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Original Article

The Protective Effect of Different Methods of Exercise Training on Plasma Levels of Nesfatin-1, Cardiorespiratory Endurance and Body Composition in Overweight and Obese Females

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Abstract

Background and Aim: Nesfatin-1 is a newly discovered anti-appetite protein which is expressed in adipose tissue and appears in plasma. It has a significant role in energy homeostasis and metabolism. The aim of this study was to assess the effects of an 8-week endurance and resistance training on the plasma level of nesfatin-1, cardiorespiratory endurance, and body composition of overweight and obese females.

Methods: A sample of 34 overweight and obese females were recruited and randomly allocated to the control (10 students), endurance training (12 students), and resistance training (12 students) groups. Females in the experimental groups did either endurance or resistance training for 8 weeks—four sessions per week and 32 sessions in total—with a severity of 65%–80% of maximum heart rate (HRmax) and 65%–80% of one repetition maximum (1RM). A blood sample was obtained from each participant before and after the study intervention and after twelve hours of fasting. The enzyme-linked immunosorbent assay (ELISA) method was employed for measuring the plasma level of nesfatin-1. The normality of the study variables was assessed by conducting the Kolmogorov-Smirnov test. Moreover, within-group comparisons were performed via the paired-samples *t* test while between-group comparisons were made by conducting the one-way analysis of variance (ANOVA) and the least significant difference (LSD) post hoc test at a significance level of less than 0.05.

Results: The means of participants' age, weight, and body mass index (BMI) were respectively 22.29 ± 2.49 years, 77.23 ± 10.00 kg, and 30.19 ± 2.79 kg/m^2. Compared with the control group, the plasma level of nesfatin-1 as well as cardiorespiratory endurance increased after the study intervention in both of the experimental groups. Besides, weight, body fat mass, BMI, and waist-to-hip ratio decreased significantly in the experimental groups ($P < 0.05$).

Conclusion: As non-invasive non-pharmacological procedures, both endurance and resistance training can exert protective effects on overweight and obese females' health through increasing the level of nesfatin-1 anti-inflammatory agent and improving obesity-related indices.

Keywords: Endurance training, Resistance training, Obesity, Nesfatin-1.

Introduction

A wide spectrum of behavioral, biological, ecological, and genetic factors can contribute to obesity. The most significant factor behind obesity is imbalanced energy intake and consumption due to restricted physical activity and high calorie intake (1). Obesity is associated with several health problems such as hyperlipidemia, diabetes mellitus, and increased risk for developing chronic conditions like cardiovascular disease and hypertension. Accordingly, the importance of developing programs for obesity prevention and management is increasing progressively (2).

Adipose tissue has been recognized as an endocrine gland secreting bioactive mediators which contribute to blood pressure regulation, fat and glucose metabolism, insulin resistance, inflammation, and vascular occlusion (3). These mediators are protein substances which are called adipokines. One of the adipokines is nesfatin. Nesfatin

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contributes to appetite stimulation, energy homeostasis, and body metabolism (4).

Nesfatin-1 is a newly discovered anti-appetite protein which is derived from nucleobindin-2 (NUCB2) (5). Oh-I et al (4) separated the derivatives of NUCB2 to nesfatin-1, 2, and 3—nesfatin-1 from number 1 to 82; nesfatin-2: from number 85 to 163; and nesfatin-3 from number 166 to 396. Nesfatin-1 contributes to the activity of the digestive system and gastric emptying and its plasma level changes after feeding, fasting, physical activity, and diabetes mellitus (6-8). Moreover, its secretion is affected by insulin and the inflammatory cytokines which regulate appetite and energy consumption (9).

The main effect of nesfatin-1 is to decrease food intake and appetite (7). Reports showed a relationship between the plasma level of nesfatin-1 and weight, fitness, body fat percentage (BFP), and fat-free mass (10). In addition, there is a negative correlation between body mass index (BMI) and fasting plasma level of nesfatin-1 (6). Ramanjaneya et al found that nesfatin-1 is mainly expressed in subcutaneous adipose tissue and it is correlated with BMI (9). Given the roles of nesftatin-1, the plasma level of this neuropeptide is probably affected by obesity (11).

