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# A comparison between the effects of one circuit-resistance and one aerobic exercise session on ghrelin to obestatin ratio in healthy young women

**[*]Mahdiyeh Ebrahim Nia and Amir Rashid Lamir**

*Faculty of Physical Education and Sport Sciences, Ferdowsi University of Mashhad, Iran*

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**ABSTRACT**

*Ghrelin and obestatin are orexigenic and anorexigenic peptides, respectively, which are believed to be important in energy homeostasis regulation and body weight control. The purpose of the present study was to compare the effects of aerobic and anaerobic exercises on ghrelin to obestatin ratio of plasma in healthy women. Twenty-four subjects were randomly divided into three groups: two experimental and one control group. Experimental group 1 performed circuit resistance exercises (CRE) (9 exercises, 25 seconds per exercise, at 60% of 1RM), experimental group 2 completed 1.5 miles running and the control group remained sedentary during this time. Blood samples were collected before and immediately after performing the exercise protocol. Plasma ghrelin, obestatin and GH levels were measured using the enzyme-linked immunosorbent assay (ELISA) method. The data were analysed by Leven, Kolmogorov-Smirnov and Repeated measure.. Results showed a significant increase in the growth hormone, ghrelin, and ghrelin to obestatin ratio, following both exercise protocols, however no significant increase was observed in plasma obestatin levels. It seems that both aerobic and resistance exercise decrease energy reservoirs and increase ghrelin secretion and ghrelin to obestatin ratio, in response to energy deficit, in order to provide the required amount of energy in the most efficient way.*

**Keywords:** Aerobic Exercise, Circuit- resistance Exercise, GH, Ghrelin to obestatin ratio, Females.

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## INTRODUCTION

Obesity and its related disorders are among the leading causes of illness and mortality in the developed world [1]. Additionally, chronic imbalance between energy intake and energy expenditure leads to obesity [2, 3]. Energy homeostasis is a complex and tightly regulated process controlled by neural and endocrine inputs. Ghrelin is a 28-aminoacid peptide discovered by Kojima et al. in 1999 [4], produced mainly in the X/A cells of the stomach and, to a lesser extent, by the intestines, pancreas, hypothalamus and pituitary. Initial studies on rodents [5] followed by humans studies revealed that ghrelin stimulates GH, ACTH and prolactin release, and increases appetite and food intake [6, 7] by stimulating the hypothalamus arcuate nucleus and increasing secretion of neuropeptide Y (NPY) and agouti-related peptide (AgRP). Circulating ghrelin levels rise during fasting and hypoglycemia, and decline upon refeeding [8] and oral or IV administration of glucose [9]. Circulating ghrelin increases in individuals with anorexia nervosa [7] and decreases in case of obesity [10]. Ghrelin is also involved in body weight regulation [11]. Continuous or repeated ghrelin administration in animals increases food intake and decreases energy expenditure, leading to weight gain [12, 13]. In addition to the ghrelin mature peptide, Zhang et al. [14] identified a 23–amino acid peptide with a flanking conserved glycine residue at the C-terminus. This compound was named ghrelinassociated peptide, or obestatin, and it has a negative effect on appetite. However, the anorexigenic properties of obestatin are still controversial [14-16]. It is interesting that obestatin, although derived from the same peptide precursor, suppressed food intake, inhibited jejuna contraction, decreased body-weight gain, and antagonized the actions of ghrelin when both peptides were coadministered [14]. These facts may suggest that the intricate balance of ghrelin and obestatin is important in the regulation of energy homeostasis and body weight control [17]. The distribution and synthesis of obestatin as well as its role in energy balance has been studied in rodents [18-20]. While physical exercise and its effect on plasma ghrelin and obestatin levels has been reported [21-24], the information about the effects of physical exercise on ghrelin to obestatin ratio is lacking [17, 25, 26]. It might be speculated that the exercise-induced suppression of plasma ghrelin [21, 27] also results in a significant change in plasma obestatin concentration. Thus, this study was conducted to investigate the effects of one bout of circuit resistance and one session of aerobic exercise on GH and plasma ghrelin to obestatin ratio.

