Possible Role for Growth Hormone in Suppressing Acylated Ghrelin and Hunger Ratings During and After Intermittent Exercise of Different Intensities in Obese Individuals

Majid Gholipour[1], Mohamad Reza Kordi[2], Mohamad Taghikhani[3],

Ali Asghar Ravasi[2], Abas Ali Gaeini[2], and Arezoo Tabrizi[1]

[1] Physical Education Center, Sharif University of Technology, Tehran, Iran

[2] Faculty of Physical Education and Sport Sciences, University of Tehran, Tehran, Iran

[3] Department of Clinical Biochemistry, Faculty of Medicine Sciences, Tarbiat Modares University, Tehran, Iran

Abstract- Body weight is influenced by both food intake and energy expenditure. Acylated ghrelin enhances appetite, and its circulating level is suppressed by Growth Hormone. Data on the acylated ghrelin responses to exercise of different intensities in obese individuals are currently not available. This study examined the effects of an intermittent exercise protocol on acylated ghrelin levels and hunger ratings in obese people. Nine inactive male ran on the treadmill at 0900 with progressive intensities of 50, 60, 70, and 80% of VO_2max for 10, 10, 5, and 2 min respectively. Blood samples were collected before the exercise at 0845 (-15 min as the resting values), after each workload (10, 23, 31, and 36 min during exercise), and at 30, 60, and 120 min thereafter. The control trial was conducted under identical conditions with the exception of exercise. Compared to the baseline, both acylated ghrelin levels and hunger ratings were suppressed at 70% of VO_2max during exercise (17.74 vs. 9.80 pmol/L and 4.84 vs. 2.96 unit respectively) and remained significantly lower than the control trial 2 h after the cessation of exercise (13.95 vs. 20.32 pmol/L and 3.33 vs. 6.04 unit, respectively). Growth Hormone increased during the exercise period and peaked at 80% of VO_2max. These findings indicate that acylated ghrelin concentrations and hunger ratings are suppressed during exercise and two hours thereafter in obese individuals, and it is possible that Growth Hormone caused the suppression of acylated ghrelin.
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Keywords: Acylated ghrelin; Appetite; College students; Obesity; Running

Introduction

The prevalence of obesity has risen enormously over the past few decades (1), and both food intake (Appetite) and energy expenditure can influence body weight (2). The endogenous stomach-released ligand of type 1a growth hormone secretagogue receptor (GHS-R-1a) was identified and named ghrelin (3,4). Ghrelin is a peptide hormone in which the serine 3 residue is modified by a fatty acid, and this acylation is deemed to be essential for ghrelin to cross the blood–brain barrier (5) to enhance appetite (6). Therefore, Just acylated ghrelin has pituitaric activity[7]. Furthermore, circulating levels of ghrelin in obese individuals are lower compared to those of people with a normal body weight (8). Plasma ghrelin levels increase back toward normal values after weight loss, which may contribute to the weight regain experienced by many obese people (9).

Exercise is also an effective method to increase energy expenditure (10), and it may lead to a short-term hunger suppression (11). Most of the studies reported that aerobic exercise has no effect on ghrelin (12-15). Although just acylated ghrelin stimulates appetite, total ghrelin were measured in these studies.

Relatively few studies have measured acylated ghrelin levels during exercise. They have shown that plasma acylated ghrelin concentrations and hunger ratings are suppressed during treadmill running in physically active and healthy men (16-19). In addition, it has been revealed that acute exercise with sufficient intensity increases the plasma GH concentration (14,20-23) and that GH may feedback-inhibit systemic ghrelin

Corresponding Author: Majid Gholipour
Physical Education Center, Sharif University of Technology, Tehran, Iran
Tel: +98 912 1361544, Fax: +98 21 66005710, E-mail: gholipour@sharif.ir

release, whereas systemic ghrelin is not involved in the exercise-induced stimulation of GH secretion (24). A better understanding of how acylated ghrelin and, consequently, appetite is affected by different intensities of exercise may help to design a more effective exercise training programme for obese patients.

