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Comparison of single circuit-resistance and aerobic exercise impact on gene expression of obestatin in lymphocytes among trained young females

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ABSTRACT

Ghrelin and obestatin are orexigenic and anorexigenic peptides, respectively, which are derived from preprogherlin and are believed to be important in the regulation of energy homeostasis and body weight control. The aim of this study was investigation of effect of a single session of circuit-resistance exercise and aerobic exercise on gene expression of obestatin in lymphocytes. In this study twenty-four trained female (age 20.91±3.11 yr, and body mass index 21.69±3.71 kg/m^2 (mean±SE)) were randomly divided into two experimental groups and one control group. Experimental group 1 were asked to perform running a distance of 1.5 mile at a fixed speed (70% VO_2max), experimental group 2 were asked to perform nonstop weight circuit exercises (9 exercises at 60%1-RM, 20s time for each, 3 circuits with 3min rest between circuits). Blood samples were collected before and immediately after exercise session. Lymphocyte separated and using lymphocyte density gradient centrifugation method and m-RNA purification performed by semi-quantitative PCR. Data's analyzed by one-way ANOVA test. The data indicated that although gene expression in experimental groups was increased a little, there was no significant increase in gene expression of obestatin in lymphocytes in these groups. In conclusion, It seems that it was due to the short length of training period and its intensity.

Key words: obestatin, lymphocyte, gene expression, aerobic exercise, circuit-resistance training, females.

INTRODUCTION

Nowadays, obesity has been known as public health problem and is a cluster of related risk factors formetabolic syndrome and type2 diabetes [1, 2]. Ghrelin is a newly discovered endogenous hormone and an important factor in obesity [3]. and is a 28-amino-acid peptide which is mainly produced by X/A-like epithelial cells lining the fundus of the stomach and is released into circulation [4, 5] .It is recognized as a novel player in the gut–brain regulation of growth hormone and energy balance [6]. Through blood circulation, ghrelin affects the hunger and satiety center in hypothalamus and stimulate food intake and weight gain. In fact plasma level of ghrelin decreases in positive energy condition, and increases in balanced negative energy condition [7, 8]. In addition to ghrelin, another peptide, obestatin, is encoded by the ghrelin gene. It has been suggested that intraperitoneal injection of obestatin suppressed food intake in a time and dose-dependent manner [9]. It has also been reported that intracerebroventricular treatment with obestatin decreases food intake in rat [10]. Thus some researchers concluded that ghrelin and obestatin have opposite effects on weight adjustment and the reduction of obestatin could be one of the contributing factors to obesity pathophysiology [9, 11]. The effects of obestatin on feeding have been confirmed by independent investigators [11-13]. However, the majority of studies suggest that obestatin does not have anorexigenic effects, regardless of injection site [14, 15]. The initial description of obestatin also reported that it inhibited gastrointestinal motility [9]. however, a number of subsequent studies failed to replicate this effect [16, 17] ; recent studies, however, demonstrated that intravenously administered obestatin could inhibit gastrointestinal motility, acting via

corticotropin-releasing factor (CRF) receptors in the hypothalamic nuclei of the brain [18, 19]. Since ghrelin and obestatin have been linked to energy balance, revealing the effects of exercise on these hormones is warranted. Wang and colleagues (2008) reported that ghrelin, but not obestatin, affected the exercise-training-induced reduction in appetite in obese rats [20]. Studies of exercise-induced effects on obestatin, especially in humans, are limited.

In a study, intraperitoneal injection of obestatin to mice resulted in food intake and weight gain regulation. This is a U-form relation and the disagreement between the findings of the above mentioned studies could possibly be justified by this relation [6]. Manshouri and colleagues (2008) in a study examined plasma obestatin levels in response to short-term anaerobic exercise. There was no significant change in plasma obestatin levels after 24 hour, 48 hour and 7 days after returning to baseline condition [21]. However, few studies have been conducted to investigate the impact of exercise and diet on plasma levels of obestatin. Moreover, to our knowledge, no investigations have determined the effect of aerobic exercise and circuit-resistance training on gene expression of obestatin in lymphocytes. Since obestatin is produced by stomach and considering the difficulties of investigating human body tissues (muscle etc.) and its metabolites, it was decided to investigate the blood cells, particularly lymphocytes which contain the required nucleus and could respond properly and interestingly to exercise. Some studies has been made on different lymphocytes gene expressions and their subset [22]. Therefore, it has been necessary to evaluate the impact of aerobic exercise and circuit-resistance training on gene expression of obestatin in lymphocytes in trained young females.

MATERIALS AND METHODS

Subjects and Research Design

This study was approved by the local ethical committee of Ferdowsi University of Mashhad, Iran. Written consent was obtained from the 24 trained young females (age 20.91 ± 3.11 years, and body mass index 21.69 ± 3.71 kg/m^2) (table 1). Subjects were randomly assigned to three groups; aerobic group (n = 8), circuit-resistance training (n = 8) and control group (n = 8). All subjects were asked to complete a medical questionnaire with a medical examination to ensure that they were not taking any regular medication, were free of cardiac, respiratory, renal, and metabolic diseases, and were not using steroids. Also, all the subjects were completely familiarized with all of the experimental procedures and had their one repetition maximum (1-RM) determined for each of the 9 exercises used in the circuit resistance exercise protocol. Subjects were all in luteal phase of their menstrual cycle.

