

EFFECT OF EXERCISE ON APPETITE-REGULATING HORMONES IN OVERWEIGHT WOMEN

■ Accepted
for publication
10.03.2013

AUTHORS: Tiryaki-Sonmez G.¹, Ozen S.², Bugdayci G.³, Karli U.², Ozen G.², Cogalgil S.⁴, Schoenfeld B.¹, Sozbir K.², Aydin K.²

¹ Department of Health Sciences, Lehman College, The City University of New York, Bronx, New York, USA

² School of Physical Education and Sports, Abant Izzet Baysal University, Bolu, Turkey

³ Department of Biochemistry, Faculty of Medicine, Abant Izzet Baysal University, Bolu, Turkey

⁴ Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Abant Izzet Baysal University, Bolu, Turkey

Reprint request to:

Gul Tiryaki -Sonmez

Department of Health Sciences

Lehman College

The City University of New York

250 Bedford Park Blv. West

Bronx NY 10468, USA

Tel: 718-960-7755

Fax: 718-960-2254

E-mail: gul.sonmez@lehman.cuny.edu

ABSTRACT: Over the past decade, our knowledge of how homeostatic systems regulate food intake and body weight has increased with the discovery of circulating peptides such as leptin, acyl ghrelin, des-acyl ghrelin and obestatin. These hormones regulate the appetite and food intake by sending signals to the brain regarding the body's nutritional status. The purpose of this study was to investigate the response of appetite-regulating hormones to exercise. Nine overweight women undertook two 2 h trials in a randomized crossover design. In the exercise trial, subjects ran for 60 min at 50% of maximal oxygen uptake followed by a 60 min rest period. In the control trial, subjects rested for 2 h. Obestatin, acyl ghrelin, des-acyl ghrelin and leptin concentrations were measured at baseline and at 20, 40, 60, 90 and 120 min after baseline. A two-way ANOVA revealed a significant ($P < 0.05$) interaction effect for leptin and acyl ghrelin. However, changes in obestatin and des-acyl ghrelin concentration were statistically insignificant ($P > 0.05$). The data indicated that although acute treadmill exercise resulted in a significant change in acyl ghrelin and leptin levels, it had no effect on plasma obestatin and des-acyl ghrelin levels.

KEY WORDS: obesity, exercise, obestatin, acyl ghrelin, des-acyl ghrelin, leptin

INTRODUCTION

The incidence of obesity is increasing rapidly in developed and certain developing countries. Obesity is related to several serious chronic diseases including diabetes mellitus, coronary heart disease and cancer. Physical activity and diet are the two most important modifiable behaviours to regulate body weight and to prevent and/or reduce obesity. Body weight regulation is a complex process, involving various systems. While the mechanism by which physical activity may counteract obesity is well known, modifying the energetic balance by increasing energy expenditure, the mechanisms behind regulation of appetite and food intake are poorly understood. However, over the past decade, our knowledge of how homeostatic systems regulate food intake and body weight has increased with the discovery of circulating peptides such as leptin, ghrelin and obestatin. These hormones regulate the appetite and food intake by sending signals to the brain regarding the body's nutritional status [9].

The discovery of leptin has improved our understanding of the relationship between adipose tissue and energy homeostasis. Leptin is synthesized and secreted by adipocytes, and informs the brain

about the status of energy stores present in adipose tissue [15]. At present, much attention has been focused on gut hormones, which are generated in the gastrointestinal tract and integrated in the brain to regulate appetite and energy balance. Ghrelin, a 28 amino acid acyl peptide, was isolated from the human and rat stomach as the endogenous ligand for the growth hormone (GH) secretagogue receptor type 1-a (GHS-R1a) [11,18]. Ghrelin has two forms in the circulation: acyl and des-acyl ghrelin [11,18].

