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SHORT COMMUNICATION

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The effect of high intensity aerobic exercise training on plasma levels of ghrelin in male rats

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Abstract

The aim of the present study was to investigate the effects of 8 weeks of high-intensity endurance training on total plasma ghrelin concentrations in male Wistar rats. Seven rats were placed in the experimental group and 5 were placed in the control. Exercise was performed 5 times a week, each session lasting 60 minutes with intensity of 80 - 85% VO2max. Blood samples were taken 48 hours after the last training session following 12 hours of fasting. The results showed that 8 weeks of high-intensity endurance training significantly increased total plasma ghrelin together with significant reduction in weight. The result of the study confirms the role of ghrelin in energy homeostasis.

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Introduction

Issues such as the regulation of weight and energy balance are always important and complicated matters. Changes in energy balance and disturbance of it, if sustained can bring about life-threatening consequences, such as obesity, impotence, diabetes and cardiovascular complications (Woods *et al.,* 2004). Since the discovery of the neuropeptide, in particular ghrelin, human knowledge in relation to the regulation of body weight and energy balance has increased significantly.

Ghrelin is a 28 amino-acid and appetizing peptide discovered by Kojima in 1999 (Kojima *et al.,* 1999). The main source of this peptide is the stomach but in addition to the stomach, pancreas, kidneys, intestine, pituitary and placenta are also able to secrete this hormone (Kojima *et al.,* 2001; Hosoda *et al.,* 2003). Ghrelin in researches is known as an effective factor in brain - gut regulation of growth hormone and energy balance (St-Pierre *et al.,* 2003; Lagaud *et al.,* 2007).

Research has shown that this peptide is involved in weight gain and fat accumulation (Tschöp *et al.,* 2000), stimulation of appetite and food intake (Kojima *et al.,* 1999), increased adipogenesis (Choi *et al.,* 2003; Unniappan *et al.,* 2008; Wren *et al.,* 2001), stimulation of gastrointestinal motility (Asakawa *et al.,* 2001) as well as memory and anxiety (Carlini *et al.,* 2004).

Studies showed that serum levels of ghrelin change in some feeding and energy balance conditions. In fact, serum levels of ghrelin decrease under positive energy balance and increase under negative energy balance (Nakazato *et al.,* 2001; Ariyasu *et al.,* 2001). For example, ghrelin levels increase in patients with anorexia nervosa and extreme thinness (Nagaya *et al.,* 2001) and decrease in obesity (Cummings et al., 2002; Otto *et al.,* 2001; Tschop *et al.,* 2001). Reductive regulation of plasma ghrelin concentrations is the possible consequence of positive energy balance in obese subjects (Tschop *et al.,* 2001).

Despite the conducted researches there are still many questions about ghrelin especially following sports activities. Given that exercise represents the most variable component of energy expenditure in humans one reason to study the effects of exercise on ghrelin may be due to the effect of exercise on energy balance which is one of the functions of ghrelin, as stimulation of appetite by ghrelin is affected by exercise and energy balance changes correspondingly (Kraemer *et al.,* 2007).

Researchers have been conducted on this hormone and physical activity and conflicting findings on ghrelin response to exercise have been obtained. Most studies on human subjects have shown that in the course of endurance training, especially when accompanied by losing weight, reduced BMI and reduced body fat, levels of total ghrelin increase (Jurimae *et al.,* 2007; Foster-Schubert *et al.,* 2005; Leidy *et al.,* 2004; Littman *et al.,* 2006). Also, few studies show no change (Morpurgo *et al.,* 2003; Ravussin *et al.,* 2001) or decrease (Ramson *et al.,* 2012; Hyun *et al.,* 2008) in plasma total ghrelin concentration. In most studies, sampling of subjects has been done under overnight fasting and in these studies high and medium intensity has been used. However, studies on the effect of endurance training on plasma total ghrelin concentrations in Wistar rats report no change (Nasiri *et al.,* 2013; Keikhosravi *et al.,* 2011; Wang *et al.,* 2008), increase (Ghanbari-Niaki *et al.,* 2010) and decrease (Ghanbari-Niaki *et al.,* 2011; Ghanbari-Niaki *et al.,* 2009). In all these studies, low to moderate intensity exercise (25-18 meters per minute) have been employed and reviewing the literature the researcher did not find any research looking into the effect of high intensity endurance training on the plasma total ghrelin concentrations in Wistar rats. Another point about these researches is that no mention is made of the nutritional status of the subjects during sampling (Keikhosravi *et al.,* 2011; Ghanbari-Niaki *et al.,* 2009) and in cases where nutritional status is discussed, the subjects have been in a 4-hour short-term fasting state (Nasiri *et al.,* 2013; Ghanbari-Niaki *et al.,* 2011) while the present study, looks into the effect of 12 hours of fasting on plasma total ghrelin levels.

