Rigorous Running Increases Growth Hormone and Insulin-Like Growth Factor-I Without Altering Ghrelin

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It has been suggested that ghrelin may play a role in growth hormone (GH) responses to exercise. The present study was designed to determine whether ghrelin, GH, insulin-like growth factor-I (IGF-I), and IGF-binding protein-3 (IGFBP-3) were altered by a progressively intense running protocol. Six well-trained male volunteers completed a progressively intense intermittent exercise trial on a treadmill that included four exercise intensities: 60%, 75%, 90%, and 100% of Vo_2max. Blood samples were collected before exercise, after each exercise intensity, and at 15 and 30 mins following the exercise protocol. Subjects also completed a separate control trial at the same time of day that excluded exercise. GH changed significantly over time, and GH area under the curve (AUC) was significantly higher in the exercise trial than the control trial. Area under the curve IGF-I levels for the exercise trial were significantly higher than the control trial. There was no difference in the ghrelin and IGFBP-3 responses to the exercise and control trials. Pearson correlation coefficients revealed significant relationships between ghrelin and both IGF-I and IGFBP-3; however, no relationship between ghrelin and GH was found. In conclusion, intense running produces increases in total IGF-I concentrations, which differs from findings in previous studies using less rigorous running protocols and less frequent blood sampling regimens. Moreover, running exercise that produces substantial increases in GH does not affect peripheral ghrelin levels; however, significant relationships between ghrelin and both IGF-I and IGFBP-3 exist during intense intermittent running and recovery, which warrants further investigation. Exp Biol Med 229:240–246, 2004

Key words: ghrelin; growth hormone; insulin-like growth factor-I; insulin-like growth factor–binding protein-3; exercise

A number of factors have led to interest in the effect of exercise on the growth hormone/insulin-like growth factor-I (GH/IGF-I) axis, including its possible role in maintenance of lean mass (1–5); involvement in metabolism of glucose, lipid, and protein (6, 7); and its alteration with age (2, 8). Although most modes of exercise stimulate an increased GH secretory response that is linear with exercise intensity (9), evidence suggests IGF-I responses are independent of GH (10). Whether previous studies have reported exercise-induced alterations of IGF-I seems to depend on several factors, including exercise mode and intensity as well as the blood sampling protocol used in the study.

Both low- and high-intensity cycling have been shown to increase IGF-I concentrations (11, 12). However, neither low-volume (13) nor high-volume resistance exercise (14–16) has been shown to change total IGF-I concentrations. Moreover, no change in IGF-I concentrations has been found following a marathon, a 20 km run, and treadmill exercise at 60% of Vo_2max (17–20). Exercise may affect other components of the GH/IGF-I system. Most circulating IGF-I is bound to one of six IGF-binding proteins (IGFBP), and more than 75% of IGF-I is carried by IGFBP-3 and a protein produced by the liver, an acid-labile subunit (21). Insulin-like growth factor–binding protein-3 has been shown to acutely increase after cycling exercise (22) and may be important in this regard.

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The recent discovery of ghrelin adds an additional catalyst for exercise-induced alteration of the GH/IGF-I system. Ghrelin is the recently isolated endogenous ligand of the GH secretagogue (GHS) receptor that is secreted primarily by the stomach (23), but also in the arcuate nucleus of the hypothalamus (23) as well as the pituitary (24, 25), kidney (26), and placenta (24). Intravenous ghrelin administration has been shown to stimulate GH in a dose-dependent fashion in healthy individuals (27) and to be related to IGF-I levels (28). Moreover, ghrelin is known to affect feeding behavior and energy metabolism (29). The effects of exercise on metabolism and feeding behavior have important implications for prevention of obesity and obesity-related diseases; thus an examination of the effects of exercise on ghrelin warrants investigation.

In a recent study investigating GH responses to strenuous exercise, it was concluded that GH responses are only partially due to complete inhibition of hypothalamic somatostatin and that other factors such as ghrelin and growth hormone–releasing hormone (GHRH) may play a role (30); however, only two studies have examined ghrelin responses to exercise (31, 32). Both studies found no change in ghrelin concentrations, but neither examined acute responses of ghrelin to a rigorous running protocol.

