



ORIGINAL ARTICLE

Effect of Aerobic Program in the Morning and Afternoon on Obestatin and the Body Composition of Overweight and Obese Women

Ramin Aghajani¹, Nematollah Nemati^{*1}, Zahra Hojjati Zidashti², Tahereh Bagherpour¹

¹ Department of Sport Sciences, Damghan Branch, Islamic Azad University, Damghan, Iran

² Department of Physical Education, Rasht Branch, Islamic Azad University, Rasht, Iran

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KEYWORDS

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ABSTRACT: This study was conducted with the aim to investigate the effect of morning and afternoon aerobic program on obestatin and the body composition in overweight and obese women. This quasi-experimental study was done in Rasht in 2018. In this study, 36 obese and overweight volunteers were randomly divided into 3 groups of training group in the morning (N=12), training group in the evening (N=12), and control group (N=12). The aerobic group did the aerobic exercise three 60-70 minute sessions for eight weeks. Exercise intensity was 55-70% MHR for the exercise period. Obestatin level and body composition of the subjects were measured before and after eight weeks of intervention. Data were analyzed using dependent t-test and analysis of variance (ANOVA) test. The results revealed no significant differences between the effects of aerobic exercise program in the morning and afternoon on obestatin in overweight and obese women. No significant difference was observed in obestatin between the training group at the morning and evening times, while a significant difference was observed in the body composition of the participants in the training group. In general, it can be concluded that morning and evening exercise have no significant effect on obestatin.

INTRODUCTION

over the past years, obesity, and infectious diseases and malnutrition used to be known as the public health issues in developed, and developing countries, respectively, but now, obesity has become a serious problem all around the world [1]. As obesity increases the risk of many chronic diseases such as hypertension, cardiovascular disease, diabetes, mental problems, arthritis, gout, gallbladder diseases and cancer, the prevalence of obesity is very worrying. Therefore, studying factors affecting obesity is of paramount importance [2].

It is unknown what is the best exercise time during the day to control weight in obese and overweight patients. The time of exercise may affect appetite and food intake. The biological rhythm is related to cyclic changes that regularly happen over certain times of the day, affecting the biological processes. In humans, the day clock is inhabited in two classes of nerve cells also known as suprachiasmatic nucleus (SCNs). These cells are in the side of the brain called the anterior hypothalamus. SCN starts a biological

*Corresponding author: nnemati258@gmail.com (N. Nemati)

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rhythm in the body, which coordinates all body organs rhythm during 24 hours [3].

Studies have shown that the daily rhythm controls some metabolic and hormonal indices affecting metabolism. The body temperature and oxygen consumed at rest at 4 AM are the lowest, with the peak at 3 and 4 PM [4]. Gastrointestinal motility pattern, intestinal absorption, gastrointestinal enzyme activity, and gastric acid secretion also have a daily rhythm. A complex system regulates the energy balance which contains central and peripheral factors. Ghrelin and obestatin peptides are known as environmental factors which play a significant role in regulating dietary intake and body weight [5]. Scientists have recently found another 23-amino acid peptide which is called obestatin. This peptide is derived from the ghrelin-constituting gene, which has undergone different changes after translation. The results of this study indicated that treating rodents with obestatin causes a negative energy balance because it reduces food intake and empties gastric. Obestatin is another peptide synthesized from a gene in common with ghrelin. Over recent years, it was found that obestatin effects are opposite to those of ghrelin in terms of gastrointestinal functioning and homeostasis. Some studies, has shown that obestatin, as an inhibitory peptide, can affect ghrelin function [6]. The results show that in simultaneous injection of ghrelin and obestatin, the inhibitory effects of obestatin on the ghrelin function are seen in food intake, intestinal contraction inhibition, slow food intake from the stomach and weight loss or gain. These facts show that there is probably a complex balance between ghrelin and obestatin, and it might be involved in different conditions of energy in regulating body weight [7].

