ORGINAL ARTICLE

Effect of 6 Weeks of High Intensity Interval Training with Nano-curcumin Supplement on Antioxidant Defense and Lipid Peroxidation in Overweight Girls- Clinical Trial

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Abstract

Objective: Thepurpose of this study was to investigate the effect of 6 weeks of HIIT training combining nano-curcumin supplement on antioxidant defense and lipid degradation in overweight girls.

Materials and Methods: The research method was semi-experimental study. Accordingly, 48 overweight girl students were randomly divided into four groups: training (n=12), training-supplement (n=12), supplement (n=12) and control group (n=12). Supplement groups consumed 80 mg nano-curcumin capsule daily. Training groups performed an exercise protocol of HIIT training with maximum heart rate for 6 weeks (three sessions per week). The control group did not have any regular exercise. Blood samples were obtained before and after training period for antioxidant indicators and lipid degradation measurement. T-test and one-way analysis of variance were used for the evaluation of within-group and between-group differences, respectively.

Results: A significant increase was observed in serum levels of Malondialdehyde (*P*-value= 0.004) in the training group after 6 weeks. Also, there was a significant increase in serum Glutathione (*P*-value= 0.001), Superoxide dismutase (*P*-value= 0.006) and Catalase indexes (*P*-value= 0.01) in the supplement group. Moreover, a significant increase in catalase (*P*-value= 0.001), glutathione (*P*-value= 0.006), superoxide dismutase (*P*-value= 0.015) and glutathione peroxidase indexes (*P*-value= 0.05) and a significant decrease in malondialdehyde (*P*-value= 0.009) were observed in the training supplement group.

Conclusion: A positive antioxidant effect was seen, so taking curcumin supplement along with exercises may have beneficial effects on reinforcement the antioxidant system and prevention of lipid peroxidation in overweight individuals.

Keywords: Turmeric, Overweight, High-Intensity Interval Training, Antioxidants

Introduction

Obesity and overweight are major health problems all over the world (1). Researchers have mentioned the decrease in physical activity and maladaptation as the main causes of obesity and overweight (2). One of the most effective

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exercise plans in the treatment of obesity and overweight is high-intensity interval training (HIIT). These exercises with less time cause more physiological stimulation than medium-intensity continuous training (3) and may cause similar or even greater changes in the range of physiological, functional and health-related changes in adults and patients. (4) However, a large increase in metabolism during HIIT may increase the production of reactive oxygen species and nitrogen, which may be associated with inefficient antioxidant defense systems and cause oxidative stress (5). The obese people have a higher level of oxygen free radicals (6). Consequently, HIIT in obese individuals may cause double damage to various molecules, including lipids, proteins, and DNA (7). One of the defense mechanisms of the body against free radicals is the antioxidant defense system and this defense system has more defensive power in tissues with higher oxygen consumption than other tissues (8). The antioxidant defense system consists of two enzymatic and non-enzymatic sections (9). The body's antioxidant enzyme system consists of superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) (10). The most important components of the non-enzymatic system are glutathione (GSH), vitamin C, vitamin E, and uric acid (11).

One of the herbal antioxidants can be curcumin, which is a major active ingredient in turmeric, with a wide range of biological and pharmacological activities. The most important biological effects of this substance are its anti-inflammatory and anti-tumor properties. In addition, curcumin as an antioxidant is one of the strongest free radical cleansers that can prevent the production of various free radicals in the biological environment (12). Curcumin can reduce the peroxidation of lipids or malondialdehyde (MDA) by maintaining antioxidant activity such as high levels of superoxide dismutase, catalase, and glutathione peroxidase (13). Takashi et al reported that curcumin could undermine the effects of oxidative stress produced by exercise activities (14). Therefore, both intense exercise and overweight and obesity lead to an imbalance of antioxidants and oxidative systems. Therefore, the researcher seeks to answer the question of whether consuming curcumin supplement with high-intensity interval training affects the antioxidant and lipid degradation of overweight girls or not.

