Comparison of the Agouti Dependent Protein and Neuropeptide Y Response to the High Intensity Interval Training in Obese and Underweight Men

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Abstract

Objective: Appetite regulation has a great impact on energy homeostasis. This study aimed to compare the response agouti-related protein (AGRP) and neuropeptide Y (NPY) to a single high intensity interval training (HIIT) in obese and underweight men.

Materials and Methods: This semi-experimental research conducted on 40 obese and underweight men who were divided into four groups including experimental groups (10 obese, and 10 underweight men) and control groups (10 obese, and 10 underweight controls). Both groups were evaluated by biochemical measurements in two stages of pre-test and post-test. HIIT was carried out to the experimental groups during one session. Data were analyzed using one-way ANOVA and T-test using SPSS 21 software.

Results: NPY levels showed a significant difference in pre and post-test in both experimental groups, NPY increased 6.14% in obese men and 25.50% in underweight men, which was significant in both obese (*P*-value= 0.002) and underweight (*P*-value= 0.007) groups. In addition, AGRP levels increased 1.91% in obese men and 6.65% in underweight men, although this was not significant (*P*-value= 0.098). However, no significant differences were observed among groups in the levels of NPY and AGRP (*P*-value= 0.114).

Conclusion: The results of the present study showed that HIIT can increase the amount of AGRP and NPY in obese and underweight men. Therefore, appetite neuropeptides increase after a training session, which should be considered in the balance of energy and food intake after exercise.

Keywords: Agouti dependent protein, Neuropeptide Y response, High intensity interval training, Obesity

Introduction

The weight balance is an important determinate factor of life expectancy (1). Obesity is caused by increased energy intake, which this disorganizing in energy balance leads people to weight gain and slimming is due to the decrease in energy intake, and energy consumption, thus it is logical that adjusting the energy intake and the

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energy consumption can reduce or increase body weight (2). Therefore, the balance of energy intake and consumed by people should be carefully controlled**.** The energy homeostasis system is regulated by central and environmental factors (3). The hypothalamus is important in controlling nutrition and energy balance by secreting various neuropeptides and chemical transmitters (4). Agouti-related protein (AGRP) and neuropeptide Y (NPY) are two well-known environmental and central factors in regulating energy intake and body weight. AGRP as one of the most potent appetitive peptides enhances centrally controlled food intake behavior, when centrally controlled. On the other hand, deficiency in the amount or expression of AGRP leads to increased metabolism (5). NPY, a peptide hormone, is also produced in different parts of the body, especially the hypothalamus (Appetite adjustment area) (6). It is the most abundant hypothalamic peptide and its most important effect is the stimulation of dietary behavior (7). In addition, studies have shown that inhibition of NPY, appetite suppression and underweight (8). In some studies the role of exercise examined in preventing, controlling, and reducing body fat. Exercise can help regulate body weight by affecting appetite hormones and altering energy intake (9). In the meantime, high intensity interval training (HIIT) is one of the most effective physical activity (10,11). HIIT increases the concentration of energy substrates and the activity of anaerobic metabolism-related enzymes. To compensate the positive energy balance, exercise and physical activity are capable of stimulating appetite hormones such as NPY and AGRP, which are secreted from the environmental and central tissues (10). Recent studies have shown that exercise can produce negative energy balance by increasing energy consumption and adjusting some appetite-related hormones such as AGRP and NPY (12,13). The aim of the present study was to compare the response of some serum neuropeptides to a HIIT in obese and underweight men.

Materials and Methods

This study was quasi-experimental. The statistical sample consisted of obese and underweight men in Isfahan city between individuals who were between the ages of 18 and 35 years, body mass index of 30 to 35 kg/m for obese individuals and those below 18.5 kg/m were chosen. G * power software was used to evaluate the sample size and research power. The underweight sample inclusion criteria were no chronic diseases such as heart and lung disease, cigarette smoking, alcohol and a special diet to lose weight. At the coordination meeting and after the description of the goals and methods of measurement by the researcher, they were examined by completing the informed consent. The eligible volunteers, were randomly selected based on height, age, and body composition and were divided into four groups of two experimental groups (obese, and underweight) and control group were divided into two groups (obese and underweight).

