Growth hormone and muscle function responses to skeletal muscle ischemia

Joseph R. Pierce, Brian C. Clark, Lori L. Ploutz-Snyder, and Jill A. Kanaley

Department of Exercise Science, Syracuse University, Syracuse, New York

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Pierce, Joseph R., Brian C. Clark, Lori L. Ploutz-Snyder, and Jill A. Kanaley. Growth hormone and muscle function responses to skeletal muscle ischemia. J Appl Physiol 101: 1588–1595, 2006. First published August 3, 2006; doi:10.1152/japplphysiol.00585.2006.—We examined the effects of ischemia (ISC) alone and with low-intensity exercise (ISC+EX) on growth hormone (GH) and muscle function responses. Nine men (22 ± 0.7 yr) completed 3 study days: an ISC day (thigh cuff inflated five times, 5 min on, 3 min off), an ISC+EX day [knee extension at 20% maximal voluntary contraction (MVC) with ISC], and a control day. MVCs and submaximal contraction tasks (15 and 30% MVC) were performed before and following the perturbations. Surface electromyogram signals were collected from thigh muscles and analyzed for median frequency and root mean square alterations. Blood samples were collected every 10 min (190 min total) and analyzed for GH concentrations. Peak GH concentrations and GH area under the curve were highest (P < 0.01) on the ISC+EX day (7.5 μg/l and 432 μg·l⁻¹·min⁻¹, respectively) compared with the ISC (0.9 μg/l and 76.4 μg·l⁻¹·min⁻¹), and CON (1.1 μg/l and 83.8 μg·l⁻¹·min⁻¹) days. A greater GH pulse amplitude, mass/pulse, and production rate were also observed on the ISC+EX day (P < 0.05). Following the intervention, force production decreased on the ISC and ISC+EX days by 16.1 and 55.8%, respectively, and did not return to baseline values within 5 min of recovery. During the submaximal contractions, median frequency shifted to lower frequencies for most of the muscles examined, and root mean square electromyogram was consistently elevated for ISC+EX day. In conclusion, ISC coupled with resistance exercise acutely increases GH levels and reduces MVC, whereas ISC alone decreases force capacity, without alterations in GH levels.

Skeletal muscle is highly dependent on proper endocrine function for its growth and development throughout the life cycle. In particular, growth hormone (GH) is known to stimulate muscle growth and maintenance by regulating protein turnover, either directly or indirectly (14, 18). Of the various factors that affect GH levels, exercise is one of the most physiologically relevant and potent stimulators of GH release (10). However, in situations of spaceflight and rehabilitation, where large amounts of muscle loss can occur, exercise may not be a viable option due to an inability to load joints with sufficient force. Interestingly, recent research has shown that, in the absence of contractile activity, inducing blood flow restriction in humans preserves skeletal muscle mass following surgically induced bed rest (29) and mitigates the slowing in the evoked muscle fiber action potential commonly observed in unweighted skeletal muscle (5). In rats, surgical crush occlusion of hindlimb vasculature has been observed to enhance skeletal muscle hypertrophy (15). Speculation exists on whether GH may play a role in this ischemia (ISC)-induced maintenance of skeletal muscle in the absence of an exercise stimulus (29, 31).

Investigations coupling ISC with resistance exercise have shown that GH concentrations are dramatically increased over the GH levels observed when the same relative exercise intensity is performed without ISC (25, 27, 31). More specifically, five sets of bilateral knee extension at a low intensity of ∼20% one repetition maximum (1 RM) while in an occlusive state (∼214 mmHg) elevated GH concentrations 290 times above baseline, while exercise alone at 20% 1 RM did not increase GH concentrations to the same magnitude (27). Similarly, Takano, et al. (25) reported that three sets of low-intensity resistance exercise (20% 1 RM) performed under an occlusive stimulus (1.3 times greater than systolic blood pressure) was capable of elevating GH concentrations to near 100 times that of baseline values, but exercise at the same intensity without occlusion did not result in similar GH responses. Interestingly, these studies reported that ISC plus low-intensity exercise conditions resulted in GH responses similar to those reported during resistance exercise at much higher intensities (∼75% 1 RM) without occlusion (16).

