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One-month of high-intensity exercise did not change the food intake and the hypothalamic arcuate nucleus proopiomelanocortin and neuropeptide Y expression levels in male Wistar rats

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Objective. The hypothalamic arcuate nucleus proopiomelanocortin (POMC) and neuropeptide Y (NPY) circuitries are involved in the inhibition and stimulation of the appetite, respectively. The aim of this study was to investigate the effects of one-month lasting high-intensity exercise on the POMC mRNA and NPY mRNA expression in the above-mentioned brain structure and appetite and food intake levels.

Methods. Fourteen male Wistar rats (250±50 g) were used and kept in the well-controlled conditions (22±2 °C, 50±5% humidity, and 12 h dark/light cycle) with food and water ad libitum. The rats were divided into two groups (n=7): 1) control group (C, these rats served as controls) and 2) exercised group (RIE, these rats performed a high-intensity exercise for one month (5 days per week) 40 min daily with speed 35 m/min. The total exercise time was 60 min. The body weight and food intake were recorded continuously during the experiments.

Results. The results showed relative mRNA expression of POMC and NPY estimated in the hypothalamic arcuate nucleus. There were no significant differences in the NPY and POMC mRNAs expression levels and food intake between C and RIE groups.

Conclusions. The present data indicate that one-month regular intensive exercise did not alter the levels of NPY and POMC mRNAs expression (as two important factors in the regulation of appetite) in the hypothalamic arcuate nucleus and food intake suggesting that this type of exercise itself is not an appropriate procedure for the body weight reduction.

Key words: proopiomelanocortin, neuropeptide Y, exercise, food intake, hypothalamic arcuate nucleus

Recently, it has been shown that the hypothalamic arcuate nucleus plays an important role in the regulation of the appetite and body weight. POMC is an anorexigenic and NPY an orexigenic neuropeptide (Klenke et al. 2010). The hypothalamic arcuate nucleus is a key component of the brain's POMC and NPY circuitries and these neuronal sets are involved in the inhibition and stimulation of the appetite, respectively (Klenke et al. 2010).

NPY that belongs to the pancreatic peptides' family, have 36 amino acid residues in its linear structure and its precursor is composed of 97-amino acid peptide that is modified by enzymatic cleavage. NPY is a highly conserved peptide occurring in various species (Cerda-Reverter and Larhammar 2000) and its binding to the NPY receptor results in the stimulation of appetite (Baskin et al. 2000; Han et al. 2011).

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POMC is an important protein that is expressed in the unicellular organisms, invertebrates, and mammals. In the pituitary corticotropic cells, POMC is cleaved to ACTH, β-endorphin, and a polypeptide of unknown function – called β-lipotropin (LPH) (Harbach and Hempelmann 2005). In the pituitary intermediate lobe cells, this neuropeptide is hydrolyzed to corticotropin-like intermediate-lobe peptide (CLIP), γ-LPH, β-endorphin, and melanotropins α- and β-MSH. It seems that in the pituitary intermediate lobe of adult humans, α-MSH and β-MSH are not secreted. Evidence suggests that melanotropins-α, via binding to its receptor known as MCR4, inhibits appetite (Harbach and Hempelmann 2005). The hypothalamic arcuate nucleus has a remarkable role in the appetite regulation (Klenke et al. 2010).

The appetite stimulation and energy expenditure reduction are two main mechanisms involved in the obesity and weight gain (World Health Organization, WHO, 2000). Scientists believe that the lifestyle associated with lower physical activity and higher food intake may play the most important role in the body weight gain leading to obesity (WHO 2000). In addition, obesity, as a worldwide problem underlying many of cardiovascular and metabolic diseases, may also increase the rate of the mortality (Bilski et al. 2013). Actually, sport, diet, and weight loss may positively affect the lifestyle quality (WHO 2000). Over the past decade, exercise and physical activities are considered to be inexpensive ways in the body weight reduction and management of appropriate body composition (Blundell et al. 2015). However, there is a discrepancy in the results of studies about the effect of sport on the appetite regulation and body weight. Some studies have shown that the effect of a physical exercise on the appetite and regulation of body weight depends on the intensity, duration, and type of exercise, because all these factors may increase the energy expenditure, change the hormonal activity in the digestive system, and food absorption (Blundell et al. 2015). In some studies, it has been shown that energy expenditure is increased in response a long-term exercise and via stimulation of food intake it can maintain the body weight and prevent from the weight loss (Bilski et al. 2013; Blundell et al. 2015). However, other studies have suggested that an intensive exercising can suppress the appetite for food and food intake (Thompson et al. 1988; King 1999).

