

The effect of resistance training with and without blood flow restriction on serum follistatin and leptin levels in active obese girls

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Abstract

Purpose: Plasma follistatin and leptin proteins are important indicators in energy and metabolism regulation that also affect body weight. The aim of this study was to investigate the effect of resistance training with and without blood flow restriction on serum follistatin and leptin levels in inactive obese girls. **Method:** Thirty inactive obese girls with an age range of 20 to 28 years participated in this study. The subjects were randomly divided into 3 groups: resistance training with blood flow restriction and resistance training without blood flow restriction, and a control group (10 subjects in each group). Data analysis was performed using paired t-tests and one-way ANOVA at a significance level of less than 5%. **Results:** After 12 weeks of participation in sports activity, follistatin levels in both resistance training groups with and without blood flow restriction increased significantly after training ($p < 0.05$). On the other hand, leptin levels decreased significantly in these groups after training interventions ($p < 0.05$). Regarding the main effect of groups, the results showed that there was a significant difference between the types of resistance training and the control group in follistatin ($P = 0.036$) and leptin ($P = 0.015$) levels. **Conclusion:** The findings indicate that resistance training can lead to an increase in plasma follistatin levels and a decrease in leptin in inactive obese girls. It is recommended that obese individuals use such resistance training with blood flow restriction as a non-invasive, low-cost, and side-effect-free method to promote health and lose weight.

Keywords: Resistance Training, Follistatin, Leptin, Blood Flow Restriction.

Introduction

Obesity is associated with high blood pressure, high cholesterol, heart disease, and tumors (Powell-Wiley et al., 2021). In addition, obesity increases the risk of diseases such as musculoskeletal disorders, hyperlipidemia, and liver disease, thereby reducing quality of life and shortening life expectancy (El Meouchy et al., 2022). Obesity also imposes costs in lost productivity and projected economic growth due to lost workdays, reduced workplace productivity, mortality, and permanent disability (Armstrong et al., 2022).

Resistance training combined with blood flow restriction can be an effective method of treating obesity and achieves similar results to high-intensity exercise (Zhuang, Shi, Liu, He, & Chen, 2023). In this type of training, the blood flow to the active muscle during exercise is restricted or stopped by tying a flexible rubber cuff or tourniquet around the proximal part of the arm or thigh (Ipavec, Žargi, Jelenc, & Kacin, 2019). The intensity of these exercises is usually considered to be 20 to 30 percent of one repetition maximum (approximately equivalent to the intensity of daily activity of individuals), so it is tolerable for people with different physical characteristics (Jønsson et al., 2024). In sports exercises with blood flow restriction, the reduction in blood flow leads to a decrease in oxygen flow; therefore, in these exercises, the force produced by fast-twitch muscle fibers plays an important role in increasing strength and muscle hypertrophy (Pearson & Hussain, 2015). In other words, in exercises with blood flow restriction, fibers that have a greater potential for increasing muscle growth (fast-twitch fibers) are called upon (Wernbom & Aagaard, 2020). Therefore, this type of training in young people may be a more suitable method for controlling obesity because it does not interfere with other treatments. Follistatin protein is another factor associated with muscle mass (Khalafi, Aria, Symonds, & Rosenkranz, 2023). Follistatin is a glycosylated plasma protein and a member of It is a large family that plays a role in the regulation of skeletal muscle mass by binding to myostatin (Parfenova, Kukes, & Grishin, 2021). Follistatin, by binding to myostatin, prevents

