Bed rest suppresses bioassayable growth hormone release in response to muscle activity

G. E. McCall, C. Goulet, R. E. Grindeland, J. A. Hodgson, A. J. Bigbee, and V. R. Edgerton. Bed rest suppresses bioassayable growth hormone release in response to muscle activity. J. Appl. Physiol. 83(6): 2086-2090, 1997.—Hormonal responses to muscle activity were studied in eight men before (-13 or -12 and -8 or -7 days), during (2 or 3, 8 or 9, and 13 or 14 days) and after (+2 or +3 and +10 or +11 days) 17 days of bed rest. Muscle activity consisted of a series of unilateral isometric plantar flexions, including 4 maximal voluntary contractions (MVCs), 48 contractions at 30% MVC, and 12 contractions at 80% MVC, all performed at a 4:1-s work-to-rest ratio. Blood was collected before and immediately after muscle activity to measure plasma growth hormone by radioimmunoassay (IGH) and by bioassay (BGH) of tibia epiphyseal cartilage growth in hypophysectomized rats. Plasma IGH was unchanged by muscle activity before, during, or after bed rest. Before bed rest, muscle activity increased (P < 0.05) BGH by 66% at -13 or -12 days (2,146 ± 192 to 3,565 ± 197 µg/l) and by 92% at -8 or -7 days (2,162 ± 159 to 4,161 ± 204 µg/l). After 2 or 3 days of bed rest, there was no response of BGH to the muscle activity, a pattern that persisted through 8 or 9 days of bed rest. However, after 13 or 14 days of bed rest, plasma concentration of BGH was significantly lower after than before muscle activity (2,594 ± 211 to 2,085 ± 109 µg/l). After completion of bed rest, muscle activity increased BGH by 31% at 2 or 3 days (1,807 ± 117 to 2,379 ± 473 µg/l; P < 0.05), and by 10 or 11 days the BGH response was similar to that before bed rest (1,881 ± 75 to 4,160 ± 315 µg/l; P < 0.05). These data demonstrate that the ambulatory state of an individual can have a major impact on the release of BGH, but not IGH, in response to a single bout of muscle activity.

Although immunoassays are routinely employed to measure growth hormone (GH), there is evidence in both rats and humans of a dichotomy between circulating GH concentrations measured by immunoassay (IGH) and bioassay (BGH) (3). Recently, reports from our laboratory have suggested that, in the rat, low-threshold muscle afferent activity stimulates the release of BGH, but not IGH, from the pituitary (4, 5). Although circulating IGH increases in humans during and after exercise (12, 19, 22), there have been no comparisons of BGH and IGH responses to muscle activity. Given the evidence that BGH release can be stimulated by low-threshold muscle afferents in rats (4, 5), it was hypothesized that voluntary muscle contractions would stimulate BGH release in humans.

Consistent with the concept that there is some aspect of neuromuscular activity that might affect IGH, and especially BGH, release are observations that regulation of both hormones is disrupted in rats subjected to spaceflight or hindlimb suspension (8–10, 17). For example, the release of BGH from cultured pituitary cells obtained from rats that had flown in space was attenuated (8, 9). Additionally, hypothalamic expression and protein concentration of GH-releasing hormone (GHRH) was reduced in rats that had flown in space (17), as was the GHRH-stimulated release of BGH from cultured rat pituitary cells after spaceflight (10). Thus chronic alterations in neuromuscular activity/loading affect important components of GH regulation, which could result in a decreased capacity for exercise-induced GH release. The present study was designed to compare the IGH and BGH responses to a single bout of muscle activity when individuals were in different ambulatory states.

Methods

Eight healthy men participated in 17 days of -6° head-down-tilt bed rest. Subjects were 42.3 ± 8 yr of age, 182 ± 6 cm in height, and 82.3 ± 12.1 kg in body wt. A detailed description of the experimental conditions can be found elsewhere (1). In conjunction with other investigations, subjects underwent 45 days of extensive physiological testing before, during, and after the bed-rest period. Some of the results from other investigators have been presented at national scientific meetings and published in abstract (7, 15, 16, 20, 21, 24) or nonabstract (23) form. A nursing staff provided 24-h care and monitored the subjects throughout the study. During the control and recovery periods, all subjects were ambulatory and active. The experimental procedures reported here were approved by the UCLA Human Subjects Protection Committee and the National Aeronautics and Space Administration Ames Human Research Institutional Review Board, and the participants gave written informed consent.

Subjects performed the exercise task depicted in Fig. 1. The subjects lay supine with the joint angles of the tested leg fixed at 160° for the knee and 90° for the ankle. With the use of identical joint positions and work-rest intervals, pilot tests of four male subjects not participating in the bed-rest study were undertaken to determine the temporal dynamics of BGH and IGH release in response to either 30% maximal voluntary contractions (MVCs) performed for 5 min or 80% MVCs performed for 1 min (Figs. 2 and 3). Although the BGH elevations were greater in the 30% MVC protocol, the temporal pattern of elevation was similar to the 80% MVC protocol (Fig. 2, A and B). These results indicated that plasma BGH significantly increased soon after the exercise was initiated and were maximally elevated by approximately two- to threefold at 5 min postexercise. At 10 min postexercise, the BGH concentration had decreased but remained significantly higher than preexercise in the 30% MVC protocol. By 30 min
postexercise, no values were different from preexercise. These pilot studies did not find any exercise-induced changes in IGH concentrations measured within 30 min of the completion of the prescribed exercise (Fig. 3, A and B).