Exercise Testing Procedures

The aerobic group ran 1.5 miles at a controlled intensity (70%VO_2max) [23, 24]. Before the main trial, participants of group 2 were taken to the weight room three times. The first and second visits of all the participants performed strength test to determine their one repetition maximum (1-RM) for each of the 9-resistance exercises, employed in the study. The 1-RM value was determined by trial and by adding or removing weight after each attempt as per required. The subjects were allowed to take as long time as they felt necessary to recover from each attempt. On the third visit, the subjects completed a practice session to insure that each participant was able to complete the entire exercise session and also to confirm that the weight lifting was producing fatigue at the end of the session. This was confirmed by visual and verbal feedback from the participants. The resistance exercise session was started at 08:30 AM to 11:00 AM to avoid the effects of circadian rhythm. It included 3 circuits of 9 exercises (arm curl, triceps extension, back extension, squat 90^0, leg curl, bench press, overhead press, dead lift, seated row) with 8–12 repetitions at 60% 1-RM. All the exercises were conducted after an overnight fast state and also with the use of free weights [25]. The subjects were instructed to follow a normal lifestyle maintaining daily habits, to avoid any medications, and to refrain from exercise 3 days before the experiment session.

Blood Collection and Lymphocyte Preparation

Blood samples were obtained from an antecubital vein before and immediately after the protocol. A 10 ^{cc} fasting venous blood from brachial vein was obtained. Blood samples were collected in test tubes and anti coagulated with EDTA. Peripheral blood mononuclear cells were isolated by lymphocyte (Cedarlane Laboratories Limited, Burlington, ON, Canada) density gradient centrifugation at 900 g according to the manufacturer's instructions and the pellet containing lymphocytes were used for further analysis.

Study Design

Subjects' weights were measured with a (sensitivity of 0.1 kg) digital scale before the protocol. Heartbeat was constantly monitored by the polar device (F1tm model). Body fat percentage was measured by Lipid Caliper Lafayette using three fold thickness methods [26].

mRNA of Lymphocytes Purification

In order to purify mRNA, the semi-quantitative RT-PCR method was used. In this method, lymphocytes were placed in liquid Nitrogen and were completely powdered by mortar and pestle. The powdered tissue was homogenized in RLT buffer; using rotor-stator homogenizer will produce more RNA. The tissue powder and liquid nitrogen are poured into 2ml RNase free microcentrifuge tubes, and liquid nitrogen was allowed to be evaporated but lymphocyte remained in freeze condition. RLT buffer was added sufficiently. Lysate was directly transferred into QIAshredder column in the tube and centrifuged for 2 minutes at a high speed; it was then moved into PCR and finally, in order to take photos, they were placed on agarose gel. At last, when the results were obtained (using UVP model: Gel Doc-It Ts310 made in USA), and Beta-actin value for each blood sample was measured, the resulted numbers were divided by Beta-actin values and multiplied by 100 [27].

Statistical Analysis

The data were analyzed using t-student dependent test (paired comparison) for pre- and post-values, while between-group comparisons were analyzed using one-way ANOVA. Statistical significance was accepted at $P < 0.05$. All statistical analysis was performed by SPSS (Version 16).

RESULTS AND DISCUSSION

The descriptive results of the subjects are presented described in Table 1. The findings obtained from the present study showed that immediately after the training protocol, although gene expression of obestatin in lymphocytes was a little increased in aerobic and circuit-resistance groups, there was no significant increase in gene expression of obestatin in lymphocytes (respectively $p = 0.473$ and $p = 0.860$, $F= 0.146$) (Table 2 and Figure1).

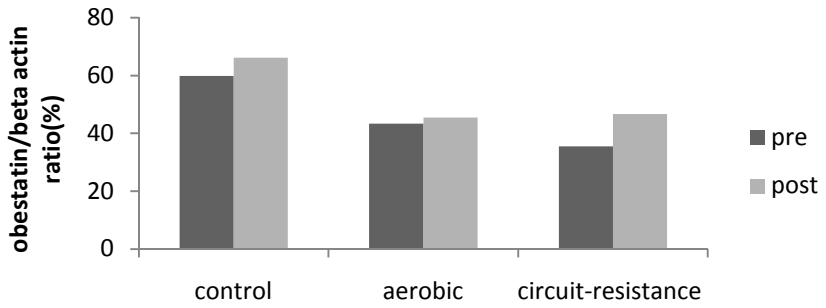
Table 1: Descriptive results

Variables	Control group (n= 8)	Aerobic group(n= 8)	circuit-resistance group(n= 8)
Age(year)	21.75±2.5	19.5±2.5	21.25±4.5
Height(kg)	160.5±2.34	158.12±4.09	166.5±5.65
Weight(m)	61±11.14	61.5±10.52	48.32±5.19
BMI(kg/m^2)	23.66±5.1	22.11±2.86	19.32±1.84
%BF	22.15±6.43	19.67±5.51	12.74±2.18

Table 2: Changes in gene expression of obestatin in lymphocytes in the three groups.