## MATERIALS AND METHODS

Before administrating this study, the researchers obtained approval from the ethical committee of Ferdowsi University of Mashhad, Iran.

### Study population

Written consent was obtained from 24 female, college student athletes (Table 1). Subjects were randomly divided into three groups of eight participants: (1) control (no exercise); (2) Circuit resistance group [resistance training at 60% of a one-repetition maximum (1-RM)]; (3) Aerobic group (1.5 miles run at 70% of $VO_2$max). Subjects completed a medical examination and a medical questionnaire to ensure that they were not taking any medication, were free of disease and were not using steroids. All groups were completely familiarized with experimental procedures and had their 1-RM determined for each of the nine exercises used in the CRE protocol. Subjects were all tested in the luteal phase of their menstrual cycle.

**Table 1. Anthropometric characteristics of participants**

| Variables | Control group (N=8) | Circuit resistance group (N=8) | Aerobic group (N=8) |
|---|---|---|---|
| Age (years) | 21.37±1.76 | 21.87 ± 3.04 | 21.12±2.35 |
| Height (m) | 161.69±1.85 | 160.5 ± 3.99 | 163.12±5.20 |
| Weight (kg) | 59.68±7.65 | 56.35 ± 9.31 | 58.25±7.80 |
| BMI($kg/m^2$) | 22.88±3.49 | 21.8 ± 3.01 | 21.82±1.92 |
| Body fat (%) | 19.67±1.65 | 18.29 ± 3.14 | 19.67±5.51 |

*Values are means ±SEM.*

### Exercise testing procedures

Before the main trial, participants were taken to the weight training room three times. On the first two visits, all of the participants performed strength tests to determine their 1-RM for each of the nine free-weight resistance exercises (arm curl, triceps extension, back extension, squat, leg curl, bench press, overhead press, dead lift, seated row). On the third visit, the subjects completed a practice session to make sure that each participant was able to complete the entire exercise session. Subjects were asked to perform 3 sets of 9 non-stop circuits (9 exercises with 60% of one- repetition maximum for each exercise at their maximum speed) with a 5-minute rest between the sets. The aerobic group was asked to run for 1.5 miles at 70% of their $VO_2$max. The employed procedure was the same as the previously reported one [21, 28, 29]. The training session for both the aerobic and anaerobic groups consisted of a 10-minute warm-up, followed by the main exercises, and a-10 minute cool-down period in the end. The duration of the entire session was 35-45 minutes, and the control group remained sedentary during this time. The heart rate of the subjects was constantly checked (70-85% of the maximum heart rate) using the polar device (F1tm, Finland). The exercise sessions were held in the morning between 08:30 and 11:00, to avoid the effects of circadian rhythms. Subjects were instructed to follow normal lifestyle habits, avoid medications, refrain from exercise three days before the study,12 hours of fasting and visit the lab after a 12-hour fast (only allowed to drink pure water) for the blood sampling.

### Controlling nutrition

In order to control the effects of nutrition on the ghrelin levels, all of the subject they were provided with specially-designed diaries and were asked to write down the quantity and the time of what they ate, for three days before the administration of blood sampling. After dietary assessment to determine the total amount of calories for each subject, a dietician prescribed each participant an individualized diet based on the 3-day diary form. This diet included the prescribed quantity and type of the food to be consumed in the meal before the blood sampling.

**Anthropometric measurements**
The subjects were weighted using a digital scale with 0.01 kg accuracy. Fat percentage of the subjects was measured with a caliper, applying the three skinfold thickness method (subscapular, abdominal, and triceps) [30].

**Blood sampling and Laboratory measurements**
Blood samples (5 cc from brachial vein) were collected between 8:00 and 9:00 AM (following an overnight fast), 24 hours before and immediately after completing the exercise protocol. Blood samples were then drawn into a pre cooled tube containing EDTA. The collected samples were immediately centrifuged at 3000 g for 10 minutes. The plasma was stored at -80°C before the analysis. Plasma ghrelin, obestatin and growth hormone (GH) levels were measured using special kits.