A recent study reported that morning exercise sometimes causes higher levels of satiety compared to afternoon exercise [5]. Also another study reported that performing exercises in the evening is better in reducing or maintaining weight [4]. Similarly, some studies reported no significant changes in the metabolic and hormonal responses in the morning and evening exercises. A recent study showed that obestatin has no effect on food intake and weight loss [8-10].

As obesity is one of the greatest problems in modern societies today, recognizing factors and mechanisms to combat obesity would help with promoting community health and saving medical costs. Some studies have revealed the significant role of ghrelin and obestatin in the energy balance and weight control [11, 12]. Thus, studying the relationship between changes in levels of these peptides and performing aerobic exercises seems to provide appropriate strategies for weight control by appropriate exercises. The researchers have shown that a diet-based exercise program reduces ghrelin and increases base metabolism. Hence, some researchers have concluded that ghrelin and obestatin have opposite effects on weight regulation, and the malfunction of obestatin may contribute to obesity pathophysiology [13].

In this study, the researcher made attempts to examine the effect of aerobic exercises in the morning and evening on obestatin and body composition in obese women, to answer the question of whether there are differences in aerobic exercises in the morning and evening, or which variables (obestatin and body composition) are improved more with morning and evening exercises.

MATERIALS AND METHODS

This study was a quasi-experimental research study. The population consisted of 120 obese students of Payam-e Noor University of Guilan, who were studying in 2017. The statistical sample of size 36 was selected using non-random convenience sampling method. First, the obese and overweight people interested in participating in the research were informed about the research objectives and the way of doing it, and the informed consent was then obtained from all participants. After that, health evaluation questionnaire (Appendix A) was distributed among them. A total of 36 female obese women with an average age of 20-35 years with no regular physical activity during the past 6 months were selected to participate in the study. After this stage, the height, weight, and BMI of the subjects were obtained. Subjects were then assigned to three exercise groups of morning, evening, and control (n=12 per group) according to homogenization based on age and fat percentage. The

inclusion criteria of the study included as not suffering from diseases like cardiovascular disease, hypertension, renal diseases and non-use of drugs, and lack of regular physical activity in the past six months. The subjects who did not meet these criteria were excluded from this study.

The subjects of the morning exercise group did their exercise at 9 AM indoors. The exercise was done for 8 weeks in three sessions per week; each session lasted 60 to 70 minutes. The exercise program included warm up (10 minutes) and running on a smooth surface in the gym area that increased gradually by increasing the number of exercise sessions. At the end of each training session, the cool down phase was considered for 5 minutes including slow running and stretching movements. Exercise intensity was 55-70% of maximum heart rate during exercises. Given the low readiness of the subjects, the intensity of exercises was considered to be 55% of maximum heart rate in the first two weeks. Then considering the principles of extra load and regarding that each person ran at intensity related to her heart rate, the intensity of exercise increased sharply in the eighth week to 70% of maximum heart rate (5% every two weeks) [14]. The intensity of the exercise was controlled by heart rate and telemetry device during the exercise. Overall, each training session consisted of warming up for 10 minutes, light training with no resistance work, main training program, and cooling for 5 minutes.

The exercise group subjects worked out indoors at 18:00. The exercise program was conducted for 8 weeks in three sessions per week, each session lasted 60 to 70 minutes. The warm-up program for exercise group was held in the morning. A 5-minute cool down period at the end of each training session, including gentle running and stretching exercise was considered. Exercise intensity was the same as the morning exercise program. It should be noted that the control group subjects did their daily activities [14].

The initial venous blood samples were taken using a 10 cc syringe in the 48 hours before the first exercise session

from all subjects in the fasting state. In addition, 48 hours after the last training session, 10cc of blood was taken from the arm vein of all subjects in the fasting state to eliminate the effect of exercise. The subjects were asked to avoid food intake for 12 hours before blood sampling. Sampling was done at 8 AM and finished at 9 AM for similarity of blood samples in the pre-test and post-test. Blood samples were collected in ethylene diamine tetraacetic acid (EDTA-containing) tubes, and then, were rapidly centrifuged and the plasma was stored at 80°C for subsequent experiments in separate tubes.