myostatin from binding to the activin receptor, and as a result, neutralizes myostatin in the blood circulation (Rodgers & Ward, 2022). The neutralization of myostatin by follistatin has a significant effect on skeletal muscle growth (Zhu et al., 2011). Increased expression of follistatin increases muscle hypertrophy through the activation of satellite cells, inhibition of myostatin, and interaction with other regulatory proteins (Gilson et al., 2009). Leptin is derived from the Greek word leptos, meaning thin, and was discovered in 1994 with the isolation of the leptin gene (Murawska-Ciałowicz, Kaczmarek, Kałwa, & Oniszczyk, 2022). This substance is a protein hormone with a helical structure similar to cytokines, which is mainly secreted by subcutaneous fat cells in a pulsatile manner (Boa, Yudkin, Van Hinsbergh, Bouskela, & Eringa, 2017). It is synthesized and released at a steady state, with a peak secretion near midnight (Becerril et al., 2019). Since the increase in plasma leptin concentration is proportional to the fat content of fat cells and the degree of obesity is influenced by dietary interventions or daily exercise activity, leptin can report the long-term status of body fat tissue accumulation to the brain (De Blasio et al.). Potential regulators of leptin secretion include exercise-induced stress, changes in fuel metabolism, systemic hormone concentrations, and the effect of energy expenditure. A decrease in fat mass is among the reasons why leptin levels change (Huh, 2018). Although some studies have shown that short-term exercise cannot affect leptin secretion, short-term changes caused by exercise (Ilias, Pantea, Căiță, & Pantea-Roșan, 2023). Exercise has an effect on energy balance and leptin secretion at night (Hagobian, Sharoff, & Braun, 2008).

However, there is still no general consensus regarding the intensity and duration of such exercises and achieving optimal adaptations with the minimum duration of exercise, as well as the applicability of these exercises (Kao et al., 2021). Although a few studies have confirmed the beneficial effects of resistance training with blood flow restriction on reducing obesity, the benefits of resistance training with blood flow restriction in obese individuals are still unclear. Therefore, examining the effectiveness of such exercises can determine the effectiveness of

this therapeutic strategy at the appropriate stage of obesity control and can be considered as a pre-treatment or a complementary treatment method (Ismail, Tolba, & Felaya, 2021). Therefore, the present study investigated the effect of a session of resistance training with and without blood flow restriction on serum levels of follistatin and decorin in middle-aged obese women.

Methods

The statistical sample of this study was 30 inactive obese girls who volunteered through a call in the city's administrative and public centers with an age range of (20-28 years) who were selected purposefully and participated in a 12-week study. Participants were randomly assigned to an equal number of 10 in the resistance training group without blood flow restriction, resistance training with blood flow restriction, and control. Then, the participants were asked to complete a medical information questionnaire and sign a written consent form. Inclusion criteria included obese girls with a body mass index above 30, non-smoking, no metabolic diseases such as cardiovascular diseases, diabetes, kidney problems, thyroid and rheumatic diseases, an age range of 20 to 28 years, and no history of participating in regular sports. Exclusion criteria included the occurrence of conditions that restricted exercise performance, such as neurological, muscular, or skeletal problems during the tests, failure to participate in the exercise program for more than 3 consecutive sessions or a total of 4 sessions, and possible dissatisfaction of the subject with continued participation. In the first session, the correct way to perform the exercises was explained to the participants. Data were collected before and after 8 weeks.

Exercise program

The exercise protocol included 3 sessions of resistance training per week for 12 weeks. Resistance training in both groups was 10 biceps strength exercises with a barbell. Resistance training with blood flow

restriction included performing forearm movements with an intensity of 30% of one repetition maximum (1-RM) with a cuff pressure (circumference of the area close to the arm trunk) of about 120 to 160 mm Hg, and the cuff pressure that restricted blood flow for each individual depended on their systolic pressure. Resistance training without blood flow restriction (exercise with an intensity of 75% of one repetition maximum) was performed for the group. For follistatin and leptin measurements, the first blood sample (5 ml) was taken from the right antecubital vein before exercise. The second blood sample was taken 48 hours after 12 weeks. Each training session lasted 50 minutes, starting with a 10-minute warm-up including slow walking and stretching, followed by the main body of the exercise for 30 minutes, and ending with a 10-minute cool-down including slow walking, stretching, and muscle relaxation. The performance characteristics of resistance training are shown in Table 1. The subjects in the control group also continued their normal lives during the study and were advised to refrain from regular physical activity and unusual diets during the study period.

