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Original Article

Diverse long-term exercise intensity effects on appetite and body weight regulation: Arcuate Neuropeptide -Y and Proopiomelanocortin gene function

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ABSTRACT

Introduction: The effectiveness of various extrinsic and intrinsic regulatory signals on food intake and body weight can be influenced by hypothalamic neuropeptide-Y (*NPY*) and proopiomelanocortin (*POMC*) neurons. While several studies emphasize the vital role of regular physical activity in effective weight management, how these molecular and cellular processes interact with physical activity remains an area in need of further exploration. Hence, this study aims to investigate the impact of various long-term physical activities intensities on the regulation of body weight and appetite.

Methods: Twenty-one Wistar rats (n=7) were randomized into three groups: 1) Control group, 2) a group engaged in regular exercise at moderate intensity for 24 weeks (24-ME, 5 days each week), and 3) a group frequently and intensively exercising over 24 weeks (24-IE, 5 days each week). Subsequently, Reverse transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) methods were performed to measure gene expression of hypothalamic arcuate nucleus *NPY* and *POMC*, as well as serum levels of acyl-ghrelin and leptin.

Results: The *POMC* mRNA level decreased in the 24-ME group compared to the control rats. However, intensive regular exercise increased *NPY* expression compared to the control rats. Inversely, body weight and food intake levels were considerably higher in the 24-ME and 24-IE groups than in the control group. Different intensities of prolonged exercise seem to heighten appetite, eventually increasing body weight through distinct molecular pathways.

Conclusion: Hence, it can be concluded that prolonged intensive exercise may not be a practical approach for weight loss.

Keywords:
Neuropeptide-Y
Pro-opiomelanocortin
Long-term exercise
Bodyweight
Appetite

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Introduction

A body mass index (BMI) of 30.0 kg/m^2 or higher categorizes individuals as obese (Ezzati et al., 2004). This phenomenon is considered one of the most prominent health-related issues globally. Based on the universal estimate published in 2016 by the World Health Organization, above 1.9 billion adults over 18 are classified as overweight, with at least 650 million adults falling into the obese category. In 2016, only 13% of the total adult population of the world (15% of females and 11% of males) were classified as obese (Aronne et al., 2009a). Past literature revealed that with a BMI of approximately 21·0 kg/m^2 the risk of diabetes, dyslipidemia, hypertension increases, and life expectancy decreases (Ezzati et al., 2004).

The sources of obesity have been the center of various research over the past decades. The most common cause of obesity is excessive energy consumption (diet intake) in response to energy loss through physical activities (Aronne et al., 2009b). Dietary intake and body weight are extensively regulated by the arcuate nucleus of the hypothalamus, primarily through the neuronal populations of pro-opiomelanocortin (*POMC*) and neuropeptide-Y (*NPY*), representing the main anorexigenic and orexigenic pathways for conserving energy and regulating food intake (De Bond and Smith 2014). According to past research, feeding behaviors are considerably regulated by the *POMC* and *NPY* neurons that consolidate the central and peripheral inputs (Han et al., 2011). The excitation of *POMC* neurons and the suppression of *NPY* neurons somewhat mediate the leptin's anorexigenic effects (Sartin et al., 2010). Orexigenic effects of the ghrelin hormone are mediated by *POMC* neuron suppression and *NPY* neurons' indirect stimulation (Chen et al., 2017).

A reasonable approach to physical training primarily aims at bodyweight maintenance. However, there are various conflicting outcomes exist regarding the influence of exercise on body weight control and appetite regulation (Stubbs et al., 2002). Several factors, including the intensity, length, and type of exercise, are responsible for increased energy intake, changes in digestive hormone activity, and food intake (Douglas et al., 2015; Holliday and Blannin, 2017).

Therefore, these practical exercise replacements can manage bodyweight and appetite. Past literature stated that long-term exercise could increase the energy intake and stimulate food absorption. Consequently, body weight can be maintained and weight loss prevented (Bilski et al., 2013). Other researchers recommended that intense exercise may suppress appetite and food absorption (Blundell et al., 2003). Hence, the comprehension of molecular and cellular pathways affecting appetite and body weight should be emphasized if various physical activities impact them differently (Casanova et al., 2019).

