ORIGINAL ARTICLE

Effects of aerobic training on asprosin levels in adipose tissue of obese rats

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ABSTRACT

Introduction. The asprosin hormone, which has been identified as a type of adipokine, is secreted from adipose tissue during fasting with a glucogenic effect on the liver. It increases glucose secretion into the blood, leading to a rise in blood insulin levels. In the hypothalamus, asprosin has an orexigenic role, helping to increase appetite which has a direct relationship with fat mass and obesity. The purpose of the present study was to evaluate the effect of aerobic exercise at different intensities on adipose tissue asprosin levels, thereby facilitating the determination of optimal training methods for controlling and treating obesity.

Material and Methods. Male Wistar rats were randomly divided into four groups (n=10 per group) low (LOW), moderate (MOD), and high-intensity aerobic training (HIGH), and the control group (CON). The training intervention was conducted over 8 weeks, and surgical sampling from the rats' visceral adipose tissue was performed 48 hours after the final training session. Asprosin content of the adipose tissue was measured via ELISA.

Results. Statistical comparisons between the groups revealed a difference in asprosin levels between the intervention groups and the control group (p=0.006). While low-intensity training already reduced asprosin levels significantly compared to controls and still was significant with intensified exercise levels, no further differences between moderate and high-intensity groups were visible anymore (p=0.410).

Conclusions. Aerobic training at low, moderate, and high-intensity levels is effective in decreasing adipose tissue asprosin levels, which is accompanied by a reduction in fat mass and the treatment of obesity.

Keywords: aerobic exercise, asprosin, obese male Wistar rats, obesity

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INTRODUCTION

Obesity is associated with many diseases and increases the risk of conditions such as diabetes, heart disease, hypertension, depression, stroke, osteoarthritis, respiratory problems, and many cancers [1-9], it is recognized as the second major cause of preventable mortality [10].

Studies have shown that one of the main causes of these rising figures in the past two decades across different societies is the reduction of physical activity due to lifestyle changes, technological advancements, and the elimination of high-activity manual labor [5, 6, 8, 11]. For this reason, public health institutions and organizations have proposed investing in exercise as an indispensable strategy for governments, and find it vital for the public to be encouraged toward engaging in increased physical activity, thereby preventing the diseases related to obesity and over weightiness, and boosting public well-being [5, 9, 11]. In line with these goals, sports sciences professionals suggested that regular exercise (aerobic or resistance exercise or a combination of both) accompanied by diet control is the best way to treat obesity [12, 13].

In 2016, Romere et al. identified the peptide hormone called asprosin and reported its involvement in glucose homeostasis. These researchers described asprosin as the product of the c-terminal cleavage of the profibrillin protein, which is transcribed by the FBN1 gene[14]. By activating the cyclic adenosine monophosphate (cAMP) signaling pathway via the OLFR734 receptor, asprosin induces the release of glucose from the liver into the blood, which consequently induces a rise in blood insulin level [15]. It is demonstrated that increased levels of circulating asprosin have a significant relationship with the incidence of type two diabetes, as well as increased fasting blood glucose and triglyceride levels, and suggested the possibility of treating obesity and type two diabetes by controlling asprosin levels [16]. In the same year, Duerrschmid et al. reported that asprosin, in addition to its glucogenic effects in the liver, can cross the blood-brain barrier and increases appetite by acting on the hypothalamus, thereby having an orexigenic role and causing a rise in fat mass over time. The results of their research showed cooperation and coordination between the hypothalamus and the liver in appetite stimulation and hepatic glucose release during the fasting state [17]. Further studies reported increase of asprosin levels in some diseases such as obesity, insulin resistance, type 2 diabetes, and polycystic ovary syndrome[18-22].

In a study on the effect of exercise on asprosin levels, Schumann et al. (2017) measured the impact of a high-intensity aerobic training session on the plasma asprosin levels of 12 obese women and 6 obese men and reported that the asprosin levels were not sensitive to acute exercise. [23]. However, Wiecek et al. (2018) measured the effect of a high-intensity cycling session on the plasma asprosin levels of two groups of men and women and reported that asprosin levels increased significantly among the female subjects [24]. In another study, Ko et al. (2019) showed that aerobic exercise can decrease asprosin levels in diabetic rats' hepatic cells [25]. Nakhaei et al (2019) also concluded that continuous and interval swimming exercise can decrease asprosin levels in rats with metabolic syndrome [26].