We recently examined the effects of a strenuous running protocol on glucoregulatory hormones (including insulin and amylin; Ref. 33) and extended this study to determine the effects of a progressive, intermittent, rigorous running protocol on ghrelin, GH, IGF-I, and IGFBP-3 concentrations. Greater increases in IGF-I have been documented from higher versus lower cycling workloads (12). Since transient changes in circulating IGF-I from exercise have been attributed to hemodynamic or metabolic effects of exercise (34), we hypothesized that a rigorous bout of running would lead to increases in IGF-I and IGFBP-3 with concomitant increases in GH. Moreover, since it has been suggested that ghrelin may play a role in GH release during strenuous exercise (30), we hypothesized that an exercise protocol this demanding would increase ghrelin concentrations. The present study differs from these previous studies in that running, rather than cycling, provided the GH/IGF-I axis stimulus, the protocol was more rigorous than those in the previous two studies, and exercise responses were compared with a nonexercise control trial.

Subjects and Methods

A description of the methods of the study is described in detail in our earlier report (33). In brief, the six male subjects' mean ± SE age, weight, percent fat, and Vo_2max were 27.7 ± 3.2 years, 72.0 ± 4.6 kg, 11.3% ± 1.2%, and 61.8 ± 2.6 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively. The subjects had no history of cardiovascular or metabolic diseases, were between the ages of 18 and 39 years, were following a normal dietary regimen, and were not taking any medications. All had previous running experience (personal record <36 mins for a 10-km run) and were well trained (Vo_2max > 52.0 $ml \cdot kg^{-1} \cdot min^{-1}$). The study was approved by the Southeastern Louisiana University Institutional Review Board.

Preliminary Trial. Subjects completed a preliminary trial to determine standard fitness measures that included body composition (skinfold measures) and cardiorespiratory fitness (Vo_2max). Subjects completed a graded exercise test to exhaustion at a constant grade. The treadmill speeds (at a 4% treadmill grade) that corresponded with 60%, 75%, 90%, and 100% Vo_2max were calculated from a regression equation generated from the treadmill speeds and corresponding Vo_2 readings during the graded exercise test to exhaustion. Specifically, the Vo_2 for each percentage of Vo_2max was determined and then entered into the regression equation to calculate the treadmill speed for that particular Vo_2. At the end of the preliminary trial, subjects were asked to maintain their normal eating regimen throughout the study, including the week before the exercise and control trials. Before these trials, subjects were questioned about the food that they ate during the prior week to screen for unusual dietary patterns (e.g., abnormal amounts of carbohydrates, fats, and proteins, as well as hypocaloric patterns); all subjects reported normal dietary patterns.

Exercise and Control Trials. Subjects refrained from exercise and alcohol 24 hrs before testing. Subjects reported for the exercise trial at 0745 hr following an overnight fast. An intravenous catheter (Travenol, 22 g, 32 mm) was inserted into an antecubital vein, and a physiological saline lock was attached. At 0830 hr, 40 mins prior to exercise (–40) and at 0900 hr, 10 mins prior to exercise (–10), resting blood samples were collected from the catheter. Subjects then completed an intermittent treadmill exercise protocol at four speeds predicted to elicit a specific Vo_2: 60% Vo_2max for 10 mins, 75% Vo_2max for 10 mins, 90% Vo_2max for 5 mins, and 100% Vo_2max for 2 mins. After each workload was completed at the prescribed intensity and duration, treadmill speed was reduced to a walking speed for 3.5–4 mins to allow a blood sample to be collected. Gas samples were collected continuously and confirmed that the actual Vo_2 corresponded with the predicted Vo_2 for each workload. The protocol was previously shown to be a provocative stimulus of glucoregulatory hormones (33), which may affect ghrelin concentrations as well as the GH/IGF-I axis. A control trial was conducted 1 month after the exercise trial under identical conditions with the exception that subjects rested, rather than completed, the bout of treadmill exercise.

In addition to blood samples collected from the intravenous catheter after each workload (60%, 75%, 90%, and 100% Vo_2max), samples were collected every 15 mins during a 1-hr recovery (R15, R30, R45, and R60). Sera from blood samples were stored at –80°C until assayed.