Due to the effect of exercise on body weight and appetite, it can be prescribed as a component in health examination processes. *NPY* and *POMC* neurons may change the impact of various extrinsic and intrinsic regulatory signals relative to appetite (Lauterio et al., 1999). Given the considerable number of contradictory studies in this regard, this research attempts to determine the influence of long-term training sessions on the molecular mechanisms of appetite.

Material and methods

Groups

In the current study, twenty-one male Wistar rats (250±50 g) were obtained from the Pasteur Institute of Tehran, Iran. They were held in a well-supervised environment, maintaining a temperature of 22±5^{0}C, a relative humidity of approximately 50±5%, and a controlled 12-hour light / dark cycle. Also, they have free access to food and drinking water.

All experimental protocols followed the guidelines outlined in the "Guide for the care and use of animals" (NIH Guide for Care and Procedure of Laboratory Animals, 8th Edition, 2010). Our procedures were also accepted by the animal care institute and the Research and Ethics Committee at the Medical Plants Research Center, Shahrekord University of Medical Sciences (IR.SKUMS.REC.1400.019).

Experimental design

Twenty-one rats (230 ± 20 g, aged 2-3 months) were randomly separated into three distinct groups (n=7/Group): 1) Control group (this group did not have physical activity), 2) This group performed moderate-level exercises for 24 weeks (over 24 weeks five times each week for 40 min each morning (the speed of 22 m/min), and the rats performed in an intensive level of activity on a treadmill (24-ME). 3) This group performed intensive repetitive physical activity (for 24 weeks five times

TABLE1: Sequence of primers for studied genes

Gene	Accession number	Primer sequence [a]
GAPDH	NM_017008.4	F: 5'TGCCGCCTGGAGAAACCTGC3' R: 5'TGAGAGCAATGCCAGCCCCA3'
POMOC	NP_000930.1	F: 5'GAGGAGAAAAGAGGTTAAGGAG3' R:5'TATGGAGGTCTGAAGCAGGAG3'
NPY	A4D158	F: 5'ACTACATCAATCTCATCACCAG3' R: 5'GTTTCATTTCCCATCACCAC3'

each week for 40 min each morning (the speed of 35 m/min), and the rats participated in an intensive level of activity on a treadmill (24-IE). The total duration of the exercises was around one hour, following a protocol with three stages: 1) At the start of the 60-min exercise, 5 m/min was determined to warm up. The speed rate of the treadmill was gradually increased from 5 m/min to 35 m/min. 2) For the second step, the speed rate at 35 m/min for 40 min was fixed, and 3) In the last stage, the speed rate decreased from 35 m/min to 5 m/min to provide recovery sessions for the rats. Rats in the control group remained stationary on the treadmill for one hour (Khajehnasiri et al., 2021; Khajehnasiri et al., 2018).

Body weights were measured at 11:00 A.M. using precision scales. Food consumption was checked each morning by manually weighing food dishes before and after a feeding period (24h) for each rat (Ali and Kravitz, 2018). At the end of the study, the animals were anesthetized using a combined injection of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (100 mg/kg) (intraperitoneal injection). Blood samples, arcuate nucleus, and hypothalamus were then collected (Khajehnasiri et al., 2019).

Acyl- ghrelin and leptin enzyme-linked immunosorbent (ELISA) assay

After inducing deep anesthesia, blood samples were collected via the left ventricle using a 19-21-gauge needle to determine levels of acyl ghrelin and leptin. Similarly, serum acyl ghrelin and leptin levels were estimated using the rat acyl ghrelin and leptin ELISA kits obtained from Cusabio, China.

Isolation of arcuate nucleus

According to the previous approach, the arcuate nucleus and hypothalamus were isolated from the brain, immediately placed in liquid nitrogen (Salehi et al., 2012), and subsequently stored in a freezer at -80°C.

