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Effect of moderate and high intensity aerobic exercise training on plasma levels of obestatin in male rats

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Abstract

Obestatin is a 23-Amino Acid peptide which secreted from the fundus of stomach and can play an important role in regulation of food receiving and body weight. The aim of the present study was to investigate the Effect of moderate and high intensity aerobic exercise training on plasma levels of obestatin in male wistar rats. Nineteen male Wistar rats (8 weeks old, 200 to 225 g) randomly divided to control and training. After two weeks of familiarization speed and time gradually increased to determined intensity for each group. Aerobic training program included 8 weeks, 5 sessions per week for 60 min. the intensity of the exercise was 80% - 85% VO_2max for high intensity training group (HIT group) and 70% - 75% VO_2max for moderate intensity training group (MIT group). Rats were anesthetized intraperitoneally fourty-eight hours after the final training session (after 12 h fasting), and Blood samples was collected directly from the right ventricle. The mean weight in HIT group reduced significantly than to control group. Plasma obestatin levels didn't have any significant differences between these three groups. However the obestatin levels in Exercise training groups were higher than control group.

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Introduction

In General, regulation and balance of energy apparently seem so simple but these processes are extremely complex. One of the effective factors is peptides which are secreted from the stomach and intestine which their functions have been considered by so many researchers during recent years (Kojima *et al.,* 1999; Zhang *et al.,* 2005). Obestatin is a 23-Amino Acid peptide which secreted from the fundus of stomach then fall into the bloodstream. This peptide will be coded by Ghrelin gene (Broglio *et al.,* 2006; Zhang *et al.,* 2005).

It is sounded that the Obestatin can play an important role in regulation of food receiving and body weight. The findings indicated that expressing the Ghrelin gene in stomach during fasting increased and decreased postprandial. As a matter of fact the plasma level of ghrelin decreased in state of positive energy balance and increased in state of negative energy balance. The Obestatin activity is versus Ghrelin function which this action causes to suppress appetite and lose weight (2006; Zhang *et al.,* 2005; Nogueiras *et al.,* 2007).

The studies on Obestatin indicated that this peptide has different functions such as impact on bodyweight, energy consumption, receiving water, glucose homeostasis, hormone secretion, gastrointestinal movements, sleep, anxiety and memory (Nogueiras *et al.,* 2007; Bresciani *et al.,* 2006; Brunetti *et al.,* 2010; Dun *et al.,* 2006; Lagaud *et al.,* 2007; Tang *et al.,* 2008, Unniappan *et al.,* 2008). Plasma obestatin concentrations will be regulated by hunger and satiety (Guo *et al.,* 2008), high carbohydrate meal (Sedlácková et al., 2008), losing weight and fatness (Wang *et al.,* 2008). In the studies observed that the plasma obestatin level and also rate of appetite inhibition is significantly lower in fat people than normal people (Guo *et al.,* 2007). It is sounded however the reduction of Obestatin function involved into Pathophysiology of obesity (Lagaud *et al.,* 2007). In a study one year physical activity with diet program caused to lose weight in obese children and it is interesting that this matter contained with increasing plasma obestatin (Reinehr *et al.,* 2008). In addition that the exercise training can improve the healthy condition of fat people can cause to loss body weight (Kraemer *et al.,* 2007). Therefore, research on the impact of exercise training on Obestatin is interesting. On the other side, changing in peripheral tissue energy state due to various Metabolism factors and physical activity will cause to change in peripheral signals which mean secreted hormones by peripheral tissues (Woods et al., 2004; Hillebrand et al., 2002). So with this kind of situation, the physical activities that impact the energy balance can also influence the obestatin secretion.

With regarding to this matter that Very little research has been done on the impact of exercise training on the peptide, different uncertain issues remained in this field.

Also it seems that there is no published subject about the impact of 8 weeks endurance training with moderate and severe intensity on plasma obestatin levels in Male Rats. So in this current study we attempted to investigate this matter.

Material and Methods

Animals

All experiments were in accordance to the principles of the declaration of Helsinki, and the protocol was approved by the Ethics Committee of the School of physical education and sport sciences at Islamic Azad University, Central Tehran Branch, Tehran, Iran. Nineteen 8-weeks old male Wistar rats (200 to 225 g) from Pasteur's Institute were used for this study. Animals maintained in the Animal House Center, Department of physical education and sports sciences of the University of Tehran. The animals were housed 3 per cage (40 × 20 × 20 cm) and they had a 12-hour, 12-hour light–dark cycle. Temperature and humidity of room were controlled and maintained at 22°C ±3°C and 40-60%, respectively. Subjects were fed a pellet rodent diet (10 gr/100 gr bW) and had free reach to water. Animals were randomly divided to control (n =

5), high intensity training (n = 7) and moderate intensity training (n = 7) groups.

The protocol for the assessment of aerobic capacity in rats

With regarding to the lack of access to direct tools such as respiratory gas analyzer device, the indirect protocol with high accuracy was used as follows:

First, rats warmed up for 10 min and the intensity of 40% or 50% VO2max, then after warm up, the tests started with rats running at the speed of 15m/min for 2min. Then, the treadmill speed increased 0.03 m/s (1.8 to 2 m/min) every 2 min till the animals were not able to run anymore. The criterion for reaching to VO_2max was the lack of increased VO_2max despite increased speed.