Although the specific importance of the GH response to exercise during vascular occlusion is unclear, it suggests that skeletal muscle is capable of potential growth without high mechanical stress (as low as 20% of maximal strength) placed on the joints, and it still remains to be elucidated whether these adaptations are attributed to hypophysal output of GH. Furthermore, the mechanisms behind GH release during exercise combined with ISC remain to be elucidated. Exercise studies have suggested that the GH response to exercise may be due to adrenergic stimulation (37), cholinergic stimulation (33), etc., but this low-intensity exercise with the addition of ISC suggests that another mechanism may drive the GH response (e.g., metaboreflex).

In addition to hormonal responses, a relationship between ISC and muscle function has been observed. Muscular fatigue, defined as a decrease in maximal muscle force, has been reported to occur concomitantly with a decrease in muscle oxygenation (21), similar to ISC. Other studies have demonstrated that ISC exercise results in decreased maximal voluntary force production (7) and significant alterations in electromyographic (EMG) activity, measured by median frequency shifts toward lower frequencies (2). More recent studies have reported that alterations in muscle function under applied ISC include increases in EMG amplitude activity, suggesting that increased motor unit recruitment occurs (20, 27, 30). Interestingly, as muscle function is altered, there is a simultaneous increase in GH concentrations (27), suggesting that they may somehow be functionally linked. Limited research exists where...
GH concentrations and muscle function are studied in the same protocol, and no study has examined if there is an association between these data. Additionally, the effects of ISC alone on muscle maintenance and/or hypertrophy responses are currently limited, and data regarding a GH response to such a perturbation are also incomplete, as previous investigations have not fully recognized the pulsatile nature of GH (31). Therefore, the purpose of this study was to examine the effects have not fully recognized the pulsatile nature of GH (31). perturbation are also incomplete, as previous investigations muscle maintenance and/or hypertrophy responses are cur- between these data. Additionally, the effects of ISC alone on protocol, and no study has examined if there is an association GH concentrations and muscle function are studied in the same.

**Materials and Methods**

**Subjects**

Nine healthy young men were recruited from the university community for participation in this study. All subjects signed an informed consent approved by the Syracuse University Institutional Review Board. The subjects were 22 ± 0.7 yr, had a mean height of 1.79 ± 0.02 m, weighed 79.9 ± 4.4 kg, and had an average percent body fat of 15.7 ± 2.6%. Subjects were excluded if they had a family history of blood clotting disorders, were taking any medications known to alter hormone production, or smoked (defined as smoking ≥2 cigarettes/day). Additionally, participants were excluded if their thighs were too large to be surrounded by the tourniquet cuff used in the occlusion.

**Experimental Design**

The study design required subjects to visit the Musculoskeletal Laboratory on three separate occasions (separated by at least 1 wk each). The ordering of the study days was randomly assigned, and each testing day lasted ~4 h. Immediately before each study day, subjects were required to refrain from strenuous exercise for at least 24 h and fast from 1900 the evening prior. The subjects all reported to the laboratory at ~0700 and had their blood pressure assessed. The average systolic blood pressure before beginning was 119 ± 5 mmHg. At the start of each testing day, a catheter was inserted into a forearm vein, followed by 30 min of quiet supine rest, 30 min of baseline blood samples, and muscle function testing. Immediately after the muscle function testing, the randomized portion was initiated and lasted 40 min. Upon completion of the randomized protocol, muscle function testing was repeated. Blood samples were taken for 0.5 h before, during, and for 2 h following the randomized portion at 10-min intervals. During the randomized portion, samples were drawn at 5-min intervals. During one of the study days, body composition was measured using air-displacement plethysmography using the BodPod (Life Measurements, Concord, CA) (9).

**Experimental Trials**

There were 3 randomized study days. During the CON day, a tourniquet cuff was placed on the upper thigh without being inflated to serve as a sham. During the ISC alone day, the subjects had ISC applied in five sets (duty cycle: 5 min on, 3 min off) by inflating a tourniquet cuff (E20 Rapid Cuff Inflator, D. E. Hokanson, Bellevue, WA) on the upper thigh to the highest pressure attainable from the apparatus (mean pressure of 280.4 ± 1.2 mmHg). This pressure was 163 ± 4.6 mmHg above the individuals’ mean systolic blood pressure using Doppler flow technology, and our laboratory has previously confirmed that this ISC protocol results in blood flow cessation to the limb (4). On the ISC + EX day, the cuff was inflated to the same pressure in the same duty cycle (5 min on; 3 min off) used in the ISC day, and the subjects performed a unilateral knee-extension exercise at a low intensity (~20% maximal voluntary contraction (MVC)), contracting at a fixed cadence (approximately a 2-s concentric contraction and a 2-s eccentric contraction) with the ISC leg until volitional fatigue. Strong verbal encouragement from the investigators was provided to maintain proper speed and to perform the contractions until subjects were unable to complete another contraction. After completion of the exercise, the cuff remained inflated until the 5 min of ISC were complete.

