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Ghrelin and Other Glucoregulatory Hormone Responses to Eccentric and Concentric Muscle Contractions

R. R. Kraemer,[1] R. J. Durand,[2] D. B. Hollander,[1]
J. L. Trynieckl,[1] E. P. Hebert,[1] and V. D. Castracane[3]

[1]Department of Kinesiology and Health Studies, Southeastern Louisiana University, SLU10845, Hammond, LA 70402; [2]Pennington Biomedical Research Center, Health and Fitness Center, Baton Rouge, LA 80808; and [3]V Daniel Castracane, Department of Obstetrics and Gynecology, Texas Tech University Health Sciences Center, 1400 Wallace Blvd., Amarillo, TX 79106

Objective: Heavy resistance exercise increases growth hormone (GH) and blood glucose levels. Ghrelin is an endogenous ligand for the GH secretagory receptor that stimulates growth hormone release. Circulating ghrelin levels are suppressed by insulin and glucose. The study was conducted to determine effects of concentric (CON) and eccentric (ECC) muscle actions at the same absolute workload on circulating ghrelin and glucose as well as related glucoregulatory peptides.

Methods: Ten-RM loads for bench press, leg extension, military press, and leg curl were obtained from nine males, mean age 25. ± 1.2 yr and body fat 17.2 ± 1.6%. Subjects then completed two experimental trials of either CON or ECC contractions at the same absolute workload. Subjects performed four sets of 12 repetitions for each exercise at 80% of a 10-RM with 90 s rest periods. A pulley system or steel levers were positioned on each machine to raise or lower the weight so only CON or ECC contractions were performed. Pre-, post-, and 15-min post-exercise blood samples were collected.

Results: Ghrelin did not increase in response to either muscle action and actually declined during the CON trial. Glucose and insulin increased regardless of the form of muscle action, but amylin and C-peptide did not change.

Conclusions: Data indicate that ghrelin does not contribute to moderate resistance exercise-induced increases in growth hormone, whether from CON or ECC muscle actions. Results suggest that with a moderate loading protocol both CON and ECC muscle actions performed at the same absolute workload elevate glucose and insulin concentrations, but are not related to post-CON exercise ghrelin suppression.

Key Words: Ghrelin; glucose; insulin; amylin; resistance exercise.

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Author to whom all correspondence and reprint requests should be addressed: R. R. Kraemer, Department of Kinesiology and Health Studies, Southeastern Louisiana University, SLU10845, Hammond, LA 70402. E-mail: rkraemer@selu.edu

Introduction

Ghrelin is a 28-amino-acid peptide recently isolated from human and rat stomach that is also present in human and rat pancreatic alpha-cells *(1)*. The peptide has been shown to be a potent growth hormone (GH) secretagogue that stimulates increases in blood glucose *(2–4)* and strongly affects feeding behavior by increasing hunger *(1)*. Recent research has demonstrated that intragastric infusions of glucose in laboratory rats result in 50% reduction of ghrelin levels when gastric emptying is permitted *(5)*. In humans, insulin and glucose have been shown to suppress circulating ghrelin *(6,7)*. Amylin is a 37-amino-acid peptide that is co-secreted with insulin from pancreatic beta-cells *(8)*. Amylin inhibits insulin and glucagon release and reduces gastric emptying *(9)*, although its involvement with ghrelin is unknown. A better understanding of the effects of ghrelin on GH and the role of glucoregulatory factors affecting ghrelin under conditions of a GH stimulus could be beneficial.