In addition to ghrelin, obestatin is a newly discovered hormone that originates from the preproghrelin gene, and results from post-translational processing of amino acids 76–98 of the preproghrelin peptide [13]. Obestatin is mainly produced in the stomach and other organs in the gastro-intestinal tract and has a variety of functions. Studies indicate that obestatin, an anti-hunger peptide, plays an important role in energy balance and body weight [13]. While ghrelin and obestatin have the same gene structure, their effects on food intake and appetite are opposite [26]. Ghrelin stimulates appetite and gastric motility whereas obestatin has been reported to reduce appetite, food intake and gut motility in rats [9]. Although

the mechanisms by which obestatin carries out its actions are not clearly known, there is evidence that it may modulate ghrelin's effect [9].

Exercise is an essential part of any weight management programme. Physical activity improves the health status of obese individuals and helps to reduce body weight. It is possible that obestatin, ghrelin forms and leptin may provide a better understanding of how physical activity contributes to weight management and health improvements.

Several studies have investigated the acute effects of exercise on appetite-regulating hormones in women [3,14,19,20,23,26] and the majority of these studies have focused on ghrelin and leptin. Currently, little is known about the influence of acute exercise on appetite-regulating hormones in overweight women. Therefore the objective of this study was to investigate obestatin, acyl-ghrelin, des-acyl ghrelin and leptin levels and their relation with each other during and after acute exercise in overweight women.

MATERIALS AND METHODS

Subjects. Nine overweight/obese women participated voluntarily in the study and written informed consent was obtained from all subjects before participation. All women were between the ages of 20 and 24 and had a BMI between 26.5 and 32.4 kg·m⁻². Table 1 summarizes descriptive statistics for the subjects. Participants completed a health screen and a physical activity questionnaire. Subjects were excluded from participation in the study if they had a history of a chronic disease, uncontrolled hypertension or taking blood pressure medication, any condition that would alter one's metabolism or ability to exercise, diagnosed psychological disorders, recent weight loss of greater than 5 kg, irregular menstrual cycles (<25 days or >35 days between cycles), or low levels of sleep (<6 h·night⁻¹). Participants had regularly occurring menstrual cycles, and were considered "low exercise risk" as per the American College of Sports Medicine (ACSM) guidelines. The study was approved by the ethical board of the Abant İzzet Baysal University School of Medicine Clinical Laboratory Research, Bolu, Turkey, and Lehman College, The City University of New York, NY, USA, and it was performed in accordance with the principles of the Declaration of Helsinki.

Specific procedures

Dietary protocol: Participants were asked to maintain their normal dietary and physical activity programmes throughout the duration of the study. Subjects refrained from physical exercise, alcohol and caffeine for 24 h before testing. Because energy balance and macronutrient intake can influence appetite-regulating hormones, it was important to provide the same diet during the 24 h before each testing day. Throughout the day before the first main trial, participants' weight and food intake were recorded. Participants then replicated this food intake during the day before the second main trial. Participants were also provided with a standardized evening meal and asked to eat it at around 8:00 pm on the day preceding each of

the study days. Dietary intake was analysed by using diet analysis software (Diet Analysis Plus[®], Version 4.0, ESHA Research, Salem, OR).

Baseline testing: Approximately two weeks before initiation of the experimental protocol, $\dot{V}O_{2max}$ was determined on a motor-driven treadmill (HP Cosmos Mercury Med 4.0) using standard Bruce protocol testing in accordance with ACSM recommendations [4]. Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured throughout the entire exercise session using a computer controlled breath-by-breath analyser, Cortex II Metalyser (Cortex, Biophysik, Leipzig, Germany). The highest achieved value for oxygen consumption was considered the subject's $\dot{V}O_{2max}$. Heart rate (HR) was measured continuously throughout the test using a commercially available HR monitor (Polar S725X, Polar Electro, Finland). The test was terminated when subjects stated they could no longer continue with the maximum workload. At the terminal workload, all subjects had to meet at least two of the following criteria for a valid test: (1) a final respiratory exchange ratio (RER) > 1.0, (2) O₂ consumption increased by <2 ml·kg⁻¹ with an increase in exercise intensity (3) with attainment of >85% of age-predicted maximal heart rate.

