

## The effect of rope jump training on serum levels of lipocalin-2, anthropometric parameters, and aerobic power in obese adolescent boys

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Received; 16/12/2019 Revised; 16/03/2020 Accepted; 28/05/2020

### Abstract

**Introduction:** Lipocalin-2 derived from adipose tissue is associated with insulin resistance, systemic inflammation, and cardiovascular disease in obese people. The current study aimed to investigate the effect of rope jump exercise training on serum lipocalin-2 levels, anthropometric parameters, and maximal oxygen uptake (VO<sub>2</sub>max) in obese adolescent boys.

**Materials and methods:** Twenty obese adolescent boys participated voluntarily in the study and were equally assigned to control and rope jump groups. Rope jump exercise protocol was practiced in 8 weeks, 3 days per week. Blood samples were taken before and after the program. The VO<sub>2</sub>max of each participant was estimated using Cooper's 12-minute run test. Serum lipocalin-2 levels were measured using a commercial ELISA kit.

**Results:** Within-group comparisons revealed that rope jump exercise training resulted in a significant reduction in weight (P = 0.005), BMI (P = 0.002), abdominal circumference (P = 0.001), body fat percentage (P = 0.001), and serum levels of lipocalin-2 (P = 0.002). In contrast, lean body mass (LBM) (P = 0.047) and VO<sub>2</sub>max (P = 0.007) increased significantly in the rope jump group. After the intervention, abdominal circumference (P = 0.001), body fat percentage (P = 0.001), weight (P = 0.001), and BMI (P = 0.001) were significantly lower in the rope jump group than in the control group. VO<sub>2</sub>max in the rope jump group was significantly higher than in the control group at the end of the exercise training protocol (P = 0.001). However, no significant differences were found between the groups concerning lipocalin-2 level (P = 0.105), waist-to-hip ratio (P = 0.461), and LBM (P = 0.053) at the end of the protocol.

**Conclusion:** While rope jump exercise training enhanced anthropometric parameters and aerobic power in obese adolescents, it failed to significantly alter the serum lipocalin-2 level, as an inflammatory marker.

**Keywords:** Rope jump training, Lipocalin-2, Adolescents, Obesity

### Introduction

Obesity is widely regarded as the most significant health challenge in the 21st century (1). Increased obesity in humans is associated with an increased risk of developing type 2 diabetes, cardiovascular

disease, liver disease, airway disease, neurological disease, biliary disease, and cancer. Obesity-related diseases lead to lower life expectancy and, consequently, premature death (2, 3). The risk of obesity is immense and frightening worldwide, as it can affect all age groups, including children and

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adolescents (4, 5). According to the World Health Organization, the number of obese children and adolescents has increased from about 11 million in 1975 to 124 million in 2016. Iran has been shown to be one of the seven countries with the highest prevalence of childhood obesity (6). From 1995 to 2007, 8.82 percent of Iranian adolescents were reported as overweight and 4.5 percent as obese (7). The rates were 10.8% and 5.1% for overweight and obesity, respectively, during the 2007-2012 period, indicating that the prevalence of obesity among Iranian adolescents has increased in recent years (8, 9). Obesity has become widespread in many developed and developing countries. Studies have revealed a strong relationship between the risk of mortality from cardiovascular disease and obesity, which is not merely confined to adults but also affects obese children and adolescents (10). Research suggests that obese children over the age of 10 are at an increased risk for metabolic disorders, heart disease, and dyslipidemia (11). Obesity is associated with the secretion of inflammatory cytokines and with chronic inflammation in the adipose tissue, which results from fat accumulation in adipocytes (12). The adipose tissue secretes bioactive molecules called adipokines, which are conducive to maintaining stable whole-body conditions (13).

Over the past two decades, the interrelationships between adipokines, metabolic syndrome, and inflammatory diseases have been better understood. In this regard, lipocalin-2 has been suggested as one of the mediators responsible for systemic inflammation and obesity-related metabolic syndrome (14, 15). Lipocalin-2 (neutrophil gelatinase-related lipocalin), a 25-kDa protein belonging to the large family of lipocalins, is produced as an inflammatory marker in large quantities in adipocytes and is a marker associated with inflammation, ischemia, infection, and kidney damage (15).