Heavy resistance exercise is known to increase GH and blood glucose levels *(10)*. It has recently been suggested that GH responses to strenuous exercise are only partially due to complete inhibition of hypothalamic somatostatin and that other factors such as ghrelin and GHRH may play a role *(11)*. Resistance exercise typically is performed using both concentric (CON) contractions that shorten the muscle and eccentric (ECC) contractions that lengthen the muscle; however, the physiological responses to these contractions may differ during standard resistance exercise. As such, resistance exercise protocols have been developed to favor the utilization of one contraction form over the other. We have recently shown that GH responses to CON muscle contractions were much greater than those produced by ECC muscle contractions at the same absolute workload. We attributed the lesser GH responses to ECC muscle actions at the same absolute workload to greater torque-producing capacity and thus reduced metabolic response compared to CON muscle actions *(12)*. We extended this study by using a subset (9 out of 10) of the same subjects to determine whether ghrelin responses to CON and ECC contraction modes result in different ghrelin responses to exercise and recovery. Moreover, we have examined glucose and glucoregulatory

Table 1
Mean (± SE) Lactate and Growth Hormone Concentrations
Before, Immediately After, and 15 Min Following Exercise for the Concentric and Eccentric Trials

	Concentric Trial			Eccentric trial		
	Time			Time		
	Pre	Post	15-Post	Pre	Post	15-Post
Lactate (m*M*)	0.89 (0.15)	7.47 (1.10)	4.87 (0.69)	1.02 (0.10)	1.86 (0.33)	1.27 (0.19)
Growth hormone (ng/mL)	0.17 (0.07)	7.42 (1.17)	7.38 (1.12)	0.13 (0.35)	2.73 (1.02)	2.13 (1.04)

responses that may be associated with ghrelin levels, including insulin, amylin, and C-peptide. CON exercise has previously been shown to elicit greater GH responses than ECC exercise at the same absolute workload *(12)*. Because ghrelin is known to be a potent GH secretagogue and because ghrelin has been suggested along with GHRH to be a synergistic stimulator of GH release during exercise *(13)*, we hypothesized that CON muscle actions would produce greater increases in ghrelin concentrations than ECC contractions. Concomitantly, we investigated whether CON muscle actions would result in greater glucoregulatory responses than ECC contractions and whether glucose and glucoregulatory hormone concentrations would be associated with changes in ghrelin levels during recovery from exercise.

Results

Lactate and Growth Hormone

The subjects in the present study were a subset of a group of subjects whose lactate and GH values were reported previously *(12)*. Similar to the previous study, subjects in the present study showed a significant time effect for lactate and GH (Table 1), both increasing significantly across all trials. Both analyses also revealed a significant trial by time interaction. Follow-up comparisons of values at each time point between CON and ECC trials indicated no significant differences at pre-exercise, but significantly higher lactate and GH concentrations during CON exercise immediately post-exercise and 15 min post-exercise than during ECC exercise.

Glucose, Insulin, Amylin, and C-Peptide

There was a significant time effect for glucose, with increases across trials, but no significant differences between trials (Fig. 1). Similarly, there was a significant time effect for insulin concentrations, with insulin increasing across trials, but no significant differences between trials (Fig. 2). Analysis of amylin concentrations failed to reveal a significant change across trials ($p = 0.23$), nor differences between trials ($p = 0.78$) (Fig. 3). C-peptide did not change over time ($p = 0.066$), nor was there a difference between trials (Fig. 4).

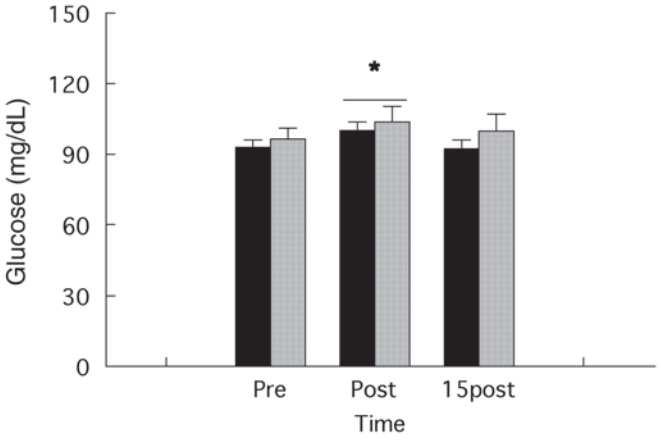

Fig. 1. Glucose responses for concentric (solid) and eccentric (hatched) trials before exercise, immediately after exercise, and after 15 min of recovery. Values are mean ± SE. *$p < 0.05$ compared with pre-exercise value.