Experimental protocol and main trials

Participants were given at least one week to recover from the preliminary exercise tests before performing two main trials (exercise and control) in a random, crossover design with an interval of at least one week between trials. The two test trials were scheduled in the follicular phase of the participants' menstrual cycle (between days 1 and 11) and spaced either 2 to 10 days or 1 menstrual cycle (3 to 5 wks) apart to control the interference of reproductive hormones with appetite-regulating hormones.

After an overnight fast, participants arrived at the laboratory at 8:00 am and an intravenous catheter was inserted into an antecubital vein (8:15 am). At 8:50 am, 10 min prior to exercise, resting blood samples were collected from the catheter. The exercise test day consisted of a 60 min treadmill exercise at 50% $\dot{V}O_{2max}$ followed by 1 h of rest, whereas the control day consisted of 2 h of rest. Adjustments were made to the treadmill speed if it was deemed necessary.

Blood samples were collected at baseline and at 20, 40, 60, 90 and 120 min after baseline for the determination of leptin, acylated ghrelin, des-acylated ghrelin and obestatin. Heart rate was recorded every 5 s during the treadmill exercise by Polar heart monitors (Polar S725X, Polar Electro, Finland). Oxygen consumption and carbon dioxide production were measured every 10 s during the 60 min exercise using a Cortex II Metalyser (Cortex Biophysik, Leipzig, Germany) and the mean respiratory exchange ratio (RER) was calculated from the recorded measurements. The analyser was calibrated before the test with gases of known concentration according to the manufacturer's guidelines (15.06% O₂, 5.11% CO₂& bal. in N₂).

After the resting/exercise intervention, participants stayed in the clinical investigation unit but were free to write/read quietly (t=60-120 min).

Blood samples and hormone analysis

Blood samples were drawn into chilled tubes containing Na₂EDTA (1.25 mg · ml⁻¹) and p-hydroxybenzoic acid (p-HBA). Immediately after collecting blood samples, the sample tubes were centrifuged at 1500 g for 15 min at 4°C for hormones (obestatin, acylated ghrelin and des-acylated ghrelin). The obtained plasma samples were mixed with 1 mol · L⁻¹ HCl (hydrochloric acid) at a ratio of 1/10 and were stored at -70°C until assayed. Blood samples for leptin were drawn into red cap tubes and centrifuged after completion of clot formation, and the serum samples were stored at -70°C until the day of the hormone measurements. Serum leptin concentration was measured using a commercial sandwich enzyme-linked immunosorbent assay (EIA-2395; DRG Diagnostics, Marburg, Germany) with a limit of detection of 1.0 ng · ml⁻¹. The intra- and inter-assay coefficients of variation were 11.6% and 6%, respectively. Plasma acylated ghrelin assay was performed using a commercially available ELISA (EIA-A05106; Biotech Centre, SPI Bio & Ellipse Pharmaceuticals; Bertin Pharma, Montigny le Bretonneux, France) with a detection limit of 1.5 pg · ml⁻¹. The intra- and inter-assay coefficients of variation were 6.7% and 5.9%, respectively. Plasma des-acylated ghrelin assay was performed using a commercially available ELISA (EIA-A05119; Biotech Centre, S PI Bio & Ellipse Pharmaceuticals; Bertin Pharma, Montigny le Bretonneux, France) with a detection limit of 2.0 pg · ml⁻¹. The intra- and inter-assay coefficients of variation were 4.4% and 3.8%, respectively. Plasma obestatin assay was performed using a commercially available ELISA (EIA-S-1284; Peninsula Laboratories, San Carlos, CA, USA) with a detection limit of 1.5 ng · ml⁻¹. The intra- and inter-assay coefficients of variation were 11% and 6.5%, respectively. At each blood sampling point, duplicate 20 µL blood samples were collected into micropipettes for the measurement of haemoglobin concentration and triplicate 20 µL blood samples were collected into heparinized micro haematocrit tubes for the determination of haematocrit. Haemoglobin and haematocrit values were used to assess plasma volume changes [7].