Considering the key role of the hypothalamic arcuate nucleus POMC and NPY in the regulation of the appetite and a consequent body weight, this study was carried out in order to clarify the molecular mechanisms involved in the control of appetite and body weight during exercise. In the present study, we examined the effects of one-month regular intensive exercise on the expression of POMC- and NPY mRNAs, body weight, and appetite, in the male rats.

Materials and methods

Animals. In the present study, fourteen male Wistar rats (Institute of Pasteur, Tehran, Iran) weighing 250±50 g were used and kept in the well-controlled conditions at 22±2 °C, relative humidity of 50±5%, and 12 h dark/12 h light cycle. The animals had food and water *ad libitum*. The composition of food was: protein19.5–20.4%, fat 3.5–4.5%, Crude fiber 4.0–4.5%, ash 10%, tryptophan 0.25%, threonine 0.75% methionine+cysteine 0.63%, lysine 1.15%, moisture 10%, salt 0.5–0.55%, calcium 0.95–1%, phosphorus 0.65–7%).

All procedures for the maintenance and use of the experimental rats were directed based on the guide for the care and use of laboratory animals (NIH Guide for Care and Use of Laboratory Animals, 8th Edition, 2010) and conducted with the approval of the institutional animal care and by the committee at Research and Ethics Committee of Shahid Beheshti University of Medical Sciences.

Experimental design. The rats were randomly divided into two groups (n=7+7): 1) control group (C, these rats served as controls without any treatment) and 2) intensively exercised group (RIE, these rats performed a high-intensity exercise on a treadmill for one month (5 days per week) 40 min daily in the morning with speed 35 m/min (Hahn et al. 2007). The total exercise time was 60 min. The test was made up of three stages respectively as follows: I) In the initial stage, the speed of the treadmill reached from 5 m/min to 35 m/min for 10 min to allow animals to warm up and prepare for exercise; II) In the second step, the speed was 35 m/min for 40 min; and III) In the last stage, in order to the recovery of the rats, the speed of the treadmill reached from 35 m/min to 5 m/min for 10 min. The animals in the control group were placed on the turned off treadmill for 60 min (Hesari et al. 2014).

The body weight was recorded at the end of the experimental period at 10:00 a.m. The food intake was monitored every morning. One day after the final exercise treatment, when the rats were not allowed to have a food intake, all the animals were anesthetized by intraperitoneal injection (i.p.) of a combination of ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (5 mg/kg) (Hesari et al. 2014).

RNA isolation. The hypothalamus with the arcuate nucleus was excised from the brain based on the previous method (Salehi et al. 2013) and placed in the liquid nitrogen and stored at −80 °C for real-time PCR analysis. Isolation of total RNAs from the hypothalamic samples was performed using the TRIzol reagent (Life Technologies, U.S.A.). A NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE) was used for measurement of the RNA concentrations.

Reverse transcription and quantitative real-time polymerase chain reaction analysis (qRT-PCR). The RT-Enzyme (BIONEER, Korea) was used to synthesize cDNA. The reverse transcription reactions were done in a DNA thermal cycler at 42 °C for 60 min and 70 °C for 10 min and the cDNA samples were stored at −20 °C. The qRT-PCR was performed by a Corbett Research RG 3000 thermal cycler (CR CORBETT, Australia). The sequences of primers and thermal schedule used in the present study are presented in Tables 1 and 2. To certain that all the primers were special for the target mRNA transcript, they were examined by NCBI BLAST. The triplicate PCRs were carried out on each cDNA sample synthesized from the arcuate nucleus of each rat.

The comparative threshold cycle (C_T) method was used for gene expression analysis. The mean ± SEM for the relative amount of target genes in two groups of animals using the $2^{-\Delta\Delta CT}$ method was calculated (Livak and Schmittgen 2001). The relative mRNA expression of POMC and NPY with respect to the internal control gene GAPDH was estimated. The fold change in the internal control of the treated samples compared to the untreated sample was $2^{-\Delta\Delta CT}$.

Statistical analysis. The SPSS Software 16 (SPSS Inc., Chicago, IL, U.S.A.) and independent t-test and paired-sample t-test were used for data analysis. Data are presented as means ± SEM. The p-values less than 0.05 were considered statistically significant.