Obestatin measuring kit with ELISA method, made in China, Glory Science Co., was used to measure plasma obestatin concentration in nano-milligrams units. The sensitivity of the method and the coefficient of in-test variation were 78pg/ml and 6.9%, respectively. BMI was obtained through dividing body weight (kg) by height squared (m). To measure subcutaneous fat, calipers were used (pinching in the three areas: triceps, upper-pelvic area and thigh), and the fat percentage of the three points of the body was measured using Jack son & Pollcok method.

In this study, descriptive statistics was used to describe the variables and inferential statistics was applied to analyze the results. Kolmogorov–Smirnov test was used to evaluate the normality of variables in each group. Additionally, in order to determine the effect of the exercises and compare the pre and post-tests in each group, dependent t-test was used. Moreover, ANOVA test was used to compare the variables changes before and after the intervention, and post hoc Turkey test was used to compare the changes of the variables in time intervals in each of the studied groups and to test the hypotheses. The significance level of $p < 0.05$ was considered as statistically significant.

RESULTS

The mean of the variables in the pre and post-intervention periods is presented in Tables 1 and 2.

Table 1. Description of the variables examined before intervention (mean \pm standard deviation)

Variable	Groups		
	Morning group	Evening group	Control
Age (year)	29.9 \pm 7.65	32.3 \pm 5.14	25.3 \pm 7.4
Height (Cm)	170.12 \pm 5.34	168.88 \pm 13.28	167.97 \pm 32.13
Weight (kg)	76.53 \pm 22.31	79.11 \pm 13.24	80.31 \pm 12.14
BMI(kg/m ²)	31.32 \pm 4.01	30.8 \pm 2.54	31.27 \pm 0.04
WHR	0.78 \pm 0.06	0.79 \pm 0.04	0.82 \pm 0.04
Body fat percentage	47.83 \pm 1.61	47.23 \pm 3.68	44.86 \pm 3.89
Lean Body Mass (kg)	40.02 \pm 8.46	41.26 \pm 3.04	44.6 \pm 4.47
Vo ₂ max (ml/Fg/min)	31.53 \pm 6.23	41.26 \pm 3.46	36.6 \pm 3.3
Systolic blood pressure (mmHg)	12.0 \pm 0.66	11.8 \pm 0.63	12.11 \pm 0.6
Diastolic blood pressure (mmHg)	8.0 \pm 0.66	7.9 \pm 0.56	8.33 \pm 0.5
Obestatin (ng/mg)	1.16 \pm 0.67	2.24 \pm 0.9	0.9 \pm 0.14

Table 2. Description of variables examined after intervention (mean \pm standard deviation)

Variable	Groups		
	Morning group	Evening group	Control
Weight (kg)	75.53 \pm 18.41	78.12 \pm 7.23	81.44 \pm 8.25
BMI(kg/m ²)	30.6 \pm 4.43	30.09 \pm 2.49	31.35 \pm 2.07
WHR	0.76 \pm 0.06	0.77 \pm 0.04	0.82 \pm 0.04
Body fat percentage	45.4 \pm 2.01	42.63 \pm 4.01	44.9 \pm 3.82
Lean Body Mass (kg)	40.7 \pm 8.81	43.9 \pm 4.09	44.6 \pm 4.15
Vo ₂ max (ml/Fg/min)	37.93 \pm 6.57	38.5 \pm 3.9	36.8 \pm 3.2
Systolic blood pressure (mmHg)	12.0 \pm 0.66	11.8 \pm 0.63	12.1 \pm 0.6
Diastolic blood pressure (mmHg)	8.0 \pm 0.66	7.9 \pm 0.56	8.33 \pm 0.5
Obestatin (ng/mg)	1.07 \pm 0.63	1.25 \pm 0.5	0.91 \pm 0.1