Cardiorespiratory endurance is one the components of physical fitness which is closely related to BMI and BFP and is essential for maintaining muscular endurance. It can be measured by the maximum oxygen consumption (VO_2max) index (12). Another component of health-related physical fitness is body composition which is assessed by measuring indices such as BFP, weight, BMI, and waist-to-hip ratio (WHR). Body composition indices have been shown to strongly correlate with the type of physical training. On the other hand, BFP has a negative significant correlation with aerobic capacity and endurance running ability (13). Furthermore, increases in BFP not only cause weight gain, but also decrease oxygen presence in the muscles and hence, reduce cardiorespiratory endurance (14).

Currently, lifestyle modifications, such as increased physical activity, are recommended as the first measures for decreasing excess body fat and preventing cardiovascular and metabolic disorders (15). Physical activity and training can also change the plasma levels of some adipokines (16). Different training (both endurance and resistance) can cause beneficial physiologic changes and improve physical fitness through different mechanisms. For instance, endurance exercises increase VO_2max, energy consumption, and fat oxidation while resistance exercises can increase absolute muscle strength and mass (17) and improve body composition. Consequently, endurance and resistance exercises are expected to positively affect factors which control metabolic abnormalities, such as nesfatin-1, through improving body composition indices.

Evidence about the effects of physical activity on the plasma level of nesfatin-1 is limited and conflicting. For example, some studies have shown that two types of single-session aerobic exercise for athletes (8) and a single-session aerobic exercise for elderly people (18) had no significant effects on the plasma level of nesfatin-1. However, the findings of another study indicated that a twelve-week circuit resistance training significantly increased the level of nesfatin-1 among overweight adolescents (19).

The aim of this study was to assess the effects of an eight-week endurance and resistance training on the plasma level of nesfatin-1, cardiorespiratory endurance, and body composition of overweight and obese females.

Methods

This pretest-posttest quasi-experimental study was conducted on 34 overweigh and obese female students who lived in the dormitories of Sistan and Baluchestan University, Zahedan, Iran. The mean of students' BMI was 30.19 ± 2.79 kg/m^2. The students voluntarily participated in the study and were randomly allocated to the control (10 students), endurance training (12 students), and resistance training (12 students) groups. The eligibility criteria were having no participation in any regular physical activity program during the 6 months prior to the study, having no history of health problems (particularly cardiovascular disease and diabetes mellitus), following no regular dietary regimen, and neither smoking cigar nor using medications which could affect the study findings.

Initially, the process of the study as well as its potential advantages and disadvantages were explained to the participants. Then, they were asked to complete a researcher-made questionnaire about their personal characteristics, medical, medicinal, and sport histories, as well as previous history of skeletal disorders. Then, we measured their height (by using a wall ruler with a precision of 0.01 meter), weight (by using the CAMRY FE551BW digital weight scale with a precision of 0.1 kg), and BFP. Weighing was performed while the participants had worn light clothing and no shoes. To calculate BFP, participants' subcutaneous fat was measured by caliper (SAEHAN, Korea), at the three points of triceps, suprailiac, and right thigh skin folds. Finally, formula 1 was used to calculate BPF (20). 'Db' in formula 1 stands for body density which was calculated by formula 2. 'S' in formula 2 is the sum of subcutaneous fat at the triceps, suprailiac, and right thigh skin folds.

$$BFP = [(4.95 / Db) - 4.5] \times 100 \quad \textbf{(1)}$$

$$Db = 1.099421 - (0.0009929 \times S) + (0.0000023 \times S2) - (0.0001392 \times Age) \quad \textbf{(2)}$$

Thereafter, we calculated body fat mass (BFM) through multiplying BFP by body weight. On the other hand, WHR was calculated before and after training through dividing waist circumference by hip circumference, both of which were measured by a non-stretchable measuring tape. For BMI calculation, weight (kg) was divided by squared height (m^2). Moreover, VO_2max was measured before and after training by employing the One Mile Walking test (the Rockport test) and using formula 3.