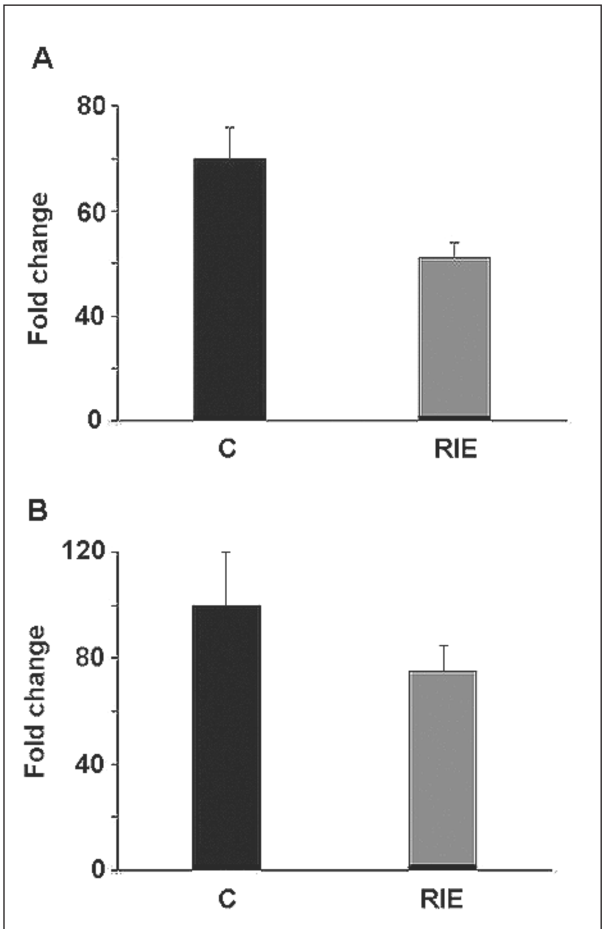

Figure 1. Effects of regular high-intensity exercise on the expression of NPY mRNA (a) and POMC mRNA (b) in the arcuate nucleus. C – Control group (n=7): animals of this group served as controls and had no treatment; RIE – regular high-intensity exercise group (n=7): the rats walked on a treadmill at a target regular high-intensity exercise for one month. The fold change of the internal control in the treated samples compared to the untreated sample was $2^{-\Delta\Delta CT}$.

Results

The results of this study showed that in the hypothalamic tissue, mRNA expression of NPY in the RIE group was lower than in the control group but not significantly (p=0.45) (Figure 1a) and the expression of POMC mRNA was not significantly different (p=0.26) (Figure 1b).

Initial body weight compared to final body weight of control and trained groups rats did not change (p=0.21). As presented in Figure 2a, the mean of food intake in the RIE group was similar to the control group (p=0.07). There was no significant difference between the mean of body weight in the control and RIE groups (p=0.12) (Figure 2b).

Discussion

This study was carried out to evaluate of the effect of one-month high-intensity exercise on appetite and food intake levels. In this regard, the relative expression of POMC and NPY mRNA that are involved in regulation of appetite were determined. Results showed that this type of exercise did not alter the expression of POMC mRNA in the hypothalamic sample containing the arcuate nucleus. Similar studies investigating the effect of exercise on POMC expression have reported that arbitrary exercise for 6 weeks caused a significant reduction in POMC expression (Levin and Dunn-Meynell 2004). In another study,