RNA isolation and real-time-polymerase chain reaction (RT-PCR)

The hypothalamic arcuate sample's total RNAs were extracted using *TRIZOL* (Yekta Tajhiz, Iran). The next step was determining their purity and concentration using a *NanoDrop* device. Then, reverse transcription of RNA was performed using a reverse transcription kit (BIONEER) and gene-specific primers (Table 1). The following stages comprised utilizing the SYBR green PCR Master Mix to administer the real-time PCR test (Takara Bio Inc). Lastly, a comparative Ct procedure was implemented to analyze real-time PCR test data, and the arithmetic formula $2^{-\Delta\Delta CT}$ was applied to calculate the relative expression of the target mRNAs regarding the reference values (Sadeghian et al., 2021).

Data analysis and statistics

The normal distribution of the collected data for the relative expression of target genes and proteins was confirmed using a Kolmogorov–Smirnov test was performed. To compare the performances of the three groups, statistical analysis employed one-way ANOVA computation within the SPSS v 24.0 statistical software package. Also, Tukey's post hoc test was used to discern significant differences among the groups. Results are presented as mean ± standard error of mean for each group, and $P<0.05$ was considered significant.

Results

Effects of 24-Week Regular Moderate and Intensive Exercise on Acyl-Ghrelin and Leptin Serum Levels

An ELISA test was performed on serum samples at the end of the 24-week moderate and intensive physical activity experiment. The findings indicated a significant difference between groups [$F(2, 18) = 62.354$; $P<0.001$;