A positive relationship has been demonstrated between serum lipocalin-2 levels and the variables of obesity, hypertriglyceridemia, hyperglycemia, and C-reactive protein (CRP) levels. The lipocalin-2 mRNA and protein expression increase substantially in the adipose tissue and are positively correlated with the expression of proinflammatory cytokines (16). In obesity and the related disorders, increased expression of various proinflammatory cytokines such as interleukin-6, interleukin-1 beta, and tumor necrosis factor-alpha is strongly involved in the regulation of lipocalin-2 (15). A significant increase in lipocalin-2 levels has been reported in coronary heart disease and inflammatory diseases (17, 18, 19). Serum concentrations of lipocalin-2 are positively correlated with body mass index (BMI), body fat percentage, waist-to-hip ratio (WHR), and waist circumference and are higher in obese than in lean individuals (16, 17, 20). It has also been shown that serum levels of lipocalin-2 are higher in obese than normal-weight children and adolescents and that its concentration is closely related to obesity and its associated metabolic complications so that the measurement of this marker is vital for the evaluation of metabolic syndrome in children and adolescents (21).

Concerning the effect of exercise on lipocalin-2 levels, researchers have shown that after 8 weeks of endurance and resistance exercise, lipocalin-2 levels decrease significantly in healthy young men (22). Another study has found that 8 weeks of aerobic exercise decreases lipocalin-2 levels in healthy overweight men (23). Choi et al. (2009) reported no significant difference in lipocalin-2 levels after 3 months of aerobic and strength training (17). In one study, a decrease in serum levels of lipocalin-2 has been demonstrated in overweight and obese women with polycystic ovary syndrome after six months of a weight loss program (24).

Immediately after vigorous exercise, Lippi et al.'s study (2012) showed a 1.6-fold increase in serum lipocalin-2 and a 7.7-fold increase in urine lipocalin in marathon runners (25).

One of the exercises that can be used by adolescents is rope jumping. Studies have indicated the enjoyment, safety, and convenient learning of rope jump exercises by adolescents (26). Rope jumping is not only a game for children but is also a common exercise. Among the benefits of rope jumping is that it can be performed by individuals irrespective of time, place, and season. It can be practiced in a variety of ways, both individually and in groups. In terms of facilities, rope jumping merely requires one rope and is relatively inexpensive compared to other sports; it is also one of the most appropriate types of basic fitness training (27). Rope jump exercises have been shown to enhance physical fitness and improve BMI in children and adolescents (28). Given the increased urbanization and lack of space in homes and schools, rope jump exercises can be an excellent alternative to activities such as running in limited spaces; it is useful to promote physical fitness at home and in schools and training centers that are spatially confined, especially during childhood and adolescence (29). Cardiovascular disease and type 2 diabetes are rooted in childhood, and it is essential to provide early intervention in lifestyle, especially for obese children and adolescents (30). There is limited information concerning the effect of rope jump training on inflammatory markers, especially lipocalin-2. What is known is that a positive association holds between serum lipocalin-2 levels and anthropometric parameters such as BMI, body fat percentage, waist circumference, and WHR (16, 17). Therefore, the present study sought to answer the question of whether or not rope jump exercise can have a significant effect on anthropometric indices, aerobic power, and,

subsequently, the lipocalin-2 inflammatory marker, which is reported to be of a higher level in obese than normal-weight adolescents.