VO_2max (ml/kg/min) = 132.853 – (0.0769 × Weight) –

(0.3877 × Age) + (6.315 × Gender) – (3.2649 × Time) – (0.1565 × Heart rate) **(3)**

The training protocol was implemented for eight weeks—four sessions per week and 32 sessions in total. Each training session initiated by a 10-minute warm-up through jogging until reaching about 60% of maximum heart rate (HRmax) as well as doing stretching exercises. Then, specialized exercises were done by the participants based on the developed protocols and finally, the session was ended by a 10-minute jogging and stretching exercises. Several days before pretest, orientation sessions were held for students in the resistance exercise group to familiarize them with exercise stations, the principles of working with weights, the severity and the length of exercises, the number of repetitions per station, as well as inter-station and inter-set rest times. The one repetition maximum (1RM) was measured indirectly for the first four weeks in the pretest period by Brzycki's formula (21) (formula 4). Also, the 1RM for the second four weeks was calculated based on the 1RM of the fourth week.

1RM = Halter (kg) / [1.0278 – (Number of repetitions until experiencing fatigue × 0.0278)] **(4)**

An 8-station circuit resistance training was developed for the students in the resistance group. The stations were lat pull-down, bench press, leg press, calf exercise, biceps curls, leg curls, lateral raise by dumbbell, and overhead press. Exercises were performed in 2–4 whole circuit sets and at 65%–80% of 1RM. The number of repetitions per station was 8–12 and the inter-station and the inter-set rest times were respectively 60–90 seconds and 2–3 minutes. The trend of load increment was stepwise and a low decrement was induced in the fourth week in order to prevent overtraining (22).

In the endurance group, the HRmax (220 – Age) was determined before the intervention. The first session of endurance training included a 20-minute running with a 65% of HRmax. The severity and the length of training were increased weekly according to the overload principle. In other words, the length of training sessions was increased 2 minutes per week and the severity of training was increased every 2 weeks by 5%. Consequently, the length of training session at the eighth week was 34 minutes and the severity was 80% of HRmax (22). Training severity was measured by a pulsemeter (POLAR F92ti, Finland). During the course of the study, the participating students were required to avoid any kind of physical activity other than the study intervention. Students in the control group were asked to perform usual activities of daily living without receiving any training intervention.

In order to assess plasma chemistries and the plasma level of nesfatin-1, a blood sample was obtained from each participant's right hand by a laboratory technician 24 hours before the first training session and after the last one. Post-intervention sampling was performed 48 hours after the last session in order to remove the effects of training-induced acute inflammation on blood indices. Blood samples from all participants were obtained after twelve hours of fasting and at the same time everyday (08:00–09:00) in order to prevent daily fluctuations on the level of nesfatin-1. The plasma of the each blood sample was separated through centrifuging samples for five minutes at 3000 rpm and then, separated plasmas were frozen and stored at –70°C. Chemical analysis and the assessment of the plasma level of nesfatin-1 were performed by Eastbiopharm human kit (made by a Chinese-American Company) with a sensitivity of 0.15 ng/ml and the Anthos 2020 ELISA Reader (Austria).

The SPSS software (v. 16.0) was employed for data analysis at a significance level of 0.05. The normality of the study variables was assessed by conducting the Kolmogorov-Smirnov test. Moreover, the paired-samples *t* test was conducted for assessing pretest-posttest within-group variations while between-group comparisons were made via the one-way analysis of variance (ANOVA) and the least significant difference (LSD) post hoc test.

Results

The means and the standard deviations of the pretest values of the study variables in three study groups are shown in Table 1. The one-way ANOVA revealed no significant difference among the groups regarding participants' personal characteristics ($P > 0.05$).