Table 1: Exercise protocols

Resistance training variables	Training group without blood flow	restriction Training group with blood flow restriction
Exercise intensity	20-80%	20-30%
Number of movements	6	6
Number of sets	3	3
Number of repetitions	10-12	10-12
Rest interval between sets	1 minute	1 minute

Data collection and statistical method

First, the subjects' anthropometric indices, including height (using a Seca model 235 height gauge) and weight (using a Seca model 767 digital scale), were measured. Blood samples of 5 cc were taken from the left arm vein of each subject 24 hours before the first training session and 48 hours after the last training session and after 12 hours of fasting at a certain time of day (between 8 and 10 am). Then, the ELISA method (Enzyme-linked immunosorbent assay) and laboratory kits manufactured by Axis_shield diagnostic company, Germany, were used.

To examine the normality of the data in different groups, the Shapiro-Wilk statistical test was used, and to examine the homogeneity of variances, the Levine test was used. Also, to examine the intragroup changes of the measured variables, the paired t-test was used, and the one-way ANOVA statistical test was used to examine the differences between the groups in the two pre-test and post-test stages. Post-test was used. The significance level of the present study was considered to be $P < 0.05$. All data were analyzed using SPSS version 26 software.

Results

Before testing the research hypotheses, the normality of the data distribution and also the homogeneity of variance between groups were examined. The results showed that the data distribution for all variables in the experimental and control groups was normal. The results of the Leven test also showed that the assumption of homogeneity of variance was valid ($P < 0.05$). The anthropometric characteristics of the two groups in the pre-test and post-test assessments are presented in Table 2. The groups were also homogeneous in terms of age, body mass index and fat percentage.

Table 2: Anthropometric and physiological indices of the studied groups

Variable	group	Stages	Mean + SD	P Value between groups
Age (years)	Exercise with blood flow restriction	Pre-test	24.5 ± 12.71	0.451
		Post-test	-----	
		P within group		
	Exercise without blood flow restriction	Pre-test	24.5 ± 87.52	
		Post-test	-----	
		P within group		
	Control	Pre-test	24.5 ± 17.12	
		Post-test	-----	
		P within group		
Body mass index (kg/m ²)	Exercise with restricted blood flow	Pre-test	33.0 ± 61.54	0.035
		Post-test	31.0 ± 50.52	
		P within group	0.001	
	Exercise without restricting blood flow	Pre-test	32.1 ± 85.24	
		Post-test	31.2 ± 98.11	
		P within group	0.022	
	Control	Pre-test	31.1 ± 15.26	
		Post-test	31.2 ± 18.90	
		P within group	0.252	
In body fat (%)	Exercise with restricted blood flow	Pre-test	36.1 ± 87.15	0.039
		Post-test	33.1 ± 42.19	
		P within group	0.034	
	Exercise without restricting blood flow	Pre-test	37.1 ± 31.92	
		Post-test	35.1 ± 20.95	
		P within group	0.003	
	Control	Pre-test	35.1 ± 24.36	
		Post-test	35.1 ± 17.12	
		P within group	0.287	

Exercise intervention for subjects based on different groups are shown in Table 3. The results of this table show that there is no significant difference between the groups at baseline. Considering the main effect of time (after 12 weeks of training compared to pre-test), follistatin levels in both resistance training groups increased significantly after training ($p < 0.05$). On the other hand, leptin levels in these groups decreased significantly after training interventions ($p < 0.05$). In addition, considering the main effect of groups, the findings also showed that there is a significant difference between the resistance training groups and the control group in follistatin ($p < 0.036$) and leptin ($p < 0.015$) levels. (Table 3)

Table 3: Comparison of mean and standard deviation of dependent variables of the study before and after 12 weeks of training intervention

Variable	group	Stages	Mean + SD	P Value between groups
Follistatin (ng/L)	Exercise with blood flow restriction	Pre-test	16.3 ± 10.82	0.036
		Post-test	20.7 ± 33.44	
		P within group	0.026	
	Exercise without blood flow restriction	Pre-test	20.3 ± 08.46	
		Post-test	25.3 ± 25.29	
		P within group	0.005	
	Control	Pre-test	17.1 ± 24.36	
		Post-test	17.1 ± 17.12	
		P within group	0.387	
Leptin (ng/ml)	Exercise with restricted blood flow	Pre-test	24.12 ± 42.93	0.015
		Post-test	17.10 ± 33.55	
		P within group	0.016	
	Exercise without restricting blood flow	Pre-test	32.13 ± 28.41	
		Post-test	16.11 ± 25.24	
		P within group	0.028	
	Control	Pre-test	22.11 ± 24.96	

