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Research Article

Effect of Moderate Intensity Exercise on Ghrelin and Leptin Hormones

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ABSTRACT

This study examined the effect of exercise on acylated ghrelin and serum leptin levels. Twelve overweight/obese women participated voluntarily in two, 1-h trials (exercise and control) in a random crossover design. Trials began at 08:00 in the morning after an overnight fast. In the exercise trial, subjects ran for 60 min. at 50 % of maximum oxygen uptake between 09:00 and 10:00. In the control trial, subjects rested for 1 h. Blood samples were collected before and immediately following the exercise and control protocols. Plasma acylated ghrelin and serum leptin values were significantly ($p<0.05$) decreased immediately after the exercise. These results suggest that acute negative energy balance induced by 60 min. acute moderate aerobic exercise elicits a metabolic response with changes in acylated ghrelin and leptin concentrations inoverweight/obese women.

Keywords Obese Women; Exercise; Ghrelin; Leptin Hormones.

INTRODUCTION

Previous obesity researches were mostly on hypothalamic control in food intake [22]. However, today it is known that, not only central nervous system (CNS) but also adipose tissue, adrenal glands, pancreas, and gastrointestinal system are effective on short-term and long-term food intake control. Primarily the discovery of leptin which is the product of Obese gene (16kDa, ob) secreted by adipose tissue and called as satiety hormone in 1994 [40]; the discovery of ghrelin which is released from gastrointestinal tract and named as hunger hormone in 1999 [19,20], and the discovery of obestatin which is the product of the same gene with ghrelin and thought to fight against the effects of ghrelin on food intake in 2005 [5-14,41] started a new era for the remarks about obesity. All these discoveries and new information attracted researchers' attention to arrangement of energy homeostasis and accelerated the researches in the subject field. Ghrelin was first discovered as an endogen ligand for the growth hormone secretagogue receptor (GHS-R) [19]; further researches revealed that it strongly stimulated food intake and body weight [36, 39]. Ghrelin exists in blood circulation in two forms as acylated and desacylated[23]. Due to these effects, later researches focused on the role of ghrelin in energy homeostasis. Ghrelin precursor (preproghrelin) consists of 117 amino acids and before its release it goes under an enzymatical process in cytoplasm, and n-octanoyl is added to serine in the tertiary position to form acylated ghrelin (active ghrelin); and this is required for ghrelin to connect to GHS-R type 1 and its effect on food intake [19]. After the discovery of leptin, by the Zhang and their team, many researches have been conducted on obesity gene, which is a hormonal protein product with 167 amino acids. At first leptin was defined as related to satiety and energy balance, and as an anti-obesity factor that had a feedback effect on hypothalamus of adipocytes [40]. Ghrelin and leptin have opposite effects on food intake [10]. Contrary to ghrelin, leptin is a protein released by adipose tissues and it decreases food intake, increases energy expenditure, with weight loss primarily due to loss of adipose tissue, although some loss of lean mass may occur. In case of hunger, ghrelin levels in plasma increase and leptin levels decrease. After leptin is released, ghrelin concentration decreases and energy expenditure increases [34]. 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. 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 effects of 1-hour moderate aerobic exercise on acylated ghrelin and leptin levels, which play important roles in energy homeostasis in overweight/obese women.

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METHODOLOGY

SUBJECTS

Twelve overweight/obese women participated voluntarily in the study and written informed consent was obtained from all subjects before participation. Table 1 sum¬marizes descriptive statistics for the subjects. Participants complet¬ed 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 pres¬sure 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 con-sidered "low exercise risk" as per the American College of Sports Medicine (ACSM) guidelines. The study was approved by the ethical board of the AbantIzzetBaysal University School of Medicine Clinical Laboratory Research, Bolu, Turkey and it was performed in accordance with the principles of the Declaration of Helsinki.

PRELIMINARY TEST

ANTHROPOMETRIC MEASUREMENTS

Height was measured to the nearest 0.1 cm using a Holtain fixed wall stadiometer. Body mass was measured to the nearest 0.01 kg using a beam balance.

VO2MAX TEST

Maximal oxygen uptake test was performed one week before the main trials. The subject's VO_{2max} was assessed during a graded exercise treadmill (HP Cosmos Mercury Med 4.0) test using standard Bruce protocol (Bruce et al. 1973) in the morning hours. 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) O_2consumption increased by < 2 ml/kg with an increase in exercise intensity, attainment of >85% of age-predicted maximal heart rate. Respiratory gases were collected and analyzed throughout the entire exercise session with the use of a computer controlled breath-by-breath analyzer Cortex II Metalyser (Cortex Biophysik, Leipzig, Germany). The highest achieved value for oxygen consumption was considered the subject's VO_{2max}. Heart rate (HR) was measured continuouslythroughout the test using a commercially available HRmonitor (Polar S725X, Polar Electro, Finland).