Endurance training group's protocol

In order to familiarize with continuous training, in the first two week (7-10 days), rats walked on the treadmill for 20 min at 5m/min and then speed and time gradually increased to determined intensity for each group. Aerobic training program included 8 weeks, 5 sessions per week for 60 min. the intensity of the exercise was 80% - 85% VO_2max for high intensity training group (HIT group) and 70% - 75% VO_2max for moderate intensity training group (MIT group). At the begining and end of exercise in each session, 5 min at 50-60% vo_2max taken into account for warm up and cool down.

At the end of each two-week, a test of maximal Oxygen consumption and a new training speed for next week were calculated. The treadmill speed initially was 16 m/min and 20 m/min in the first week, and then in 8^{th} week it reached to 30 m/min and 34 m/min in moderate intensity endurance training group and high intensity endurance training group, respectively. The treadmill incline was zero in all of exercising steps. All of training sessions implemented under red light at 8 -9 PM (due to their activation cycle were in darkness).

The living conditions of animals in the control group except daily exercise in different times was so similar to exercise group even in order to further simulation of control group with in training groups, they put three times a week on treadmill for 15 min at 0.15 m/s. This exercise did not make any response.

Blood sampling

Rats were anesthetized intraperitoneally Fourty-eight hours after the final training session (after 12 h fasting), with injection of ketamine (30 to 50 mg/kg bw) and xylazine (3 to 5 mg/kg bw). Blood was collected directly from the right ventricle. Collected plasma poured in EDTA-containing tubes and was centrifuged during 10 min at 3500 round/min, then the plasma was frozen at -80°C. Total plasma obestatin concentration was determined by rat ELISA kit (EIA; Bachem, Peninsula Laboratories Inc., CA, USA). The sensitivity of kit was 0.02 ng/mL.

Statistic analysis

Evaluation of normality was performed with the Kolmogorov-Smirnov test, and values were found to be normally distributed. Repeated measures ANOVA was used for analayzed changes in mean body weight between groups over time. One-way ANOVA and Tukey post-hoc tests were used to assess differences between obestatin concentration values. Results are reported as means ± SE. Significance was defined as $p<0.05$. Data were analyzed using the SPSS software (version 19; SPSS, Chicago, IL).

Results

The mean weight in HIT group reduced significantly than to control group. In contrasting, were not any significant differences between MIT groups with control group (Table 1).

The yielded result from one- way ANOVA indicated that the exercise didn't have any meaningful impact on the plasma obestatin levels and the amount of the plasma obestatin didn't have any significant differences between these three groups. Of course it is notable that the obestatin in Exercise training groups

was higher than control group even their differences between HIT and control groups reach near to meaningful level (P=0.142).

Table 1. Mean body weight, and plasma obestatin concentrations in male wistar rats.

Variables	Control	MIT	HIT	F Value	P Value
Body Weight, g	318.6±11.78	306±8.86	293.71±8.84	4.731	0.024[a]
Obestatin, ng/ml	2.05±0.12	2.15±0.11	2.19±0.11	2.066	0.159

Values are mean±S.E.M.
MIT: Moderate intensity training, HIT: High intensity training
[a] HIT vs Control

Discussion

In the present study it was observed that plasma obestatin levels increased after moderate and high - intensity endurance exercise but this increase was not significant in any of the training groups compared to controls. The results showed that the mean plasma obestatin after high-intensity exercise was larger than the mean obestatin after moderate exercise.

Increased plasma obestatin subsequent to endurance exercise has also been observed in Ghanbari-Niaki studies (Ghanbari-Niaki *et al.,* 2008; Ghanbari-Niaki *et al.,* 2010). However in Ghanbari-Niaki studies increased plasma obestatin following training has not been significant. In this study, it was found that the type and duration of training programs have been effective on how to respond and adapt.

The results of the present study are similar to those of Wang et al (Wang *et al.,* 2008) where the effect of 8 weeks of training session of treadmill running with a slope of 5 degrees and a speed of 20 meters per minute, 5 days a week and each session 40 minutes, on plasma obestatin levels in obese mice had been examined, and results showed no change in plasma obestatin levels following acute and chronic exercise. However, it was seen that diet coupled with a weight loss exercise program would increase plasma obestatin levels in overweight children (Reinehr *et al.,* 2008; Zou *et al.,* 2009). In this respect, the researchers concluded that obestatin increase following weight loss may be an essential mechanism developed for the maintenance of weight loss (Lagaud *et al.,* 2007).

Thus, factors such as the fasting state of subjects during the study, subjects' weight, body mass index, type of program being employed and even the sampling time after the exercise have been different in the studies and have played a role in the incidence of different findings.

It should be noted that a possible reason for the change of obestatin levels is the effect of certain hormones. It is likely that changes in the levels of certain hormones during exercise have caused obestatin levels to change, because previous studies have shown that levels of plasma obestatin and ghrelin and stomach are regulated by several hormones such as growth hormone, (Murakami *et al.,* 1997), insulin, somatostatine (Harada *et al.,* 2008), and glucagon (Andersson *et al.,* 2005). Given that in the present study the levels of these hormones were not examined, hormone changes may be effective in changing obestatin. To clarify this issue requires further and more comprehensive studies.

It should be noted that up to now no studies have examined the effect of endurance training on the level of obestatin and this study is the first research on this topic. The findings of this study show that although obestatin levels following moderate and high intensity endurance exercise have no significant difference compared with the control group, the obestatin levels in the subjects following high intensity exercise is higher than those in control group with moderate intensity. So exercise intensity may also be effective in this matter.

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