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Plasma obestatin, estradiol, and liver ATP concentrations in response to endurance exercise training at different durations in male rats

Abbass Ghanbari-Niaki [1*]

[1] Exercise Biochemistry Division, Faculty of Physical Education and Sports Sciences, University of Mazandaran, Baboulsar, IR Iran

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ABSTRACT

Background: Obestatin is secreted from the stomach and has effects on energy balance and food intake.
Objectives: The purpose of the present study was to investigate obestatin, estradiol, and liver ATP responses after 8 weeks of a treadmill exercise running program at different durations.
Materials and Methods: 37 male Wistar rats (8 to 10 weeks old, 145 to 160 g) were assigned to control (n = 9) and training (n = 28) groups. After an accommodation period of 3 weeks, the training group was further divided randomly into 30-minute (T30), 60-minute (T60), and 90-minute (T90) groups. Animals ran on a motor-driven treadmill at 20 m/min (0% grade) for various durations 5 days/week for the subsequent 5 weeks and were sacrificed 72 h after the last exercise session. Livers were excised and immediately washed in ice-cold saline and frozen in liquid nitrogen to determine liver ATP concentrations. Plasma was also collected for obestatin, glucose, and estradiol determinations. One-way analyses of variance were used for data analysis.
Results: Plasma obestatin concentrations were significantly higher after training in the T90 group compared with the T30 group. Higher and significant resting plasma estradiol levels were observed in the T60 and T90 trained rats when compared with the control group. Changes in plasma glucose and liver ATP concentrations were not significant.
Conclusions: It seems that long-duration, high-volume training increases plasma obestatin to a greater degree than short-duration, short-volume training. The results also show that long-term exercise induced an increase in plasma obestatin and was accompanied with a higher level of estradiol.

Implication for health policy/practice/research/medical education:
Due to high prevalent obesity, incorrect weight reduction-Induce nutritional disorders in regular people and athletes, the results of present study could help athletes , behavior therapist , pharmacologist, and someone who desire to focus on the duration of exercise for weight management.

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1. Background

In 2005, Zhang *et al.* initially isolated a ghrelin-associated peptide derived from preproghrelin, which bound selectively to the orphan receptor GPR39. They named the peptide obestatin (1). It has been reported that interaperitoneal and intracerebroventricular injection of obestatin suppresses food intake in a time and dose-dependent manner (1-3). Obestatin has been shown to have multiple functions, including effects on gastrointestinal motility, energy homeostasis, cell proliferation, hormone secretion, thirst, sleep, memory, anxiety, water

* *Corresponding author at:* Abbass Ghanbari-Niaki, Exercise Biochemistry Division, Faculty of Physical Education and Sports Sciences, University of Mazandaran, Baboulsar, IR Iran. Tel: +98-1212263326, Fax: +98-1125342202
Email: ghanbara@umz.ac.ir

intake, body weight, energy expenditure, cardioprotection, and liver metabolism (4-12). Obestatin has also been found to induce early response gene expression in the stomach, intestine, and white adipose tissue, and as a metabolic hormone capable of binding to GPR39, it is able to regulate the functions of diverse gastrointestinal and adipose tissues (13) Plasma obestatin and ghrelin, both products of preproghrelin, are altered by fasting and refeeding (14), a high-carbohydrate meal (15), weight reduction (16), and obesity (17, 18). Electrical stimulation of the stomach or intestine (for 2 h) has been shown to reduce ghrelin concentration but not obestatin concentration in the rat fundus (19). It has been also reported that plasma ghrelin, not obestatin, is regulated by several hormones such as insulin (20, 21) and estrogen (estradiol; 22-24). Estrogen (estradiol) is classically viewed as a hormone that binds to intracellular receptors, which then acts as a transcription factor to modulate gene expression, and estradiol production is most commonly thought of as an endocrine product of the ovary; however, there are many tissues that have the capacity to synthesize estrogens from androgen and to use estrogen in a paracrine or intracrine fashion. In addition, other organs, such as the adipose tissue, can contribute significantly to the circulating pool of estrogens (25-28). Estrogen plays a variety of biological roles such as regulating food intake, body weight, adiposity, and plasma-ghrelin concentration in both human and rodent subjects (22-24). The effects of different forms of physical exercise on plasma obestatin in humans (29, 30) and plasma as well as tissue obestatin in rats (31-33) have been studied by several investigators, with some conflicting findings. Some investigations have reported no change in plasma obestatin (29, 30), and one study reported a reduction in plasma obestatin (16) after chronic exercise. Moreover, exercise-training studies have revealed significant reductions in obestatin concentrations in the fundus, small intestine, and hypothalamic tissues (31, 32). With respect to estrogen or estradiol and exercise training, there is some information on the effects of estrogen on skeletal muscle neutrophil infiltration, calpain activity, ATP content, CPK activity, lipoprotein lipase activity, and tissue glycogen metabolism (34-41), but data regarding exercise training on plasma estrogen are sparse. Although there is no clear-cut and direct information on the relationships between exercise, obestatin, and estradiol, but on the basis of above mentioned results, which have been elicited from exercise training effects on food and energy intake, appetite, weight reduction, plasma and tissue obestatin concentrations might be provide some indirect clues to the relationship between these variables (obestatin, exercise and estradiol).