**Muscle Function Assessment**

After the baseline blood samples were taken (minutes 0–30), the subject was seated in a leg extension dynamometer (MedX, Ocala, FL). The back rest was adjusted to create a hip joint angle of 100° from flexion, and a seat belt was secured across the subject’s hips to prevent movement of the hip joint and to minimize assistance from other muscle groups. For all isometric force measurements (MVC and submaximal forces), the knee joint angle was set at 60° from flexion. To obtain an MVC (both extension and flexion), the subjects were instructed to push as hard as possible against an immovable arm of the apparatus attached to a force transducer three times. The highest force of the three trials was recorded as their MVC. Isometric force was measured by a force transducer (model U1T, HBM, Marlborough, MA; sensitivity of 0.002 V/N), amplified, and recorded at 1,000 Hz using a 16-bit data-acquisition card (MP150, BioPac).

The first muscle function test (immediately before the randomized portion) included the initial MVC determination. Following the MVC determination, participants were asked to exert a constant force against the force transducer, matching target lines at submaximal intensities corresponding to 15 and 30% of their knee-extension MVC for 8 s. The subjects had visual feedback for matching the target lines displayed on a computer screen placed 1 m directly in front of them. On each experimental day, 1 min before the completion of the randomized portion of the protocol, the subject was asked to repeat the MVC and the submaximal muscle function testing. Upon completion of ISC and after muscle function testing, the cuff was released. Additional knee-extension MVC testing occurred 1, 2, and 5 min after cuff release.

During all tasks, subjects had small areas of the thigh prepared for EMG recording, as previously described by our laboratory (4). Briefly, bipolar surface electrodes (Ag-AgCl, 10-mm diameter, 25-mm interelectrode distance) were placed on the agonists vastus lateralis (VL), vastus medialis (VM), and rectus femoris (RF), as well as the antagonist biceps femoris (BF) muscle. A reference electrode was placed on the patella. The EMG signals were amplified 1,000 times, band-pass filtered between 10 and 500 Hz, and sampled at 1,000 Hz (MP150, BioPac Systems, Santa Barbara, CA).

**EMG Signal Processing**

Two main parameters were extracted from the interference EMG signal collected: the root mean square (RMS) EMG and the median frequency EMG, as previously described (6). Data were processed using AcqKnowledge software version 3.7.2 (BioPac Systems, Goleta, CA).

The RMS EMG of the signal was calculated around a 1-s portion centered on the peak force (0.5 s in each direction, ~1,024 points total) during the MVCs. RMS EMG data during the submaximal contractions were then normalized to the MVC data and are reported as a percentage of maximum. For the quadriceps muscles (agonists:
GH

Blood sampling was conducted via a heparin lock catheter placed in a forearm vein at the start of the experimental day. Following catheter placement, a 30-min rest period was given, after which three samples separated by 10 min were taken (baseline measurements). Subsequently, blood samples were taken every 5 min until the end of the random intervention of the protocol, at which time blood sampling resumed at 10-min intervals. Blood was sampled for a total of 190 min.

Blood drawn from the forearm vein was placed into Vacutainers prepared with EDTA and chilled to preserve the integrity of the samples. All samples were centrifuged at 2,300 rpm for 15 min at \(-10^\circ C\). Upon separation, the plasma was aliquotted to microcentrifuge tubes and frozen at \(-80^\circ C\) until analysis.

**Assay**

GH concentrations were analyzed using a commercially available multiplex bead-based assay (LINCOplex, Linco Research, St. Charles, MO), which is an antibody sandwich assay using capture and detector antibodies (coupled to phycoerythrin). Samples were run in duplicate, and the GH kits are reported to have a sensitivity of 0.004 \(\mu g/l\). Intra- and interassay coefficients of variation were 7.8 and 5.3\%, respectively. All samples were analyzed on a Luminex 100 System (Luminex, Austin, TX) using Bioplex Software (Bio-Rad Laboratories, Hercules, CA).

**Hormone Data Analysis**

The mean GH concentration calculated from the duplicate data at each time point was used in the data analysis. Peak GH concentration was recorded as the highest GH value on the study day. Integrated area under the curve analysis (\(\mu g/ml^{-1}min^{-1}\)) was employed to detect significant elevations above baseline measures using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA).