Table 2 shows the results of between- and within-group comparisons of plasma chemistry and nesfatin-1 by

Table 1. Comparison of the Study Groups Regarding Participants' Personal Characteristics

Variable	Group			*P* Value (The One-Way ANOVA)
	Endurance Training Mean±SD	Resistance Training Mean±SD	Control Mean±SD	
Age (y)	22.81 ± 2.44	22.50 ± 2.67	21.50 ± 2.41	0.54
Height (cm)	158.55 ± 5.33	161.60 ± 7.74	159.20 ± 6.40	0.47
Weight (kg)	76.17 ± 6.81	77.96 ± 9.71	77.69 ± 13.72	0.91
BMI (kg/m^2)	30.31 ± 2.48	29.73 ± 1.49	30.51 ± 3.99	0.81
BFM (kg)	26.82 ± 5.88	26.38 ± 5.84	25.33 ± 9.60	0.89
WHR	0.82 ± 0.04	0.84 ± 0.04	0.85 ± 0.02	0.20
VO_2max (ml/kg/min)	36.32 ± 4.69	37.31 ± 3.63	36.93 ± 3.22	0.84

Abbreviations: BMI, body mass index; BFM, body fat mass; WHR, waist-to-hip ratio; ANOVA, analysis of variance.

conducting the one-way ANOVA and the paired-samples *t* test. Within-group comparisons showed that in both experimental groups, VO_2max and the plasma level of nesfatin-1 increased significantly while obesity-related indices such as weight, BFM, BMI, and WHR decreased significantly after the study. Moreover, in the control group, participants' weight, BFM, and BMI increased significantly and the level of nesfatin-1 decreased significantly after the study ($P<0.05$)

The one-way ANOVA (Table 2) indicated that pretest-posttest mean difference values of WHR, VO_2max, and nesfatin-1 in both experimental groups were significantly different from the control group ($P<0.05$), while the mean difference values of other body composition indices in the experimental groups did not significantly differ from the control group ($P>0.05$). The results of the LSD post hoc test (Table 3) showed that VO_2max in both experimental groups was significantly higher than the control group ($P<0.001$); however, the difference between the experimental groups regarding VO_2max was not statistically significant ($P=0.23$). Besides, WHR in the endurance training group was significantly lower

Table 2. The Results of Between- and Within-Group Comparisons Regarding the Level of Nesfatin-1, Cardiorespiratory Endurance, and Body Composition Indices

Variables	Groups	Pretest (Mean ± SD)	Posttest (Mean ± SD)	*P* Value (Paired-Samples *T* Test)	Mean Differences (Mean ± SD)	*P* Value (One-Way ANOVA)
Weight (kg)	Endurance	76.17 ± 6.81	74.36 ± 7.26[a]	0.003	-1.809 ± 1.511	0.627
	Resistance	77.96 ± 9.71	76.30 ± 9.43[a]	0.005	-1.660 ± 1.315	
	Control	77.6 9 ± 13.72	78.72 ± 13.36[a]	0.006	1.030 ± 0.910	
BFM (kg)	Endurance	26.82 ± 5.88	24.38 ± 5.56[a]	0.001	-2.434 ± 1.841	0.397
	Resistance	26.38 ± 5.84	24.58 ± 5.38[a]	0.001>	-1.801 ± 0.983	
	Control	25.33 ± 9.60	26.30 ± 9.68[a]	0.024	0.975 ± 1.135	
BMI (kg/m^2)	Endurance	30.31 ± 2.48	29.63 ± 2.51[a]	0.002	-0.739 ± 0.604	0.347
	Resistance	29.73 ± 1.49	29.12 ± 1.49[a]	0.004	-0.613 ± 0.468	
	Control	30.51 ± 3.99	30.92 ± 3.91[a]	0.008	0.975 ± 1.135	
WHR	Endurance	0.82 ± 0.04	0.81 ± 0.03[ab]	0.011	-0.011 ± 0.012	0.023
	Resistance	0.84 ± 0.04	0.83 ± 0.04[a]	0.013	-0.013 ± 0.013	
	Control	0.85 ± 0.02	0.86 ± 0.02	0.343	0.002 ± 0.006	
VO_2max (ml/kg/min)	Endurance	36.32 ± 4.69	43.39 ± 3.98[ab]	0.001>	7.076 ± 3.600	0.001
	Resistance	37.31 ± 3.62	41.76 ± 3.04[ab]	0.003	4.443 ± 3.581	
	Control	36.93 ± 3.22	35.97 ± 1.42	0.298	-0.967 ± 2.766	
Nesfatin-1 (ng/ml)	Endurance	13.07 ± 1.62	14.38 ± 1.70[ab]	0.001>	1.311 ± 0.666	0.004
	Resistance	12.02 ± 0.79	14.17 ± 0.64[ab]	0.001	2.142 ± 0.979	
	Control	13.24 ± 0.76	12.08 ± 0.98[a]	0.010	-1.157 ± 0.830	