Considering that the data on the acylated ghrelin responses to exercise of different intensities in obese individuals are currently not available, we decided to investigate the effects of acute exercise on acylated ghrelin concentrations and, therefore, appetite in inactive obese males. The primary purpose of the present study was to determine plasma acylated ghrelin concentrations and alterations in hunger ratings during and after exercise with different intensities. We hypothesised that this intermittent exercise protocol with a progressive intensity would cause a temporary suppression of plasma acylated ghrelin concentrations and, consequently, hunger ratings in obese individuals and that these would be associated with plasma GH elevation. Furthermore, lactate concentrations were measured to demonstrate the degree of metabolic stress.

Materials and Methods

Subjects

Ten obese male students (BMI > 30) were recruited from the Sharif University of Technology. They were advised of the nature and purpose of the study and were then provided written informed consent for participation in the present study, which was approved by the National Research Ethics Committee. All volunteers were not involved in any form of regular physical activity, were non-smokers, were not taking any medications, and had no diet restrictions or history of metabolic or cardiovascular diseases or any surgery for at least six months prior to the study. The medical/descriptive data were collected through electrocardiogram (ECG) and cardiac echocardiography tests, medical history screening, and a questionnaire. The characteristics of the participants are reported in Table 1.

Table 1. Characteristics of the 9 subjects. Data are mean±SD

Age (years)	20.6±1.4
Height (cm)	174.7±2.8
Weight (kg)	99.6±6.4
BMI (kg/m^2)	32.7±2.5
Waist circumference (cm)	109.3±6
Hip circumference (cm)	112.6±3.4
Waist to Hip Ratio (WHR)	1±0.1
Body fat (%)	25.4±4
VO$_2$max (ml.kg^{-1}.min^{-1})	34.2±4.4

Preliminary trials

The subjects attended the laboratory and were familiarised with treadmill running. Then following parameters were measured: hip and waist circumferences, skinfold thickness at 3 sites (chest, abdomen, and thigh) on the right-hand side of the body using calipers (Harpenden - CE 1020 - England) to calculate the body density for estimation of body fat percentage[25], height to the nearest 0.1 cm using a stadiometer, and weight to the nearest 0.01 kg using a digital scale (Seca, Hamburg, Germany). BMI was calculated as weight in kilograms divided by the square of height in meters. Cardiorespiratory fitness (VO$_2$max) was assessed on a treadmill by measuring respiratory gases with an automated system (Cosmed, Quark b^2, Italy). The equipment was interfaced to a personal computer, and the values were recorded. VO$_2$ (breath-by-breath) and heart rate (short-range telemetry) were monitored throughout the test.

The subjects completed a graded exercise test to exhaustion at a constant grade (0% treadmill grade) that began with a workload of 4 km/h (evaluated in the preliminary session). The workload increased 1 km/h every 2 min. All subjects reached VO$_2$max when either the primary criterion of a plateau in VO$_2$ with an increase in workload or two of three secondary criteria were met. The secondary criteria were the following: (i) reaching the predicted maximal heart rate (ii) a respiratory exchange ratio greater than 1.1, and (iii) a rating of perceived exertion (15-point Borg Scale) of 19 or 20. The treadmill speed values (at 0% treadmill grade) corresponding to 50, 60, 70, and 80% of VO2max were calculated using a regression equation based on the treadmill speeds and corresponding VO$_2$ readings during the graded exercise test to exhaustion in the preliminary trial. Heart rate values corresponding to 50, 60, 70, and 80% of VO2max were estimated as well. A subject was selected randomly to perform the exercise protocol for examination the accuracy of the predicted treadmill speeds and heart rates. There were approximately no differences between the results of this test and the predicted values for 50, 60, 70, and 80% of VO2max (predicted: 15.51, 18.61, 21.71, 24.82, ml.kg^{-1}.min^{-1}; evaluated: 15.53±0.06, 18.29±0.07, 21.61±0.09, 24.68±0.13 ml.kg^{-1}.min^{-1}, respectively). Then, because of being more familiarised with the main exercise protocol and probability of a more economical performance, he was eliminated from the sample group, and the nine remaining subjects participated in the exercise and control trials. Considering the limited access to equipment for oxygen consumption

measurements in the main exercise trial, predicted treadmill speeds and heart rates of nine remained subjects were used to determine exercise intensity.