Summarizing these findings, asprosin might represent a potential biomarker for predicting obesity-related diseases, such as type two diabetes, metabolic syndrome, and even coronary artery disease. Therefore, controlling the levels of this hormone given its role in

appetite modulation may be a way to prevent and even treat obesity. Undoubtedly, regular exercise can be one of the most effective strategies for achieving the mentioned goals. Therefore, in this study, we aimed to investigate the effect of aerobic exercise at different intensities as a non-pharmacological treatment to reduce asprosin levels with its implication for obesity.

MATERIALS AND METHODS

Ethical considerations

All stages of experimentation on the animals were conducted following the ethical standards adopted by the Ferdowsi University of Mashhad Research Ethics Committee (Code IR.MUM.FUM.REC.1397.039) and in compliance with international ethical guidelines for work with laboratory animals.

Statistical population and sampling method

A total of 40 male Wistar rats were purchased at an age of five weeks with a mean weight of 70.15 ± 12.84 g, and a mean body mass index (BMI) of 0.17 g/cm^2. Since the BMI value was considered a measure of obesity the BMI of the rats reached 0.84 g/cm^2 at the age of 14 weeks and the end of the fattening stage before intervention.

Measurement of weight, height, and BMI

The weight and height of all subjects were measured between 8:00 and 9:00 AM on a specific day once per week. These measurements were obtained from the date of purchasing until the end of their twenty-third week of life.

The process of fattening the rats

The rats were fed and maintained on a standard diet for one week after purchase to allow familiarization. From week 6 to week 14 (rat age), the rats have given a high-caloric diet with high lipid content and were finally considered obese rats given the rise in BMI.

Training intervention

The obese rats were divided into four groups: (i) low-intensity aerobic exercise group (LOW, n = 10); (ii) moderate-intensity aerobic exercise group (MOD, n = 10); (iii) high-intensity aerobic exercise group (HIGH, n = 10); (iv) untrained control group (CON, n = 10). The subjects were then familiarized with the training environment while obesity was consolidated during week 15 in terms of rat age.

From the sixteenth week until the end of the twenty-third week (rat age), the training intervention was conducted across the low, moderate, and high-intensity groups according to Table 1. Over eight weeks, the rats were trained at different speeds on rodent treadmills five days a week, with one 60-minute session held each day between 8:00 am and noon.

	LOW			MOD			HIGH		
Week	**Training duration (min)**	**Speed (m/min)**	**Maximum oxygen consumption (%)**	**Training duration (min)**	**Speed (m/min)**	**Maximum oxygen consumption (%)**	**Training duration (min)**	**Speed (m/min)**	**Maximum oxygen consumption (%)**
1	15	10	23-25	15	10	25-26	15	10	23-25
2	27	15	35-39	27	15	37-40	27	15	35-37
3	34	16	39-43	34	20	50-53	35	20	47-50
4	40	17	42-46	40	21	52-56	45	22	51-55
5	46	18	46-51	46	23	57-61	54	24	56-60
6	52	19	50-55	52	24	60-65	59	27	63-67
7	58	20	55-60	58	26	65-70	60	31	73-77
8	60	21	60-65	60	28	70-75	60	34	80-85

Table 1. Aerobic exercise program at three different intensity levels [27].

Sampling of visceral adipose tissue from the rats

After 48 hours had passed since the final training session, including 12 hours of fasting, the rats were transferred to the dissection room. Next, the rats were anesthetized using an intramuscular injection of a combination of ketamine (30-50 mg/kg body weight) and xylazine (3-5 mg/kg body weight).

After unconsciousness was confirmed, a 5-6 cm incision was made in the abdominal region of each rat, and samples of visceral adipose tissue were rapidly obtained and transferred to 1.5 mL microtubes containing liquid nitrogen.

Measuring the amount of asprosin hormone

The asprosin hormone content in an equal amount of adipose tissue was measured using the special ELISA kit for rats provided by Hangzhou Eastbiopharm Co. following the manufacturer's instructions.