### Materials and methods

This quasi-experimental study incorporated voluntarily 20 obese inactive male subjects from Bojnord city (age:  $13.40 \pm 1.09$  years; height:  $154.70 \pm 8.31$  cm; BMI:  $27.12 \pm 2.19$  kg/m<sup>2</sup>; body fat:  $26.78 \pm 3.19\%$ ). Obesity was determined according to the US Centers for Disease Control and Prevention (CDC) criteria. At baseline, the subjects completed the Baecke Physical Activity Questionnaire. Inclusion criteria consisted of obesity (having a BMI above 95% of the CDC criteria as per age and male gender), inactivity (not participating in a regular exercise program for the past 6 months), absence of cardiovascular and metabolic disease, and non-consumption of exercise and nutritional supplements. After the subjects' guardians were informed about the program, its benefits, and potential risks, they signed a consent form for the participation of their children in the study. Subjects pledged to have persistent participation in the program, to observe what was required of them, and to refrain from participating in other exercise programs. They were given the right to leave the program as long as they felt anxious about performing the protocol. All subjects were instructed on how to jump rope in the course of two weeks (three sessions per week). After their height and weight were measured and their BMI calculated, the subjects were matched for BMI and were divided into control (n = 10) and rope jump (n = 10) groups.

Height and weight were measured by digital and calibrated height and weight gauges in Javad Al-A'emeh Hospital in Bojnourd (Iran) at baseline and 48 hours after the last training session. Subjects' weight was measured in fasting conditions with their bladder empty

and the subjects having minimal clothing coverage (31). Moreover, when assessing body composition, the subjects were asked not to consume food or caffeine (32). BMI was calculated by weight ratio in kg / m<sup>2</sup>. Waist circumference was measured at the end of a natural expiration at the point between the last rib and iliac crest. Hip circumference was measured around the widest portion of the hips, and WHR was calculated as waist measurement divided by the hip measurement. Abdominal circumference was measured using a tape meter in the most prominent abdominal area. Jackson and Pollock's three-point method was employed to calculate body density. In this method, the subcutaneous fat thickness of the chest, thigh, and abdomen was measured using a Yagami caliper (made in Japan). Finally, the Siri equation was used to calculate body fat percentage (33). Lean Body Mass (LBM) is a component of body composition, which was calculated from the fraction of body fat weight from the total body weight. Moreover, Cooper's 12-minute test was applied to measure aerobic power (34).

The subjects in the rope jump group performed the exercise protocol for 8 weeks, 3 sessions per week. The exercise protocol of

this study is modeled after the ones used in similar studies on the effect of rope jump training in obese adolescents (35), with slight changes considered in the current study after the pilot study (Table 1). The subjects were familiarized with and trained as to the manner of rope jumping in the first two weeks of the program. Instructions involved issues such as rolling the hands in circles, rotating the rope with the left and right hands on the side, rotating the rope with both hands in the front and over the head, turning the rope with the hands on the sides to form an '8', turning the rope with the right hand and left hand in sequence and jumping simultaneously, and turning the rope in the front and jumping. Moreover, tips were highlighted such as jumping for 3 to 5 cm above the ground so that the rope passes below the feet and non-landing on the heel, similar to the recommendations made in other studies (28). At the beginning and at the end of each training session, five minutes of warm-up and five minutes of cooling down were performed by stretching. Subjects in the control group performed daily activities and were prohibited from performing any exercise.

**Table 1.** Rope jumping exercise training protocol.

<b>Week</b>	<b>The main body of the exercise</b>	<b>Intensity (number of jumps / min)</b>
1	Training how to jump rope	Instructions on the technique
2	Training how to jump rope	Instructions on the technique
3	Twenty 1-minute sets with a 30-second rest between sets	40-50
4	Twenty 1-minute sets with a 30-second rest between sets	50-60
5	Fifteen 1.5-minute sets with a 30-second rest between sets	50-60
6	Ten 2.5-minute sets with a 30-second rest between sets	60-70
7	Eight 3-minute sets with a 30-second rest between sets	70-80
8	Six 4-minute sets with a 60-second rest between sets	80-90
9	Six 4-minute sets with a 60-second rest between sets	90-100
10	Five 5-minute sets with a 60-second rest between sets	100-110