		Post-test	22.11 ± 17.82	
		P within group	0.421	

Discussion

The aim of this study was to compare two types of resistance training with blood flow restriction on serum follistatin and leptin levels in inactive obese girls. The results of the present study show that resistance training with blood flow restriction for 12 weeks and three sessions per week showed a significant decrease in leptin levels and a significant increase in follistatin levels.

The first results of the present study showed that resistance training for 12 weeks and three sessions per week causes a significant increase in serum follistatin levels. Therefore, it seems that resistance training with blood flow restriction and resistance training without blood flow restriction increase the anabolic environment in the trained muscles. The results of the present study are consistent with the results of Sheikhi Pirkohi et al. (2019) (Shikhi Pir Kohi, Zakeri, Dehkhoda, Mirakhori, & Amani-Shalamzari, 2019) and Bagheri et al. (2018) (Bagheri, Rashidlamir, & Attarzadeh Hosseini, 2018). In these studies, it was also shown that 6 weeks of functional training with blood flow restriction and 8 weeks of resistance training with blood flow restriction lead to an increase in follistatin levels. It has been shown that follistatin plays an important role in reducing myostatin signaling. Follistatin acts as one of the important inhibitors of myostatin and neutralizes its function. In the presence of follistatin, myostatin is unable to bind to its receptor and its atrophic activity is reduced (Khalafi et al., 2023). It can be said that these factors were probably effective in the increase in follistatin levels in both the blood flow restriction and non-blood flow restriction training groups in the present study.

Other results of the present study showed that resistance training for 12 weeks and three sessions per week causes a significant decrease in serum leptin levels. Reducing leptin concentration through exercise,

with changes in energy balance, improves sensitivity. Insulin is accompanied by changes in hormones related to carbohydrate and fat metabolism. It is of particular importance to examine leptin changes associated with obesity; therefore, in order to change the levels of leptin and its related hormones such as (insulin, cortisol, growth hormone, catecholamines, aldosterone, sex hormones, testosterone, triiodothyronine, thyroxine, etc.), the intensity and duration of the exercise program should be appropriate (Qaid & Abdelrahman, 2016). In general, most studies have shown that a balanced, low-fat diet and physical exercise reduce blood leptin levels (Cipryan, Dostal, Plews, Hofmann, & Laursen, 2021). Exercise can reduce fat mass and play an important role in energy expenditure. It can also affect metabolism (free fatty acids, lactic acid, triglycerides, etc.) (Abbenhardt et al., 2013). It has been reported that after 3 months of resistance training in obese men, plasma leptin levels after exercise decreased significantly by 21%. While there are studies whose findings are inconsistent with the present study, Weltman et al. (Weltman et al., 2000) recently reported showed that 30 minutes of exercise with different intensities and caloric intake (from 1150 ± 11 to 455 ± 529 kcal) in 7 healthy young men did not cause changes in leptin levels during exercise and during the recovery period (3.5 hours). Kramer et al. (2002) reported no significant changes in leptin concentrations after short-term exercise. There are several factors that could explain this change in the leptin response to muscular exercise (Kraemer, Chu, & Castracane, 2002). This is likely related to the time of blood sampling, the intensity and duration of exercise, the individual's nutritional status, the circadian rhythm of leptin, and the caloric imbalance caused by exercise.

Conclusion

According to the findings of the present study, it can be said that resistance training with blood flow restriction is an effective factor in increasing follistatin and reducing leptin. However, further research is

needed to accurately determine the effect of this training method on reducing obesity and other adaptations related to exercise. Also, the limitations of this study are the lack of measurement of the degree of hypertrophy of the involved muscles and functional changes of the subjects. He was present at the investigation.

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