EXPERIMENTAL PROTOCOL AND MAIN TRIALS

Participants were given at least one week to recover from the pre¬liminary 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 acylated ghrelin and leptin hormones.

After an overnight fast (no food or drink except water), participants arrived at the laboratory at 8:00 am and an intravenous catheter was inserted into an antecu¬bital vein (8:15 am). For the exercise condition, subjects ran at 50% VO_{2max} with an RER (VCO_2/VO_2) between 0.7-0.8 for 60 minutes. During the exercise, subjects breathed through a facemask. Respiratory gas exchange variables were measured throughout the test in a breath by breath mode and data were stored in 10-second intervals. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were continuously measured using a Cortex II Metalyser (Cortex Biophysik, Leipzig, Germany). The mean respiratory exchange ratio (RER) was calculated from the recorded measurements. The treadmill speed was adjusted every few minutes to maintain the speed at 50% of the VO_2 max according to the subject's preliminary test results. Blood samples were collected at baseline and immediately after exercise for the determination of leptin and acylated ghrelin. Heart rate was recorded every 5 s during the treadmill exercise by Polar heart monitors (Po¬lar 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, Ger¬many) and the mean respiratory exchange ratio (RER) was calcu¬lated from the recorded measurements. The analyser was calibrated before the test with gases of known concentration according to the manufacturer's guidelines (15.06% O_2, 5.11% CO2& bal. in N_2).

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 immunosorbentassay with a limit of detection of 1.0 ng • ml-1. Plasma acylated ghrelin assay was performed using a commercially available ELISA with a detection limit of 1.5 pg • ml-1. At each blood sampling point, duplicate 20 µL blood samples were collected into micropipettes for the measurement of haemoglobin concentration and tripli-

cate 20 µL blood samples were collected into heparinized micro haematocrit tubes for the determina¬tion of haematocrit. Haemoglobin and haematocritvalues were used to assess plasma volume changes [11].

Statistical Analyses

Means and standard deviations (±SD) were determined. The differences between pre-test and post-test measurements and differences between baseline measurements of exercise and control testing were analyzed with the Paired Samples T-test. The level of significance was set at $P < 0.05$. The results were analyzed using statistical software (SPSS 20.0, SPSS Inc. Chicago, IL, USA). Plasma volume changes did not differ significantly between trials, and the unadjusted values are presented.

RESULTS

The physical characteristics of the subjects (means ±SD) were as follows: age 21.35 ± 2.15 yr, height 1.63 ± 0.13 m, body mass 79 ± 2.20 kg, body mass index (BMI) 29.81 ± 1.30kg/m^2, maximum oxygen uptake 31.50 ± 3.40 ml .kg-1 .min -1. The subjects' characteristics are presented in Table 1.

Table 1.Physical characteristics of the participants.

Age (yr)	21.35 ± 2.15
Body weight(kg)	79 ± 2.20
Height (m)	1.63 ± 0.13
BMI (kg · m-2)	29.81 ± 1.30
$VO2_{max}$(ml · kg-1·min-1)	31.50 ± 3.40

ACYLATED GHRELIN AND LEPTIN CHANGES OVER TIME

BASELINEVALUES

Baseline fasting acylated ghrelin and leptin concentrations did not differ significantly (Paired Samples T-test, P>0.05) between trials.

EXERCISE EFFECT

Statistical analysis of data showed that there were statistically significant differences between the pre-test and the post-test of both the leptin exercise group (p=.003) and the ghrelin exercise group (p=.000). On the other hand, no statistical differences were observed for leptin control (p=.833) and ghrelin control (p=.111) groups (Table 2).

Table 2. Effect of treadmillexercise at an intensity of 50% VO_2max for a period of 60 min on serum leptin and plasma acylated ghrelin with in thetrials.

	Pre-Test	Post-Test	t	p
LeptinExercise (ng/ml)	5.77±1.06	4.90±1.05	4.289	.003*
Leptin Control (ng/ml)	5.37±1.12	5.42±.87	-.218	.833
AcylatedGhrelinExercise (pg.ml-1)	54.5±9.19	44.88±8.47	6.982	.000*
AcylatedGhrelin Control (pg.ml-1)	54.55±10.36	51.11±8.60	1.791	.111

* Significantd ifferences with in the groups (p<0.05). Valuesaremean ± SD (n=12).

DISCUSSION

The purpose of the present research is examining the effects of 1-hour exercise on ghrelin and leptin in overweight/obese women. According to the findings obtained in the present research, 1-hour moderate intensity exercise suppresses hunger hormone ghrelin and satiety hormone leptin in overweight/obese women.