Materials and Methods

The research method was a semi-experimental type with before-after design.The subjects were 48 overweight girl students who were purposively selected among all overweight girl students of Shahid Chamran University. The mean age and the body mass index (BMI) of the participants were 21.88 (± 0.94) years and 28.12 (± 2.10) kg / m^2, respectively. A physician examined all subjects in terms of drug use, general health, cardiovascular health, and blood pressure prior to entering the study. Then, the subjects were randomly divided into four groups: training (n= 12), training-supplement (n= 12), curcumin supplement (n= 12) and control group (n=12). Prior to beginning training intervention, subjects completed medical questionnaire and written consent was taken from them. In the same session, anthropometric measurements (height, weight) and body composition were measured using Body composition analyzer (Olympia model 3/3 Javan Company, South Korea). Also, the maximum oxygen consumed (VO_{2max}) was measured using the Bruce test on treadmill and subjects were advised to avoid heavy physical activity before the assessment sessions. Subjects of the supplement and training-supplement groups received an 80 mg nano-curcumin capsule every day before lunch (15). The training and control groups received no supplementation and the control group didn't have regular physical activity.

Training Protocol: The exercise protocol used for training and training-supplement groups was the Shuttle-Run test which was performed for 6 weeks and 3 sessions per week in a 20-

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meter distance indicated by three cones. The start of the training protocol, subjects ran at the top of the starting point (cone 1) to cone 2 (route A), then returned in the opposite direction, running 20 m to the cone 3 at maximum speed (path B). Finally, they reverted to the starting point (cone 1) at maximum speed (route C) to a distance of 40 meters. Subjects continued this process at maximum speed to complete the training period of 30 seconds. After 30 seconds of rest, the exercise protocol was repeated. Exercise progression was increased by increasing the number of 30-second repetitions from 4 times in the first and second weeks, 5 times in the third and fourth weeks, and 6 times in the fifth and sixth weeks. From the beginning of the exercise protocol, in each session up progression, subjects carried out a warmed-up program for 5 to 10 minutes, and at the end of each session for 5 to 10 minutes, they carried out a cooled-down program. According to the formula (maximum heart rate = age- 220), the maximum heart rate of the participants was obtained. Polar heart rate monitor was used to measure heart rate during all training sessions (16). Exercise intensity was controlled through the Borg index.

Subjects were present at the Shahid Chamran University of Ahvaz after a 12-hour fasting period and 5 ml of blood were taken to measure the malondialdehyde, glutathione peroxidase, glutathione, catalase, and superoxide dismutase. The first blood sample was taken 48 hours before the beginning and the second one was taken 48 hours after the end of 6-week period of training and supplementation. Blood samples were centrifuged at 3000 rpm and kept at -70 °C after separating the serum.

Descriptive statistics were used to calculate the mean and standard deviation of data. The Shapiro-Wilk test was used to determine the variables normality and the paired T-test was used to analyze the data and whitin group comparison. To analyze the changes between the groups, one-way ANOVA and Bonferroni post hoc test were used. All of the statistical tests were performed using SPSS - 23 and the significance level of the tests was considered *P*-value≤ 0.05.

Ethical considerations

This study was approved by Committee of Ethics in Shahid Chamran University of Ahvaz, Ahvaz, Iran with number of EE/96.24.3.85899/scu.ac.ir, then was registered in the Iranian Clinical Trial Registration Center (www.irct.ir) with IRCT20180927041150N1 code. At first session, all subjects sign the inform consent.

Results

The results of the anthropometric and body composition indices in before and after are presented in table 1. Based on paired t-test (the results are shown in Table 2), MDA index significantly increased in the training group (*P*-value= 0.004) and non-significant change in antioxidant indices (SOD, GPX, CAT, and GSH indices) was observed in the training groups after the 6-weeks training period (*P*-value≥ 0.05). In the supplement group, there was a significant increase in SOD (*P*-value= 0.006), CAT (*P*-value= 0.019) and GSH (*P*-value=0.001) While no significant changes were observed in GPX and MDA (*P*-value≥ 0.05). In the supplement-training group, there was a significant increase in SOD (*P*-value= 0.015), GPX (*P*-value= 0.05), CAT (*P*-value= 0.001), GSH (*P*-value= 0.006) and MDA decreased significantly (*P*-value= 0.009). Based on One-way analysis of variance, there was between-group change at the serum levels of SOD, CAT, GSH, and MDA (*P*-value≤ 0.05). Based on Bonferroni post hoc test, there was a significant increase in serum SOD levels in the supplement-training group compared to the training group. Also, the CAT index showed a significant increase in the supplement-training group compared to the control group and GSH index increased significantly in the two groups of supplement-training and supplement compared to the training group (*P*-value≤ 0.05). Also, MDA index was significantly increased in the