2. Objectives

However, to the best of my knowledge, data regarding plasma obestatin and exercise training of different durations and corresponding exercise volumes are lacking and possibly could account for differences found in previous studies. Thus, this investigation was conducted to investigate the effect of an 8-week treadmill exercise-training regimen at different training durations (30, 60, and 90 minutes) and corresponding exercise volumes on plasma obestatin and estradiol concentrations. A second aim was to determine whether any changes in plasma obestatin concentrations are accompanied with a significant change in plasma glucose or liver ATP concentrations after completing different treadmill running durations and total exercise volumes Based on a previous study from our lab, i hypothesized that there would be a reduction in obestatin concentrations in the T60 and T90 groups, but no change would exist in the T30 group.

3. Material and Methods

3.1. 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 the School of Medicine Sciences at Tarbiat Modares University (TMU), Tehran, Iran. Thirty-seven male Wistar rats (8 to 10 weeks old, initial weight 145 to 160 g) were used for this study. Animals were obtained from Pasteur's Institute (Amol, Mazandaran) and maintained in the Animal House Center, Department of Biological Sciences of the University of Mazandaran. The animals were housed 5 per cage (46 L in volume, 24.5 × 15 × 8 in) with a 12-hour, 12-hour light–dark cycle. Temperature and humidity were maintained at 22°C ± 1.4°C and 55.6% ± 4.0%, respectively. Animals were fed a pellet rodent diet ad libitum and had free access to water. Strewment was changed every 4 days, and the same person handled the rats throughout the study. Animals were randomly assigned to control (n = 9) and training (n = 28) groups. The control group remained sedentary, whereas the training group underwent a running exercise program.

3.2. Exercise training protocol

Treadmill training began by familiarizing the rats with the apparatus for 4 days by placing them on the motorized-driven treadmill (Iranian Model, 10 lanes, designed by Dr. Abbass Ghanbari-Niaki, Department of Physical Education & Sports Sciences, University of Mazandaran, Babolsar, Mazandarn, Iran). The training group exercised 5 days/week for 8 weeks. In the first week, the rats exercised on the treadmill at a speed of 15 m/min. The angle of inclination was 0%, and the running time was gradually increased to 30 min/day. In the second week the speed was increased to 20 m/min with a 0% gradient, and the duration was increased to 60 min/day. In the third week, the speed remained constant at 20 m/min, the angle of inclination was 0%, and the exercise duration increased to 90 min/day. In the next 5 weeks the speed and dura-

tion remained constant. At the end of the third week, the rats in the training group were randomly divided into 30 min (n = 9, T30), 60 min (n = 10, T60), and 90 min (n = 90, T90) groups (37). This exercise condition corresponded to a moderate intensity of approximately 50 to 55% of maximal oxygen consumption (42). The total distance run by the animals each week for the T30, T60, and T90 groups was 3 km, 6 km, and 9 km, respectively.