Given the inconsistent results of researches and the key role of this peptide in the homeostasis and weigh regulation it is necessary to study the impact of exercise as an important factor affecting energy balance and weight regulation on serum levels of ghrelin.

Material and methods

Animals

All experiments involving the animals followed the policies of the Iranian Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes, and the protocol was approved by the Ethics Committee of Islamic Azad University, Central Tehran branch, Iran. 12 male Wistar rats (8 weeks old, initial weight 200 to 225 g) were used for this study. Animals were obtained from Pasteur's Institute and 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) with a 12-hour, 12-hour light–dark cycle. Temperature and humidity were maintained at 22°C ±3°C and 40-60%, respectively. Animals were fed a pellet rodent diet ad libitum (10 gr/100 gr bW) and had free access to water. Animals were randomly assigned to control (n = 5) and training (n = 7) groups. The control group remained sedentary, whereas the training group underwent a running exercise program.

Protocol for the assessment of aerobic capacity in rats.

Due to lack of access to direct instruments such as Respiratory Gas Analyzer, indirect yet highly accurate protocol was used as follows:

At first there was a 10-minute warm-up with intensity of 40 to 50 percent VO2max, after warming up, the test started with rats running at the speed of 15 meters per minute for 2 minutes, then treadmill speed was increased at a rate 0.03 m/s once every two minutes (1.8 to 2 m/min) until the animals were no longer able to run and reached exhaustion (Wisloff *et al.*, 2001). Exhaustion is defined as wasting time without attempting to get back on the treadmill for 15 seconds (Lira et al., 2011). The recorded VO2max speed is the speed at which VO2 reaches a plateau. Researches show there is a high correlation between treadmill speed and VO2max in the rats (r=0.98-0.94, p<0.0005). Hence the VO2max of the rats can be obtained according to the running speed.

Exercise training protocol

In the first two weeks, the exercise group exercised for 7-10 days in order to get used to continuous training. The exercise protocol was designed based on Burniston's study and included 8 weeks of exercise; five sessions a week and each session included running for 60 minutes. 5 minutes at the beginning of exercise with the intensity of 50-60 vo2max for warm-up and 5 minutes with the intensity of 50-60 vo2max at the end to cool off while the main part involved 50 minutes, with intensity of 80-85% Vo2max (Burniston, 2008). Treadmill inclined zero degrees during all phases of the workout. Treadmill speed was 20 meters per minute in the first week and was increased to 34 meters per minute in the eighth week. All training sessions were held from 8 to 9 PM under red light (due to their activity cycle being in the dark).

Living conditions of the animals in the control group except when performing exercises were the same at other times, even to ensure more simulation during the exercise period the control group was placed on the treadmill at a speed of 0.15 meters per second twice a week, each time for 15 minutes. The intensity of this activity is approximately 45% of Vo2Max in rats and does not cause any exercise response (Kemi *et al.,* 37).

Blood sampling

Fourty-eight hours after the final training session (after 12 hour fasting), the rats were anesthetized intraperitoneally with a mixture of ketamine (30 to 50 mg/kg bw) and xylazine (3 to 5 mg/kg bw). Blood was collected directly from the heart into test tubes containing EDTA and was separated by centrifugation, and the plasma was frozen and stored at -80ºC until biochemical analyses were performed. Total plasma ghrelin concentration was determined by a rat enzyme immunoassay (EIA; Cusabio, china). The sensitivity of kit was 1.95 pg/mL.