Intra-group analysis showed no statistically significant differences between obestatin in obese women in the aerobic exercise group before and after the intervention ($P = 0.733$). In addition, there were no significant differences between the value of blood obestatin in obese women in the aerobic exercise group before and after the intervention ($P=0.059$). Additionally, there were no significant differences between blood obestatin in obese women before and after intervention ($P = 0.91$) (Table 3). Moreover, ANOVA showed no significant differences between obestatin level changes in three groups ($P = 0.817$) (Table 3).

The in-group analysis showed no significant differences between the BMI of obese women in the exercise group before and after the intervention ($P = 0.091$). Furthermore,

there was a significant difference between the BMI of obese women before and after intervention ($P = 0.0001$), but no significant differences was observed between the BMI of obese women in the control group before and after intervention ($P = 0.151$) (Table 3). In addition, the results of intra-group study showed a significant difference between the WHR of obese women in the morning aerobic exercise group before and after intervention ($P = 0.003$). Additionally, there was a significant difference between WHR of obese women in the aerobic training group before and after the intervention ($P=0.001$) while there were no significant differences between WHR of obese women in the control group before and after intervention ($P = 0.81$) (Table 3). However, ANOVA showed a significant difference between \pm BMI and WHR in three groups ($P =$

0.035 and $P = 0.0001$, respectively) (Table 3). The results of Post hoc Tukey test showed that there is a significant difference between the level of BMI in the evening exercise group and the control group ($P = 0.049$), but there was no significant differences between the level of BMI of other groups ($P > 0.05$). The results of Post hoc Tukey test showed significant differences between the changes in WHR level in the morning exercise group and control group ($P = 0.008$), as well as the evening exercise sessions and control group ($P = 0.0001$). In addition, ANOVA showed a significant difference between the changes in body fat percentage in three groups ($P = 0.0001$).

Likewise, intra-group analysis showed a significant difference between the fat percentage of obese women in the morning aerobic exercise group and in the evening aerobic training group before and after the intervention ($P = 0.0001$ and $P = 0.0001$). However, there were no significant differences between the fat percentage of obese women before and after intervention ($P = 0.53$) (Table 3). The results of Post hoc Tukey test showed a significant difference between the level of body fat percentage in the morning training group and the control group ($P = 0.003$), and the evening group and control group ($P = 0.0001$). There is a significant difference in the level of BMI in the morning and evening exercise groups ($P = 0.009$).

Table 3. Changes in variables in pre and post intervention between the three study groups

Variable	Group	Pre-intervention	Post-intervention	Statistical estimate	Differences in pre and post-intervention	Statistical estimate
BMI(kg/m ²)	Morning group	31.32±4.01	30.6±4.43	t= 1.89 P= 0.091	-0.68±1.13	F= 3.8 P= 0.035**
	Evening group	30.8±2.54	30.09±2.49	t= 5.59 P= 0.0001*	-0.74±0.41	
	Control	31.27±0.04	31.35±2.07	t= 1.59 P= 0.151	0.08±0.16	
WHR	Morning group	0.78±0.06	0.76±0.06	t= 4.11 P= 0.001*	-0.01±0.01	F= 13.43 P= 0.0001**
	Evening group	0.79±0.04	0.77±0.04	t= 4.81 P= 0.001*	-0.02±0.01	
	Control	0.82±0.04	0.82±0.04	t= 2.00 P= 0.081	0.003±0.005	
Body fat percentage	Morning group	47.83±1.61	45.4±2.01	t= 5.73 P= 0.0001*	-2.39±132	F= 23.00 P= 0.0001**
	Evening group	47.23±3.68	42.63±4.01	t= 6.78 P= 0.0001*	-4.6±2.14	
	Control	44.86±3.89	44.9±3.82	t= 0.65 P= 0.53	0.12±0.58	
Obestatin (ng/mg)	Morning group	1.16±0.67	1.07±0.63	t= 0.35 P= 0.733	-0.08±0.79	F= 0.203 P= 0.817
	Evening group	2.24±0.9	1.25±0.5	t= 0.54 P= 0.59	0.05±0.32	
	Control	0.9±0.14	0.91±0.1	t= 0.11 P= 0.91	0.007±0.2	