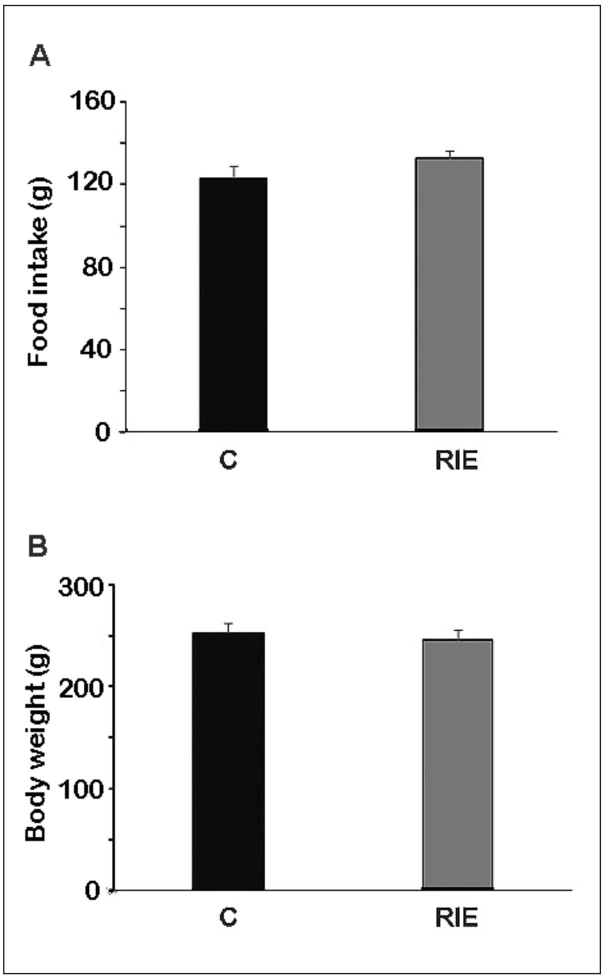

Figure 2. Effects of regular high-intensity exercise on the food intake level (a) and body weight (b). C – Control group (n=7): animals of this group served as controls and had no treatment; RIE – regular high-intensity exercise group (n=7): the rats walked on a treadmill at a target regular high-intensity exercise for one month. The fold change of the internal control in the treated samples compared to the untreated sample was $2^{-\Delta\Delta CT}$.

Table 1
The sequences of primers used in the present study

Gene			Amplicon Length (BP)
GAPDH	Forward	5'CGGCCAAATCCGTTCACACCGA3'	122
	Reverse	5'GGCTCTCTGCTCCTCCCTGTTC3'	
POMC	Forward	5'GAGGAGAAAAGAGGTTAAGGAG'3	167
	Reverse	5'TATGGAGGTCTGAAGCAGGAG'3	
NPY	Forward	5'ACTACATCAATCTCATCACCAG'3	147
	Reverse	5'GTTTCATTTCCCATCACCAC3'	

Table 2
The thermal schedule used for real-time PCR in the present study

Primary denaturation, 1 cycle		**95 °C, 5 min**
45 cycle	Denaturation	95 °C, 30 s
	Annealing	60 °C, 25 s
	Extension	72 °C, 30 s
Final extension, 1 cycle		**72 °C, 5 min**

physical exercise for 7 weeks (the rats change adapted to the treadmill in the first five weeks, then they run on the treadmill with the speed 35 m/min, for 25 min per day) resulted in an increase in the POMC expression (Chen et al. 2007). These results are not in accordance with our findings. The observed differences may be due to the differences in the duration and type of the performed exercises, because researchers believe that duration, type, and intensity are the most important factors of the exercise procedure that may influence POMC and NPY gene expressions (Benite-Ribeiro et al. 2016).

In the present study, one-month regular high-intensity exercise did not affect the NPY expression. Likewise, in the previous study in the rats, the arbitrary exercise (rotating wheel) for eighteen weeks did not cause a significant change in the expression of NPY. However, in another study in the diabetic rats, the mild exercise neutralized the increase in NPY expression mRNA in the arcuate nucleus. Also, the acute exercise caused an increase in NPY expression (Benite-Ribeiro et al. 2016). These conflicting results are likely due to differences between the protocols of the training, animal gender, and physical status of the animals used in the experiments (intact or diabetic). NPY and POMC of hypothalamus have an axial role in the regulation of food intake, energy balance, and modulation of the endocrine system (Hill et al. 2008). On the other word, NPY and POMC in the arcuate nucleus belong to the main mediators in the regulation of appetite (Medici Lorenzeti et al. 2013). Hormones such as leptin, insulin, and ghrelin, via alteration in the expression of POMC and NPY regulate appetite and appetite, in turn, affects the energy levels (Benite-Ribeiro et al. 2016). In the present study, one-month regular high-intensity exercise did not change food intake and the body weight significantly. Previous studies have shown that exercise may cause an increase in appetite (in 19% of reports).

However, in 65% of them, appetite did not change through exercise (Pomerleau et al. 2004). These results are in accordance with our finding. It should be considered that the level of food intake after exercise is different depending on the intensity, duration, and the gender of individuals. For example, it has been reported the exercise intensity reduced the level of food intake in men and increased the level of food intake in women (Pomerleau et al. 2004).

In the present study, one-month of high-intensity exercise did not change the levels of NPY and POMC expressions in the hypothalamic arcuate nucleus and food intake in the male Wistar rats. Our results indicate that these hypothalamic factors may play an important regulatory role because no alteration in the expression of NPY and POMC levels was associated with any alteration in food intake and body weight. It is believed that there is not any correlation between the energy expenditure and the food intake, because in the previous studies, it had been shown that when the intensity of exercise was reduced, the food intake level did not change significantly. Our finding also clearly indicate that one-month regular intensive exercise did not affects the food intake significantly.

In summary, the results of the present study showed that in the rats treated one-month with regular intensive exercise, NPY and POMC mRNA levels and food intake did not change significantly. This might suggest that this type of exercise itself is not an appropriate procedure for reduction in the body weight because it did not change NPY and POMC (i.e. two important factors in the regulation of appetite), expression levels in the hypothalamic arcuate nucleus.

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