The total level of plasma ghrelin was measured applying sandwich ELISA method, using American made human kits ELISA phoenix, pharmaceuticals, Belmont.USA, with the sensitivity of 6/25 pg ml. The coefficient Percent of variation within a test was determined 4/7%. Plasma obestatin levels were measured using sandwich ELISA method and American made human kits. The sensitivity of the mentioned method was 78 pg ml. Coefficient Percent of variation within a test was determined 9/6%. Growth hormone (ELISA kit dbc Canada) in the obtained samples was measured according to manufacturer's instructions. The Sensitivity of the mentioned method was 20 pg ml.

**Statistical analysis**
Normality of the data was tested using K-S test and the equality of the variances of the groups in different factors was tested using levene test. Variance analysis test with repeated measure and LSD were used to examine the difference between the groups. Statistical calculations of this study were done using the SPSS software (Ver.16).

## RESULTS AND DISCUSSION

Results showed that plasma ghrelin, GH and ghrelin/obestatin ratio increased significantly after aerobic and circuit resistant exercises in the experimental groups ($p<0.05$) but plasma obestatin ,despite a minor increase in the aerobic group, did not change significantly in three groups (table 2 and 3).

**Table 2: Comparison of hormone levels in the participants' plasma (repeated measure)**

| Variables | Group Time | Control group | Circuit resistance group | Aerobic group | P-Value | |
|---|---|---|---|---|---|---|
| | | | | | Factor | Factor*group |
| Ghrelin (ng/ml) | Before protocol | 0.71 ± 0.03 | 0.70 ± 0.41 | 0.75 ± 0.03 | 0.001 | 0.001* |
| | After protocol | 8.30 ± 0.16 | 8.15 ± 0.19 | 1.30 ± 0.21 | | |
| Obestatin (ng/ml) | Before protocol | 4.35 ± 0.73 | 3.65 ± 0.71 | 4.20 ± 1.08 | 0.693 | 0.537 |
| | After protocol | 4.32 ± 0.49 | 3.62 ± 0.44 | 4.32 ± 0.83 | | |
| GH (ng/ml) | Before protocol | 0.67 ± 0.46 | 0.65 ± 0.41 | 0.70 ± 0.43 | 0.001 | 0.001* |
| | After protocol | 0.70 ± 0.43 | 4.30 ± 1.35 | 8.17 ± 1.35 | | |
| Plasma ghrelin to obestatin ratio | Before protocol | 0.16 ± 0.02 | 0.19 ± 0.04 | 0.18 ± 0.06 | 0.002 | 0.002* |
| | After protocol | 1.93 ± 0.2 | 2.27 ± 0.32 | 1.97 ±0.36 | | |