The effects of exercise on acylated ghrelin is fairly controversial and depend on the intensity and duration of exercise [1,6,12,15,18,29,35,37]. We here report a significant reduction of plas¬ma acylated ghrelin after acute moderate intensity exer¬cise. The decrease in acylated ghrelin during exercise in our subjects is in agreement with previous research [2,6,27,28,29,38]

Broom et al. [2] conducted a research on 9 healthy male at the age of 19-25, and they reported that 60-minute exercise at 72% of Max. VO_2 suppressed plasma

acyl ghrelin and hunger. Similarly, Martins et al. [27] reported that acute modarete intensity exercise suppressed ghrelin among overweight and obese individuals. 40-minute exercise didn't change plasma ghrelin level and decreased hypothalamus ghrelin levels, and it recovered in 12-24 hours among rats fattened through diet [38]. 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 muco¬sal ischaemia resulting from redistribution of blood flow from the splanchnic circulation towards the skeletal muscles during exer¬cise [30].

Besides researches that report that exercise decreased ghrelin, there are some other researches that reported that exercise didn't affect, or even increased ghrelin. Erdmann et al. [13] conducted a research on 14 participants (4 female, 10 male) and studied the effects of different duration and intensity of cycling exercises (1.group: below anaerobic threshold 30. 60 and 120 minutes; 2. group: above anaerobic threshold 30 minutes) on ghrelin, and they reported that only 30 minute exercise below anaerobic threshold (60rpm. 50 Watt) increased ghrelin. Jüramai et al. [16] reported in their research that ghrelin was 24.4 % higher than the basal values after 6000 m. (average: 19 min.52 sec.) rowing exercise among 9 elite rowers. Christ et al. [7] reported that ghrelin increased at a significant level after 3-hour cycling exercise conducted at 50% of VO_{2max}. Sartorio et al. [32] reported that Growth Hormone (GH) increased among both female and male athletes while ghrelin hormone increased only among male athletes after 60-90 min. exercise done at 80% of VO_{2max}. This finding indicates that ghrelin release is different among male and female individuals.

Burns et al.[4] reported that during and 2 hours after 1-hour treadmill exercise done at 73.5% of $VO_{2max}$by male and female participants, there wasn't a significant difference between total plasma ghrelin levels of control and exercise groups. Martin et al. [25] studied the effects of 60-minute cycling exercise done at 65% of maximal heart beat rate on ghrelin, feel of hunger, and food intake on 12 participants. According to the findings of this research, modarete intensity acute exercise reduced feel of hunger temporarily, this temporary reduction contributed a short-term negative energy balance. However, this temporary effect of appetite couldn't be explained by the changes in the ghrelin levels. Jüramai et al.[17] reported that after 30-minute exercise conducted below and above anaerobic threshold by 9 elite rowers, GH increased but total ghrelin concentration didn't change at any intensity. Similarly, Bailey et al. [1] conducted a research on 9 healthy male participants and reported that 90-minute moderate intensity exercise suppressed leptin, but didn't change ghrelin. Pomerant et al. [31] conducted a research on male children of various pubertal developments, and reported that 30-minute medium intensity exercise didn't change ghrelin at any pubertal periods. Schmidit et al. [33] reported that GH increased but ghrelin didn't change during, and 40 minutes after various intensity 20-minute exercises done in different days at 50%, 70%, and 90% of Max.VO2 (maximal oxygen consumption capacity). As in the researches mentioned above, GH and IGF-1 concentrations were reported to increase but ghrelin didn't change after treadmill exercise done for 10 minutes at 60% of VO_{2max}, for 10 minutes at 75% of VO_{2max}, for 5 minutes at 90% of VO_{2max}, and for 2 minutes at 100% of VO_{2max}. [21].Sartorio et al. [32] reported that GH increased but ghrelin didn't change among male participants who conducted 30-minute exercise done 2 times at different times at 80% at Max. VO_{2max}. The findings mentioned above show that total ghrelin didn't affect BH release during exercise.

As can be seen from the researches above, the effects of acute exercise are very contradictory. The main reasons for these differences between the findings can be the differences in the intensity and durations of the exercises, participants, gender, and methodology. Further researches with more controls are required in order to understand the effects of acute exercise on ghrelin hormone, especially among female individuals.

In the current study, modarate intensity exercise resulted in a decrease of serum leptin. Most of the current studies elaborating on the effects of ex¬ercise on leptin support this finding [8,9]. 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 [26,27].

In conclusion, this study demonstrated that energy deficiency induced by acute moderate-intensity exercise lowers leptin and acylated ghrelin. The present findings of decreased ghrelin concentrations after acute treadmill exercise at moder¬ate intensity in overweight/obese women may be important for weight loss and weight maintenance. Further research is needed to determine the significance of this finding. Further well-controlled research is required to determine the influence of exercise on acylated ghrelin and leptin 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.

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