

## The Effect of Endurance Training on Ghrelin, Insulin, Glucose and Estrogen in Male Rats

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Article information	Abstract
<p>Article history: Received: 10 Jan 2013 Accepted: 27 Feb 2013 Available online: 26 May 2013 ZJRMS 2014; 16(2): 42-44</p> <p>Keywords: Rat Ghrelin Aerobic training</p> <p>*Corresponding author at: Department of Physical Education and Sport Sciences, University of Mazandaran, Babolsar, Iran. E-mail: sara.nasiri64@gmail.com</p>	<p><b>Background:</b> Ghrelin is a 28-amino-acid peptide hormone that is secreted primarily by stomach cells with lesser amounts secreted by other cells (including the hypothalamus). The aim of present study was to examine the effects of 8 weeks aerobic training with different durations on resting plasma Ghrelin levels in male rats.</p> <p><b>Materials and Methods:</b> Fifty adult Wistar male rats (6-8 weeks old, 270±10 g) were selected and randomly divided into five groups: 30, 60 and 90 min training, sham and control groups. All experimental groups performed an 8-week treadmill running program at the same velocity at 0 gradients for 30, 60 or 90 min/day, 5 days/week. The concentration of ghrelin in blood samples was assessed after 8 weeks and 72 hours following the final training session. The ghrelin concentration was measured by ELISA. Possible statistically significant differences between groups after the exercise training intervention was determined by one way ANOVA, and LSD test was used for a post hoc analysis.</p> <p><b>Results:</b> Resting levels of ghrelin concentration were unchanged after training. Similarly, there was no observed change in the insulin and glucose concentrations compared with the control group. However there was a significant difference in estrogen when compared with the control group.</p> <p><b>Conclusion:</b> The data suggest that body weight reduction is amplified by exercise-induced and increases in plasma estradiol and a moderate duration exercise program.</p> <p>Copyright © 2014 Zahedan University of Medical Sciences. All rights reserved.</p>

### Introduction

Energy hemostasis is a complex process that central and environmental factors impact on at different conditions. The main place of energy regulation is in the central part of hypothalamus. Recently, it is believed that the regulation of energy not only take place by central part, but also influenced by environmental factors of the gastrointestinal (GI) tract and adipose tissue [1]. In spite of existence wide information about central part of energy hemostasis, appetite, and weight there is a few researches about environmental peptides.

Recent studies have revealed much concerning about the pathophysiological complexities of obesity and metabolic diseases. Ghrelin recently has been discovered as a peptide expressed by different tissues and has different actions. Ghrelin facilitates growth hormone (GH) expression and increases appetite, with rising and falling plasma levels before and after meals. Thus, ghrelin effectively is associated with regulation of energy balance and insulin action. Ghrelin is produced mainly in the stomach with more ghrelin-producing cells found in the fundus than pylorus. Ghrelin is also found in other portions of the GI tract, including the duodenum, jejunum, ileum, and colon. Since the original observation of ghrelin in the GI tract, numerous studies have been reported the ghrelin secretion from other tissues,

including pancreas, hypothalamus, testis, placenta, lung, cardiomyocytes, and chondrocytes. Also studies show that hormones such as insulin, GH and glucagon have a main role in the ghrelin regulation.

As exercise training improves the health status of obese individuals and is associated with reduction of body weight, it may impact on ghrelin and this peptide may provide better understanding of how exercise improves health. More ever, endurance training leads to weight reduction. Wang et al. [2] studied the effect of one session and 8 weeks training on plasma and hypothalamus ghrelin levels. They observed ghrelin levels did not change by one session training but after 8 weeks training the amount of hypothalamus ghrelin decreased in experimental groups.

So in favor of spread roles of ghrelin and multiple effects of exercise on cells changes, energy balance and weight regulation, the aim of this study was to investigate the effect of 8weeks endurance training on ghrelin. Will these changes can effect on other factors that play important roles in body metabolism such as insulin and glucose? Will ghrelin changes can effect on estrogen secretion? And finally will these changes depend on another or not?

## Materials and Methods

In this empirically study, 50 male rats were selected (6-7 weeks, 270±10 g), from Iran north institution. The testing groups randomly were divided into 4 groups: 3 experimental groups and 1 sham group. Control groups did not participate in any activity.

Experimental groups exercised for 8 weeks, 5 days a week. All period of exercise divided into 3 stages: familiarization, overload, and establishment. In familiarization stage (first week) rats walked on the treadmill (for 10-15 min, with 5 m/min) every day. In overload stage (second and third week) firstly, rats ran on the treadmill (for 15 min, with 15 m/min) and gradually intensity and duration increased to 20 m/min and 90 min. In establishment stage (from forth week to eight week) experimental groups ran for 4 weeks within a period of 30, 60, and 90 min with 20 m/min [2]. Incidentally, in every period of exercise, 5 min, 9 intensity, and 6-8 m/min taken into account for warm up and cool down. Sham group did familiarization and overload stages.

Rats were anesthetized with injection of ketamine (30-50 mg/kg) and xylazine (3-5 mg/kg) 72 hours after last session of exercise (they were feed, 4 hours before killing food taken from the cage but water was available). Blood samples were collected from right ventricle and were centrifuged within 10 min with 300 round/min. Collected plasma poured in micro tubes and placed in -80°C. All stages were done within a period of 5 days from 7:00 to 11:30 O'clock.

The ghrelin concentration was measured by ELISA method. Plasma glucose, insulin and estrogen were measured by colorimetric enzymatic methods, by rat insulin ELISA and rat estradiol ELISA, respectively.

Statistical analysis between groups after intervention was determined by one way ANOVA. The LSD test was used for a post hoc analysis. All statistical calculations were performed using the statistical software SPSS-16 and a significance level of  $p < 0.05$  was considered.