Five cc blood samples were taken from subjects in fasting conditions (12 hours) at baseline and 48 hours after the last training session at Javad Al-A'emeh Hospital (Bojnord, Iran) from 8 to 9 a.m. after a 30-minute rest on the chair. Blood samples were

centrifuged at 4 °C for 10 minutes at a speed of 3000 rpm (24T centrifuge, Aram Gostar Co., Iran). The centrifuged sera were poured into 0.5 ml microtubes (Kariz Mehr Co., Iran) and transferred under refrigerated conditions to Gonabad University of Medical Sciences

for biochemical evaluation and stored at  $-80^{\circ}\text{C}$ . Biochemical evaluation of lipocalin-2 serum content was performed by a commercial 96-well lipocalin-2 human kits (ZellBio GmbH, Germany, Catalog No: ZB-11429C-H9648) with a sensitivity of  $10\text{ ng/L}$  and a detection range of  $200$  to  $6400\text{ ng/L}$  using the Sandwich ELISA based on the antibody-antigen reaction. Serum concentrations of lipocalin-2 were read at  $450\text{ nm}$  by the BioTek microplate reader (Epoch2 Model, USA).

### Statistical analysis

Statistical analysis was performed using SPSS software version 16 (SPSS Institute, Chicago, USA). The significance level was set at  $P < 0.05$ . Given the low number of subjects, the normal distribution of the data was assessed by the Shapiro-Wilk test. The dependent t-test was employed to examine within-group differences of the variables with a normal distribution. Wilcoxon statistical test was used to assess within-group differences in variables that did not have a normal distribution. Also, the analysis of covariance (ANCOVA) test was applied to better control for between-group differences. Results are presented as mean  $\pm$  standard deviation.

### Results

Concerning anthropometric variables, within-group assessments revealed no significant change in weight ( $P = 0.261$ ), BMI ( $P = 0.257$ ), WHR ( $P = 0.226$ ), abdominal circumference ( $P = 0.917$ ), body fat content ( $P = 0.430$ ) and percentage ( $P = 0.375$ ), and LBM ( $P = 0.867$ ) in control group. However, within-group assessments showed that 8 weeks of rope jump training reduced weight ( $P = 0.005$ ), BMI ( $P = 0.002$ ), abdominal circumference ( $P = 0.001$ ), fat percentage ( $P = 0.001$ ), and body fat ( $P = 0.001$ ) in obese adolescents, although it did not affect WHR values ( $P = 0.296$ ). In

contrast, the LBM of obese subjects increased significantly after rope jump training ( $P = 0.047$ ). Between-group evaluations showed that there was a significant difference between abdominal circumference size ( $P = 0.001$ ), body fat content ( $P = 0.001$ ) and percentage ( $P = 0.001$ ), weight ( $P = 0.001$ ), and BMI ( $P = 0.001$ ) of the two groups at the end of the training. However, there was no significant difference in WHR ( $P = 0.461$ ) and LBM ( $P = 0.053$ ) between rope jump and control groups at the end of the training period (Table 2).

Regarding aerobic power, within-group assessments showed that  $\text{VO}_2\text{max}$  value was significantly higher in the rope jump group at post-test ( $26.68 \pm 6.37\text{ ml/kg/min}$ ) than baseline ( $21.12 \pm 3.41\text{ ml/kg/min}$ ) ( $P = 0.007$ ). However, there was no significant difference between  $\text{VO}_2\text{max}$  values before ( $22.38 \pm 3.60\text{ ml/kg/min}$ ) and after the intervention ( $21.68 \pm 3.26\text{ ml/kg/min}$ ) in the control group ( $P = 0.113$ ). Between-group assessments showed that the values in the rope jump group were higher than in the control group at the end of the training period ( $P = 0.001$ ) (Figure 1).