Analyses. Lactate was determined using an enzymatic, colorimetric method (Sigma Chemical, St. Louis, MO). Ghrelin was determined by radioimmunoassay (Linco Research, Inc., St. Charles, MO). Growth hormone was measured by a sensitive chemiluminescent assay (Immulite, Diagnostic Products Corp., Los Angeles, CA). Serum IGF-I was measured following ethanol extraction by an IRMA procedure (Diagnostic Systems Laboratories, Inc., Webster, TX). Serum IGFBP-3 was determined by an IRMA procedure (Diagnostic Systems). For the ghrelin assay there were low, middle, and high pools that ranged from 1000 to 2000, and the average value was 16.17% for the interassay CV and 7.07% for the intraassay CV with a sensitivity of 10 pg/ml. Interassay coefficients of variation for GH, IGF-I, and IGFBP-3 were 8.8%, 9.5%, and 13.1%, respectively; sensitivity was 0.5 ng/ml, <5.0 ng/ml, and <2.0 ng/ml, respectively; intraassay coefficients of variation for all three assays were <5.0%. Hematocrit was determined using the microhematocrit method; colorimetric analysis was used to determine hemoglobin (Sigma Chemical). Hematocrit and hemoglobin were then used to determine the degree of hemoconcentration (35).

Statistics. Raw hormone values were corrected for plasma volume shifts, and corrected data were analyzed statistically using three different approaches. First, to examine total response of hormones/binding protein to exercise, integrated area under the curves (AUC) for exercise and control trials were computed using a trapezoidal method after subtracting average baseline concentrations for each subject. Mean AUC values for exercise and control trials were compared using dependent *t* tests. Second, 2 × 8 (trial × time point) repeated measures analyses of variance (ANOVAs) were used to examine hormone/binding protein changes over time and hormone/binding protein concentrations between trials. *Post hoc* analyses were computed to determine Eta-squared, an indication of the percent of variance in the hormone attributed to group differences, and observed power to detect differences of this size as significant. Third, to examine the relationship between ghrelin and GH, IGF-I, and IGFBP-3 concentrations in response to exercise, Pearson correlation coefficients were computed.

Results

Lactate values increased approximately 10-fold from pre-exercise to a peak value after 100% Vo_2max (Fig. 1). This indicated that substantial metabolic stress was produced by the exercise protocol.

For GH there was a significant time effect [$F(6,60) = 9.03$, $P < 0.001$] and time by trial interaction [$F(6,60) = 10.83$, $P < 0.001$]. During the exercise trial, GH rose after 75% Vo_2max and peaked after 100% Vo_2max, then declined sharply during recovery (Fig. 2); values did not change significantly during the control trial. The Eta-squared value indicated 52% of the variance in GH was attributed to

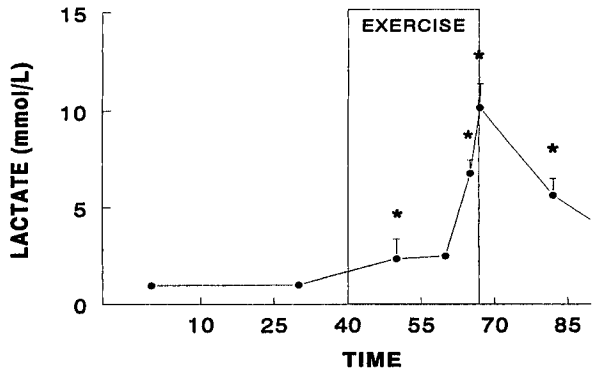

Figure 1. Data depict mean ± SE blood lactate for exercise and control trials before exercise, after each exercise intensity, and for 30 mins of recovery. Concentrations are for 40 and 10 mins before exercise; after each exercise intensity (10 mins of 60% Vo_2max, 10 mins of 75% Vo_2max, 5 mins at 90% Vo_2max, and 2 mins at 100% Vo_2max); and 15 and 30 mins of recovery. The asterisk (*) represents significantly different values for exercise versus control values.

changes during exercise and control trials (the interaction), and power to detect these differences as significant was 0.99. Growth hormone AUC for the exercise trial was significantly higher than the control trial ($P < 0.05$).