Statistical analysis

Statistical analysis was carried out using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). All the variables were checked regarding their normal distribution using the Shapiro–Wilk test and data are presented as means ±SD. Repeated-measures, two-factor ANOVA was used to examine differences between the two trials over time for obestatin, acylated ghrelin, des-acylated ghrelin, leptin and plasma volume change. Between-trial differences at each time point were examined using one-way ANOVA and Bonferroni post hoc tests when significant interactions were found. Differences in energy and macronutrient intakes of the controlled diet and differences between baselines values of hormones were assessed using paired sample

t-tests. Pearson correlation coefficients were calculated to examine relationships between variables. Plasma volume changes did not differ significantly between trials, and the unadjusted values are presented. Statistical significance was accepted at the 5% level.

RESULTS

Baseline characteristics

Nine subjects volunteered for this study. Descriptive statistics are shown in Table I. Subjects were overweight or obese (BMI: 28.32±1.82 kg · m²), had a mean age of 22.83±1.38 years, and were relatively sedentary, with an average $\dot{V}O_{2max}$ of 32.66±6.12 ml · kg⁻¹ · min⁻¹.

TABLE I. DESCRIPTIVE STATISTICS

Age (yr)	22.83 ± 1.38
Body weight(kg)	78 ± 5.31
Height (m)	1.64 ± 0.03
BMI (kg · m ²)	28.32 ± 1.82
$\dot{V}O_{2max}$ (ml · kg ⁻¹ · min ⁻¹)	32.66 ± 6.12

Note: values are mean±SD

The energy and macronutrient intakes of the controlled diet were similar (paired t-test, P>0.05) before exercise and control trials.

Baseline obestatin, acylated ghrelin, des-acylated ghrelin and leptin concentrations did not differ significantly (paired t-test, P>0.05) between trials (Table II).

TABLE 2. BASELINE HORMONE CONCENTRATIONS BEFORE EXERCISE AND CONTROL TRIALS

	Exercise*	Rest*	P value [^]
Obestatin (ng · ml ⁻¹)	5.27 ± 0.86	5.04 ± 0.57	0.53
Acylated Ghrelin (pg · ml ⁻¹)	54.55 ± 9.19	54.12 ± 10.99	0.93
Des-acyl Ghrelin (pg · ml ⁻¹)	388.77 ± 59.50	393.37 ± 64.53	0.88
Leptin (ng · ml ⁻¹)	5.77 ± 1.06	5.19 ± 1.05	0.28

Note: *Mean±SD, ^ Based on paired t-test.

Responses to treadmill running

During exercise, average heart rate was 141.55±5.68 beats · min⁻¹, mean % $\dot{V}O_{2max}$ was 53.05±3.34 % ml · kg⁻¹ · min⁻¹ and mean respiratory exchange ratio was 0.88±0.09.

Plasma obestatin, acylated ghrelin, des-acylated ghrelin and serum leptin, changes over time

Two-factor ANOVA revealed a main effect of trial (P<0.05), a main effect of time (P<0.05) and a trial and time interaction (P<0.05) for acylated ghrelin and leptin concentrations. Post hoc analysis indicated that there were significant differences between exercise and control trials at 40 min and 60 min for acylated ghrelin and leptin (P<0.05) (Figure 1).