*Values are means ±standard Deviation,* ***: p<0.05***

The present study focused on the adaptations made in the concentration of ghrelin, obestatin and GH, due to training. The results showed that plasma ghrelin increased after one session of exercise in both of the experimental groups. Short-term aerobic exercise does not appear to affect total ghrelin [31]. Erdmann and colleagues [32] found that total ghrelin levels rose after low-intensity exercise but did not change after high-intensity exercise. Total ghrelin levels were not affected by the duration of exercise. Broom *et al*. [21] found that acylated ghrelin levels decreased after 1 hour of treadmill running. Ghrelin levels were lower in the exercise group, compared to the control group, for at least 9 hours after exercise. In addition, acylated ghrelin levels were directly related to self-reported hunger. The authors suggested that inconsistent results in past research might be due to measuring total ghrelin instead of acylated ghrelin. Consistent with this notion, Marzullo *et al*. [33] found that acute aerobic exercise reduced acylated ghrelin but did not affect total ghrelin. However, discrepancies still exist; Ueda *et al*. [34] reported that acute aerobic exercise did not produce significant changes in acylated ghrelin. Ghanbari-Niaki [35] found that a single episode of resistance exercise decreased immunoreactive ghrelin, immediately after exercise and increased ghrelin 24 hours after exercise. Ballard *et al*. [36] reported that one session of resistance exercise reduced total ghrelin as well as food intake. Further research [37] investigating resistance exercise differentiated between concentric (lifting weights) and eccentric (lowering weights) muscle contractions. There were no changes in total ghrelin after eccentric contraction exercises; but total ghrelin decreased after concentric exercises. On the other hand, Takano and colleagues [38] found no changes in ghrelin within 30 minutes after short-term, low-intensity resistance exercise. The results of the present study indicating the increase in plasma GH concentrations after exercise is in agreement with the findings of the previous studies [4, 35, 37, 38]. According to previous studies, performing resistance exercises with high intensity would probably cause muscle damage (and decrease Glut4 protein content) and as a result of a delay in restoring muscle glycogen stores bring about negative energy balance in the body. In response to this possible energy shortage, the obestatin levels decrease and the ghrelin to obestatin ratio levels increase in order to restore the lost energy supplies of body.Kojima in 2001 [5] indicated that ghrelin and GHs-R stimulate growth hormone release by increasing levels of intercellular calcium through activating the phospholypase IP3 pathway. Among the researches carried out in a single session, Ghanbari-Niaki [35] indicated that despite the increase in GH after resistance exercise, Ghrelin levels decreased. He claimed that there was probably a reverse pattern in GH and ghrelin secretion. Some studies have reported that plasma ghrelin levels increase approximately twofold in fasting [39], And fasting is one of the factors creating a negative energy balance which can lead to stimulating peptides such as ghrelin [28, 29, 40]. The present data indicate that a single bout of circuit-resistance and aerobic exercise had no effect on plasma obestatin which has been introduced as a stomach anorexignic peptide. This change in the level of obestatin has also been reported in other previous studies [18, 21]. Some studies have shown that ghrelin and obestatin have an important role in energy balance and weight control. Survey findings show that ghrelin is sensitive to the negative energy balance and plays a significant role in the short and long term energy balance and glucose homeostasis [40]. Vicennati et al. [26] observed that obese females compared to their normal weighted counterparts, have a higher obestatin, and a lower ghrelin level. They also have lower ghrelin to obestatin ratio. It was also observed that the ghrelin to obestatin ratio has a negative correlation with body mass and abdominal fat distribution. In general, decrease of ghrelin to obestatin ratio in obese women supports the hypothesis that ghrelin and obestatin imbalance may be involved in pathophysiology of obesity.

**Table3: LSD test for comparing variables changes in 3 groups**

| Variables | group | Mean difference | P value |
|---|---|---|---|
| Ghrelin | 1 | 6.86 | 0.001* |
| | 2 | 6.96 | 0.001* |
| | 1 | 6.86 | 0.001* |
| | 2 | 0.09 | 0.545 |
| Obestatin | 1 | 0.001 | 1.00 |
| | 2 | 0.15 | 0.59 |
| | 1 | 0.001 | 1.00 |
| | 2 | 0.15 | 0.59 |
| GH | 1 | 3.64 | 0.001* |
| | 2 | 7.47 | 0.001* |
| | 1 | 3.64 | 0.001* |
| | 2 | 3.83 | 0.001* |
| Ghrelin/Obestatin ratio | 1 | 1.94 | 0.001* |
| | 2 | 1.64 | 0.001 |
| | 1 | 1.94 | 0.001* |
| | 2 | 0.30 | 0.034* |

*: $p<0.05$

The results of the present study demonstrated that one session of aerobic exercise and a session of circuit resistance exercise lead to stability of the resting plasma obestatin levels and increase ghrelin and GH levels. According to the obtained results, it can be concluded that performing resistance and aerobic exercise with the intensity of 60% of 1-RM and 70% $V0_2$ max may result in negative energy balance in the body and as a result increase ghrelin levels and gherlin to plasma obestatin ratio. The results of the present study showed that exercise will probably cause a negative energy balance in the body stimulating ghrelin secretion, which in turn increases ghrelin to obestatin ratio levels to stimulate food intake behavior, restore lost sources of energy, and re-establish energy balance.

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