IGF-I levels rose slightly during exercise and remained elevated during recovery (Fig. 3). Comparison of concentrations via ANOVA failed to reveal significant differences between exercise and control trials nor significant changes during trials. The Eta-squared value indicated 13% of variance in IGF-I was due to differences in changes during exercise versus control trials; observed power for this effect was 0.53. Area under the curve values for IGF-I were significantly higher during the exercise trial than during the control trial ($P < 0.05$), indicating a greater overall response during the exercise trial.

The IGFBP-3 values rose during exercise and remained elevated after 15 mins of recovery, then declined at 30 mins of recovery (Fig. 4). The ANOVA comparing values during exercise and control trials revealed a significant time effect [$F(6,60) = 3.67$, $P < 0.01$], but no significant differences between trials. Eta-squared calculations indicated 9% of the variance was attributed to differences in changes in IGFBP-3 during exercise versus control trials; observed power for this effect was 0.36. The IGFBP-3 AUC was somewhat higher during the exercise than the control trial; this difference approached statistical significance ($P < 0.10$). (Although our initial experiment involved four recovery blood samples [i.e., 15, 30, 45, and 60 mins postexercise], serum and plasma samples for all six subjects were only of sufficient quantity for the 15- and 30-min blood draw time points. However, observed hormone concentrations for three subjects with sufficient serum for 45 and 60 mins postexercise was essentially unchanged for ghrelin, IGF-I, and IGFBP-3 compared with samples at 15 and 30 mins postexercise).

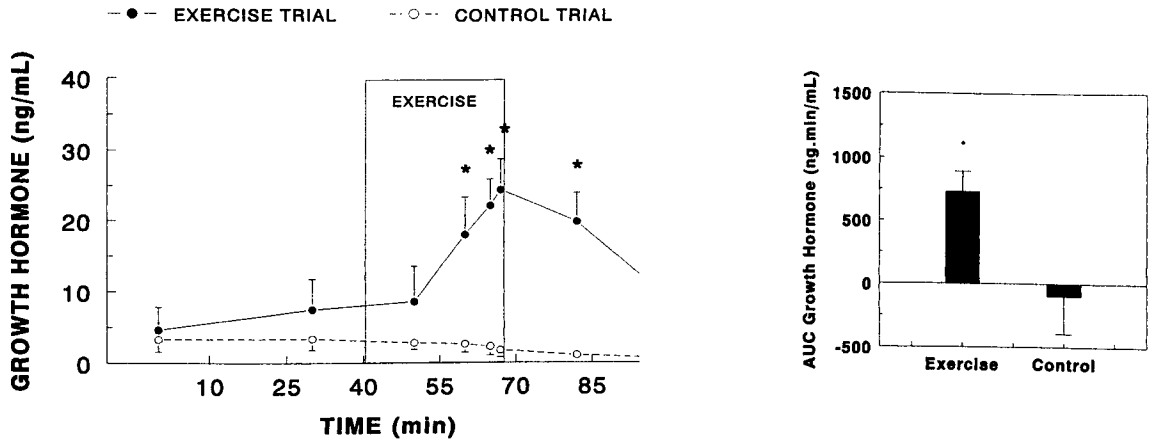

Figure 2. (Left) Data represent mean ± SE growth hormone (GH) concentrations for exercise and control trials before exercise, after each exercise intensity, and for 30 mins of recovery. Concentrations are for 40 and 10 mins before exercise; after each exercise intensity (10 mins of 60% Vo_2max, 10 mins of 75% Vo_2max, 5 mins at 90% Vo_2max, and 2 mins at 100% Vo_2max); and 15 and 30 mins of recovery. (Right) Values are area under the curve (AUC) concentrations for growth hormone for exercise and control trials. The asterisk (*) represents significantly different values for exercise versus control.

Ghrelin concentrations during exercise were stable and not significantly different than during the control trial (Fig. 5). The size of the mean difference between ghrelin during exercise and control was small: less that 1% of the variance in this hormone was explained by the trial factor. Comparison of AUC values similarly did not indicate any differences between the exercise and control trials.