Exercise and control trials

The participants were given 2 weeks to recover from the preliminary exercise tests before performing two main trials (exercise and control) in a counterbalanced, randomised design with an interval of 7 days between trials. They also remained inactive throughout the tests and were asked to refrain from ingesting caffeine and alcohol 24 h prior to the main trials. On the exercise trial days, participants arrived at the laboratory between 0730 and 0745 after having fasted for 12 h. Water was permitted ad libitum, except during the trial days. An intravenous catheter (NovaFlon, 45 mm, Medikit) was inserted into an antecubital vein at 0800, and a physiological saline lock was attached. Then, subjects rested in a semi-supine position. At 0845 (-15 min before the exercise), the first blood samples were collected from the catheter as the resting values. At 0900, subjects completed a supervised-intermittent treadmill exercise protocol at 4 speeds estimated to elicit a specific VO$_2$: 50% of VO2max for 10 min, 60% of VO2max for 10 min, 70% of VO2max for 5 min, and 80% of VO2max for 2 min. Treadmill speeds were adjusted if the heart rate was above or below the predicted value. After each workload was completed at the prescribed intensity and duration, the treadmill speed was reduced (3 km/h for 3 min) to allow a blood sample to be collected. Thus, blood samples were collected at 10, 23, 31, and 36 min during the exercise period (on the treadmill) and 30, 60, and 120 min thereafter (R30, R60, and R120) during the recovery period (sitting position). Given the fact that this protocol, which was used by

Kraemer *et al.* (14), could increase GH levels in well-trained subjects; was modified to be performed by our inactive obese subjects with lower physical fitness (Figure 1).

Blood samples were collected in tubes containing clot activators and were then centrifuged at 3,500 rpm for 10 min. Then, supernatants were transferred into separate tubes and were stored at -20°C for analysis of growth hormone later. Separate blood samples were collected in monovettes containing EDTA and p-hydroxymercuribenzoic acid to prevent the degradation of acylated ghrelin by protease. Samples were centrifuged at 3,500 rpm for 10 min at 4°C, and then, supernatants were transferred into separate tubes, following which 100 μl of 1 M HCl per ml of collected plasma was added. Samples were then centrifuged at 3,500 rpm for 5 min at 4°C. The supernatants were then transferred into separate tubes and were stored at -20°C for analysis of acylated ghrelin later. At each workload, an additional 1 ml of whole blood was collected in EDTA-containing venoject tubes for the measurement of the haemoglobin concentrations and determination of haematocrit values and additional 2.5 ml of whole blood was collected in a venoject tubes containing K2 EDTA for the measurement of lactate concentrations. Haemoglobin and haematocrit values were used to assess plasma volume changes (26). A clock was on display in the laboratory throughout the trials, and subjects were aware of the time. Environmental temperature and humidity were not monitored. All subjects remained fasting throughout the trial days, and at the time each blood sample was drown, subjects were asked to rate their appetite - how hungry they felt - by placing a mark on a 100-mm visual analogue scale (VAS) (27,28).
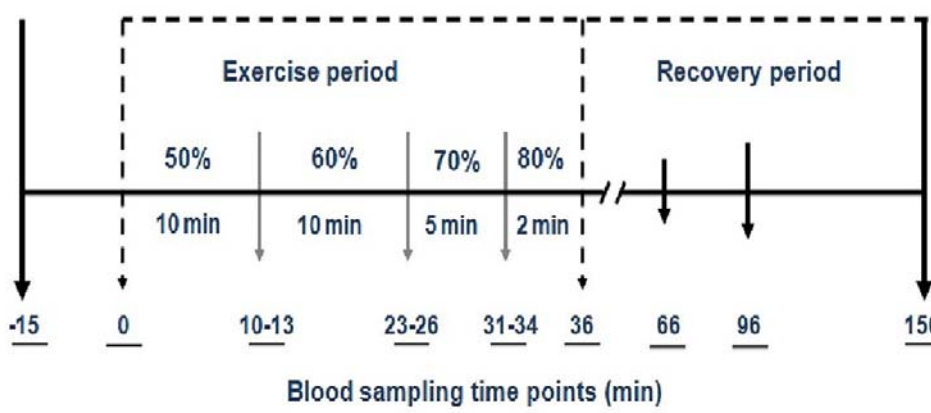