In the case of lipocalin-2, within-group assessments showed that rope jumping decreased serum levels after the intervention ( $1113.54 \pm 205\text{ ng/l}$ ) as compared with baseline ( $1247.72 \pm 211\text{ ng/l}$ ) ( $P = 0.002$ ). However, there was no significant difference in serum lipocalin-2 levels at baseline ( $1104.05 \pm 182\text{ ng/l}$ ) and after the protocol ( $1129.38 \pm 99\text{ ng/l}$ ) in the control group ( $P = 0.647$ ). Moreover, between-group assessments indicated that there was no significant difference as for serum lipocalin-2 levels between the two groups at the end of the training period ( $P = 0.105$ ) (Figure 2).

### Discussion

Within-group results showed that rope training increased LBM and  $\text{VO}_2\text{max}$  levels,

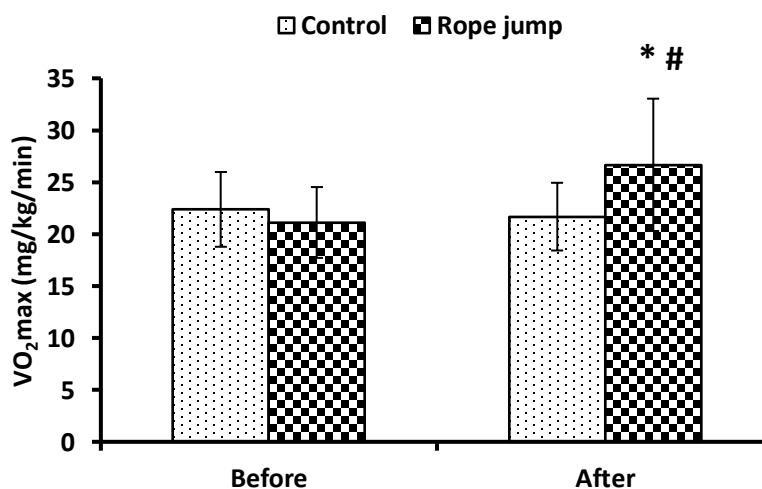
while it significantly reduced the weight, BMI, abdominal circumference, body fat content, body fat percentage, and serum lipocalin-2 levels of obese adolescents. Between-group evaluations showed no significant difference in lipocalin-2, WHR, and LBM levels between the rope jump and control groups at the end of the training

period. However, abdominal size, body fat content, body fat percentage, weight, and BMI at the end of the training session were significantly lower in the rope jump group than in the control group, and VO<sub>2</sub>max mean value of the rope jump group were significantly higher than that of the control group.

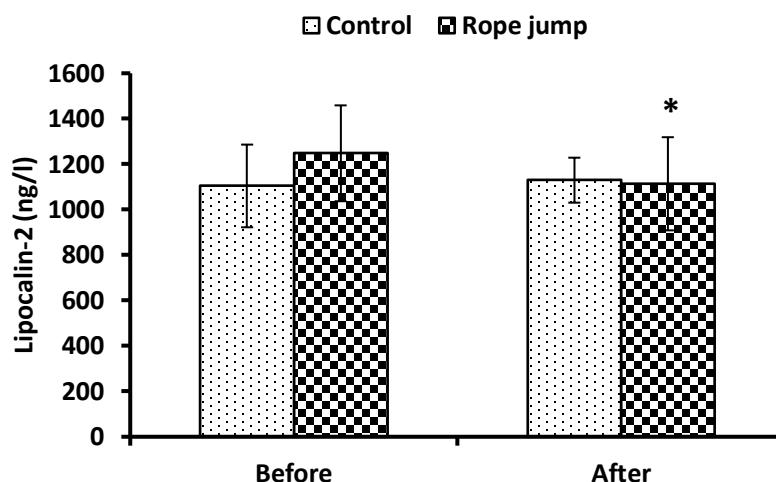
**Table 2.** Within-group and between-group assessment results for anthropometric variables.