3.3. Liver and blood sampling

Seventy-two hours after the final training session, the rats were anesthetized intraperitoneally with a mixture of ketamine (30 to 50 mg/kg bw, ip) and xylazine (3 to 5 mg/kg bw, ip), and part of the liver was excised, divided into two pieces, washed in ice-cold saline, and immediately frozen in liquid nitrogen to determine the ATP concentrations. All frozen liver pieces were stored at -80°C until the analyses were performed. All liver samples (100 mg) were weighed immediately, then grounded into a fine powder within a porcelain mortar previously frozen in liquid nitrogen, and kept cold with the repeated addition of liquid nitrogen. All samples were then extracted (deproteinized) with 1 w/3.5% vol of perchloric acid (0.58 N). The frozen liver powder and frozen acid were rapidly ground together, and the frozen mixture was transferred to a glass homogenizer while still dry and frozen. The frozen mixture was slowly allowed to thaw (30 min) while resting in crushed ice and then homogenized for 10 s using a Potter-Elvejheim homogenizer set at 800 rpm, cooled in ice, and centrifuged for 10 min at 4,000 × g (4°C). The supernatant was obtained and neutralized by the addition of a solution of 5 M K2CO3 (1 vol/0.85 vol). Methyl orange was used as an indicator of pH (1 vol/ 0.0025 vol). The neutralized solution was allowed to stand for 10 min in crushed ice and centrifuged for 10 min at 4,000 × g (4°C). The KClO 4 precipitate was discarded, and the supernatant was used for the ATP measurements. 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 -80°C until biochemical analyses were performed.

3.4. Plasma glucose, total obestatin, estradiol, and liver ATP concentrations

Plasma glucose was determined by an enzymatic, colorimetric method (glucose oxidase–amino antipyrine; Pars Azmoun, Tehran, Iran), and the intra-assay coefficient of variation and sensitivity of the method were 1.3% and 1 mg/ dL, respectively. Total plasma obestatin concentration was determined by a rat enzyme immunoassay (EIA; Bachem, Peninsula Laboratories Inc., CA, USA). For obestatin, the intra-assay coefficient of variation and sensitivity were 5.4% and 0.02 ng/mL, respectively. Plasma estradiol concentration was determined by an ELISA (Diagnostics Biochem Canada Inc., Ontario, Canada), and the intra-assay coefficient of variation and sensitivity were 5.8 % and 0.10 pg/mL, respectively. Liver ATP concentration was determined using a Biaffin (Kassel, Germany) ATP-sensitive bioluminescence kit, and the amount of ATP in the samples was calculated according to the manufacturers' protocol.

3.5. Statistics

The Kolmogorov-Smirnov test was used to determine the normality of the distribution, and variables were found to be normally distributed. Statistical analyses were performed using a one-way analysis of variance (ANOVA). A Sheffe post-hoc test was used in the event of a significant ($P < 0.05$) F value. All of the data are reported as means ± SE. All statistical analyses were performed with SPSS (version 13; SPSS, Chicago, IL).

4. Results

The mean body weights were significantly lower following training programs ($F = 6.396$, $P < 0.002$), and the T60 and T90 trained groups had lower mean body weights when compared with the control group *(Table 1)*. The results revealed that total obestatin concentrations in the plasma were significantly different between groups and that resting plasma obestatin was significantly higher in the T90 group than in the T30 group, but the difference was not significant between the other groups *(Table*

Table 1. Mean Body weight, plasma glucose, obestatin, estradiol (E2), and liver ATP concentrations in wild male rats separated into a control group and exercise groups that were trained for 30 (T30), 60 (T60), and 90 minutes (T90).

Variables	Control	T30	T60	T90	F value	*P* value
Body weight, g	360 ± 10	339 ± 4.6	320 ± 5.4	328 ± 5.6	6.39	0.002 [a]
Plasma Glucose, mg/dL	209 ± 10.4	186 ± 6.6	197 ± 9.0	207 ± 9.5	1.48	0.24
Obestatin, ng/mL	2.56 ± 0.12	2.07 ± 0.09	2.36 ± 0.11	2.7 ± 0.12	3.89	0.019 [b]
Estradiol, pg/mL	38 ± 6.6	62 ± 5	69.2 ± 5.8	65.7 ± 8	4.84	0.007 [c]
Liver ATP, μmol/g	2.67 ± 0.7	2.63 ± 0.06	2.79 ± 0.17	2.57 ± 0.1	0.63	0.59

Values are mean ± S.E.M. 8-10 rats per group. NS = no significant difference between groups.
[a] T30 and T90 vs C
[b] T90 vs T30
[c] T30 and T60 and T90 vs C

1). Resting plasma estradiol concentrations were significantly higher following the exercise training programs in all durations when compared with the control group, but there were no significant differences among the trained groups *(Table 1)*. Glucose and ATP concentrations in the plasma and liver did not differ significantly among groups *(Table 1)*.