Abbreviations: BMI, body mass index; BFM, body fat mass; WHR, waist-to-hip ratio; ANOVA, analysis of variance.
[a]The result of the paired-samples *t* test for within-group comparison is significant at a *P* of less than 0.05.
[b]The result of the one-way ANOVA for between-group comparison is significant at a *P* of less than 0.05.

Table 3. The Results of the LSD Post Hoc Test for Between-Group Comparisons

Variable	Groups		Mean Difference	Standard Error	*P* Value
VO_2max (ml/kg/min)	Endurance	Resistance	1.634	1.332	0.23
		Control	7.424	1.332	0.001[a]
	Resistance	Control	5.790	1.363	0.001[a]
WHR	Endurance	Resistance	-0.014	0.015	0.351
		Control	-0.044	0.015	0.007[a]
	Resistance	Control	-0.030	0.015	0.066
Nesfatin-1 (ng/ml)	Endurance	Resistance	0.217	0.633	0.735
		Control	2.303	0.633	0.002[a]
	Resistance	Control	2.085	0.671	0.006[a]

Abbreviations: WHR, waist-to-hip ratio; LSD, least significant difference
[a]Statistically significant at a *P* value of less than 0.05.

than the control group ($P = 0.007$), whereas there was no statistically significant difference regarding WHR between the resistance training and the control groups ($P = 0.066$) and between the experimental groups ($P = 0.351$). On the other hand, the study groups differed significantly from each other regarding the posttest values of nesfatin-1 ($P = 0.004$). The LSD post hoc test revealed that compared with the control group, the plasma level of nesfatin-1 was significantly higher in the endurance ($P = 0.002$) and resistance ($P = 0.006$) training groups while the difference between the experimental groups regarding the posttest values of nesfatin-1 was not statistically significant ($P = 0.753$).

Discussion

This study was among the few quasi-experimental studies in which the effects of endurance and resistance training on the plasma level of nesfatin-1 were assessed independently and concurrently. The important findings of the study were significant increase in the plasma level of nesfatin-1 and cardiorespiratory endurance as well as significant decrease in body composition indices (such as weight, BFM, BMI, and WHR) in both experimental groups. Moreover, in the control group, the plasma level of nesfatin-1 also decrease significantly and the means of weight, BFM, and BMI increased significantly after the study. Significant increase in the plasma level of nesfatin-1 in the endurance training group agrees with the findings that Chaolu et al (23) and Haghshenas et al (10) reported. All these findings denote that changes in weight, BFM, and BMI are associated with changes in plasma level of nesfatin-1. Similarly, Tsuchiya et al (6) reported a negative correlation between BMI and nesfatin-1. Haghshenas et al (10) also noted that the level of nesfatin-1 is probably correlated with physical fitness, BFP, and fat-free body mass.

We also found a significant decrease in BFM in the experimental groups which could have been a cause for significant increase in the level of nesfatin-1. Previous studies have shown that a 5%–10% decrease in visceral and subcutaneous adipose tissues following physical exercise increases the level of adiponectin adipokine (24). The findings of the present study revealed that after doing endurance and resistance training BFM decreased respectively by 9% and 6.82%, denoting that both types of training could provide adequate stimulations for decreasing BFM and the level of nesfatin-1. Nesfatin-1 is a strong anti-appetite neuropeptide which contributes to the regulation of metabolism, induces satiety, restricts food and fluid intake, and thus, causes weight loss (25). Haghshenas et al (26) found that through increasing plasma level of nesfatin-1, endurance training reduced food intake and body weight among obese male rats. Chaolu et al (23) also noted that training and a high-fat diet reduced food intake and body weight of mice through increasing the level of nesfatin-1.