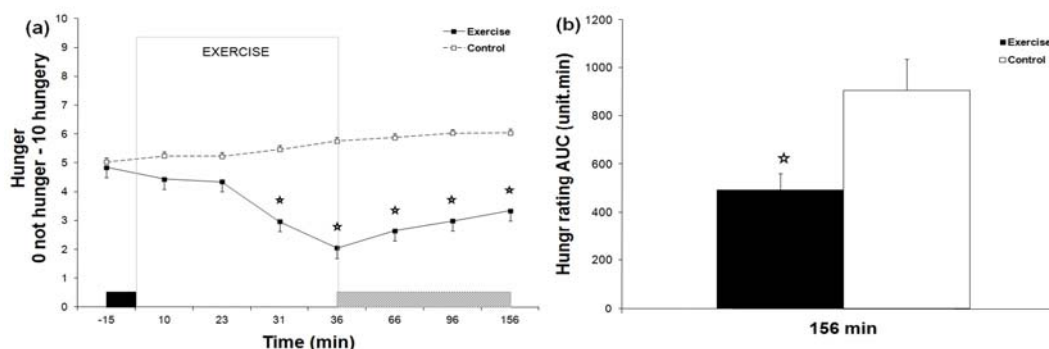**Figure 1.** Exercise protocol and blood sampling time points.

Figure 2. Data are mean±SE for 9 subjects. (a) Hunger ratings during exercise and control trials before exercise as resting values (solid rectangle), after each workload of exercise, and for 30, 60, and 120 min of recovery (hatched rectangle). (b) Values are total area under the curve (AUC) for hunger ratings for exercise and control trials. The star (*) represents a significant difference between exercise and control trials.

The control trial days were conducted under identical conditions with the exception of the exercise (sitting, reading, working at a computer).

Blood biochemistry

Plasma acylated ghrelin and GH concentrations were measured by enzyme immunoassays (RD194062400R, BioVender - Laboratory medicina, a.s., CAN-GH-4070, Diagnostics Biochem Canada Inc, respectively). Plasma lactate concentrations were measured by enzymatic, colorimetric methods (Randox Laboratories, Conuty Antrim, UK). Plasma haemoglobin concentrations and haematocrit values were determined using an automated haematology analyser (Sysmex, KX-21, Japan). To eliminate inter-assay variation, samples from each participant were analysed in the same run. The within-batch coefficients of variation (CV) for the assays were as follows: acylated ghrelin 6.7%, growth hormone 4.4%.

Statistical analysis

Data were analysed using the Statistical Package for the Social Science (SPSS) software version 16.0 for Windows. Exercise, recovery, and total area under the curve (AUC) values for hunger rating and plasma acylated ghrelin and GH concentration versus time curves were calculated using the trapezoidal rule. Dependent t-tests were used to assess differences between resting values (baseline), values at R120, between values at baseline and R120, and between AUC values for hunger, acylated ghrelin and GH for the exercise and control trials. A 2 x 8 (trial x time point) repeated-measures, two-factor ANOVA was used to examine hunger, acylated ghrelin, GH and plasma volume changes over time, and where appropriate, post-hoc pairwise comparisons were performed using the

Bonferroni method for each time point between trials. The Pearson correlation coefficient was used to examine the relationships between resting values and anthropometric data as well as between variables. Statistical significance was accepted at the 5% level. There were no significant differences between trials for plasma volume changes, and the unadjusted values are reported. Results are given as means±SE, unless otherwise stated.

Results

Lactate

Plasma lactate levels were elevated with progressive increases in workload from baseline (1.31±0.12 mmol/L) to its peak value at 80% of VO2max (3.63±0.24 mmol/L).

Hunger

There was no significant difference (P=0.613) between baseline hunger ratings in the exercise and control trials. Two-factor ANOVA revealed a main effect of trial (P<0.0005), a main effect of time (P<0.0005), and a trial x time interaction effect (P<0.0005) for hunger. Post-hoc analyses indicated between-trial differences at 70%, 80%, R30, R60, and R120. However, after adjustment, differences at 70% (P=0.007), 80% (P=0.002), and R30 (P=0.023) remained significant. In the exercise trial, the hunger ratings decreased from the baseline during treadmill running, increased during recovery, but remained significantly lower than baseline and control trial (both P<0.0005). AUC values during treadmill running, recovery, and total periods for hunger ratings in the exercise trial and the same time in the control trial were used to evaluate the between-trial differences. All AUC

values (exercise, recovery, and total periods) for hunger ratings were significantly lower (*P*=0.008, *P*<0.0005, *P*<0.0005, respectively) in the exercise trial compared with the control trial (Figure 2).