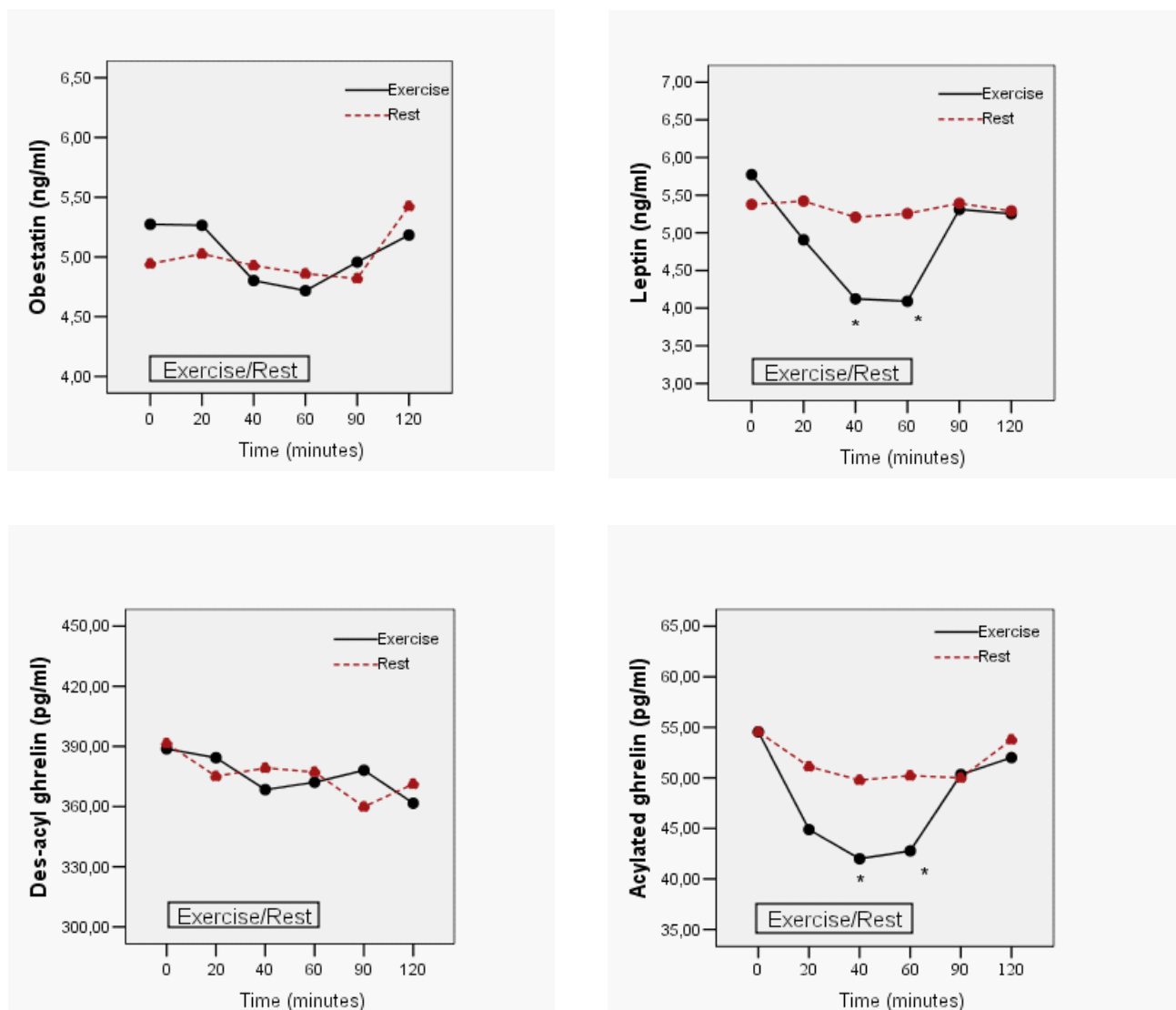


FIG. 1. ACYLATED GHRELIN, OBESTATIN, DES ACYL GHRELIN AND LEPTIN CONCENTRATIONS OVER 120 MIN DURING THE EXERCISE AND CONTROL TRIALS. THERE WAS AN EFFECT OF TRIAL ($P < 0.05$), AN EFFECT OF TIME ($P < 0.05$), AND A TRIAL X TIME INTERACTION ($P < 0.05$) FOR ACYLATED GHRELIN AND LEPTIN

Note: *Significantly different from the control trial ($P < 0.05$) after Bonferroni adjustment

Two-factor ANOVA revealed a main effect of time ($P < 0.01$) for des-acylated ghrelin and obestatin but there was no main effect of trial ($P > 0.05$) and no interaction effect ($P > 0.05$) (Figure 1), indicating that des-acylated ghrelin and obestatin changed significantly during the trials but were not influenced by exercise.