To determine GH secretory events (22), a multiple-parameter model of deconvolution analysis was employed. With a hormone concentration at any given time being a reflection of both release and metabolic clearance, it has also been suggested that these two processes are related by a convolution integral (35). By simultaneously estimating all plasma concentrations and their variances and assuming a Gaussian distribution, this integral can be solved to determine secretory pulses (35). The duplicate data were first prefitted using an automated waveform-independent technique (PULSE2). These initially determined presumptive peaks were then subject to further multiparameter deconvolution analysis, where basal secretion, number of pulses, half-duration of the GH pulse, half-life, pulse amplitude, mass of GH/pulse, and GH production rate were calculated. GH secretory pulses were considered significant if the fitted amplitude could be distinguished from zero with 95\% statistical confidence (22). Half-duration of the secretory pulse was defined as the duration in minutes of the calculated secretory burst at half-maximal amplitude. Mass of GH secreted/pulse was the integral of the calculated secretory pulse (in \(\mu g/l\) of distribution volume). The GH production rate was calculated as the product of the number of pulses and the mean mass of GH/pulse (22).

**Statistical Analysis**

Interaction effects (day \(\times\) time) for GH concentrations were first examined using a two-way analysis of variance with repeated measures (RM-ANOVA). Differences in integrated area under the curve GH concentrations and GH deconvolution parameters between experimental treatment sessions (CON, ISC, and ISC+EX) were examined using one-way RM-ANOVA. For muscle function outcomes, interaction effects were examined using a three-way RM-ANOVA (day \(\times\) time \(\times\) muscle). Subsequent one-way RM-ANOVA between days were used to determine differences in RMS EMG and median frequency EMG. When a statistical difference was detected in RM-ANOVA, post hoc analysis (least significant difference) was used to determine where the difference occurred. Additionally, dependent Student’s t-tests were employed to determine pre- and postintervention differences within day/conditions.

To determine whether GH concentrations (\(\mu g/l\)) and muscle function outcomes (knee-extension force only) at the end of the intervention were related, the percent changes of each measure were analyzed using rank-order correlation analysis. Since the percent change in GH concentration and knee-extension force were not normally distributed, Spearman’s \(\rho\)-correlation coefficient, \(r\), was calculated to evaluate the associative changes between the percent change in force in relation to the percent change in GH concentration.

All data are expressed as means \(\pm\) SE. Significance was accepted at \(\alpha \leq 0.05\). All statistical tests for all dependent variables were performed using SPSS for Windows, version 12.0 (SPSS, Chicago, IL).

**RESULTS**

**GH**

*GH concentrations.* The GH analysis was based on a sample size of eight. The data from one subject were omitted because all samples consistently fell below the sensitivity of the assay (0.004 \(\mu g/l\)). The pattern of GH responses on the 3 study days is illustrated in Fig. 1. Over the course of each baseline period (the first 30 min), the values of GH concentrations were not significantly different among the 3 study days. GH concentrations were elevated above baseline values during the ISC+EX day beginning at minute 50 and remained elevated over baseline until a time point between minutes 80 and 90 (\(P < 0.05\)). Additionally, the plasma concentrations observed during the same day were higher than those on both the CON and ISC days from minutes 60 to 110 (\(P < 0.05\)), which included the peak GH concentration (7.5 \(\pm\) 1.8 \(\mu g/l\)) observed on the ISC+EX day at minute 80, 10 min after the cessation of five ISC duty cycles. By minute 120, GH concentrations on the ISC+EX day were similar to concentrations on the ISC and CON days for the remainder of the study.

Integrated GH concentrations above baseline were highest for the ISC+EX day (432 \(\pm\) 115 \(\mu g/l^{-1}min^{-1}\)), which was significantly higher (\(P < 0.01\)) than that for the ISC and CON days (76 \(\pm\) 26 and 84 \(\pm\) 36 \(\mu g/l^{-1}min^{-1}\), respectively) (Fig. 1, inset). There was no statistical difference between the ISC and CON days.