Variable	group	Mean ± standard deviation		Interquartile range	
		Before	After	Before	After
Weight (Kg)	Control	67.56 ± 11.61	67.81 ± 11.29	23.08	22.32
	Rope jump	62.93 ± 8.60	60.83 ± 9.06*#	7.60	8.65
BMI (kg/m <sup>2</sup> )	Control	27.58 ± 2.74	27.69 ± 2.64	4.84	4.42
	Rope jump	26.65 ± 1.48	25.76 ± 2.02*#	2.48	3.86
WHR	Control	0.89 ± 0.06	0.88 ± 0.06	0.12	0.14
	Rope jump	0.86 ± 0.030	0.85 ± 0.032	0.03	0.07
Abdominal circumference (cm)	Control	91.60 ± 7.78	91.65 ± 7.86	15.88	14.50
	Rope jump	87.35 ± 4.51	83.85 ± 4.69*#	7.88	8.12
Body fat (%)	Control	26.48 ± 3.23	26.75 ± 3.08	6.52	6.38
	Rope jump	27.08 ± 3.30	22.96 ± 4.02*#	4.99	7.73
Body fat (Kg)	Control	18.07 ± 4.69	18.27 ± 4.36	9.05	9.41
	Rope jump	17.13 ± 3.60	14.18 ± 4.13*#	3.71	5.28
LBM (Kg)	Control	49.51 ± 7.69	49.53 ± 7.70	12.94	12.50
	Rope jump	45.79 ± 5.76	46.64 ± 5.47*	6.22	5.81

The \* mark indicates a significant difference from baseline; the # mark denotes a significant difference with the control group at the end of the study. Abbreviations: BMI = Body Mass Index; LBM, Lean Body Mass; WHR: Waist-to-Hip Ratio.



**Figure 1.** VO<sub>2</sub>max values of inactive obese adolescents. Abbreviations: VO<sub>2</sub>max, maximal oxygen uptake. The \* mark denotes a significant difference from baseline; the # symbol stands for a significant difference with the control group.



**Figure 2.** Serum lipocalin-2 levels in inactive obese adolescents. The \* mark denotes a significant difference from baseline.

In a three-month study of an exercise protocol consisting of aerobic exercise at 60 to 75 percent of maximal heart rate (approximately 300 kcal/day) and muscle strength training (approximately 100 kcal/day) per session five times a week in inactive obese women, no significant change in lipocalin-2 levels was reported (17). In similar lines and consistent with our results, no significant change in lipocalin-2 levels was reported after 8 weeks of progressive resistance training in the range of 20% to 90% of maximal repetition four sessions a week in overweight and obese men (36). In the present study, although anthropometric parameters such as BMI, body fat content, and body fat percentage were significantly decreased, lipocalin-2 levels did not change significantly. Lipocalin-2 is expressed and produced in cells and tissues other than the adipose tissue, such as macrophages, endothelial cells, monocytes, and hepatocytes (37-39). Therefore, a lack of change in lipocalin-2 levels may be due to the different responses of these tissues to exercise activity, which requires further study. Moreover, it is likely that lipocalin-2 levels in this study were affected by WHR

and LBM indices, as they did not change significantly over time.

The results of the present study are not in line with those of a study conducted with inactive healthy young men for 8 weeks in two groups of resistance training (65-80% of maximal repetition) and aerobic training (65-80% of maximal heart rate), where reduced serum lipocalin-2 levels were reported in both groups (22). Another study showed a decrease in serum levels of lipocalin-2 after 8 weeks of aerobic exercise with an intensity of 65 to 80% of maximal heart rate in obese men aged 27 years on average (23). Following a six-month weight loss program, including low-calorie diet and exercise, another study reported lower serum levels of lipocalin-2 in obese women with polycystic ovary syndrome (24), which is inconsistent with the results of our study. Consumption of saturated fatty acids is shown to alter lipocalin-2 levels (40). Hence, the different results found in different studies may be associated with the difference in dietary types adopted by participants in the studies, which needs further investigation.