Correlations

There were no significant correlations ($P > 0.05$) between obestatin, acylated ghrelin, des-acylated ghrelin and leptin in baseline values and over other periods either for the exercise trial or the control trial.

DISCUSSION

In this study, the obestatin, leptin, acylated ghrelin and des-acylated ghrelin concentrations and their relation with each other during and after moderate intensity exercise were investigated to contribute to our understanding of the relationship between exercise and appetite-regulating hormones. The results of the present study indicated that acute treadmill exercise at moderate intensity did not change

plasma obestatin and des-acyl ghrelin concentrations. However, a significant decrease in plasma acylated ghrelin and leptin was observed after exercise and there were no correlations between variables over the exercise trial.

To the authors' knowledge, the present study is the first study to examine obestatin levels after acute moderate intensity exercise in overweight women. There are a limited number of studies that have investigated the effects of exercise on obestatin; some of these studies were not properly designed and some were conducted on animals. These studies have observed decreases [10,16], increases [1,27,30] or no alterations [8,11,20,26] in obestatin concentration following exercise. In the present study, acute moderate intensity aerobic exercise had no effect on plasma obestatin level. In agreement with our findings, obestatin was not altered by single circuit-resistance exercise, or by acute aerobic and anaerobic exercise in women [8,10,20,26]. Ghanbari et al. [10] found that acute resistance exercise with different intensities (40%, 60% and 80% of one maximum repetition) had no effect on plasma obestatin level. Ebrahimnia et al. [8]

also reported that single circuit-resistance exercise and single aerobic exercise (70% $\dot{V}O_{2max}$) had no impact on obestatin level. Manshoury et al. [20] investigated the response of obestatin levels to short-term anaerobic interval exercise training. The results showed that short-term anaerobic exercise had no effect on plasma obestatin levels. However, plasma obestatin level was decreased after 4 weeks of circuit resistance training (80% of one repetition maximum) [16] and weight reduction due to a long-term lifestyle intervention in obese children [27]. Meanwhile, other studies reported an increase in response to lifestyle intervention [1] and after long duration, high volume training in male rats [12]. Zuo et al. [30] reported that a camp that included 3 h of aerobic exercise every day resulted in higher plasma ghrelin and obestatin concentrations after weight reduction. The inconsistent findings may be due to the intensity, duration and type of exercise employed and/or the sex of the subjects.

Leptin and ghrelin are two hormones that have been recognized to have a major influence on energy balance [17]. These hormones regulate appetite and food intake by sending signals to the brain regarding the body's nutritional status. Leptin is a mediator of long-term regulation of energy balance, suppressing food intake. On the other hand, ghrelin is a fast acting hormone, seemingly playing a role in meal initiation [10]. Whether ghrelin has an influence on circulating leptin levels has not yet been demonstrated [17].

In the current study, exercise resulted in a decrease of serum leptin. Most of the current studies elaborating on the effects of exercise on leptin support this finding [5,6,29]. The mechanisms explaining the factors relating to leptin during exercise are not well understood. The reduction of leptin has been attributed to alteration in energy balance, improvements in insulin sensitivity and alteration in lipid metabolism and lipid concentration [21].

The effects of exercise on acylated ghrelin and des-acyl ghrelin are fairly controversial and depend on the intensity and duration of exercise [14,24,28]. We here report a significant reduction of plasma acylated ghrelin level but not des-acyl ghrelin during acute exercise. The decrease in acylated ghrelin during exercise in our subjects is in agreement with previous research [22,24], but des-acyl ghrelin concentrations were not examined in these studies.

At present, the cause of the decrease in acylated ghrelin during acute moderate exercise is still unclear. Reduced acylated ghrelin

concentration after acute moderate intensity exercise might be due to increased sympathetic nervous system activity and gastric mucosal ischaemia resulting from redistribution of blood flow from the splanchnic circulation towards the skeletal muscles during exercise [25]. There is also some evidence that ghrelin levels are suppressed following resistance exercise of moderate intensity and are lower with higher GH concentrations during aerobic exercise. It has been suggested that negative feedback from elevated GH causes the reductions [2]. However, Shiiya et al. [28] reported that acute moderate exercise decreased acylated ghrelin level. They also suggested that this was likely mediated by elevated sympathetic tone, but unrelated to alteration of GH secretion upon exercise. However, GH secretion and plasma catecholamines were not measured in the present study.