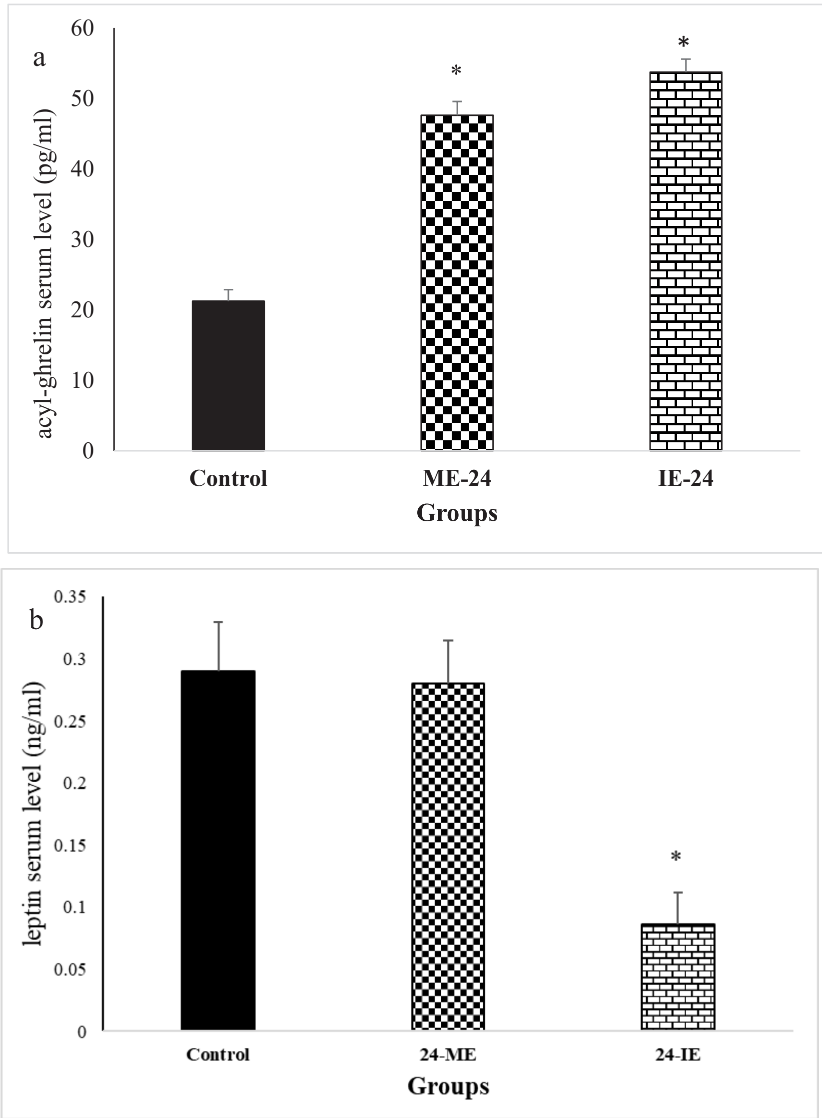

FIGURE 1. Effects of regular moderate and high-intensity exercise on levels of (a) ghrelin and (b) leptin in the serum. 1) Control group (n=7): animals in this group received no treatment). 2) 24-ME group (n=7): This group performed medium-level exercise for 24 weeks. 3) 24-IE (n=7): This group participated in intensive routine physical activity for 24 weeks, five times each week. * Showed a significant difference compared to the control group (P <0.05). Data are reported as mean ± standard error of mean.

Fig.1a]. Specifically, acyl-ghrelin serum levels increased in both the 24-ME and 24-IE groups compared to the control rats (P<0.05; Fig.1a).

In contrast, the leptin serum levels showed no significant difference within groups [F (2,18) = 1.515; P=0.247; Fig.1b]. Notably, the 24-week intensive training experiment significantly reduced serum leptin levels in the control rats, (P<0.05; Fig.1b). However, the 24-ME group could not effectively alter serum leptin levels compared to the control group (Fig.1b).

The Effects of 24-Week Regular Moderate and Intensive Exercise on Hypothalamic POMC and NPY Gene Expression

Real-time PCR analysis was conducted to evaluate the effect of 24 weeks intense and moderate exercise on arcuate *POMC* and *NPY* mRNA levels. The results indicated a significant difference in *POMC* gene expression between groups [F (2, 18) = 4.69; P<0.05; Fig.2a]. Post-hoc Tukey test indicated a significant decrease in *POMC* expression that the 24-ME group compared to the control group (P<0.05; Fig.2a). However, no differences in arcuate *POMC* mRNA levels were observed between the 24-IE and control rats (Fig.2a).

One-way ANOVA showed a significant difference in *NPY* mRNA expression among the groups [F (2, 18) = 5.04; P<0.01; Fig.2b]. In addition, the post-hoc Tukey test showed that the *NPY* mRNA level increased in the