Pearson correlation coefficients revealed significant relationships between ghrelin and both IGF-I ($r = 0.25$, $P < 0.05$) and IGFBP-3 ($r = 0.52$, $P < 0.01$). However, no relationship between ghrelin and GH was found ($r = 0.00$, $P > 0.05$).

Discussion

We examined the effects of a progressively intense intermittent running protocol on peripheral concentrations of GH, IGF-I, and IGFBP-3, as well as the most recent member of the family of GH regulators, ghrelin. Compared with concentrations of the same subjects in a nonexercise control trial, the exercise produced expected large (fivefold) increases in GH and smaller significant increases in IGF-I (AUC), but did not increase ghrelin or IGFBP-3. There were significant positive correlations between ghrelin and IGF-I as well as ghrelin and IGFBP-3; thus higher ghrelin values were associated with higher IGF-I and IGFBP-3 concentrations. However, there was not a significant relationship between ghrelin and GH.

GH has been shown to respond to most exercise modalities, including running (8, 9, 36); resistance exercise (13, 14); and cycling (37, 38). The mechanism for increases in GH may include stimulation of growth hormone–releasing hormone (GHRH) and/or reduction in release of somatostatin (39), but our findings suggest it is not stimulated by peripheral ghrelin concentrations during a strenuous running trial. It remains a possibility that ghrelin released in the

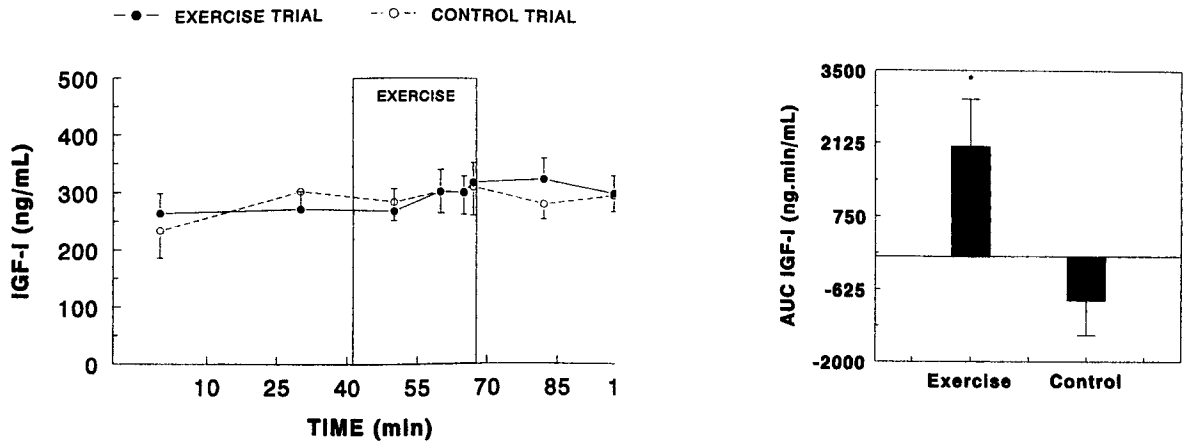

Figure 3. (Left) Data depict mean ± SE insulin-like growth factor-I (IGF-I) concentrations for exercise and control trials before exercise, after each exercise intensity, and for 30 mins of recovery. Concentrations are for 40 and 10 mins before exercise; after each exercise intensity (10 mins of 60% Vo_2max, 10 mins of 75% Vo_2max, 5 mins at 90% Vo_2max, and 2 mins at 100% Vo_2max); and 15 and 30 mins of recovery. (Right) Values are area under the curve (AUC) concentrations for IGF-I.