CONCLUSIONS

This study demonstrates that acute moderate intensity exercise significantly reduces acylated ghrelin and leptin levels but has no effect on des-acyl ghrelin and obestatin levels in overweight women. The present findings of decreased ghrelin concentrations and stable obestatin concentrations after acute treadmill exercise at moderate intensity in overweight women may be important for weight loss and weight maintenance. Further well-controlled research is required to determine the influence of exercise on appetite-regulating hormones in different intensities and duration of exercise in different subject groups. Such research could have important implications regarding the role of exercise in weight management.

Acknowledgments

This work was mainly supported and funded by the Scientific Research Projects Unit (2010.08.03.374) of the University of Abant İzzet Baysal and partly supported by a PSC-CUNY research award 42 grant of the City University of New York, NY, USA. The authors thank the institutions which collaborated and supported this research and all the participants for their participation in this study.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

- Balagopal P.B., Gidding S.S., Buckloh L.M., Yarandi H.N., Sylvester J.E., George D.E., Funanage V.L. Changes in circulating satiety hormones in obese children: a randomized controlled physical activity-based intervention study. *Obesity (Silver Spring)* 2010;18:1747-1753.
- Bilski J., Telegáów A., Bilka J.Z., Dembiski A., Warzecha Z. Effects of exercise on appetite and food intake regulation. *Med. Sportowa* 2009;13:82-94.
- Burns S.F., Broom D.R., Miyashita M., Mundy C., Stensel D.J. A single session of treadmill running has no effect on plasma total ghrelin concentrations. *J. Sports Sci.* 2007;25:635-642.
- Bruce R.A., Kusumi F., Hosmer D. Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. *Am. Heart J.* 1973;85:546-562.
- Desgorges F.D., Chennaoui M., Gomez-Merino D., Drogou C., Guezennec C.Y. Leptin response to acute prolonged exercise after training in rowers. *Eur. J. Appl. Physiol.* 2004;92:609-611.
- Desgorges F.D., Chennaoui M., Drogou C., Guezennec C.Y., Gomez-Merino D. Relationships between leptin levels and carbohydrate intake during rowing training. *J. Sports Med. Phys. Fitness* 2008;48:83-89.
- Dill D.B., Costill D.L. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J. Appl. Physiol.* 1974;37:247-248.