The hormone response to exercise during the bed-rest study was investigated on the test days indicated in Fig. 4. Blood was collected by venipuncture by using lithium-heparin vacuum tubes made from polyethylene terephthalate (Venoject II, Terumo, Somerset, NJ). Preexercise blood collection occurred shortly after the subjects awoke, at 7 AM. The exercise test occurred 1.5–3 h after the preexercise blood collection, and blood was collected immediately after completion of the test. All subjects fasted overnight for both pre- and postexercise blood collections. The experimental conditions were held constant for a given subject across all testing sessions. One subject was unable to participate in the last bed-rest test session because of illness. Difficulty with venipuncture prohibited blood collection from another subject during the first bed-rest recovery test session. An additional subject's BGH data were excluded from the first bed-rest recovery test session because of the postexercise value exceeding the assay's linear dose-response curve.

Blood samples were immediately cooled on ice and then centrifuged at 1,000 g for 20 min at 4°C. Plasma was extracted and frozen at −70°C until analyses were performed. Plasma was analyzed for concentrations of testosterone, cortisol, 3,5,3'–triiodothyronine (T3), and thyroxine (T4) by using radioimmunoassay kits obtained from Diagnostic Products (Los Angeles, CA). Radioimmunoassay for plasma GH (IGH) was accomplished by a double-antibody technique adapted from the procedures of Schalch and Reichlin (18). GH was purified within the laboratory as previously described (2), and antiserum to GH was generated in rabbits. Secondary antibodies were obtained from Antibodies (Davis, CA). A bioassay of tibia epiphyseal cartilage growth in hypophysectomized rats was performed to measure BGH as previously described (6). The tibial growth-promoting activity of plasma, i.e., BGH, was compared with a standard purified bovine pituitary IGH with a biological potency of 1.5 IU/mg. The BGH concentration of the human samples is expressed in terms of human GH (3.0 IU/mg).

Plasma hormone concentrations pre- and postexercise and differences between testing conditions were compared by
using a two-factor repeated-measures analysis of variance (ANOVA) for subjects from whom data were available for all tests sessions. Multivariate ANOVA, which adjusts for the correlation due to repeated measurements on the same subjects, was not feasible because the number of repeated measures (14 blood collections) exceeded the total number of subjects (5 or 6 with complete data sets). Therefore, univariate analyses were performed by using the Greenhouse-Geisser procedure, which corrects for repeated measurements by adjusting the F-value before the probability estimate.

**RESULTS**

The fasting preexercise plasma concentrations of testosterone, cortisol, T₃, T₄, BGH, and IGH remained unchanged throughout the study (Table 1, Fig. 4A and B). BGH was significantly elevated postexercise on both pre-bed-rest control test days (Fig. 4A). However, the exercise response of BGH was absent after 2 or 3 days and 8 or 9 days of bed rest, and by 13 or 14 days of bed rest the postexercise level was significantly lower than the preexercise level. There was a small but significant BGH exercise response 2 or 3 days after completion of bed rest; however, after 10 or 11 days the BGH response to exercise was restored to pre-bed-rest control levels. In contrast to BGH, IGH was unaffected by muscle activity regardless of the ambulatory state (Fig. 4B).

The plasma concentrations of testosterone were not affected by the exercise test, although one of the comparisons indicated a significant decrease after exercise (Table 1). There was, however, a significant main effect of test day for testosterone. Cortisol was significantly reduced by the exercise test, although one of the comparisons indicated a significant decrease after exercise (Table 1). Thyroid hormone (T₃ and T₄) concentrations before (solid bars) and after (open bars) exercise test. Values are means ± SE; n = 8 unless indicated otherwise. Exercise test days were as follows: 12 or 13 (−13/12) and 7 or 8 (−8/7) days before; after 2 or 3 (2/3), 8 or 9 (8/9), and 13 or 14 (13/14) days of bed rest; and 2 or 3 (+2/3) and 10 or 11 (+10/11) days after completion of bed rest. *Significant main effects (2-factor analysis of variance; P < 0.05) for difference from pre- to postexercise and between test days, and interaction between factors was significant (P < 0.001). †Significantly different from preexercise within same test day (pairwise contrast; P < 0.05).