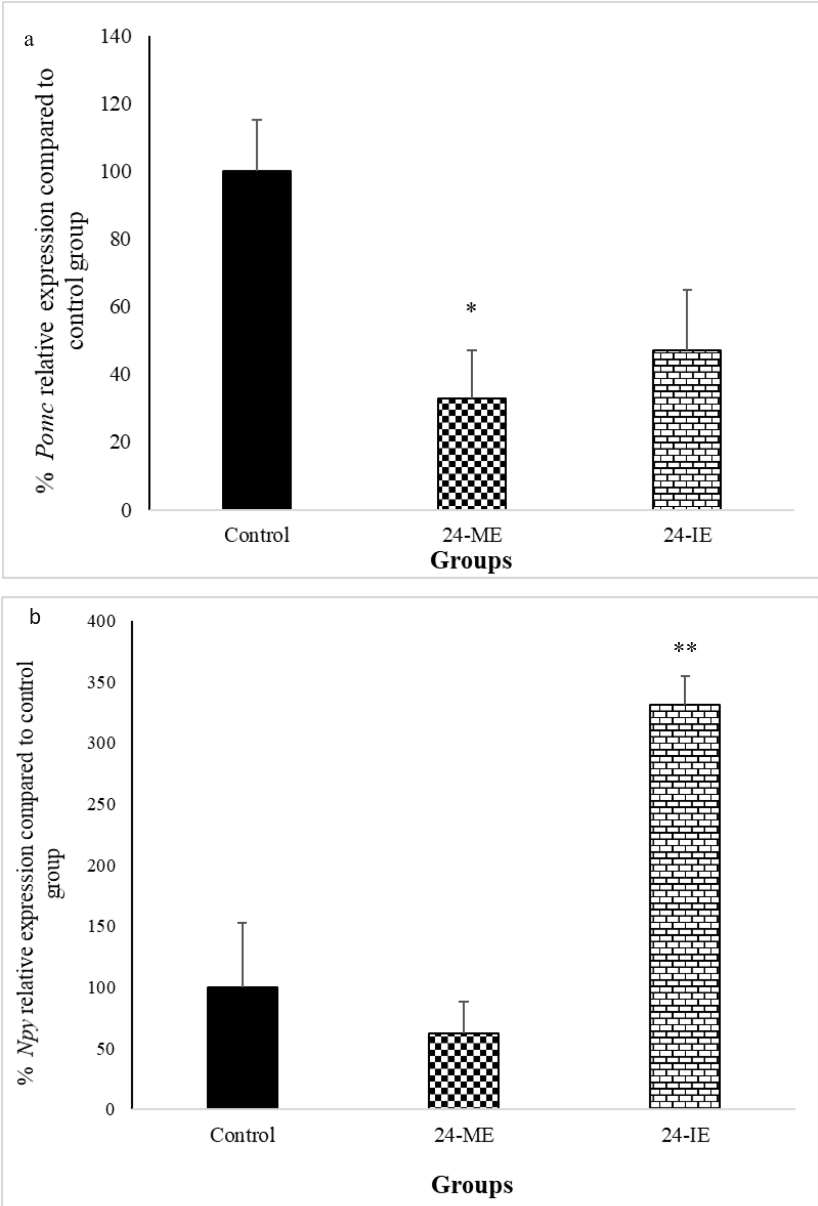

FIGURE 2. Effects of regular moderate and high-intensity exercise on the % relative expression of (a) POMC and (b) NPY genes between groups in the arcuate nucleus. 1) Control group (n=7): Animals in this group recieved no treatment). 2) 24-ME group (n=7): This group performed medium-level exercise for 24 weeks. 3) 24-IE (n=7): This group participated in intensive routine physical activity for 24 weeks, five times each week. *P<0.05 and **P<0.01 indicate significant differences compared to the control group. Data are reported as mean ± standard error of mean.

24-IE group compared to the control group (P<0.05). In contrast, there was no notable difference in *NPY* mRNA expression between the control group and the 24-ME group (Fig.2b).

The effects of 24-week regular moderate and intensive exercise on food intake and body weight

Finally, the effect of food intake and body weight on moderate and intense 24-week physical activity was evaluated. A significant increase in food intake was observed in both the 24-IE and 24-ME groups compared to the control rats (P<0.05; Fig.3a), correlating with increased body weight in the 24-IE and 24-ME rats (P<0.05; Fig.3b).

Discussion

The current study examined the effect of different intensities of long-term physical activity on appetite and body weight from a molecular perspective. The interplay among orexigenic (ghrelin), anorexigenic hormones (leptin), and the neuronal complexes in the hypothalamus and brainstem governs the energy balance