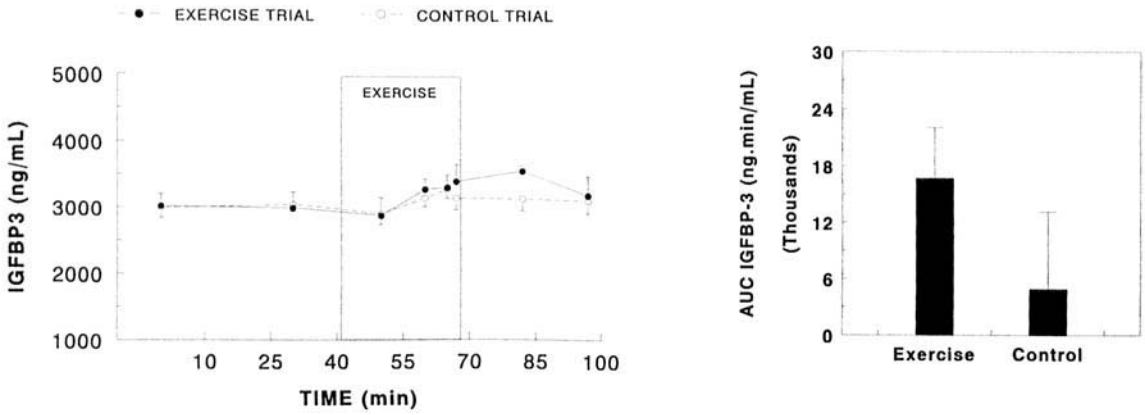

Figure 4. (Left) Data represent mean ± SE insulin-like growth factor–binding protein-3 (IGFBP-3) concentrations for exercise and control trials before exercise, after each exercise intensity, and for 30 mins of recovery. Concentrations are for 40 and 10 mins before exercise; after each exercise intensity (10 mins of 60% Vo_2max, 10 mins of 75% Vo_2max, 5 mins at 90% Vo_2max, and 2 mins at 100% Vo_2max); and 15 and 30 mins of recovery. (Right) Values are area under the curve (AUC) concentrations for IGFBP-3.

central nervous system may act upon the hypothalamus or pituitary during exercise and enhance the GH response, since ghrelin mRNA is expressed in the arcuate nucleus (23, 31). Ghrelin in the arcuate nucleus would have a paracrine action and not be detectable in plasma.

Several studies have examined the effects of exercise on total IGF-I concentrations with equivocal results that seem to be related to exercise mode. Cycling at different intensities has been reported to increase IGF-I concentrations (12, 22), as has rowing (28), but no significant increases were reported in response to low- or high-volume resistance exercise (13, 14, 40). Moreover, no significant change in total IGF-I has been reported in response to 60 mins of treadmill running at 70% Vo_2max (17) or to running a marathon using a pre/post sampling design (18, 19). However, greater increases in IGF-I have been documented from higher versus lower cycling workloads (12). Thus, we utilized a progressive running protocol that included greater intensities than previous running studies and more frequent sampling, and we demonstrated a greater AUC IGF-I response to the exercise versus the control trial of the same subjects. Perhaps the intensity of the protocol, blood sampling design, and/or use of AUC analyses explain the different IGF-I findings between the present and previous running studies.

We are aware of only two studies to date that have examined ghrelin responses to exercise. Dall *et al.* (31) reported no effect of 45 mins of cycling at approximately 62% of Vo_2max on ghrelin concentrations in normal and GH-deficient patients. Another investigation examined ghrelin responses to cycling in normal subjects and patients with the leucine 7 to proline 7 polymorphism in the signal peptide of neuropeptide Y (32). The cycling protocol progressed to 80% Vo_2max in 8 mins, maintained 80% Vo_2max for 10 mins, and declined to 20% Vo_2max for 10 mins. Although GH increased significantly in response to the cycling (with greater increase in normal subjects), there were no significant changes in ghrelin concentrations. The protocol in the present study included progressively intense