Statistical analysis

After the results were obtained, descriptive statistics were used to determine the means and standard deviations. The Shapiro–Wilk test was then used to check the normality of the data. To examine the significance of differences between means, one-way ANOVA was used. Levene's test was used to check for homogeneity of variance across the groups. Differences in means between each pair of groups were assessed using Dunnett's post-hoc test. Data analysis was done using SPSS software version 22, while figures were plotted with Excel software. Statistical significance was considered at the level of $p<0.05$.

RESULTS

Based on the results, the following descriptive indices were obtained regarding the levels of asprosin hormone in the adipose tissue of Wistar rats subjected to the control group and the three training groups (low, moderate, or high intensity) (Figures 1 and 2).

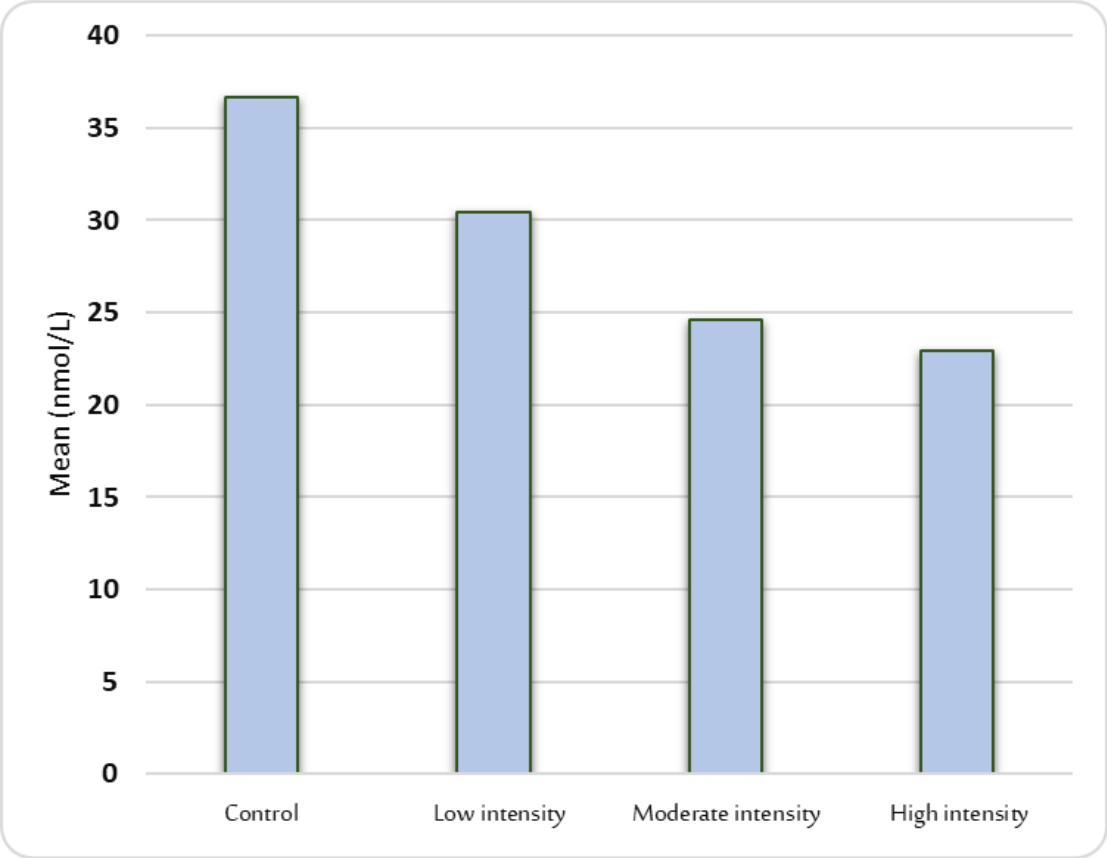

Figure 1. The mean asprosin levels in the visceral adipose tissue of rats in each of the control and intervention groups.