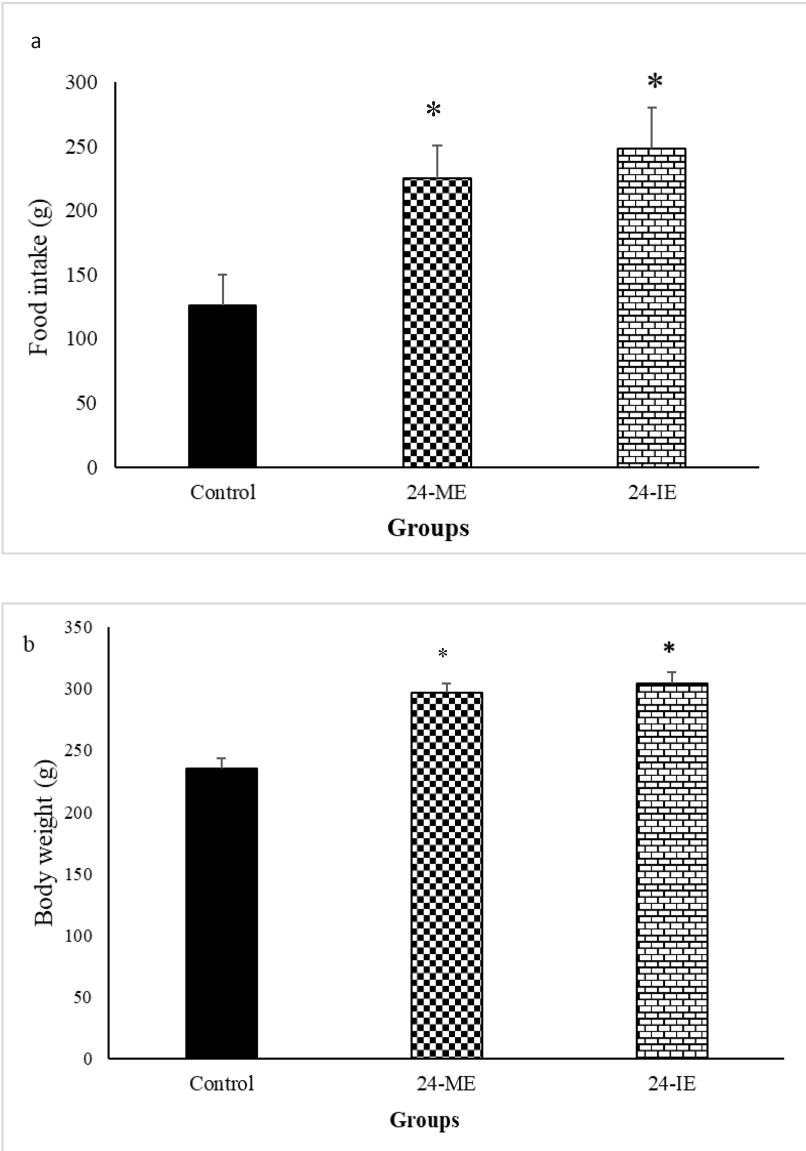

FIGURE 3. a) The amount of food intake between groups. b) Changes in body weight, 1) Control group (n=7): Animals in this group received no treatment). 2) 24-ME group (n=7): This group engaged in medium-level exercise for 24 weeks. 3) 24-IE (n=7): This group participated in intensive routine physical activity for 24 weeks, five times each week. *P<0.05 and **P<0.01 indicate significant differences compared to the control group. Data are reported as mean ± standard error of mean.

of the body. This equilibrium, managed by peripheral and central signals, relies not on a single hormone but on the interaction between various hormones, culminating in clinical implications (Owyang and Heldsinger, 2011; Wardlaw, 2001). Hence, hunger and satiety are affected by the ratio of leptin-related hormones to acyl ghrelin concentrations (Klok et al., 2007).

Due to the rapid growth of obesity, identifying how several hormones and neurotransmitters' mechanisms affect energy balance has been the subject of in-depth research (Farhadipour and Depoortere, 2021). Some studies have reported elevated circulating levels of the anorexigenic hormone leptin in obese patients, but remarkably, the orexigenic hormone ghrelin levels were diminished (Heymsfield et al., 1999).

The data obtained from our study revealed a significant elevation in serum acyl ghrelin levels across both moderate and intense physical activity formats. In contrast, serum leptin levels slightly increased in the intensive exercise routine group, indicating that the intensity of physical activity plays a significant role in serum leptin levels. However, contrary results have shown that perceived hunger or satiety and related hormones (ghrelin, pancreatic polypeptide, peptide YY, and insulin) are not affected by normal 12-week resistance activities (Guelfi et al., 2013). This discrepancy could be attributed to exercise type, duration, and intensity (Bilski et al., 2009).