* Significant intra-group change ($P < 0.05$)

** Significant difference between groups ($P < 0.05$)

DISCUSSION

After 8 weeks of aerobic exercise program, no significant change was observed in the concentrations of obestatin in none of the groups in the study. Moreover, there were no significant changes in the concentration of obestatin in none of the groups. Very few studies have been conducted on physical activity and obestatin and due to little background, it is very difficult to interpret results. Study on obese children, showed higher concentration of base obestatin [15]. A research study conducted on rats reported no significant changes in the plasma concentrations of

obestatin after 6 weeks of training on the rotating band at a speed of 25 m / min for 5 days a week [16]. Nevertheless, there was a significant decrease in obestatin of gastric and intestinal tissue. Several hormones including insulin, somatostatin, and glucagon regulate Ghrelin and obestatin in plasma and stomach. A recent study on the growth hormone amount showed a negative correlation between GH and obestatin [17]. However, studies conducted to investigate the effect of physical activity on GH and ghrelin revealed the negative feedback loop between increasing

GH and the decrease in ghrelin plasma and stomach, but no information about obestatin is available yet. The results of another study showed as subjects underwent GH-infusion, ghrelin concentration decreased after physical activity, but ghrelin concentration did not change significantly in the absence of GH [18]. The researchers examined the effect of 8 weeks of endurance training on a belt rig at a speed of 22 m / min for 5 days per week on the levels of obestatin, ghrelin plasma and hypothalamus in rats [2]. The concentrations of obestatin and ghrelin plasma did not change significantly under both acute and chronic conditions, but the activity of ghrelin and obestatin in the hypothalamus decreased after 8 weeks. Considering lack of changes in plasma obestatin, the results of the present study are in agreement with those of the three above-mentioned studies, indicating that plasma obestatin alone does not play any significant role in regulating energy balance. The researchers argued that in obese children and control group with normal weight, after 3 months of dietary intake and free aerobic exercise (basketball, swimming, running and cycling, performed at two turns in the morning and afternoon), the obestatin and ghrelin concentration in plasma increased significantly [8, 9]. According to them, the levels of ghrelin and obestatin have to do with obesity in children. Additionally, the researchers observed a significant increase in obestatin levels in obese children after one year of combined dietary intake and body weight gain, whereas they observed no significant changes with regard to ghrelin [15]. Following the lifestyle changes in the end, it was found that obestatin plays a role in regulating energy balance in children. Therefore, it is probable that the change in lifestyle for a long time leads to a change in energy balance which in turn affects the plasma obestatin concentration. Food intake, glucose uptake, high-carbohydrate diet, and negative energy balance decrease gastric and metastatic gastric abscess in some animal and human specimens. The increased levels of liver and muscle glycogen in trained rats can be attributed to lower levels of obestatin in the stomach tissue. This is because the studies have shown that endurance training increases muscle and liver glycogen (with or without carbohydrate loading) in human and animal specimens [6]. Another study reported