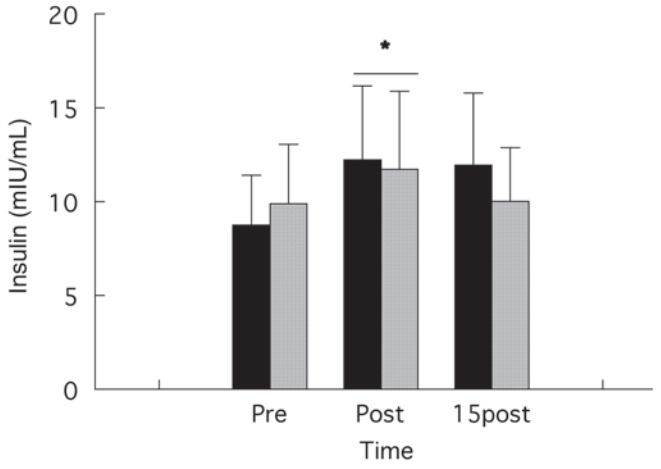

Fig. 2. Insulin responses for concentric (solid) and eccentric (hatched) trials before exercise, immediately after exercise, and after 15 min of recovery. Values are mean ± SE. *$p < 0.05$ compared with pre-exercise value.

Ghrelin

Analysis of ghrelin revealed a significant time by trial interaction (Fig. 5). Follow-up comparisons indicated ghrelin decreased significantly during the CON trial both from pre- to post-exercise and from post- to 15-min post-exercise, but did not change during the ECC trial.

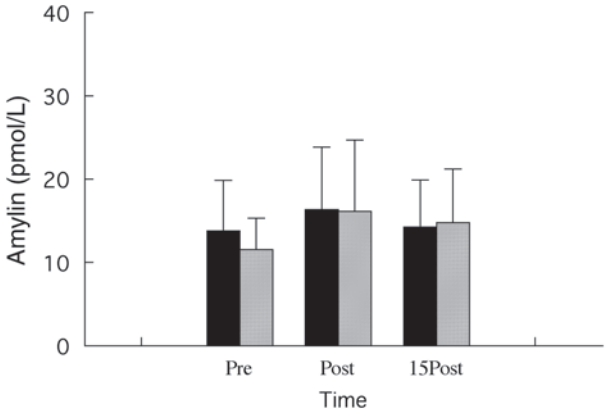

Fig. 3. Amylin responses for concentric (solid) and eccentric (hatched) trials before exercise, immediately after exercise, and after 15 min of recovery. Values are mean ± SE.

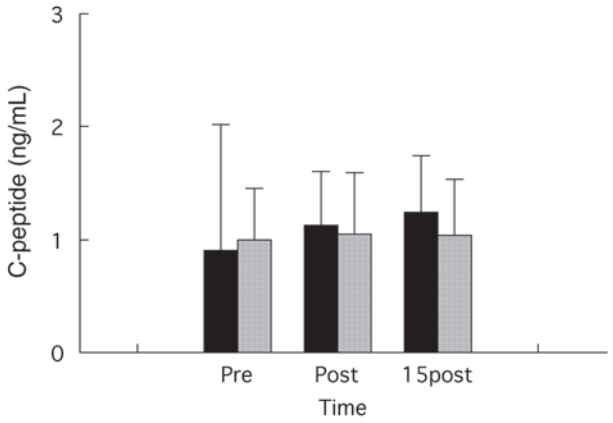

Fig. 4. C-peptide responses for concentric (solid) and eccentric (hatched) trials before exercise, immediately after exercise, and after 15 min of recovery. Values are mean ± SE.