Practice program

HIIT was performed in one session on the experimental group according to the running-based anaerobic sprint test (RAST). Exercise program consisted of 10 to 15 minutes warm-up, slow running and stretching exercises. They then traveled over 35 m 6 times at their maximum speed and 10 seconds resting between every repetition in two sets. At the end of each session, running soft, stretching and softening movements for ten minutes performed the cooling operation. Provided no intervention were performed on obese and underweight male control group.

The method of measuring blood variables

In the first stage, blood sampling was performed between 9-11 am and after 12 hours fasting. Then subjects in the experimental group performed intense intermittent activity

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and immediately after the activity such as the first step, blood sampling from the experimental and control groups was performed. Blood sampling was performed using needles and vacant tube. The blood sample was centrifuged at 3000 rpm for 15 min and the serums were separated and immediately transferred to the refrigerator at -80° C until measurement. The samples were removed from the freezer for measurement and allowed to stand at room temperature for 30 minutes. They were then turned upside down three times to eliminate the gradient resulting from freezing and thawing, and the concentration of the samples to be uniformed. NPY response was measured using (Hangzhou Eastbiopharm) kit 2.36 ng/l with sensitivity that was made in us and by ELISA method. Also, the response of AGRP was assayed using (Hangzhou Eastbiopharm) kit with sensitivity of 1.01 pg/ml made by USA and by ELISA method.

Measuring of body composition variables

Weight of people using Seca digital scale, made in Germany measured. Height of people using the Seca wall height gauge, making the German state stand by the wall without shoes were measured while the shoulders were in normal condition and body weight was evenly divided on both feet and eyes were parallel to the horizon. To measure the body mass index of the subjects, their height and weight were measured first, and then the body mass index of the subjects was obtained by dividing the chi-square weight. In this formula, weights are in kg and height in meters and body mass index in kg. Percentages of body fat were calculated using body mass index (BMI) information and age of the subjects using the following formula (1):

Body fat percentage= (1.20×BMI) + (0.23 ×Age) - (10.8×sex) -5.4

Note: in case of the BMI estimation formula, sex is 1 for males and 0 for females

Statistical Methods

The leven's test was used to check the natural distribution of data from the Kolmogorov-Smirnov test and to test the homogeneity of variance. To determine the effect of a HIIT training session on serum neuropeptide response in obese and underweight men, the dependent T-test to examine within-group differences and to check the differences between groups. One-way analysis of variance (ANOVA) was used because of the significance of the above test. Data were analyzed using SPSS software version 21 and the results were considered significant level (*P*-value< 0.05).

Ethical considerations

The study received ethical approval from University of Qom and oral consent was also obtained from all individuals before enrolling them to study. (Ethics Code: IR.QOM.REC.1399.004)

Results

The characteristics of the subjects in the research groups are shown in table 1. There was no significant difference between the groups of study in terms of age, weight, fat percentage and BMI (*P*-value< 0.05) based on the results of table 1. Also the Kolmogorov-Smirnov test has showed the natural explanation of the data between groups and the Levin test the variance homogeneity of the four studied groups.

In addition, none of the general indicators showed a significant difference between the groups.

According to the results of the study, one-way analysis of variance showed that there was no significant difference between the groups in AGRR and NPY (*P*-value> 0.05). While intra-group evaluation of dependent T showed a significant difference in NPY index from pre-test to post-test in both experimental groups, NPY increased 6.14% in obese men and 25.50% in underweight men. which was significant in both obese (*P*-value= 0.002) and

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underweight (*P*-value= 0.007) groups (Table 2).

Also, based on intra-group evaluation of the data, percent increase of AGRP from pre-test to post-test was observed in obese and underweight men. AGRP increased 1.91% in obese men and 6.65% in underweight men, although this increase was not significant (*P*-value> 0.05).

