text{ and } -0.66, \text{respectively}) \).

Table 3 displays the correlations between GH variable AUCs and body composition in all participants. bGH is significantly negatively associated with BMI \( (r = -0.63) \), body fat percentage \( (r = -0.56) \), and android-to-gynoid ratio \( (r = -0.50) \). GHBP is significantly positively associated with the body composition variables BMI \( (r = 0.76) \), body fat percentage \( (r = 0.81) \), and android-to-gynoid ratio \( (r = 0.70) \).

DISCUSSION

The findings of this investigation indicate that whereas an acute RE protocol can stimulate iGH of the same magnitude in sedentary lean and obese men, the overall concentrations of bGH differ between obese and lean men. However, in contrast to iGH, an exercise-induced increase in bGH levels was not
found. Additionally, this group difference was also reflected in overall concentrations of GHBP. Here, similar to previous studies, it was determined that GHBP concentrations were correlated to BMI, body fat, and body composition and higher intra-abdominal fat levels (12–14). GHBP concentrations were significantly greater in obese than lean men. No prior study has examined bGH and GHBP simultaneously or in obese vs. lean groups, although researchers have speculated that the currently unknown biological function of GHBP may be related to the regulation of GH bioactivity (45). Associations between GH system variables in lean men are positive and statistically significant. However, in obese men, there appears to be a disturbance of the associations between GH system variables. Therefore, our present findings are novel, in that they support assertions that GHBP concentrations and GH concentrations are inversely associated with one another in obese individuals. In addition, GHBP concentrations are positively and bGH concentrations are negatively associated with total fat mass. These associations may mediate the GH suppression in obese individuals that has been identified in prior research findings.

In contrast to our findings (Fig. 1), Ormsbee et al. (43) recently reported that lean, but not obese, men had higher serum levels of iGH after a bout of RE consisting of 2 sets of 10 repetitions and a third set to muscular exhaustion employing a load equaling 85% of the individual’s established 10 RM. Since neuromuscular recruitment during RE may be a factor influencing GH secretion (7) and since activation of even small muscle groups results in marked increases in GH release (29), we suggest that the exercise protocol used by Ormsbee et al. was not sufficient to cause a GH response in the obese participants. For this reason, our protocol utilized large (back squat) and small (bench press and shoulder press) muscle groups. Variables that influence GH secretion include 1) the amount of muscle mass recruited, which directly affects the metabolic and hormonal responses to RE (3), and 2) the total work performed, which affects GH release (1). Still other variables include intensity, volume, rest interval between sets, and magnitude of total work done.

Prior research has identified differences in GH system function between lean and obese individuals. Frystyk et al. (16) speculated that GHBP may regulate GH bioactivity via an unknown mechanism. Therefore, we examined correlations between bGH, iGH, and GHBP over the course of the exercise protocol to explore whether different feedback mechanisms may play a role in differences in GH secretion. We suggest that the inverse relationship between bGH and GHBP concentrations in obese, but not lean, men (Table 2) indicates suppression of release of a biologically active isoform of GH. Whether GHBP itself may control that GH release at the level of the pituitary gland is unknown. In addition, because of the prior findings of altered GH physiology associated with markers of obesity, we explored correlations between bGH, iGH, GHBP, and different time points in the RE protocol and between these variables and body composition variables. Others have suggested that visceral adiposity may account for lower iGH in the circulation (24), but prior findings are mixed on this point. One study found no association between 24-h circulating iGH concentrations and waist-to-hip ratio (49), while another reported associations between increased visceral fat and decreased pulsatile GH release (36). This is the first study, to our knowledge, to explore all three variables in the same sample. We found that, consistent with prior speculation on GHBP, adiposity, and iGH, GHBP was negatively associated with iGH AUC and iGH response at midexercise and immediately postexercise in obese men only. In lean men, preexercise GHBP was positively associated with the iGH response at
midexercise and immediately postexercise. These findings highlight the complexity of the associations between GH variables and the alterations associated with increased adiposity.

Increased circulating GH might be an effective way to reduce total and abdominal visceral fat mass in obese individuals and improve cardiometabolic risk (40). Administration of recombinant GH has been a form of therapy in obese adults, but it is not without associated risks (39). It has been linked to arthralgia, peripheral edema, and paresthesia, as well as increases in fasting plasma glucose and fasting insulinemia (10). Finding a method to stimulate GH in obese individuals that does not involve administration of recombinant GH and carries lower risk would, therefore, be very beneficial. The present findings are encouraging in that regard, as RE is a mode of exercise that may present as a more logical starting point for a nonpharmacological intervention.

In summary, our results argue for the importance of measuring iGH, bGH, and GHBP levels to further understand and differentiate their possible roles in obese and lean individuals. Although a functional role for GHBP in the bioactivity of GH remains speculative, it is important to measure iGH, bGH, and GHBP to further understand and differentiate their possible roles in obese and lean individuals. Overall, these results integrate with prior research to suggest that bGH represents a chronic bioactivity adaptation potentially

### Table 2. Correlations between GH variables at preexercise, at midexercise, and immediately postexercise for lean and obese men

<table>
<thead>
<tr>
<th>GHBP</th>
<th>Pre</th>
<th>Mid</th>
<th>IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean</td>
<td>0.18</td>
<td>0.19</td>
<td>0.07</td>
</tr>
<tr>
<td>Obese</td>
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<td>0.19</td>
<td>0.09</td>
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<table>
<thead>
<tr>
<th>iGH</th>
<th>Pre</th>
<th>Mid</th>
<th>IP</th>
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<tbody>
<tr>
<td>Lean</td>
<td>0.76*</td>
<td>0.81*</td>
<td>0.70*</td>
</tr>
<tr>
<td>Obese</td>
<td>0.70*</td>
<td>0.15</td>
<td>0.15</td>
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<table>
<thead>
<tr>
<th>GHBP</th>
<th>Pre</th>
<th>Mid</th>
<th>IP</th>
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<tbody>
<tr>
<td>Lean</td>
<td>0.61</td>
<td>0.62</td>
<td>0.08</td>
</tr>
<tr>
<td>Obese</td>
<td>0.07</td>
<td>0.30</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Values are Pearson’s product moment correlations ($n = 9$ in each group). GH, growth hormone; bGH, bioactive GH; iGH, immunoreactive GH; GHBP, GH-binding protein; Mid, midexercise; Pre, preexercise; IP, immediately postexercise. Significant correlation: *$P < 0.05$; †$P = 0.06$.

### Table 3. Correlations between GH variables and body composition variables

<table>
<thead>
<tr>
<th>BMI</th>
<th>%Body Fat</th>
<th>Android-to-Gynoid Ratio</th>
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</thead>
<tbody>
<tr>
<td>Lean</td>
<td>0.63*</td>
<td>0.56*</td>
</tr>
<tr>
<td>Obese</td>
<td>0.19</td>
<td>0.20</td>
</tr>
<tr>
<td>GHBP</td>
<td>0.76*</td>
<td>0.81*</td>
</tr>
</tbody>
</table>

Values are Pearson’s product moment correlations ($n = 18$). *Significant correlation: $P < 0.05$. 

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related to alterations subsequent to increased adiposity. Our current findings of overall greater bGH in lean than obese sedentary men suggest that suppression of bGH is occurring in obese individuals even when equivalent iGH response to exercise has occurred. Our findings offer a basis for follow-up studies examining these variables in a chronic RE protocol. We would also argue that RE offers a mechanism whereby individuals who are obese are able to exercise at the same intensity as lean individuals and that further investigations of prolonged RE should be conducted to understand whether RE could be a intervention to alter GH systems related to increased adiposity.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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