Deconvolution analysis revealed no statistical differences in basal secretion among the 3 study days (0.003 \(\pm\) 0.001, 0.004 \(\pm\) 0.001, and 0.002 \(\pm\) 0.001 \(\mu g/l\) for CON, ISC, and ISC+EX days, respectively; Table 1). GH pulsatility was somewhat altered by ISC, but more consistently with ISC+EX. The number of secretory pulses decreased from 2.9 \(\pm\) 0.4 on the CON day to 1.9 \(\pm\) 0.4 on the ISC day and to 1.3 \(\pm\) 0.2 pulsions on the ISC+EX day (\(P < 0.05\)). Although ISC reduced
the number of pulses relative to the CON day, no other deconvolution parameter was altered during the ISC day. In addition to its effects on the number of secretory pulses, ISC+EX resulted in a greater GH pulse amplitude (0.83 ± 0.25 μg/l), mass of GH/pulse (15.97 ± 4.53 μg/l), and GH production rate (17.05 ± 4.3 μg·L_v⁻¹·min⁻¹, where L_v is distribution volume) than observed on both ISC and CON days (P < 0.05).

**Muscle Function**

**Knee-extension force.** There was no difference in MVC force among the 3 days before the perturbations (Fig. 2). Following ISC and ISC+EX, knee-extension force was significantly lower immediately postintervention (−16.1 and −55.8%, respectively; P < 0.05), with the decrease during the ISC+EX day being lower (P < 0.05) than during both the ISC and CON days. In the three subsequent MVCs (within 5 min), knee-extension force did not return to baseline values on either the ISC or ISC+EX day (P < 0.05), and absolute force was lower (P < 0.05) at all postintervention measures on the ISC+EX day than the ISC and CON days. Although, during the last MVC, knee-extension force was still 9.8% below baseline (P < 0.05) for the ISC day and 39.4% below baseline (P < 0.05) for the ISC+EX day, the ISC trial at this time point was not different than the CON day.

**RMS EMG.** Figure 3 illustrates the increase in RMS amplitude of the EMG signal following the respective interventions during the 15 and 30% MVC contraction tasks. The analysis of the RMS EMG was based on n = 8 for all muscles except the VM, which was analyzed using n = 6. Exclusion criteria for this data were based on the inability to obtain a clean signal (e.g., minimal surrounding noise). In the case of the VM muscle, the subject excluded was the same subject excluded for the GH analysis. There was a consistent increase (P < 0.05) in the RMS EMG following ISC+EX for all of the muscles examined at 15% MVC, whereas ISC alone did not alter RMS EMG for the same contraction intensity. A similar trend was observed during the 30% MVC, in that all of the muscles examined had an elevated RMS EMG from preintervention values on the ISC+EX day. Furthermore, on the ISC+EX day

Table 1. Effects of ischemia or ischemia plus exercise on GH secretion as estimated by multiple parameter deconvolution analysis

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia</th>
<th>Ischemia + Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH basal secretion, μg/l</td>
<td>0.003±0.001</td>
<td>0.004±0.001</td>
<td>0.002±0.001</td>
</tr>
<tr>
<td>Number of GH secretory pulses</td>
<td>2.9±0.4</td>
<td>1.9±0.4*</td>
<td>1.3±0.2*</td>
</tr>
<tr>
<td>GH pulse half-duration, min</td>
<td>16.8±1.6</td>
<td>19.7±2.0</td>
<td>19.1±1.5</td>
</tr>
<tr>
<td>GH half-life, min</td>
<td>16.6±2.1</td>
<td>14.6±0.5</td>
<td>16.8±1.2</td>
</tr>
<tr>
<td>GH pulse amplitude, μg/l</td>
<td>0.06±0.03</td>
<td>0.09±0.44</td>
<td>0.83±0.25†</td>
</tr>
<tr>
<td>Mass of GH/pulse, μg/l</td>
<td>1.5±0.7</td>
<td>2.5±1.5</td>
<td>16.0±4.5†</td>
</tr>
<tr>
<td>GH production rate, μg·L_v⁻¹·min⁻¹</td>
<td>5.1±2.8</td>
<td>3.1±1.4</td>
<td>17.1±4.3†</td>
</tr>
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</table>