Acylated ghrelin

No significant difference was found (*P*=0.255) between the baseline values of plasma acylated ghrelin concentrations in the exercise and control trials. Two-factor ANOVA revealed a main effect of trial (*P*=0.001), a main effect of time (*P*=0.007), and a trial x time interaction effect (*P*<0.0005) for acylated ghrelin. Post-hoc analyses indicated between-trial differences at 70%, 80%, R30, R60, and R120, but after adjustment, significant differences were found at 70% (*P*=0.005) and 80% (*P*=0.011). In the exercise trial, acylated ghrelin decreased during treadmill running and increased sharply during recovery, especially at R30. Although it finally remained non-significantly lower than the baseline (*P*=0.091), between-trial differences were significant (*P*=0.009). Furthermore, significant differences were found for acylated ghrelin AUC over the recovery and total periods (*P*<0.0005, *P*=0.001, respectively) between exercise and control trials (Figure 3).

Growth hormone

The results showed that the baseline values of plasma GH concentrations did not differ significantly

(*P*=0.604) between exercise and control trials. Two-factor ANOVA revealed a main effect of trial, a main effect of time, and a trial x time interaction effect (all *P*<0.0005) for plasma GH concentrations. Post-hoc analyses indicated between-trial differences at 60%, 70%, 80%, R30, R60, and R120. After adjustment, differences at 70%, 80%, R30, R60 (all *P*<0.0005), and R120 (*P*=0.001), remained significant. In the exercise trial, GH rose during treadmill running and then declined during recovery; however, it remained significantly higher than baseline and control trial (both *P*<0.0005). All AUC values (exercise, recovery, and total period) for GH were significantly higher (all *P*<0.0005) in the exercise trial compared with the control trial (Figure 4).

Correlation between an acylated ghrelin and other variables

There were no significant correlations between the baseline plasma acylated ghrelin concentrations and other baseline variables. When the values of individual time points in the exercise trial were compared, strong significant positive correlations were observed between the plasma acylated ghrelin concentrations and hunger ratings at 80% (*r*=0.751, *P*=0.10) and R30 (*r*=0.811, *P*=0.004). There was also a significant negative correlation (*r*=-0.611, *P*=0.040) between acylated ghrelin and GH at 80% (Figure 5). There were no significant correlations between variables among all AUC values.
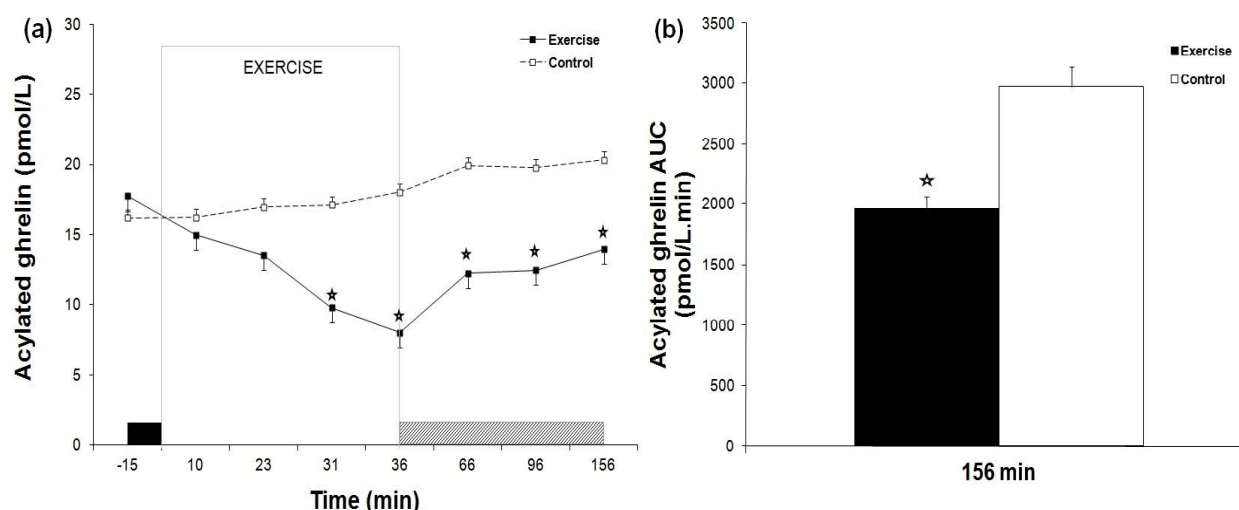