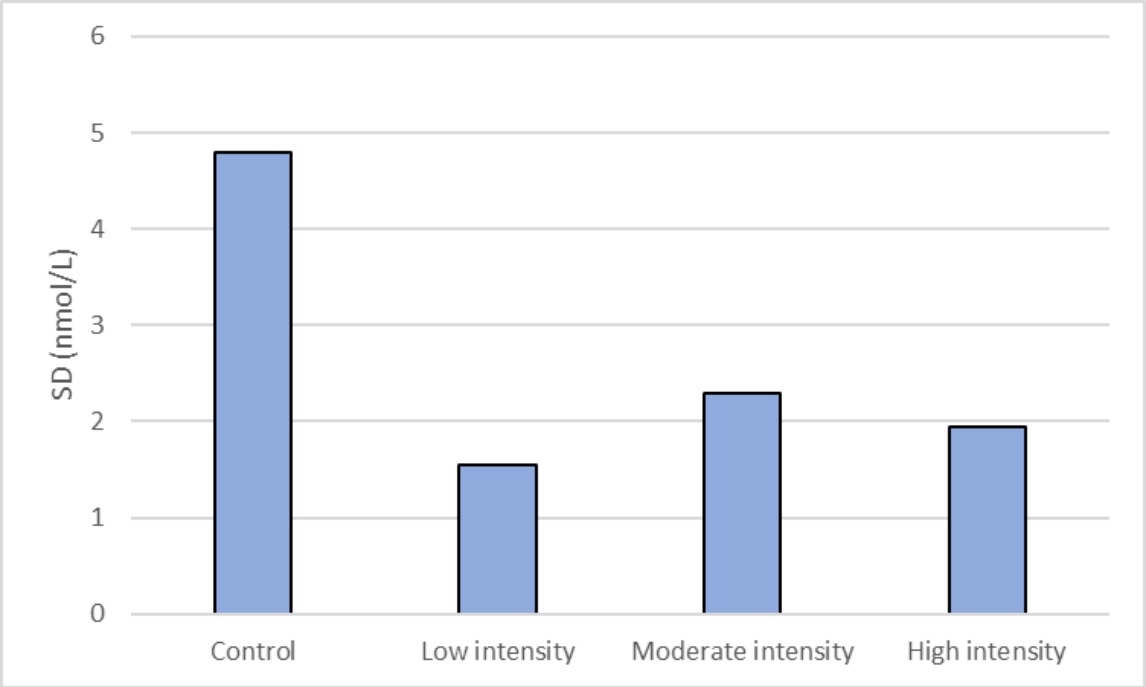

Figure 2. The standard deviation of asprosin levels in the visceral adipose tissue of rats in each of the control and intervention groups.

The normality of the data related to all groups was confirmed via the Shapiro–Wilk test. Then, Levene's test was used to assess the homogeneity of variances, with the results being presented in Table 2.

Table 2. The results of Levene's test for examining homogeneity in the variance of asprosin levels between the study groups.

Group	Population	Mean (nmol/L)	Standard deviation (nmol/L)	Test statistic	1 degree of freedom	2 degrees of freedom	Significance
CON	10	36.69	4.80	4.994	-	31	**0.006**
LOW	10	30.44	1.55				
MOD	10	24.58	2.29				
HIGH	10	22.89	1.94				

Table 3: Results from the comparison of mean asprosin levels between the study groups using the Welch tests.

Test	Test statistic	1 degree of freedom	2 degrees of freedom	Significance
Welch	**37.814**	**3**	**16.224**	**0.000**

According to the results (p=0.006), the homogeneity of the variances across the four groups was rejected. Hence, the means were compared using the Welch tests (Table 3).

According to the results obtained from the above tests, the null hypothesis regarding the equality of means was rejected (p≤0.05). In other words, the asprosin hormone levels were not the same between the control and intervention groups. Based on the non-homogeneity of the variances, Dunnett's post-hoc test was used to compare the means between each pair of groups, the results of which are presented in Table 4.

The results of Dunnett's post-hoc test demonstrated a significant difference between the control group and each of the training groups, meaning that the rats in the control group had significantly higher adipose tissue asprosin levels in comparison with all three intervention groups. Furthermore, significant differences were also observed between the low-intensity exercise group and each of the moderate and high-intensity groups, such that asprosin levels were higher in the low-intensity group. However, no significant difference was found in asprosin levels between the moderate and high-intensity training groups.

Table 4: The results of Dunnett's post-hoc test for comparing mean asprosin levels between each pair of groups.