Individual differences of subjects, age, nutritional status, and intensity and duration of exercise may be other reasons for the

inconsistency of results with those of the present study. It should be noted that in the current study, serum levels of lipocalin-2 decreased after the rope jump training protocol, although the decrease was not statistically significant. As the levels of this molecule were significantly reduced in studies with greater weight loss and body fat percentage, longer exercise duration and a more substantial decrease in body weight and fat percentage in the present study could probably lead to a significant change in lipocalin-2. This, however, requires further research. In a study by Damirchi et al. (2011), a significant increase in serum levels of lipocalin-2 was found after the implementation of the Bruce protocol in obese and inactive normal-weight men (41). Increased serum and urine levels of lipocalin-2 after 60 km of running have been demonstrated in trained male athletes (25), the results of which are inconsistent with those of the present study. Elevated serum levels of lipocalin-2 in these studies may be due to inflammation after intense exercise, which is associated with a significant increase in CRP and WBC levels (41), as inflammatory markers. The lipocalin-2 expression has been found to increase in the face of oxidative stress and inflammatory factors (38, 42) and to decrease after 48 hours toward basal levels (43). Therefore, the elevated levels of this molecule in the two studies mentioned above may indicate increased inflammation caused by vigorous exercise. In sum, in the present study, despite the decreased levels of lipocalin-2 in the exercise group, no significant differences were found in the levels of this molecule between the two groups in obese adolescents. Significant reductions in body fat percentage have been reported after 8 weeks of rope jump training in inactive overweight and obese girls, aged 20 to 25 years and boys with the mean age of 11 years (29), which is consistent with the results of our study.

Another study shows a significant decrease in waist circumference, hip circumference, weight, BMI, body fat content, and body fat percentage after 6 weeks of rope jump training, five sessions per week, in obese adolescent boys (35). Catecholamines and growth hormone have been shown to increase during exercise, whereby the lipolysis content is increased. On the other hand, aerobic exercise increases the density of beta-adrenoceptors in the adipose tissue, which improves the sensitivity of the lipolysis process (44). While these aspects have not been examined in the present study, they may be among the potential mechanisms for improvement in anthropometric parameters. A 12-week rope jump training of 13- to 15-year-old students with thought disorders and a 10-week rope jump training of 13 to 15-year-old students with visual impairments led to no significant change in body mass index (27, 45), which are inconsistent with the results of our study. The inconsistent results can be attributed to the fact that the subjects in these studies had normal BMI, while the adolescents were obese in our study. In short, rope jump training seems to be one of the best exercises to improve anthropometric parameters in overweight and obese adolescents.

Aerobic training significantly increases mitochondrial oxidative enzymes and creates conditions where body tissues can have more oxygen available, thereby increasing the  $VO_2$ max and aerobic power (44). Alongside this, studies have shown a significant increase in  $VO_2$ max after 8 weeks of rope jump training in inactive girls (46), which is consistent with the results of our study. A significant increase in aerobic power and cardiorespiratory endurance has been reported after 8 weeks of rope jump training in boys with an average age of 11 years and after 12 weeks of rope jump training in students with thought disorders (29, 44). Reports indicate increased aerobic power and



capacity after 10 weeks of rope jump training in 10- to 12-year-old boys as well as students with visual impairment ranging in age from 15 to 17 years (27, 28), which corresponds with the results of the current study. In summary, the results of the present study showed that rope jump training increased aerobic fitness in obese male adolescents.

### Conclusion

Overall, in the present study, eight weeks of rope jump training led to significant reductions in weight, BMI, abdominal size, body fat content, and body fat percentage in obese adolescents. However, serum levels of lipocalin-2, WHR, and LBM did not change significantly. It was also shown that aerobic power significantly increased after this training protocol. In fact, although anthropometric indices improved and aerobic fitness increased after 8 weeks of rope jump

training in obese adolescents, serum levels of lipocalin-2, as an inflammatory marker, did not change significantly.

### Acknowledgments

We appreciate all the subjects who assisted the researchers in conducting the study.

### Ethical approval

The study was approved by the Institutional Ethical Committee for Human Use (IR.IAU.BOJNOURD.REC.1399.003).

### Conflicts of interest

The authors declare that they have no conflict of interest.

### Funding/Support

The University of Bojnord supported this study.

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