Discussion

The aim of this study was to investigate the comparison of the response AGRP and NPY to HIIT in obese and underweight men. Previous studies have shown that inactivity may impair the mechanisms responsible for appetite regulation. On the other hand, it has been shown that physical activity induces appetite regulation (14,15). The purpose of the present study was to compare a session of HIIT on the response of serum neuropeptides in obese and underweight men. The results of this study showed that one session of intense exercise training significantly increased NPY in both obese and underweight groups. The results of this study were in line with the findings of Chen et al. and Suri et al., (16,17). While, with the results of keshtar et al. examining the effect of moderate exercise on obese rats and reporting no change in NPY (18), was inconsistent. The reason of differences in findings may be related to the training population in varied size, intensity, repetition, rest, and type of intense exercise program. Several mechanisms lead to increased NPY after an intense intermittent exercise. NPY neurons are sensitive to blood glucose changes and are activated by reduction of blood sugar. Exercise improves energy balance, reduces muscle glycogen stores, and increases glucose uptake by activated muscles, which increase NPY factors. Increase glycolysis in muscles and increase in intracellular glucose metabolism products, such as hexamines, may increase. NPY also begins to increase by suppressing the cost of extra energy after training to stop the catabolism process following exercise, making the condition anabolic (19). This will start replenishing lost energy stores during exercise, they start rebuilding and this helps rebuild carbohydrate reserve (20).

Table 1. Subject's descriptive characteristic in four groups

Variable	Group	Underweight Mean (±SD)	*P*-value	Obese Mean (±SD)	*P*-value
Age (years)	Experiential	26.50 (±5.95)	0.12	24.50 (±5.64)	0.082
	Control	28.70 (±5.27)		27.20 (±5.00)	
Weight (kg)	Experiential	52.51 (±2.91)	0.144	105.17 (±25.26)	0.074
	Control	54.43 (±7.42)		109.36 (±24.10)	
Height (cm)	Experiential	172.60 (±6.80)	0.091	174.70 (±7.51)	0.068
	Control	176.10 (±11.58)		180.40 (±11.01)	
BMI (kg/m^2)	Experiential	17.60 (±0.70)	0.171	34.10 (±6.21)	0.138
	Control	17.48 (±1.07)		33.21 (±3.46)	
Fat percentage	Experiential	11.01 (±1.62)	0.152	30.35 (±6.94)	0.145
	Control	11.37 (±1.89)		29.90 (±4.54)	

Table2. Comparison of participants' blood indices in the four study groups (data are shown as mean and standard deviation

Variable	Group		Pre-test Mean (±SD)	Post-test Mean (±SD)	*P within group	**P between groups
AGRP (pg/ml)	Underweight	Experiential	84.43 (±17.99)	90.05 (±10.99)	0.120	0.50
		Control	90.94 (±10.07)	86.60 (±8.37)	0.229	
	Obese	Experiential	73.81 (±16.12)	75.22 (±17.87)	0.676	
		Control	78.41 (±17.18)	76.95 (±14.85)	0.628	
NPY (ng/l)	Underweight	Experiential	440.24 (±99.89)	552.52 (±114.38)	0.007	0.121
		Control	489.98 (±78.67)	471.82 (±98.81)	0.392	
	Obese	Experiential	565.15 (±218.36)	599.90 (±215.04)	0.002	
		Control	553.31 (±99.88)	467.70 (±105.41)	0.239	

**P*-value for dependent T-test result (significance level *P*-value< 0.05), ** *P*-value for ANOVA test results (significance level *P*-value <0.05).