Values are means ± SE; n = 8 men. GH, growth hormone; L_v, distribution volume. *P < 0.05 vs. control day; †P < 0.05 vs. control and ischemia days.
during the 30% MVC, the increased RMS EMG values in the VL, RF, and BF muscles were all higher \((P < 0.05)\) than the same muscles examined on the ISC and CON days. Median frequency of the EMG. Changes in the median frequency of the EMG were less consistent between the muscles when compared among study days (Fig. 4). As indicated in Fig. 4, median frequency was based on \(n = 8\) for the VL, RF, and BF muscles, and \(n = 6\) for the VM due to the aforementioned reason in the RMS EMG results. On the ISC+EX day at 15% MVC, decreases in median frequency EMG from preintervention values were observed in the VL, RF, and BF muscles \((P < 0.05)\). At 30% MVC, significant decreases \((P < 0.05)\) in the median frequency of the EMG were only observed in the RF and BF muscles, both relative to preintervention values, and when compared against the ISC and CON days \((P < 0.05)\). When all of the muscles were averaged during the 30% MVC on the ISC+EX day, a 9% decrease in median frequency of the EMG was observed, similar to the 10% decrease in median frequency of the EMG during the 15% MVC contraction. Median frequency of the EMG in the VM muscle was different than that for all other muscles at both 15 and 30% submaximal contractions by not showing a significant change for any study day.

Correlation Analysis

Analyzing ISC and ISC+EX days separately revealed no significant relationship between GH concentrations and muscle force \((P = 0.61\) and 0.14, respectively). When the data from both the ISC and ISC+EX days were collapsed, Spearman’s correlation coefficient revealed the relationship between GH concentrations and knee-extension force were significantly related postintervention \((r = -0.69, P < 0.01)\).

DISCUSSION

The purpose of this study was to examine 1) if ISC would result in altered plasma GH concentrations and muscle function parameters, and 2) if these alterations were heightened with the addition of low-intensity resistance exercise (ISC+EX). ISC alone had no major effects on GH concentrations; however, ISC+EX increased GH levels approximately ninefold over baseline values. Furthermore, ISC+EX resulted in increased GH pulse amplitude, mass of GH/pulse, and GH production rate. Although ISC alone did not alter GH concentration, ISC did alter muscle function, as indicated by a decreased force production. The force decrements were exacerbated in the ISC+EX trial, with a greater amplitude and lower frequency of the EMG signal during submaximal tasks, which is commonly observed during fatigue (6).

ISC and GH

Previously, it was thought that, to observe gains in muscle mass as a result of resistance exercise, the intensity must be relatively high (e.g., >70% 1 RM), most likely due to the relationship between exercise intensity and endogenous hormonal responses (17, 23). Recent reports, however, of skeletal muscle hypertrophy under ISC conditions have illustrated that a strong mechanical stimulus may not be needed to elicit such gains (15, 26, 28–31). In fact, if the stimulus provides a sufficient signal to induce endocrine changes, this may stimulate muscle growth. Evidence suggests that ISC can mitigate muscle atrophy after surgically (29) and mechanically induced (5) disuse in humans, as well as lead to muscle hypertrophy in

Fig. 3. Changes in root mean square (RMS) electromyogram (EMG) at 15 and 30% MVC. VL, vastus lateralis; RF, rectus femoris; VM, vastus medialis; BF, biceps femoris. Values are means ± SE; \(n = 8\) for VL, RF, and BF; \(n = 6\) for VM. *\(P < 0.05\) vs. preintervention; ‡\(P < 0.05\) vs. control and ISC days.
GH-IGF-I axis, as other factors have been noted to be involved

Additional explanations may lie outside the realm of the

skeletal muscle maintenance are contradictory (15, 29, 31).

pertrophy to ISC alone involved a chronic (2–4 wk) vascular

whereas these studies reporting muscle maintenance and hy-

tration did not measure GH concentrations, it has been specu-

lated that growth factors (e.g., GH) were most likely involved

in the regulation of protein turnover (29), although a more

recent study reported that, in the 15 min following an occlusive

stimulus alone, GH concentrations were not altered (31).

In contrast to our hypothesis, ISC alone did not alter the

plasma GH concentrations compared with baseline measures or

compared with the CON day, but our data indicated that

low-intensity exercise with ISC is needed to stimulate GH

secretion, which is in agreement with a recent investigation

that examined cooperative effects of exercise plus ISC (31).

Therefore, our findings do not support previous work that GH

could be responsible for these changes from ISC alone. Unlike

previous studies (5, 15, 29), this investigation examined the

acute effects of applied ISC (1 session of 5 bouts of ISC),

whereas these studies reporting muscle maintenance and hy-
pertrophy to ISC alone involved a chronic (2–4 wk) vascular

occlusion exposure. Currently, the effects of ISC alone on

skeletal muscle maintenance are contradictory (15, 29, 31).