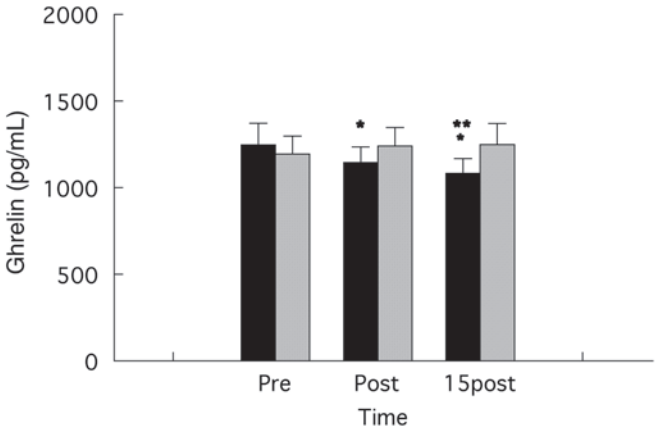

Fig. 5. Ghrelin responses for concentric (solid) and eccentric (hatched) trials before exercise, immediately after exercise, and after 15 min of recovery. Values are mean ± SE. *$p < 0.05$ compared with pre-exercise value for concentric trial; **$p < 0.05$ compared with post-exercise value for concentric trial.

Correlations

Pearson product moment correlations revealed no significant relationship between change in insulin during exercise and change in ghrelin post-exercise ($r = 0.17$, $p > 0.05$), nor was there a significant relationship between change in glucose during exercise and change in ghrelin post-exercise ($r = –0.34$, $p > 0.05$). However, there was a significant inverse relationship between change in GH during exercise and change in ghrelin post-exercise ($r = –0.58$, $p = 0.01$) indicating that muscle contraction-induced increases in GH were related to suppressed ghrelin levels during recovery.

Discussion

In the present study we investigated whether CON muscle actions would increase ghrelin concentrations to a greater extent than ECC contractions performed at the same absolute load. We also sought to determine whether CON and ECC muscle actions would result in greater glucoregulatory responses than ECC muscle actions and whether greater GH, glucose, and glucoregulatory hormone concentrations would be associated with suppressed ghrelin levels during recovery from exercise.

Lactate responses demonstrated that the CON resistance exercise trial elicited a greater metabolic load than the ECC resistance exercise trial as expected. Resistance exercise increased glucose and insulin regardless of the form of contraction employed; however, amylin and C-peptide did not change with either form of contraction. Ghrelin did not increase in response to either muscle action, regardless of an increase in GH, and was suppressed following the CON trial compared with the ECC trial using the same absolute load. There was a significant inverse correlation between pre/post-change in GH and post/15 min post-change in ghrelin, but the pre/post-change in insulin and glucose was not associated with a change in ghrelin.

Currently only two studies exist that have examined ghrelin responses to exercise, and both used an aerobic exercise stimulus *(14,15)*. This is the first study to investigate ghrelin responses to resistance exercise and we have examined separate effects of ECC and CON muscle actions at the same absolute workload on ghrelin, growth hormone, and glucoregulatory factors in healthy males.

Both glucose and insulin concentrations increased regardless of the form of muscle contraction. Increases in blood glucose are consistent with previous studies using different resistance exercise protocols *(10,16)*, but not with other resistance exercise studies *(17,18)*. Data from Robergs et al. *(16)* suggested that the glucose increases were from plasma volume shifts. Insulin increases in response to elevated levels of glucose, amino acids, and fatty acids *(20)*, which may explain the insulin increases in the present. We have previously reported increases in insulin and amylin in response to intense aerobic exercise that produced elevated blood glucose levels during exercise and recovery *(21)*. The insulin response in the present study differs from a previous resistance exercise report *(10)*, although the protocols used in the present study differ from the previous study, possibly explaining the different findings.