Figure 3. Data are mean±SE for 9 subjects. (a) Acylated ghrelin concentrations during exercise and control trials before exercise as resting values (solid rectangle), after each workload of exercise, and for 30, 60, and 120 min of recovery (hatched rectangle). (b) Values are total area under the curve (AUC) for acylated ghrelin concentrations for exercise and control trials. The star (*) represents a significant difference between exercise and control trials.
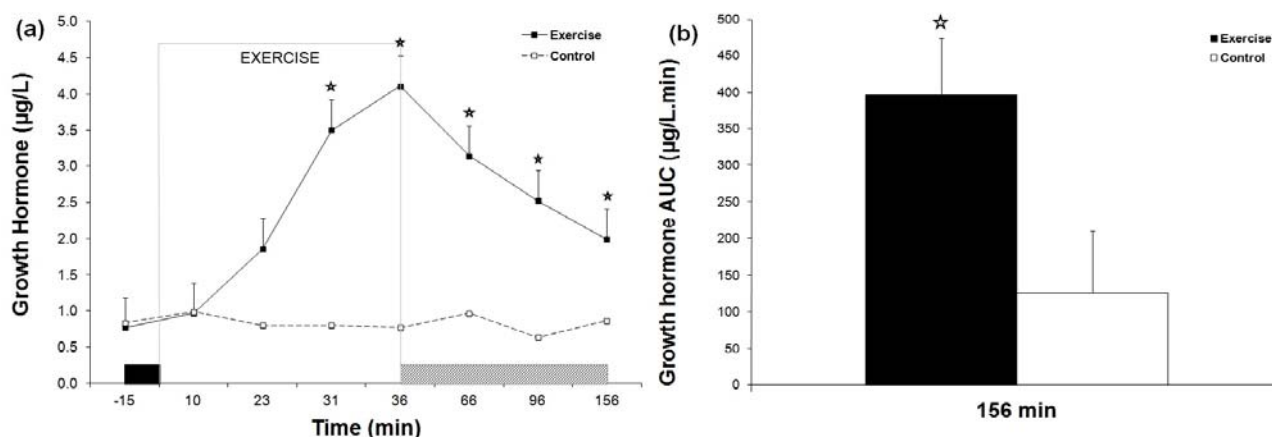
Figure 4. Data are mean±SE for 9 subjects. (a) Growth hormone concentrations during exercise and control trials before exercise as resting values (solid rectangle), after each workload of exercise, and for 30, 60, and 120 min of recovery (hatched rectangle). (b) Values are total area under the curve (AUC) for growth hormone concentrations for exercise and control trials. The star (*) represents a significant difference between exercise and control trials.
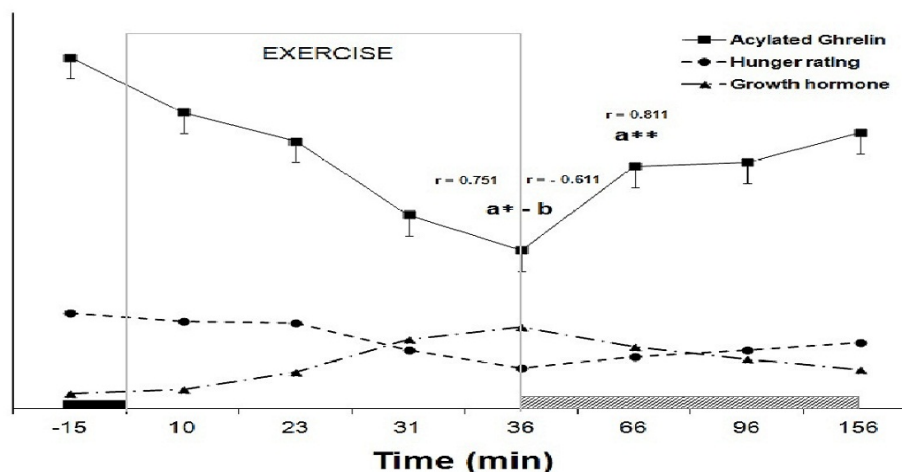

Figure 5. (a): Significant relationship between acylated ghrelin and hunger rating (* P=0.10, ** P=0.004) . (b): Significant relationship between acylated ghrelin and growth hormone (P=0.040).