Additional explanations may lie outside the realm of the

GH-IGF-I axis, as other factors have been noted to be involved

with the previously observed effects on muscle growth (heat

shock protein-72, myostatin, etc.) under ISC conditions (15).

GH studies of muscle hypertrophy using traditional resis-
tance exercise have shown that the intensity of the exercise

must be moderately high with a short rest between sets to

achieve increased plasma GH concentrations (17). The present

study along with previous reports demonstrate that plasma GH

concentrations can be elevated by coupling resistance exercise

with ISC (24, 25, 27, 31), yet the exercise intensity employed

is substantially lower than resistance exercise aimed at muscle

hypertrophy (~75% 1 RM) (17). Accordingly, we observed

plasma GH concentrations approximately nine times greater

than baseline when ISC was combined with low-intensity (20%)

1 RM) resistance exercise. The GH responses observed were

smaller with our ISC+EX perturbation than that observed in

the previous reports (25, 27, 31, 32), possibly due to the

differences between the stress load (bilateral vs. unilateral

exercise; continual vs. intermittent ISC) or GH assay tech-

niques (RIA vs. bead-based immunoassay).

ISC+EX was the only experimental condition that consist-
tently altered GH pulsatility. This intervention reduced the

number of pulses, as well as increased GH pulse amplitude,
pulse mass, and production rate. The threefold greater produc-
tion rate on the ISC+EX day compared with the CON day was
due to a 10-fold increase in pulse mass; yet there was an ~50% reduc-
tion in the number of pulses. Similarly, ISC alone re-
sulted in an ~35% decrease in the number of pulses; however,

unlike the ISC+EX day, the pulse mass did not change and,

accordingly, nor did the ISC production rate. Although the

pulse mass increased to 2.5 μg/l on the ISC alone day, this was

not enough of an increase to 1) reach significance and to 2)

match the production rate on the ISC+EX day. Although we

are unsure of an explanation, or the physiological meaningful-

ess of a reduced number of GH pulses on the ISC day, it seems

that GH would not likely explain enhanced muscle mass

in the presence of ISC alone, as trends for decreased produc-
tion rates as well as reduced integrated GH concentrations were

observed on the ISC day vs. the CON day, although neither of

these changes were significant.

There has been considerable speculation on the mechanisms

underlying elevated GH responses to exercise, including neural

stimulation, metabolite accumulation, nitric oxide, and acid-

base alterations [as reviewed in Godfrey et al. (10)]. Previous

work on afferent feedback has illustrated that low-threshold

afferents (type I and II) have led to output of bioassayable

forms of GH from the pituitary (11), whereas immunoassay-

able GH was not affected. Therefore, it is likely that type I and

II afferent feedback were not responsible for the GH response

in the present study, given that we observed an increase in

immunoassayable GH during the ISC+EX trial. Conversely,

the GH response observed during ISC exercise is most likely
due to metabolite buildup and the subsequent metaboreflex via

muscle chemoreceptor stimulation (type III and IV afferents).

Previous investigations of the metaboreflex reported that rhyth-
mic handgrip exercise followed by forearm occlusion increased

muscle sympathetic nerve activity to nonworking muscle and

sustained the response after the exercise had ceased (36).

Furthermore, increased muscle sympathetic nerve activity is

associated with increased heart rate and arterial blood pressure

(pressor response), and it is possible that increased afferent

signals (type III and IV) leading to this pressor response could
also drive GH release from the anterior pituitary. Our data support the hypothesis that the afferent stimulation of the type III and IV nerves can feedback upon the hypothalamus to either increase GH-releasing hormone or inhibit somatostatin release. Since unilateral leg ISC alone does not alter GH release, this suggests that a metabolite stimulus was not strong enough to feedback to the hypothalamus.

**ISC and Muscle Function**

Lack of blood flow to a muscle typically results in decreased oxygenation and failed substrate delivery, and these changes in the surrounding environment have been associated with muscle fatigue (21). Following the application of five sets of vascular occlusion, strength was decreased by 16.1%, and the addition of low-intensity exercise reduced strength by 55.8%. Both ISC and ISC+EX reduced voluntary force production, and this could be due to either central or peripheral mechanisms, although the more likely explanation would be the latter in this investigation. For instance, an increased H+ concentration, previously shown to result in force decrements of type II fibers (34), may have caused the force decrement observed in the current investigation. Although the effects on decreased muscle force lasted for at least 5 min during both ISC and ISC+EX relative to baseline, the postintervention force production on the ISC day other than immediately postintervention was not different from CON forces. The clinical or physiological importance of such a response is not certain, but intriguing nonetheless.