Both oral and iv administration of glucose have been shown to suppress ghrelin concentrations *(22)* as has administration of insulin *(7)*. Moreover, there is evidence that GH may inhibit ghrelin release *(14)*. Previous research has indicated that fasting will alter the diurnal rhythm of ghrelin that is followed by similar changes in serum GH *(23)*. Although there were increases in glucose and insulin concentrations from pre- to post-exercise, there was no significant relationship between these increases and the reduction of ghrelin. Moreover, ghrelin was suppressed following the CON and not the ECC trial, but glucose and insulin were significantly elevated for both trials. Collectively, these data suggest that glucose and insulin are not associated with suppression of ghrelin following CON and ECC muscle contractions. Perhaps a higher glucose or insulin response would have altered these findings.

Amylin was not altered over either trial and we are aware of no other studies examining amylin responses to resistance exercise. We have previously reported that a progressive, high intensity running protocol produces increases in insulin at the highest exercise intensities (90 and 100% V_{O_2max}) and during recovery, and amylin responses, although not as large as insulin, mirrored those of insulin *(21)*. The present resistance exercise trials resulted in smaller insulin responses than those in the previous amylin investigation that utilized running as a stimulus, leaving the possibility that a greater stimulus from a different resistance exercise protocol may be required to reach the threshold for amylin release. C-peptide also was not altered over either trial. C-peptide has a longer half-life than insulin *(24)*, and it is possible that a longer time period would be required before the C-peptide pool would increase enough to be revealed. However, these findings suggest that amylin and C-peptide do not affect ghrelin concentrations in response to separate resistance exercise muscle actions using a moderate muscle load.

Ghrelin did not increase in response to either trial. This suggests that from CON or ECC muscle contractions at the same absolute workload, ghrelin does not contribute to moderate resistance-exercise-induced increases in growth hormone. However, ghrelin was suppressed post-exercise. We have previously reported increases in GH in response to both the CON and ECC trials with a much greater GH response to the CON muscle actions *(12)*. These results were similar in the present study using a subset of the same subjects. The increase in GH in the present study was associated with a decrease in ghrelin concentrations following exercise. It is possible that greater frequency of blood sampling in an extended recovery would have revealed different ghrelin results. The effectiveness of sample timing with an immediate post-exercise sample and 15 min recovery sample assumes a relatively short half-life of ghrelin. In humans, the half-life of ghrelin has been suggested to be less than 60 min *(25)* and has shown to be 30 min in rats *(26)*. This, coupled with data demonstrating pronounced 30 min postprandial reductions in ghrelin *(22)* suggest that the sampling protocol could detect exercise responses. It is also possible that changes in ghrelin secretion are unrelated to pituitary GH secretion. Ghrelin has been shown to be a potent orexigenic agent *(27)*, and it remains to be determined whether the magnitude of the small reduction in ghrelin concentrations that occurred following the CON trial could alter post-exercise appetite or have any other physiological effect.

We previously described the changes in plasma volume that occurred in responses to CON and ECC muscle actions for these and other subjects *(12)*. In order to verify that suppression of ghrelin levels in the present study was not due to changes in plasma volume, we analyzed ghrelin using a trial × time (2 × 3) ANOVA after correcting each post- and 15-min post-exercise ghrelin concentration for plasma volume shifts using hematocrit and hemoglobin values *(28)*. Results were not different than the uncorrected values. It remains a possibility that ghrelin released in the 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 *(14)*. Ghrelin in the arcuate nucleus would have a paracrine action and not be detectable in plasma.

In summary, the data indicate that ghrelin does not contribute to moderate resistance-exercise-induced increases in growth hormone, whether from CON or ECC muscle actions performed with the same absolute load. Moreover, CON muscle actions may lead to a suppression of ghrelin concentrations. Finally, results suggest that with a moderate loading protocol, both CON and ECC muscle actions elevate glucose and insulin concentrations, but are not related to post-exercise ghrelin suppression. Future research will be required to determine the physiological significance of these findings.