The study findings also revealed a significant increase in the level of nesfatin-1 in the endurance training group. This finding contradicts the findings which Ghanbari-Niake et al (8) reported. They found that two types of single-session short-term severe physical activity programs were not effective in improving the level of nesfatin-1 among athletic individuals (8). Similarly, Bashiri et al (18) reported that a single-session aerobic training did not significantly increase elderly people's level of nesfatin-1. The contradiction between our findings and the findings of two abovementioned studies can be related to the differences in the type, severity, and length of training programs as well as the age and the gender of the participants of the studies. Moreover, non-significant change in the level of nesfatin-1 in the study conducted by Bashiri et al (18) can be attributed to low energy intake during the training program due to the short length and the great severity of their training program.

Contrary to our findings, Tofighi et al (27) found that an 8-week aerobic exercise had no significant effect on the level of nesfatin-1 among young obese men. This contradictory finding can be attributed to insignificant changes in the body weight and the BFP of their participants (27). Body weight, in turn, can be affected by the severity and the length of training as well as dietary regimen. Training severity in the study undertaken by Tofighi et al (27) was 55%–65% of VO_2max which probably could not affect these variables. In addition, Tofighi et al (27) conducted their study on young obese men with a BMI of greater than 34 while our participants were female students. Given the limited information about the expression and the functions of nesfatin-1, the exact mechanism of action of physical activity in the expression of nesfatin-1 is still unknown. Based on the findings of this and the previous studies, the level of nesfatin-1 is probably affected by long-term training, weight loss, and BFP.

As mentioned earlier, studies have shown that the level of nesfatin is affected by different factors. For instance, Stengel et al (7) found that fasting state decreased the level of nesfatin-1 in rats up to 18%. However, given the significant increase in the level of nesfatin-1 in our study, fasting (as a study limitation) had no significant effect on the level of nesfatin-1. We also found that in the circuit resistance training group, the level of nesfatin1-increased significantly. This finding is consistent with the findings that Tavassoli et al (19) reported. They found a significant increase in the level of nesfatin-1 and a significant decrease in the level of BFP after a twelve-week circuit resistance training. These findings are in line with our findings. However, while resistance training in our study significantly decreased body weight, BMI, and BFM, resistance training in their study had no significant effect on body weight and BMI (19). Given the negative correlation of BMI and the level of nesfatin-1 (6), one of the potential reasons behind significant increase in the level of nesfatin-1 in our study could be significant

decreases in BFM, BMI, and weight.

Another important finding of the present study was the significant increase in cardiorespiratory endurance in both experimental groups during the eight-week period of the intervention. Mohammadi-Damieh et al (22) and Saghebjoo et al (28) also reported a significant increase in the indicator of cardiorespiratory endurance—i.e. VO_2max. The training protocols of these two studies were similar to ours and the increase in cardiorespiratory endurance in these studies has been significant in both endurance and resistance training groups. As mentioned earlier, BFP negatively correlates with aerobic capacity (13). Accordingly, one reason behind the increased cardiorespiratory endurance in the present study could be the significant decrease in BFM.

Among the study limitations were environmental temperature, participants' motivations, psychological stress, lifestyle, endocrine hormones, genetic characteristics, and our inability to strictly manage participants' dietary regimen. All these factors might have affected the level of nesfatin-1. Given the paucity of studies on the effects of endurance training on the level of nesfatin-1 among overweight and obese human individuals, further studies are needed to understand factors affecting this adipokine during physical exercise. Future studies are recommended to assess the effects of different training programs on the levels of nesfatin-1 and other adipokines while strictly managing the intervening effects of dietary regimen.

Conclusion

The findings of the present study showed that the 8-week endurance and resistance training significantly increase the plasma level of nesfatin-1 and cardiorespiratory endurance and significantly decrease body composition indices such as weight, BFM, WHR, and BMI. As non-invasive procedures, both endurance and resistance training can exert protective effects against cardiovascular disease, type II diabetes mellitus, etc., and promote overweight and obese females' health through increasing the level of nesfatin-1 anti-inflammatory agent. Considering the significant effects of nutrition on the expression and the secretion of nesfatin-1, future studies are recommended to strictly manage participants' dietary regimens and assess their effects on the level of nesfatin-1.

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