## Results

The values of biochemical parameters are provided in table 1. It shows that ghrelin levels didn't differ significantly between the five groups with lower levels in 30 min group. Similarly, there was no observed change in the insulin and glucose concentrations compared with the control group with lower levels in 60 min and sham groups respectively. However there was a significant difference ( $p < 0.05$ ) in estrogen when compared with the control group with higher level in 60 group. Additionally, weight had a significant change after exercise ( $p < 0.05$ ).

There was negative and non significant relation between ghrelin and insulin, glucose but there was positive and non significant relation between ghrelin and estrogen, weight.

## Discussion

In this study weight significantly decreased in experimental groups in comparison with control group. Findings of our research show that resting levels of ghrelin concentration were unchanged after training.

Studies showed different results about that. Wang et al. [2] studied the effect of short term training (40 min, one session, 20 m/min speed, slope 15 degree) and long term training (40 min, 5 days in a week, 8 weeks, 20 m/min speed, slope 15 degree). Despite weight reduction after long term training, they reported plasma ghrelin levels were unchanged; however, hypothalamus ghrelin was decreased. Ghanbari-niaki et al. [3] studied the effect of running with moderate intensity for 6 weeks (speed: 25 m/min, 60 min) on the ghrelin levels. Results showed that 6 weeks training decreased ghrelin levels. According to the study, the mice were killed after 37 hours, whereas in the present study, rats were killed after 72 hours of recovery, so it might be one possible reason for the disagreement of the present paper with Ghanbari-niakis article [3]. Schmidh et al. [4] reported ghrelin levels did not change by running on treadmill with intensity 50 and 70%  $Vo_2max$  (3 minutes) and 90%  $Vo_2max$  (1 minute) in 3 difference days. Also, Ghanbari-niaki et al. [5] studied the effect of ATP and glycogen reduction on ghrelin resting levels in trained male rats. Subjects divided into two groups saline (control: 8, exercise: 8) and etionin (control: 8, exercise: 8). Exercise group ran for 60 min with speed of 25 m/min during 10 weeks. The amount of plasma ghrelin did not change after 10 weeks endurance training whether etionin infusion was effective on ghrelin levels so ghrelin levels in etionon-exercise and etionin-control were more than the other groups. Note that the decrease in liver glycogen and ATP was happened following the administration of ethionine and since ATP reduction, increased food intake behavior in rats, ghrelin increased levels seem reasonable but in the present study, a reduction in energy reserves [6] was not observed, so it might be one of the possible reason for no change in ghrelin levels. Andersson et al. [1] reported that one session running on the treadmill (60 min, speed: 22 m/min, slope 10 degree) decreased ghrelin levels whether glucose level was unchanged. In Andersson research, ghrelin decreased immediately after exercise whether in our study rats killed 72 hours after last session.

**Table 1.** Ghrelin, insulin, weight, glucose and estrogen changes after 8 weeks

Variables	Groups	Control	Sham	30 min	60 min	90 min
Weight* (g)		360.11±15	357.71±29	339.40±34	326.11±42	321.77±28
Ghrelin (ng/ml)		7.64±0.23	7.92±1.04	7.55±0.5	7.82±0.8	7.64±0.57
Glucose (mg/dl)		215.87±66	176.42±34	187.0±46	197.30±22	206.87±55
Insulin (µg/l)		0.32±0.13	0.24±0.12	0.28±0.21	0.22±0.14	0.33±0.27
Estrogen* (pg/l)		38.11±26.9	58.28±23.75	63.5±12.23	69.2±18.36	65.75±23.5

\* significant changes

In the present study, there was positive but non-significant relationship between ghrelin and estrogen. Also a significant plasma estradiol concentration was found in all trained groups in comparison with control group. Our resting plasma estradiol levels in sedentary/control group was in agreement with previously reported values by several investigators [8]; Ueyama et al. [9]; Mystkowski et al. [10]. It has been suggested that plasma estradiol levels and replacement could regulate body weight in ovariectomized and gonadectomized rats [10].

Results of this study demonstrated that ghrelin had a negative and non-significant relationship with glucose and insulin. Haltiat et al. [11] investigated the effect of glucose injection on ghrelin in thin and obese subjects. In that study 12 thin persons (age: 26 week, BMI: 19.8-23.9 kg/m<sup>2</sup>) and 13 obese persons (age: 27 week, BMI: 27.7-42.2 kg/m<sup>2</sup>) were injected 300 mg/kg glucose and saline in two days. Higher increasing was observed in obese group in comparison with thin group. In both groups glucose infusion caused significant ghrelin reduction. Broom et al. [12] observed not significant relationship between ghrelin with glucose and insulin after 60 minutes running at 72% V<sub>O<sub>2</sub></sub>max. They showed that acylated ghrelin concentration was suppressed during running.

There was limitation to the present study. Lack of controlling the amount of food taken by rats since there

were 5 rats in each cage, although 10 g per 100 g body weight pellet was poured but could not say that all rats ate equally. In the present study we focused on changes in body weight of rats so we weigh them at the beginning and end of the study.

In conclusion our findings showed that despite significant changes in ghrelin weight loss in 60 minutes group is more than other groups. Since estrogen is known as a non-orexigenic factor and suppressing effect of estrogen on ghrelin was observed in other researches, the results of our study might be suggested that body weight reduction is amplified by exercise-induced elevated plasma estradiol and the nature of moderate duration exercise program so in order to lose weight a moderate term low intensity exercise program should be considered.

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#### **Authors' Contributions**

All authors had equal role in design, work, statistical analysis and manuscript writing.

#### **Conflict of Interest**

The authors declare no conflict of interest.

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