Materials and Methods

Subjects and Research Design

The study was approved by the Southeastern Louisiana University Institutional Review Board and was conducted in accordance with the policy statement of the Declaration of Helsinki. The research design and protocol were the same as our previous report *(12)*. Written consent was obtained from nine males with recreational weight training experience. Inclusion criteria were 18–30 yr of age and a minimum of 3 yr of recreational weight training experience. Exclusion criteria were (1) participation in competitive bodybuilding or weight lifting for the previous year; (2) smoking; (3) taking medications that could alter test results (e.g., anabolic steroids, sympathoadrenal drugs); (4) history of pituitary, renal, hepatic, cardiovascular, or metabolic disease; (5) adherence to a reduced calorie or low fat diet, or ketogenic diet that could affect hormonal levels; and (6) use of over-the-counter ergogenic aids within the past month including creatine monohydrate, androstenedione, DHEA, or ephedra. Upon meeting criteria for participation, nine

healthy males gave written consent and subsequently completed three testing trials on separate days including a preliminary trial, a CON only exercise trial, and an ECC only exercise trial. The last two trials were conducted using a counterbalanced design to compare effects of CON and ECC muscle actions on ghrelin, glucose, insulin, amylin, and C-peptide. The preliminary trial served several purposes: (1) to collect anthropometric and strength data, (2) to screen subjects for dietary habits and use of ergogenic aids, and (3) to familiarize subjects with the exercise equipment and protocols. The mean ± SE for age, height, body weight and %body fat were 25 ± 1.2 yr, 176.7 ± 1.6 cm, 85.8 ± 5.5 kg, and 17.2 ± 1.6%, respectively. The subjects' 10 repetition maximum (10-RM) for bench press, leg extension, military press, and leg curl were 118.90 ± 35.68 kg, 39.09 ± 13.03 kg, 71.71 ± 21.82 kg, and 21.72 ± 7.24 kg, respectively.

Preliminary Trial (Session 1)

Height and weight were determined using a calibrated scale. Body composition was assessed with Lange skinfold calipers using a four-site (abdomen, suprailiac, triceps, and thigh) equation *(29)*. Subjects were also tested to determine the 10-repetition maximum (10-RM) for each of the four exercises: bench press (BP), leg extension (LE), military press (MP), and leg curls (LC); the exercises were performed on a resistance training machine (BK 602 Super Jungle, Body Masters, Rayne, LA). These four exercises were chosen because they represent two upper body and two lower body exercises commonly employed in normal resistance training routines. 10-RM strength measurements have been accepted as a suitable secondary choice for determining appropriate training loads when maximal strength testing is not essential *(30)*. The subjects participating in the study were well-trained recreational athletes who were not involved in sport but whose normal training regimen consisted of bodybuilding-type workouts (i.e. multiple sets, high repetition training); thus, a 10-RM was deemed more appropriate for individuals in our study. The subjects performed the lifts to determine the 10-RM in a controlled manner without bouncing or jerking movements, and position of all benches, seats, bars and subject handgrip alignment were recorded and kept constant for each exercise. For each exercise, the distance the weight was displaced was determined with a metal meter stick and pointers mounted onto the resistance exercise equipment in an effort to maintain constant work per repetition in subsequent sessions.

After completion of the 10-RM for each exercise, a brief familiarization procedure for the experimental trial was conducted. Subjects performed light-weight CON and ECC repetitions to become familiar with the movements.

Two 48-in. (10 lbs each) steel pipes were bolted on top of the machine bench and military press before each trial. The steel rods were placed on the machine before the 10-RM to simulate the conditions of the CON and ECC trials. A 5–10 min rest period after each 10-RM attempt was allowed. BP was performed first, followed by LE, MP, and LC.