**Table 1. Plasma hormone concentrations**

<table>
<thead>
<tr>
<th>Test Days</th>
<th>Testosterone, ng/ml</th>
<th>Cortisol, µg/dl</th>
<th>T₃, ng/ml</th>
<th>T₄, µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preexercise</td>
<td>Postexercise</td>
<td>Preexercise</td>
<td>Postexercise</td>
</tr>
<tr>
<td>−13 or 12</td>
<td>5.63 ± 0.68</td>
<td>5.48 ± 0.58</td>
<td>21.04 ± 1.12</td>
<td>16.55 ± 1.03‡</td>
</tr>
<tr>
<td>−8 or 7</td>
<td>5.58 ± 0.55</td>
<td>5.49 ± 0.54</td>
<td>21.33 ± 1.15</td>
<td>13.75 ± 0.81‡</td>
</tr>
<tr>
<td>2 or 3</td>
<td>5.61 ± 0.48</td>
<td>5.49 ± 0.52</td>
<td>22.10 ± 0.94</td>
<td>16.70 ± 1.11‡</td>
</tr>
<tr>
<td>8 or 9</td>
<td>5.65 ± 0.66</td>
<td>5.34 ± 0.65</td>
<td>19.58 ± 1.03</td>
<td>17.51 ± 1.22</td>
</tr>
<tr>
<td>13 or 14 (n = 7)</td>
<td>5.59 ± 0.59</td>
<td>5.10 ± 0.49</td>
<td>19.43 ± 1.41</td>
<td>16.09 ± 1.99</td>
</tr>
<tr>
<td>+2 or 3 (n = 7)</td>
<td>4.26 ± 0.48</td>
<td>4.07 ± 0.50</td>
<td>21.43 ± 1.01</td>
<td>15.91 ± 1.53‡</td>
</tr>
<tr>
<td>+10 or 11</td>
<td>5.43 ± 0.58</td>
<td>5.20 ± 0.50</td>
<td>20.04 ± 0.91</td>
<td>14.96 ± 0.90‡</td>
</tr>
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</table>

Values are means ± SE; n = 8 men unless indicated otherwise. Values are for all data collected within a test day. T₃, 3,5,3'-triiodothyronine; T₄, thyroxine. *Significant effect for test day, P < 0.05 (analysis of variance main effect). †Significant effect for acute exercise time, P < 0.05 (analysis of variance main effect). ‡Significantly different from prewithin same test day, P < 0.05 (pairwise contrast).
plasma concentrations were significantly elevated by the exercise test and also showed significant main effects for test day (Table 1). Despite these significant main effects for test day and/or the exercise test response, there were no significant interactions between test day and exercise for testosterone, cortisol, T₃, or T₄.

**DISCUSSION**

The relative changes in the IGH and BGH responses to muscle activity on the test days before and after bed rest show that BGH, but not IGH, was significantly elevated postexercise (Figs. 4A and 5). Although this is the first study to report elevated circulating BGH in response to muscle activity in humans, previous studies have reported increased circulating levels of IGH in response to resistance exercise regimens in which multiple muscle groups were worked intensely (11, 12). However, the shorter exercise duration (6–7 min) and generally lower exercise intensity and small muscle mass response to resistance exercise regimens in which multiple muscle groups were worked intensely (11, 12). Alternatively, a reduced capacity of pituitary somatotrophs to synthesize and/or secrete BGH (8, 9) also may have played a role in the exercise-induced release of BGH during bed rest. These data demonstrate that the regulation of BGH release is remarkably sensitive to the ambulatory state of an individual and perhaps more specifically to chronic levels of neuromuscular activity and loading.

There are several lines of evidence consistent with the hypothesis that chronic muscle unloading attenuates the exercise-induced release of BGH as observed in the present study. After spaceflight or hindlimb suspension of rats, the synthesis and/or secretion of GH from pituitary cells is decreased both in vivo and in vitro, particularly for a high-molecular-weight fraction rich in BGH activity (8, 9). In the 14-day Russian biosatellite Cosmos 2044 mission, there were several indications of dysfunction of BGH regulation in rats. Although no consistent effects of chronic unloading on plasma IGH were observed (13), decrements in hypothalamic-pituitary BGH regulation in rats that had flown in space were indicated by reduced secretion of BGH from cultured pituitary cells (9) and attenuation of the “hypertrophic/calcification zone” within their own tibia epiphysial plates (14). Additionally, diminished hypothalamic immunostaining for GHRH in the neurosecretory terminals of the median eminence and reduced expression of prepro-GHRH mRNA in the arcuate nucleus was observed for rats flown in space (17). Hymer et al. (10) recently demonstrated that GHRH stimulated secretion of BGH from in vitro rat pituitary cell cultures was altered after spaceflight. Collectively, these data suggest that chronically altered neuromuscular activity/loading and/or proprioception disrupts the synthesis and/or release of BGH.

In conclusion, short bouts of moderately intense muscle activity of a relatively small muscle mass elevated the plasma concentrations of BGH but not IGH. Furthermore, the exercise-induced release of BGH was inhibited by bed rest but returned to pre-bed-rest control values by 10 or 11 days of recovery from bed rest. Given the previous results from studies of rats demonstrating a role of proprioceptive afferents in BGH, but not IGH, regulation (4, 5), the present data suggest that a prolonged nonambulatory state depresses BGH responses to exercise, perhaps because of a functionally blunted muscle-afferent-pituitary axis.

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**REFERENCES**