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The results of this study also showed no significant difference in the amount of NPY between obese and underweight men. The results of the present study were aligned with the findings of Rijak et al., that no significant change was observed in the effect of 12 hour running on the NPY levels in rats that had access freely to energy stores (21). But they contradict the results of studies by Ramson et al., which showed that a combination of high intensity exercise protocol at 10 h in the first week, 15 h in the second week, and 20 h in the third week caused a significant change in NPY concentration in the male thigh (22). The observed contradiction among the findings of the present study and the studies involved can be attributed to factors such as different exercise program, number of training sessions and sample used. It sounds that when using long-term training programs, a decrease in NPY levels is observed. Due to the intensity and duration of the exercise program can be effective in the NPY response process. Among the mechanisms by which intense intermittent exercise affects NPY concentrations and possibly appetite may be influenced by this type of exercise, glucose change, nutritional deprivation, and negative energy balance may be significant. Changes in energy balance and glycogen depletion following intense exercise activities result in positive energy balance and alteration of NPY secretion in order to regenerate glycogen and create a positive energy balance. Thus, according to existing mechanisms, increased neuropeptide T stimulates the restoration of lost energy sources due to the impact of a single exercise session and restores energy balance (18). However, the difference between the effect of one session of intense exercise on the rate of NPY secretion in obese and underweight men may be due to the insufficient number of sessions and the duration of said physical activity to balance energy and subsequently stimulate the secretion of this peptide. Recovery of carbohydrate reserves and recovery of glycogen was considered.

Another result of this study was the lack of significant change of AGRP in obese and underweight men from pretest to posttest .The findings of this study are in agreement with the results of Salehi et al. and Ghiasi et al. (23,24). While, it is inconsistent with the results of Rahmani et al. (25). The most important reason for contradiction in the findings of the present study with the above studies is due to differences in the response of individuals to exercise, inadequate exercise duration, frequency and intensity of exercises, and inactivity and inactivity of samples. Exercise interventions have important effects on the intra-cell energy equation, AGRP secretion, and ultimately weight management and control (26). Evidence also suggests that the AGRP has an inhibitory effect on the hypothalamic-pituitary-thyroid axis. In general, the induction of the AGRP suppresses the TSH-secreting hormone TRH and reduces circulating thyroid hormones (27). To review the results of the studies it seems that one of the most important factors in increasing the AGRP in starvation and food restriction is the decrease of cellular energy charge. Restrictions on energy intake can negatively balance energy within the muscle cell and increase the cell's demand for energy. These factors activate several mechanisms in the body, which eventually trigger the expression of high levels of one of the strongest hormones, the neuropeptides involved in the regulation of appetite, called the agouti-dependent protein; As a result, appetite stimulation with AGRP is altered by exercise training (24). However, the lack of significant change in levels of this peptide in the present study can be attributed to the sufficient intensity and duration of exercise to strike a negative balance and subsequently stimulate the release of appetitive peptides to balance the two sides of the energy equation.

Also, the results of this study showed no significant difference in AGRP between obese and underweight men. The present, results are in line with the findings of Delphan et al., which examined the effect of two traditional

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dietary protocols with saunas and reported no significant change in AGRP in professional wrestlers (28). Therefore, exercise, increases appetite and leads to an increase in the negative energy balance and amounts of hypothalamic and extrapulmonary appetite peptides, including the AGRP (29). At the same time, the negative energy balance for stimulating appetite and enhancing food absorption as well as improving the response of the agitator-dependent protein secretion depend on the initial amount of fuel and nutritional status as well as on the intensity, duration and mode of training (27). Therefore, considering the severity, duration, and mode of vigorous exercise in the present study, it can be justified that the lack of difference arising from effect of a vigorous periodic exercise session on the amount of AGRP in obese and underweight men. Also, the low number of subjects in varied groups of the present study had an impact on the results of statistical tests and the difference with the results of other studies. The present study was a quasi-experimental study in a population of obese and underweight men, so controlling all influencing factors such as genetic factors, or others that were independent of obesity and underweight was beyond the control of the researchers, and these factors can influence the results.

Conclusions

Overall, the results of the present study showed that a session of HIIT can increase the amount of AGRR and NPY protein in obese and underweight men. However, according to the results of this study, this increase was more in the underweight group than in the obese group. Therefore, increased appetite following HIIT results from increased levels of AGRR and NPY proteins. According to the above findings that underweight individuals who complain of anorexia ،since the AGRR and NPY may increase appetite, They can use intermittent exercises especially HIIT to increase appetite.

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Conflict of Interest

No conflict of interest was declared.

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