Sessions 2 and 3 (CON and ECC Trials)

The subjects returned to the weight room 7 d later for session 2 or 3 following an 8-h fast. Sessions 2 and 3 were conducted within 1 wk of each other in counterbalanced fashion. At 8:00 AM the subject was seated quietly for 20 min followed by collection of a baseline blood sample from an antecubital vein via venipuncture. The subject then performed 4 sets of 12 repetitions of the four exercises at 80% of the previously determined 10-RM in the following order: BP, LE, MP, and LC. During the CON trial subjects lifted the weight for each repetition, whereas lowering the weight was performed by technicians using steel bar extensions or a pulley. During the ECC trial, technicians lifted the weight stack with steel bar extensions or a pulley, and then the subject lowered the weight. Repetitions were performed to the rhythm of a metronome and the weight was lifted in 2 s and lowered in 2 s. Subjects rested 90 s between all sets and exercises. A rest period of 90 s was chosen because this interval is indicative of high intensity weight training sessions commonly performed. After completion of the fourth set of LC, a blood sample was immediately collected and another blood sample was taken 15-min post-exercise. For each blood sample, blood was collected in a 10-mL whole blood tube and a 10 mL EDTA tube for hormone analysis, and a 3-mL tube with sodium fluoride and potassium oxalate for lactate determination. Sera and plasma were aliquotted and samples were stored at −80°C until hormone assays were performed.

Blood Analyses

Blood samples were analyzed for lactate, GH, glucose, insulin, amylin, C-peptide, and ghrelin. Lactate and glucose were determined using an enzymatic method (Sigma Chemical, St. Louis, MO). Ghrelin and C-peptide were determined by radioimmunoassay (Linco Research, St. Charles, MO, USA, and Diagnostic Systems Laboratories, Webster, TX, USA). Amylin was determined by immunoenzymatic assay (Linco Research Inc.). GH and insulin were determined by chemiluminescent assay (Immulite, Diagnostic Products Corporation, Los Angeles, CA). 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 coefficient of variation (CV) and 7.07% for the intraassay CV. The interassay and intraassay coefficients of variation for amylin were < 15% and < 10%, respectively. Interassay coefficients of variation for insulin, C-peptide, and growth hormone were 10.6, 12.0, and 8.8%, respectively, and the intraassay CVs were all <5%.

Lactate, GH, glucose, insulin, amylin, C-peptide, and ghrelin concentrations were analyzed using separate 2 × 3 (trial × time) ANOVAs with repeated measures on the second factor. The trial factor represented CON and ECC trials, and the time factor represented pre-, post-, and 15-min recovery

values. Statistical significance was accepted at $p < 0.05$. Significant effects were followed by appropriate planned comparisons. Because we were interested in investigating whether changes in GH and glucoregulatory factors during exercise affected ghrelin concentrations during recovery, change scores in growth hormone, glucose, and insulin from pre- to post-exercise were compared with change scores in ghrelin from post- to 15-min post-exercise by using a Pearson product moment correlational analysis.