Discussion

To our knowledge, the present experiment is the first study that examines acylated ghrelin and hunger rating changes during and after intermittent exercise in obese individuals. The novel finding of this study is the suppression of acylated ghrelin during this exercise protocol at 70%; in particular, this suppression remained significantly lower than that observed for control trial for up to 2 h after the cessation of exercise. The same changes for hunger ratings were observed. The lack of oxygen consumption measurements for all participants

in the main exercise trial is a limitation of the present study. Given the fact that during exercise, ghrelin response is modulated by the preceding intake of fat, especially after low fat (29), it is another limitation that we did not control their diet before fasting.

Most of the studies reported that aerobic exercise has no effect on ghrelin (12-15). One study, however, reported that plasma ghrelin concentrations increased, especially during the last hour of prolonged exercise with moderate intensity (29). Another study showed that ghrelin levels decreased significantly to some extent (120 min) after exercise (24). Total ghrelin was measured in the above studies, and because it does not

adequately reflect acylated and Des-acyl ghrelin concentrations (30), changes in acylated ghrelin were not demonstrated. Few studies have measured plasma acylated ghrelin concentrations during exercise (16-19). The results from the above studies show that plasma acylated ghrelin concentrations are suppressed during treadmill running. Consistently, in the present study acylated ghrelin was suppressed during the exercise trial at 70% of VO_2max. This suppression lasted 2 h after the cessation of exercise and remained significantly lower than the control trial.

Given the fact that appetite is influenced by acylated ghrelin (6), the results of the present study showed that there was a suppression of hunger as well. The hunger ratings were suppressed in parallel with a reduction of acylated ghrelin, the lower the plasma acylated ghrelin concentration, the greater the suppression of appetite. This finding is consistent with the results of the studies that measured hunger ratings (16,17). This, however, is contrary to the results of other studies that have shown that exercise-induced suppression of hunger ratings return to control values within 2 h after the cessation of exercise (11,19). Although, there was no correlation between all the AUC values for acylated ghrelin and hunger ratings, there was a positive correlation between them at 80%. All the AUC values for acylated ghrelin and hunger ratings were significantly lower for the exercise trial compared with the control trial. It is known that there is a redistribution of blood flow during exercise (31,32), and it has been suggested that this redirection of flow away from the splanchnic circulation towards the muscles might be a reason for the transient exercise-induced suppression of appetite (11), however, the inhibitory effect of GH on ghrelin secretion is probable (24).

It has been shown that acute exercise with sufficient intensity (approximately 70% of VO_2max) increases the plasma GH concentration (14,20-22), and its secretion is related to exercise intensity in a linear dose-response manner (23). These results are consistent with our findings that showed that GH levels gradually rose during exercise trial. The GH levels significantly differed from baseline at 60% of VO2max, preceded the acylated ghrelin suppression at 70% of VO_2max (24), and remained significantly higher than the corresponding values for control trial at R120. Conversely, the acylated ghrelin levels and, consequently, the hunger ratings decreased at 70% of VO_2max and remained significantly lower than the corresponding values for control trial at the same time point (R120).

Iranmanesh *et al.* (33) reported that there was a dramatic impairment of GH production (about a quarter of what was described in normal subjects) in obese people. The results of the present study showed that the mean GH concentration at baseline and even at peaked values (80%) was considerably low compared to the normal subjects (14,34). This may indicate that the amount of GH does not impact ghrelin per se. It seems that a significant increase in the resting values (more than fivefold) might be more important for suppression of acylated ghrelin than its circulating concentration per se.

In conclusion, these findings indicate that plasma acylated ghrelin concentrations and hunger sensations of inactive obese individuals are suppressed in response to intermittent treadmill running with progressive intensity and two hours thereafter. Because there was an elevation of GH before the acylated ghrelin suppression (24), GH might be influential for this suppression. Further investigation is required to examine whether this protocol could elicit the same effects in a short-term training programme.

Acknowledgments

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