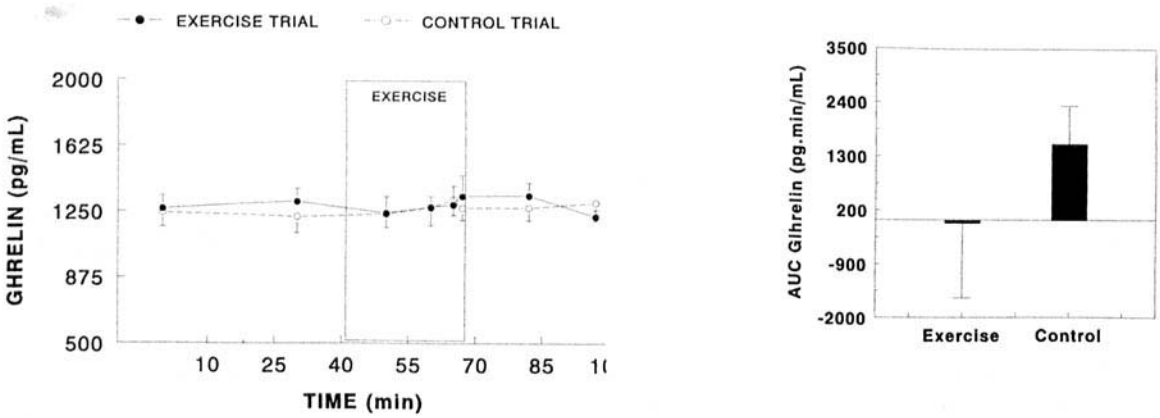

Figure 5. (Left) Data depict mean ± SE ghrelin concentrations for exercise and control trials before exercise, after each exercise intensity, and for 30 mins of recovery. Concentrations are for 40 and 10 mins before exercise; after each exercise intensity (10 mins of 60% Vo_2max, 10 mins of 75% Vo_2max, 5 mins at 90% Vo_2max, and 2 mins at 100% Vo_2max); and 15 and 30 mins of recovery. (Right) Values are area under the curve (AUC) concentrations for ghrelin.

exercise exceeding 80% of Vo_2max, including 5 mins at 90% Vo_2max and 2 mins at 100% of Vo_2max. Thus, compared with the investigation by Dall *et al.* (31), three of the four stages in the present study were conducted at a higher intensity, and compared with the study by Kallio *et al.* (32), two of the four stages were conducted at a higher intensity. Thus, the present study differs from these previous studies in that running, rather than cycling, provided a GH/IGF-I axis stimulus, the protocol was more rigorous than those in the previous two studies, and exercise responses were compared with a nonexercise control trial.

It has been shown that ghrelin gene expression is affected by the level of circulating IGF-I (40). In the present study we found significant relationships between ghrelin and IGF-I ($r = 0.25$, $P < 0.05$) as well as ghrelin and IGFBP-3 ($r = 0.52$, $P < 0.01$) across trials, which is different from the previous findings of Dall *et al.* (31). Although the design of the present study does not allow us to attribute cause, the significant increase in IGF-I in the present study may have contributed to the significant relationship between ghrelin and IGF-I levels found in the present study. Whether ghrelin is affected by IGF-I during exercise remains to be elucidated.

It could have been hypothesized that the glucoregulatory stress from the exercise would result in suppression of ghrelin concentrations during recovery. Data from Flanagan *et al.* (41) suggests that insulin may suppress ghrelin independently of glucose. We previously reported that the same exercise protocol dramatically elevated insulin concentrations at the end of the protocol (100% Vo_2max; 33), yet in the present study, ghrelin levels did not appear to be suppressed. It is possible that greater insulin levels would be required in order to suppress ghrelin concentrations in recovery.

The study examined ghrelin responses to high-intensity exercise in subjects who fasted overnight. It is possible that a fasting-induced elevation of ghrelin masked an exercise-induced effect. However, previous research has shown that ghrelin concentrations are reduced 2 hrs postprandial (42). If subjects had eaten several hours prior to exercise, a possible alternate effect of meal-induced suppression of ghrelin may have modified an exercise-induced effect on ghrelin. Moreover, feeding changes insulin/glucose dynamics, which is known to affect the GH/IGF-I axis, and we did not wish to interject a confounding variable that could modify these hormone concentrations. Since the effects of either caloric intake or restriction on exercise-induced responses of ghrelin is not currently known, we chose to use a fasting condition in the present study. Clearly this issue warrants further investigation.

In summary, the findings of the study demonstrate increases in total IGF-I concentrations in response to a progressive, rigorous, intermittent running protocol, which differs from previous studies that utilized running as the GH/IGF-I axis stimulus. These differences may be attributed to a more rigorous running protocol and/or more frequent blood sampling in the present study. Moreover, results suggest that rigorous running does not increase ghrelin concentrations and that peripheral ghrelin levels do not affect GH responses to exercise. However, ghrelin was significantly related to IGF-I and IGFBP-3, and future studies will be required to determine the implications of these findings.

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