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References

1 Date, Y., Nakazato, M., Hashiguchi, S., et al. (2002). *Diabetes* **51,** 124–129.
2 Arosio, M., Ronchi, C. L., Gebbia, C., Cappiello, V., Beck-Peccoz, P., and Peracchi, M. (2003). *J. Clin. Endocrinol. Metab.* **88,** 701–704.
3 Broglio, F., Arvat, E., Benso, A., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 5083–5086.
4 Broglio, F., Benso, A., Castiglioni, C., et al. (2003). *J. Clin. Endocrinol. Metab.* **88,** 1537–1542.
5 Williams, D. L., Cummings, D. E., Grill, H. J., and Kaplan, J. M. (2003). *Endocrinology* **144,** 2765–2767.
6 Flanagan, D. E., Evans, M. L., Monsod, T. P., et al. (2003). *Am. J. Physiol. Endocrinol. Metab.* **284,** E313–E316.
7 Mohlig, M., Spranger, J., Otto, B., Ristow, M., Tschop, M., and Pfeiffer, A. F. (2003). *J. Endocrinol. Invest.* **25,** RC36–RC38.
8. Young, A. (1997). *Curr. Opin. Endocrinol. Diabetes* **4,** 282–290.
9 Nyholm, B., Walker, M., Gravholt, C. H., et al. (1999). *Diabetologia* **42,** 1314–1323.
10 Kraemer, W. J., Hakkinen, K., Newton, R. U., et al. (1998). *Eur. J. Appl. Physiol.* **77,** 206–211.
11 de Vries, W. R., Abdesselam, S. A., Schers, T. J., Maas, H. C., Osman-Dualeh, M. I., and Koppeschaar, H. P. (2002). *Metabolism* **51,** 1093–1096.
12 Durand, R. J., Castracane, V. D., Hollander, D. B., et al. (2003). *Med. Sci. Sports Exerc.* **35,** 937–943.
13 de Vries, W. R., Schers, T. J., Ait Abdesselam, S., Osman-Dualeh, M., Maitimu, I., and Koppeschaar, H. P. (2003). *Int. J. Sports Med.* **24,** 208–211.
14. Dall, R., Kanaley, J., Hansen, T. K., et al. (2002). *Eur. J. Endocrinol.* **147,** 65–70.
15 Kallio, J., Pesonen, U., Karvonen, M. K., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 5348–5352.
16 Robergs, R. A., Pearson, D. R., Costill, D. L., et al. (1991). *J. Appl. Physiol.* **70,** 1700–1706.
17 Haff, G. G., Koch, A. J., Potteiger, J. A., et al. (2000). *Int. J. Sport Nutr. Exerc. Metab.* **10,** 326–339.
18 Haff, G. G., Schroeder, C. A., Koch, A. J., Kuphal, K. E., Comeau, M. J., and Potteiger, J. A. (2001). *J. Sports Med. Phys. Fitness* **41,** 216–222.
19. Keul, J., Haralambie, G., Bruder, M., and Gottstein, H. J. (1978). *Med. Sci. Sports* **10,** 13–15.
20 Haff, G. G., Lehmkuhl, M. J., McCoy, L. B., and Stone, M. H. (2003). *J. Strength Cond. Res.* **17,** 187–196.
21 Kraemer, R. R., Acevedo, E. O., Synovitz, L. B., et al. (2002). *Metabolism* **51,** 657–663.
22 Shiiya, T., Nakazato, M., Mizuta, M., et al. (2002). *J. Clin. Endocrinol. Metab.* **87,** 240–244.
23 Muller, A. F., Lamberts, S. W., Jansses, J. A., et al. (2002). *Eur. J. Endocrinol.* **146,** 203–207.
24 Wahren, J., Ekbert, K., Johansson, J., et al. (2000). *Am. J. Physiol. Endocrinol. Metab.* **278,** E759–E768.
25 Moller, N., Nygren, J., Hansen, T. K., Orskov, H., Frystyk, J., and Nair, K. S. (2003). *J. Clin. Endocrinol. Metab.* **88,** 850–852.
26 Tolle, V., Bassant, M. H., Zizzari, P., et al. (2002). *Endocrinology* **143,** 1353–1361.
27 Olszewski, P. K., Li, D., Grace, M. K., Billington, C., Kotz, C. M., and Levine, A. S. (2003). *Peptides* **24,** 597–602.
28 Dill, D. B. and Costill, D. L. (1974). *J. Appl. Physiol.* **37,** 247–248.
29 Jackson, A. S. and Pollock, M. L. (1978). *Br. J. Nutr.* **40,** 497–504.
30. Baechle, T. R. and Earle, R. W. (2000). *Essentials of strength training and conditioning*. Human Kinetics: Champaign, IL.