that one running session on treadmill could reduce glycogen in liver and muscle. This evacuation with high-carbohydrate intake caused an increase in the glycogen storage of liver and a reduction in the ghrelin levels of plasma [19]. Another study showed that obestatin does not play a significant role in regulating energy balance. This finding is in line with that of the present study [16]. For the first time, the researchers reported that obestatin plays a role in controlling energy balance [10]. One of the researchers found different factors related to energy metabolism such as food intake, the weight of the body, body composition, energy consumption, locomotor activity, respiratory rate, and hypothalamic neuropeptides affect energy balance regulation in response to long-term injection of obestatin in rats, but observed no significant effects [20]. Furthermore, another study investigated the effect of obestatin on the regulation of energy balance and found similar results [21]. One of the studies examined the obese children, who had 3 hours aerobic activity per day, performing in three fields of basketball, swimming and ping-pong in the morning and afternoon, and a dietary intake for one month [9]. The results revealed a significant increase in the concentration of ghrelin and plasma obestatin following a significant weight loss. These researchers believe that ghrelin and obestatin are associated with childhood obesity. In severely obese women who lost 62% of their extra weight using gastric bypass surgery, ghrelin levels decreased significantly, whereas obestatin concentrations showed no significant changes. Unlike previous results, weight loss was associated with decrease in ghrelin levels, and obestatin concentrations showed no changes.

After all, in aggressive studies, including stomach surgeries, a part of the stomach containing ghrelin producing cells is eliminated while other areas have more ghrelin secretion to make compensations [17]. One of the studies reported higher concentrations of obestatin and lower ghrelin and lower ghrelin to obestatin ratio in obese women compared to the control group [22]. These researchers reported the significant role of the imbalance between ghrelin and obestatin in the pathophysiology of obesity. The role and importance of obestatin in activity as

an anti-appetite peptide involved in energy balance to prevent weight gain is still ambiguous due to having little understanding of it. Probably, the level of obestatin is affected by exercise and may result in changes in appetite and weight. Considering the recent discovery of this peptide, only a small number of studies have investigated the effect of exercise on this peptide. As obestatin can affect energy balance, this peptide level may be influenced by physical activity, which in turn changes the appetite and weight [16]. Owing to the negative energy balance caused by exercise, it is likely that the level of obestatin is affected by exercise as well. In the studies conducted so far, different results have been reported about the effect of physical activity on the level of plasma obestatin and various tissues. The exercise type, duration, and intensity seem likely to have an influence on the level of obestatin. Moreover, different tissues may show different responses to exercise. Increasing obestatin following the loss of weight may be a necessary mechanism to maintain weight loss. Factors such as being measured in fasting or non-fasting state during the study, the body weight, BMI, the type of exercise program used, and even the time of post-activity sampling have affected the results. There has been a significant correlation between changes in BMI and changes in plasma obestatin in obese subjects. Moreover, a significant negative correlation was found between the level of growth hormone and the obestatin level of fundus and small intestine. Overall, it seems that the increase in the level of obestatin in the weight loss process is critical to maintain and prevent weight gain, because obestatin peptide is an anti-appetite and has a function opposite to that of ghrelin [18, 19].

The results of the present study showed a significant difference between the effects of aerobic exercise program in the morning and evening on the composition of the body in overweight and obese women. The results of this study are in accordance with that of studies showing no changes in BMI [23]. However, the results are inconsistent with some studies [24, 25]. One of the studies reported that an aerobic training course causes a decrease in fat percentage, BMI, waist to pelvic circumference in overweight men [24]. In addition, another study reported a significant

reduction in BMI after aerobic exercise [26]. Moreover, a positive effect on body fat percentage and cardiovascular fitness of overweight / obese individuals following a short-term course of physical activity intervention was reported by another study [25]. Furthermore, one study concluded no significant differences between weight variations and BMI following a periodic speed and endurance exercise [23]. The inconsistency and insignificant changes in WHR are probably due to the low intensity and duration of exercise, and not controlling the subjects' diet.

The results showed that aerobic exercise in the morning and evening did not make significant changes to the ghrelin and obestatin levels. In addition, there was no difference between the effects of these exercises on ghrelin to plasma obestatin ratio. Overall, one can state that aerobic exercise in the morning and evening not only does not affect obestatin but also is insufficient to affect obestatin considering its duration and intensity.

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