Our findings on several training intensities demonstrated differential effects on the expression of the hypothalamic arcuate gene. Regular activity led to a reduction in *POMC* mRNA levels, indicating moderate exercise impact. Conversely, routine intensive activity significantly increased *NPY* gene expression, emphasizing the importance of exercise intensity in the expression of hypothalamic arcuate *POMC* and *NPY* gene expression.

Notably, after the cessation of moderate training, a significant decrease in hypothalamic arcuate *POMC* mRNA levels was observed, while no changes were observed in the group involved in intense routine physical activity. According to our results, *POMC* gene expression remained unaffected after a 4-week resistance training session at a speed of 35 m/s. Conversely, seven-week training sessions (five-week adjustment, two-week intense) reduced *POMC* mRNA levels in the frontal cortex and hippocampal CA_1 regain (Jiaxu and Weiyi, 2000). In contrast, other studies have shown increased gene expression in response to voluntary training sessions (Laing et al., 2016).

Regarding alterations in *NPY* expression, our study revealed that one month of intensive training had no impact on this protein (Khajehnasiri et al., 2019). Similarly, the expression of *NPY* did not alter after an eighteen-week rotating wheel training. In contrast, another study showed that moderate activity levels counteract increased *NPY* mRNA expression in the arcuate nucleus in diabetic animals, while acute exercise improved *NPY* expression (Benite-Ribeiro et al., 2016). These differences could be attributed to variations in physical activity duration and type. It is widely presumed that the duration, intensity, and type of training are primary factors affecting the expressions of the *POMC* and *NPY* genes (Benite-Ribeiro et al., 2016; Khajehnasiri et al., 2019).

The regulation of food consumption, energy equilibrium, and the endocrine system is primarily governed by *NPY* and *POMC* in the hypothalamus (Hill et al., 2008). Specifically, NPY and POMC in the arcuate nucleus play significant roles in appetite regulation (Sohn, 2015). Hormones such as insulin, leptin, and ghrelin modulate appetite by modifying *POMC* expression, thereby influencing energy levels (Klok et al., 2007; Varela and Horvath, 2012).

In our study, routine intense and moderate training activities resulted in increased food absorption and regulated body weight, as anticipated. However, contrary to our findings, previous studies have reported increased food consumption and weight regulation following intensive exercise. In contrast, moderate exercises gradually increased consumption and appetite (in 19% of reports) but had no impact on weight regulation. Nonetheless, 65% of past studies reported that the appetite was not altered through physical activity (Pomerleau et al., 2004). Other research has revealed that physical activity intensity reduced food consumption in males but increased it in females (Hagobian et al., 2009; Pomerleau et al., 2004). According to prior studies, food consumption levels were relatively unaffected when exercise intensity was reduced. Hence, the substantial correlation between food intake and energy consumption is presumed to be absent.

In the rats of the 24-ME group, considerably increases were observed in food consumption rates, body weight, and acyl-ghrelin levels. While anorexigenic peptide *(POMC*) gene expression decreased, acyl-ghrelin continued to increase. However, it seems that increased food consumption and body weight in the 24-IE group may be mediated by improvements in acyl ghrelin and decreases in leptin, subsequently raising orexigenic peptide (*NPY*) mRNA levels in the arcuate hypothalamus.

Conclusion

To conclude, the study revealed that 24 weeks of intensive exercise in male rats led to increased *NPY* mRNA levels, increased food intake, and accelerated body mass. However, this parameter did not affect *POMC* gene expression. Conversely, moderate physical activity decreased *POMC* mRNA levels. Despite increased food consumption, there were no effects on *NPY* mRNA and leptin serum levels.

Thus, different intensity levels in long-term physical activity affect appetite and body weight through two distinct molecular pathways. This suggests that sustained high-intensity physical activity may not be a dependable method for weight loss.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgements

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Ethics approval

All experimental procedures using rats were conducted in accordance with the animal care and use guidelines approved by the institutional ethics committee at Medical Plants Research Center, Shahrekord University of Medical Sciences (IR.SKUMS.REC.1399.285) and were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Availability of data and materials

The data are available for any scientific use with kind permission.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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