Statistical analysis

The Kolmogorov-Smirnov test was used to determine the normality of the distribution, and variables were found to be normally distributed. In pre-test independent-samples T test showed that weight did not significantly different between groups. Statistical analyses were performed using an independent-samples T test ($P < 0.05$). All of the data are reported as means ± SE. All statistical analyses were performed with SPSS (version 19; IBM Company, USA).

Results

Using independent T test, the subjects' weights were compared before the exercise protocol in the groups and there was no significant difference between the groups (control: 213.80, high intensity endurance: 210 g). ANOVA Test with repeated measurements showed that the average weight in the high-intensity endurance training group significantly decreased compared to control group (control: 318.60, High-intensity Endurance: 293.71g). The results of the Independent T test (Table 1) show that the exercise has a significant effect on plasma ghrelin levels and the exercise group had higher ghrelin levels.

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

Variables	mean±S.E.M.	Mean difference	T value	P Value
control	0.7840±0.02	0.04	2.626	0.025[a]
HIT	0.8286±0.03			

HIT: High intensity training
[a] HIT vs Control

Discussion

This study shows that 8 weeks of intense endurance training can significantly increase ghrelin and can lead to a significant reduction in body weight in the rats. It appears that ghrelin is sensitive to changes in body weight and increase in Ghrelin is a compensatory behavior in response to weight loss. In other words, an increase in ghrelin may act as a compensatory mechanism to restore the body to an adjusted state (De Souza *et al.,* 2004). Most studies generally maintain that prolonged exercise increases plasma ghrelin level if weight loss has occurred (Kraemer *et al.,* 2007).

For example, the study of Leady *et al.* (2004) shows that in subjects with normal having lost more than 3 kg of weight through a combination of exercise and diet intervention, a significant increase is observed in plasma total ghrelin levels and in subjects with no weight loss, ghrelin levels remain unchanged (Leidy *et al.,* 2004). Thus, the significant increase of ghrelin in this study can be associated with the significant weight loss in subjects following exercise.

Studies have been carried out on the effects of endurance exercise on plasma ghrelin in rats (Ghanbari-Niaki *et al.,* 2010) and the results of this study are consistent with one and contrasts with other studies (Nasiri *et al.,* 2013; Keikhosravi *et al.,* 2011; Wang *et al.,* 2008; Ghanbari-Niaki *et al.,* 2011; Ghanbari-Niaki *et al.,* 2009). In all of these studies, low to moderate intensity exercise (between 18-25 min) have been employed whereas this study looks into the effect of high-intensity endurance training on the concentration of the plasma total ghrelin in Wistar rats. Another point about this study is that in some cases the nutritional status of the subjects at sampling stage is not mentioned (Keikhosravi *et al.,* 2011; Ghanbari-Niaki *et al.,* 2009) and in cases where nutritional status is mentioned, the participants have in a short-term 4-hour in fasting state (Nasiri *et al.,* 2013; Ghanbari-Niaki *et al.,* 2011) while in the present study, the effect of 12 hours of fasting on plasma total ghrelin levels has been investigated.

Given the inconsistent findings in this area the differences can be attributed to such factors as differences in fasting state of the subjects during the study, weight and BMI of the subjects, the intensity and volume of training, the impact of other hormones such as insulin, cortisol, estrogen and ... even the sampling time after the exercise.

It must be noted that studies suggest that factors such as fasting, malnutrition, low blood sugar, chronic underweight, weight loss following diet and exercise, increase total ghrelin (Kojima *et al.,* 1999; Foster-Schubert *et al.,* 2005; Broglio *et al.,* 2001; Dall *et al.,* 2002; Ghanbari-Niaki, 2006; Ghelardoni *et al.,* 2006).

The result of this study shows the role of ghrelin in the body's energy homeostasis and balance such that exercise reduces the level of energy reserves in the liver and muscle cells. Ghrelin secretion is increased in response to the lack of energy to stimulate food intake behavior and to restore lost sources of energy. This increase in ghrelin could stop the catabolic process following training exercise.

Given the ambiguities and contradictions in research findings regarding the impact of exercise on ghrelin further future studies are required on different aspects of the subject.

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