8. Ebrahimnia M., Rashidlamir A., Rashidlamir S., Alhosseini M.S., Mirzende del Z. The effect of a single circuit-resistance and aerobic exercise on plasma obestatin levels in well-trained females. *Iranian J. Health Phys. Activity* 2012;3:32-37.
9. Epelbaum J., Bedjaoui N., Dardennes R., Feng D.D., Gardette R., Grouselle D., Loudes C., Simon A., Tolle V., Yang S.K., Zizzari P. Role of the ghrelin/obestatin balance in the regulation of neuroendocrine circuits controlling body composition and energy homeostasis. *Mol. Cell Endocrinol.* 2010;314:244-247.
10. Ghanbari-Niaki A., Saghebjo M., Rahbarizadeh F., Hedayati M., Rajabi H. A single circuit-resistance exercise has no effect on plasma obestatin levels in female college students. *Peptides* 2008a;29:487-490.
11. Ghanbari-Niaki A., Jafari A., Abednazari H., Nikbakht H. Treadmill exercise reduces obestatin concentrations in rat fundus and small intestine. *Biochem. Biophys. Res. Commun.* 2008b;372:741-745.
12. Ghanbari-Niaki A. Plasma obestatin, estradiol, and liver ATP concentrations in response to endurance exercise training at different durations in male rats. *Int. J. Endocrinol. Metab.* 2010;8:147-152.
13. Grönberg M., Tsolakis A.V., Holmbäck U., Stridsberg M., Grimelius L., Janson E.T. Ghrelin and obestatin in human neuroendocrine tumors: expression and effect on obestatin levels after food intake. *Neuroendocrinology* 2012;Nov 9. [Epub ahead of print]
14. Hagobian T.A., Sharoff C.G., Stephens B.R., Wade G.N., Silva J.E., Chipkin S.R., Braun B. Effects of exercise on energy-regulating hormones and appetite in men and women. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2009;296:233-242.
15. Hausman G.J., Barb C.R., Lents C.A. Leptin and reproductive function. *Biochimie* 2012;94:2075-2081.
16. Hedayati M., Saghebjo M., Ghanbari-Niaki A. Effects of circuit resistance training intensity on the plasma ghrelin to obestatin ratios in healthy young women. *Int. J. Endocrinol. Metab.* 2012;10:475-479.
17. Klok M.D., Jakobsdottir S., Drent M.L. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. *Obes. Rev.* 2007;8:21-34.
18. Kojima M., Hosoda H., Date Y., Nakazato M., Matsuo H., Kangawa, K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999;402:656-660.
19. Larson-Meyer D.E., Palm S., Bansal A., Austin K.J., Hart A.M., Alexander B.M. Influence of running and walking on hormonal regulators of appetite in women. *J. Obes.* 2012;2012:730409.
20. Manshoury M., Ghanbari-Niaki A., Kraemer R.R., Shemshaki A. Time course alterations of plasma obestatin and growth hormone levels in response to short-term anaerobic exercise training in college women. *Appl. Physiol. Nutr. Metab.* 2008;33:1246-1249.
21. Martins C., Morgan L., Truby, H. A review of the effects of exercise on appetite regulation: an obesity perspective. *Int. J. Obes. (Lond.)* 2008;32:1337-1347.
22. Marzullo P., Salvadori A., Brunani A., Verti B., Walker G.E., Fa-nari P., Tovaglieri I., Medici C.D., Savia G., Liuzzi A. Acylated ghrelin decreases during acute exercise in the lean and obese state. *Clin. Endocrinol.* 2008;69:970-971.
23. Unick J.L., Otto A.D., Goodpaster B.H., Helsel D.L., Pellegrini C.A., Jakicic J.M. Acute effect of walking on energy intake in overweight/obese women. *Appetite* 2010;55:413-419.
24. Ozen-Vatansever S., Sonmez-Tiryaki G., Bugdayci G., Ozen, G. The effects of exercise on food intake and hunger: Relationship with acylated ghrelin and leptin. *J. Sports Sci. Med.* 2011;10:283-291.
25. Perini R., Veicsteinas A. Heart rate variability and autonomic activity at rest and during exercise in various physiological conditions. *Eur. J. Appl. Physiol.* 2003;90:317-325.
26. Rashidlamir A., Ebrahimnia M., Hashemi J.A. The effects of a single session aerobic exercise on obestatin gene expression in trained women. *Tehran Univ. Med. J.* 2011;69:426-431.
27. Reinehr T., de Sousa G., Roth, C.L. Obestatin and ghrelin levels in obese children and adolescents before and after reduction of overweight. *Clin. Endocrinol. (Oxf.)* 2008;68:304-310.
28. Shiya T., Ueno H., Toshinai K., Kawagoe T., Naito S., Tobina T., Nishida Y., Shindo M., Kangawa K., Tanaka H., Nakazato M. Significant lowering of plasma ghrelin but not des-acyl ghrelin in response to acute exercise in men. *J. Endocrine* 2011;58:335-342.
29. Witek K., Wit B., Lerczak K., Sempolska K., Glinkowski W. Influence of a monitored aerobic training and a low-energy diet on serum leptin concentration in obese women. *Biol. Sport* 2003;20:33-41.
30. Zou C.C., Liang L., Wang C.L., Fu J.F., Zhao Z.Y. The changing ghrelin and obestatin levels in obese children after weight reduction. *Acta Paediatr.* 2009;98:159-165.