Two-by-two comparison between groups		The difference in means (nmol/L)	Standard deviation (nmol/L)	Significance
CON	LOW	*6.2446	1.795	0.039
	MOD	*12.1075	1.844	0.000
	HIGH	*13.7975	1.804	0.000
LOW	MOD	*5.8629	0.931	0.000
	HIGH	*7.5529	0.847	0.000
MOD	HIGH	1.690	0.948	0.410

DISCUSSION

Among the various strategies for preventing and treating obesity, sporting activities, and diet control have been identified as fundamental and effective approaches [28, 29]. Regular exercise has been shown to reduce dyslipidemia, blood glucose, and blood pressure, thereby preventing the incidence of type two diabetes [29-32]. Recent research also suggests the positive effect of regular exercise on the regulation of the secretion of adipokines and the reduction of inflammatory factors [29]. According to the findings of the present study, low to high-intensity aerobic exercise is effective in reducing asprosin hormone levels. It has been shown that the plasma levels of asprosin in obese and insulin-resistant humans and rats are higher than the respective healthy subjects with normal weights, such that neutralization of this hormone via monoclonal antibodies leads to a large decrease in the appetite of obese mice and thereby induces their weight loss [14-17, 33].

In the liver, the specific G-protein coupled receptors OLFR734 for asprosin are present on the hepatocyte membrane and activate the cAMP-PKA axis. In this way, by activating the glycogen phosphorylase enzyme and inhibiting glycogen synthase activity, asprosin plays a glucogenic role and increases blood glucose levels, consequently causing a rise in insulin secretion [14, 15]. Asprosin also has the potential to cross the blood-brain barrier and, by activating AgRP+ via the cAMP signaling pathway, plays an orexigenic role and stimulates appetite. Simultaneously, asprosin inhibits POMC neurons in the hypothalamus, which, by increasing appetite, causes increased body fat accumulation and thus obesity [17]. Hence, it is suggested that by modulating the level of asprosin hormone, it is possible to treat obesity, reduce appetite, help regulate blood glucose, and treat people who have severe body fat deficiency.

To the best of our knowledge, our study is the first research comparing different intensities of aerobic training on asprosin hormone levels in adipose tissue of rats which shows the effectiveness of aerobic training from low to high intensity on reducing this hormone.

In another study on the effect of exercise on asprosin levels, Schumann et al. (2017) measured the effect of a high-intensity aerobic exercise session (85% of maximum heart rate) on a treadmill on the plasma asprosin levels of 12 obese women and 6 obese men and reported that the levels of asprosin were not sensitive to acute exercise [23]. However, Wiecek et al. (2018) measured the effect of a high intensity cycling session on the plasma asprosin levels of two groups of men and women. Here, participants had to peddle rapidly for 20 seconds on a stationary bicycle, after which blood samples were obtained at multiple intervals. The researchers reported that asprosin levels increased significantly among the female subjects, while no changes were observed among the men [24].

Research on the effect of exercise and diet control in both men and women has shown that there are differences in the outcomes obtained

by these two groups, which may be due to phenotypic differences in adipose tissue among the sexes [34, 35]. Thus, the difference in the results obtained between the studies of Schumann et al. and Wiecek et al. can be attributed to the varying responses of men and women to sporting activities.

In line with our research, Ko et al. (2019) reported that eight weeks of aerobic exercise on a treadmill for 60 mins at speed of 20 m/min can reduce hepatic asprosin in diabetic rats [25]. The similarity of their findings with our results can be the effect of similar exercise types and intensity.

In another related study, Nakhaei et al. (2019) concluded continuous and interval swimming training can decrease asprosin levels in rats with metabolic syndrome [26].

Our study results and these findings can be an indication of the effectiveness of different types of aerobic training on the reduction of asprosin hormone levels in obese and diabetic rats.

CONCLUSION

To date, the present study is the only study to have compared the effect of continuous aerobic training with different intensities on the levels of the asprosin hormone in the adipose tissue of obese male rats. The findings of this study suggest that continuous aerobic exercise at low, moderate, and high levels of intensity is effective in reducing adipose tissue asprosin levels. However, there was no significant difference in outcomes between the two groups of moderate and high-intensity aerobic training, we observed an increasing decline in asprosin level with increasing intensity of exercise.

Therefore, further studies are needed with extended study protocols at distinct intensities to clarify the association between exercise levels and the course of asprosin secretion including its impact on adipose tissue and BMI.

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Conflicts of Interests

The authors declare no conflicts of interest.

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