	Pre-test	Post-test	p-value
Control group	59.85±16.5	66.18±14.62	0.603
Aerobic group	43.40±14.25	45.49±18.49	0.473
circuit-resistance group	35.57±22.56	46.72±17.31	0.860

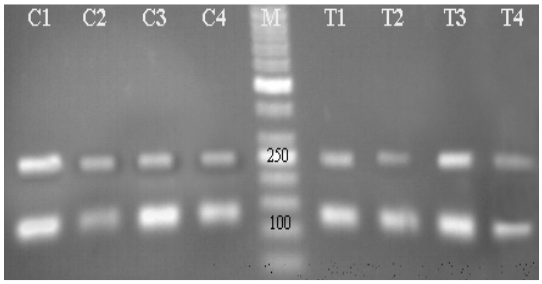
Figure 1: Comparison of gene expression of obestatin in lymphocytes in the three groups.



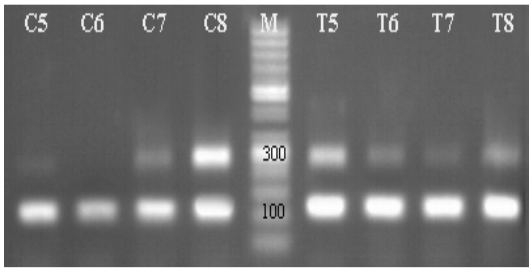
According to our knowledge, no previous studies have been made on the impact of a single session of aerobic and single circuit-resistance exercise on gene expression of obestatin in lymphocytes human blood samples. The present study findings indicate that a single bout of circuit-resistance and aerobic exercise didn't have significant increase on gene expression of obestatin in lymphocyte of experimental groups. Manshori et al. (2008) in a study examined plasma obestatin levels in response to short-term aerobic exercise. In this study it was showed that the plasma obestatin levels of participants didn't change and they concluded that obestatin level is not affected by short-term physical activity [21]. Wang et al. (2008) repotted that a short (acute) and long-term treadmill exercise for 8 weeks at 22 m/min did not change plasma obestatin levels in rat subjects. But hypothalamus obestatin level was decreased after the activity session. Remarkably, in that study, appetite declined after the activity and 12-24 hour after the

session the appetite was normal. The researchers concluded that it was not obvious that whether or not obestatin could have any impact on food intake or energy balance in hypothalamus [20].

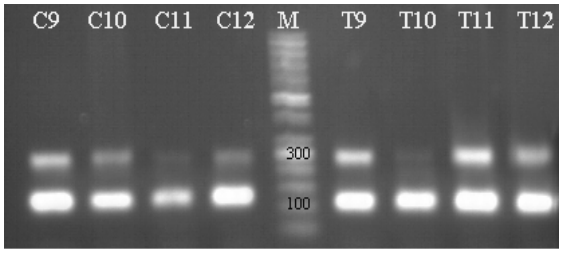
Figure2: Semi-quantitative RT-PCT of lymphocyte obestatin mRNA expression in three groups, prior and immediately after research program (mean±SD)



Control Group



Aerobic Group



Circuit-resistance training Group

Ghanbari-Niaki et al. (2008a) examined the effect of running for 6 weeks on obestatin concentrations in rat fundus and small intestine. The findings suggested that obestatin concentration reduced in fundus and small intestine [28]. Accordingly they proposed that, through negative feedback, increased GH level will lead to control obestatin level in fundus and small intestine. Ghanbari-Niaki et al. (2008b) reported that plasma obestatin levels did not change in response to a single circuit of resistance exercise at different intensities [29]. In another study examined a single circuit-resistance exercise effect, with different intensities (40%, 60% and 80% of 1-RM) on plasma obestatin levels in female college students which was not significan [30]. Since exercise training could lead to negative energy balance, obestatin level could possibly be influenced by exercise. In some researches it has been shown that a session of exercise training could not change obestatin level [20, 29]. Yet, in some others, obestatin level changed by increasing the exercise time [20, 28, 31].

It seems the sort and duration of exercise programs contribute to the kind of reaction and adaptation. Also, different tissues of body such as hypothalamus, stomach and small intestine fundus may show different reactions to exercise. It should be noted that the amounts of plasma GH was also measured in this study. After a session of exercise the amount of plasma GH in both aerobic and resistance groups increased significantly which is in agreement with the findings of other studies [20, 21, 29].

Therefore it seems, through negative feedback, increased amounts of plasma GH could lead to obestatin gene expression control [28]. The mechanisms of how exercise affects lymphocyte gene expression are generally unknown. Also, due to the fact that this peptide has been discovered recently, limited research has been reported on the impact of physical activity on plasma obestatin level and other various tissues. Apparently, to accurately specify

the role of obestatin as a hormone involving in energy balance and body weight regulation, much more research is needed.

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