5. Discussion

The major finding of the present study is that the higher training duration (T90) produced a significantly greater obestatin adaptation than the lowest (T30) training duration, but obestatin concentrations in the trained groups were not significantly different than in the control group. Thus, the first hypothesis was not tenable. Moreover, body weight was reduced in both the moderate (T60) and high (T90) training groups, and plasma estradiol was increased in all three training groups compared with the control group's values. These changes were not accompanied by any significant alterations in plasma glucose and liver ATP concentration. The lower obestatin levels may have been due to a lack of change in body weight in the T30 group. The present investigation is the first to determine the effects of exercise training durations on fed plasma obestatin levels. Reinehr *et al*. (16) examined a 1-year outpatient obesity intervention program based on a high-carbohydrate, fat-reduced diet and increased physical activity; in particular, they looked at plasma obestatin and ghrelin concentrations and reported that children with substantial weight loss demonstrated significantly lower obestatin and a tendency to have higher ghrelin concentrations at baseline. Reductions in hypothalamus, fundus, and small-intestine obestatin have been reported by several investigators (30, 32). The mechanism by which endurance exercise programs at high durations increase plasma obestatin is poorly understood. However, it has been suggested that plasma obestatin concentration could be regulated by several factors, including fasting, refeeding (14, 35), and nutrients (15). It has been also reported that plasma ghrelin is regulated by several hormones such as insulin (20, 21), somatostatin (24), estrogen (20, 22, 23), and body-weight reduction or gain (16, 17). With the exception of estradiol, in this study we did not measure the abovementioned hormones, and male rats were employed. Additionally, in this study significant weight reductions were observed in the T60 and T90 groups, but total obestatin in resting plasma was significantly higher only in the T90 group because T90 had lower weight than the other groups. Thus, it seems that weight reduction could be taken into account as an effective factor on total plasma obestatin in trained animals. Zuo *et al*. (43) reported that resting plasma ghrelin but not obestatin levels were lower in overnight fasting obese children than a control group. The camp included 3 h of aerobic exercise every day and resulted in higher plasma ghrelin and obestatin concentrations after weight reduction. In a comparison between spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKR), Li *et al*. (44) reported that the overnight-fasting SHRs had lower weight and higher plasma obestatin concentrations. Collectively, these studies suggest that weight loss is associated with increases in obestatin and support our findings that greater weight loss is associated with higher obestatin levels in trained animals.

Sakata *et al*. (23) found that estrogen treatment significantly stimulated ghrelin mRNA expression and ghrelin production in a dose-dependent manner. They also reported that ghrelin mRNA expression and production did not change in 3 weeks in gonadectomized rats, and plasma estrogen concentrations are very low in this condition. This suggests that ghrelin mRNA transcription is regulated by stomach estrogen but not by gonadal estrogen, which is thought to be a source of circulating estrogen (22). Relatedly, in the present study, higher plasma estradiol concentrations were found in all trained groups when compared with the control animals. The resting plasma estradiol levels in the sedentary (control) group coincided with previously reported values by others. According to Mystkowski *et al*. (45), Wistar rats with leydig cell tumor bearing had lower weights and food intake and 10 times higher plasma estradiol compared to sham and pair-fed animals. They also reported that estradiol administration at different doses (2.5, 7.5, and 15mg) reduced body weight and food intake. According to Ghanbari-Niaki *et al*. (32) ethionine-induced liver ATP deficiency resulted in elevated plasma ghrelin but not obestatin concentrations, and the higher liver ATP levels in trained animals were not significant. Ghanbari-Niaki *et al*. (31) reported that a significant reduction in fundus and total plasma ghrelin concentrations was accompanied with a higher liver ATP in male trained rats. However, a lower liver ATP was found only in the T90 group. Taken together, the finding might suggest that higher plasma obestatin accompanied with body-weight reduction is amplified by exercise-induced, elevated plasma estradiol and the nature of long-duration exercise programs. In summary, this is the first study demonstrating the effect of an exercise training program of different durations and corresponding total exercise amounts on total plasma obestatin, estradiol, and liver ATP concentrations. This study is also the first to demonstrate that treadmill exercise training in long durations induces a more pronounced reduction in body weight and higher total plasma obestatin than shorter duration, lower volume training. The present results also partially confirm other previously reported findings on the effect of weight loss by exercise interventions and other weight-reducing factors (e.g., estradiol and hypertension, bypass surgery) on total plasma obestatin in human and animal subjects. Further study of the effects of estradiol administration and exercise training at longer durations on obestatin gene expression and its concentration in plasma is warranted.

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Conflict of Interest

None declared.

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