Our decrement in knee-extension strength with ISC and ISC+EX is similar to those seen with high-intensity fatigue resistance exercise protocols (1, 13). Hakkinen and Pakarinen (13) reported that muscle force fell by 10.3 ± 4.7 and 24.6 ± 18.9% following two fatigue resistance exercise protocols (twenty 1 RM or 10 sets of a 10 RM, respectively). Additionally, in a study of acute responses to maximal and forced repetitions, decreases in muscle force were 38.3 and 56.5%, respectively (1), with the magnitude of change being significantly different between maximal and forced repetitions. In both of the investigations by Hakkinen and Pakarinen (13) and Ahtiainen et al. (1), a greater accumulation of lactate was observed in the protocols that resulted in greater force decrements. Additionally, a decreased ability to activate motor units by the central nervous system may have accounted for the larger decrements during the more exhausting protocols (e.g., forced repetition protocol) (1).

Muscle fatigue on the ISC+EX day was demonstrated by the concomitant increase in RMS EMG and decrease in median frequency EMG. Drastic increases in EMG amplitude, as seen in the present study, suggest that, during ISC+EX, more motor unit activity was required to maintain the same absolute force during the submaximal contractions at both 15 and 30% MVC. At 15% MVC, an overall increase of 237% in RMS amplitude of the EMG was observed, whereas in the 30% MVC, an overall increase of 175% resulted. Several other studies have observed that, under ISC conditions, additional motor unit activity is required to maintain force levels (20, 27). Takarada et al. (27) found that EMG activity of the muscle was 1.8 times greater with exercise combined with occlusion than the same exercise without occlusion, whereas Moritani et al. (20) reported increases in mean motor unit spike amplitude and frequency during the contractions with arterial occlusion compared with exercise with unrestricted blood flow. Similar responses are seen to occur during submaximal isometric contractions maintained until volitional fatigue (3, 6).

Previous studies (6, 12) have used the shift of median frequency of the EMG to lower values (spectral compression) as an indicator of local muscle fatigue. We observed spectral compression at both the 15 and 30% MVC submaximal contractions under ISC conditions, as previously reported (19). Although there was an ~10% decrease averaged across all muscles examined, the VM muscle responded differently at both submaximal contractions. At present, we are unsure of an explanation for the differential VM response, although this response could be due to different muscle articulations (monoarticular vs. biarticular) (8). Overall, variability of the median frequency of the EMG may be due to the surrounding metabolic environment of the muscle (6) or other factors not addressed in the present investigation.

**GH and Muscle Function**

As demonstrated by correlation analysis, elevated plasma GH concentrations were related to decreased voluntary muscle strength. Furthermore, it appears that the subjects who experienced the largest decrements in muscle force concomitantly experienced the highest plasma GH concentrations (Spearman’s $r = −0.69, P < 0.01$) postintervention. Presently, it is difficult to speculate on the mechanisms that underlie this relationship, but it is possible to use the relationship as a basis for developing exercise protocols to evoke a GH response in that, if a low-intensity resistance exercise protocol under ISC conditions is to be used as a means for potentially maintaining/gaining muscle mass (e.g., spaceflight and/or rehabilitation settings), it may be beneficial to design an exercise protocol that causes greater force decrements following the exercise.

In conclusion, low-intensity resistance exercise with ISC increases plasma GH concentrations, whereas ISC alone does not alter GH secretion. Furthermore, ISC alone alters the muscle MVC, but not frequency or amplitude measures of the EMG signal, whereas ISC+EX exacerbated the force decrements seen during ISC and consistently altered the frequency and time domains of the EMG signal. The mechanisms of the increased plasma GH concentrations during exercise plus ISC are not well understood, but possibly repeated ISC plus low-intensity exercise bouts may alter the endocrine milieu sufficiently to increase muscle mass and strength and may have important implications in spaceflight and rehabilitation (29). More research is warranted in developing resistance exercise programs in the presence of ISC in such situations where muscle mass and strength are needed to be maintained, yet high forces are not able to be applied to the joints.

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Present address of J. Pierce: US Army Research Institute of Environmental Medicine, Military Performance Division, Building 42, Kansas St., Natick, MA 01760 (e-mail: joseph.pierce@amedd.army.mil).
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