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Table 1. The anthropometric indices, body composition of subjects before and after the study

Variable	group	pre-test Mean (± SD)	Post-test Mean (± SD)	*P*-value Within-group	*P*-value Between-group
Age (Years)	Supplement - training	21.66 (±1.15)	-	-	-
	training	20.25 (±0.85)	-	-	
	Supplement	22.64 (±0.88)	-	-	
	Control	22 (±0.81)	-	-	
Height (cm)	Supplement - training	160.80 (±0.37)	-	-	-
	training	156.75 (±2.01)	-	-	
	Supplement	161.25 (±0.94)	-	-	
	Control	158.66 (±0.33)	-	-	
Weight (kg)	Supplement - training	72.7 (±3.23)	71.32 (±3.44)	0.05*	0.14
	training	70.22 (±6.22)	69.2 (±6.89)	0.37	
	Supplement	71.1 (±2.9)	70.12 (±3.15)	0/17	
	Control	71.66 (±6.24)	72.83 (±6.17)	0/28	
BMI (kg/m^2)	Supplement - training	28.07 (±1.22)	25.78 (±1.31)	0.07	0.37
	training	28.44 (±2.05)	28.28 (±2.47)	0.74	
	Supplement	27.37 (±2.72)	27 (±1.82)	0.11	
	Control	28.73 (±2.72)	28.97 (±2.83)	0.31	
PBF (%)	Supplement - training	37.32 (±0.91)	36.72 (±0.97)	0.01*	0.27
	training	34.75 (±2.21)	33.87 (±2.73)	0.24	
	Supplement	33.8 (±1.48)	33.45 (±1.55)	0.47	
	Control	34.63 (±2.22)	35 (±2.11)	0.14	
WHR	Supplement - training	0.89 (±0.009)	0.89 (±0.009)	0.01*	0.17
	training	0.87 (±0.023)	0.85 (±0.03)	0.21	
	Supplement	0.85 (±0.01)	0.85 (±0.01)	0.39	
	Control	0.84 (±0.02)	0.85 (±0.02)	0.63	

BMI: body mass index
PBF: peripheral body fat
WHR: waist hip ratio

training group compared to the supplement-training group, supplement, and control (*P*-value≤ 0.05) and there was a significant decrease in the supplement-training group (*P*-value≤ 0.05).

Discussion

The results of this study showed that 6 weeks of high-intensity interval training significantly increased serum levels of MDA as lipid destruction index in overweight girls. Several studies have shown a significant increase in lipid peroxidation and serum levels of MDA following intense aerobic exercise, which the results of this study are in line with the results of the mentioned studies. (17-20). Free radicals react with phospholipid layers of the cell membrane and result in cellular degradation. As a result of this reaction, measurable products are released, most notably malondialdehyde (21). Research has shown that lipid peroxidation and cell membrane degradation are affected by various factors such as exercise intensity (22). The results of the present study regarding changes in malondialdehyde levels were inconsistent with the results of some studies in this area (21,23-27). As Gupta et al. (2015) examined the effect of three weeks of regular aerobic training, MDA decreased in healthy subjects (23). Research has shown that obesity is associated with increased oxidative stress, and in obese individuals, the production of free radicals increases and the antioxidant system is weakened (27). Amirkhizi et al. (2012) indicated that the mean plasma concentrations of MDA in women with overweight and obesity were significantly higher than those with normal weight (28). Consequently, the reason for the inconsistency of the present study with Gupta is the higher levels of MDA in overweight people also the difference in the type of exercise. Soares et al. (2015) also examined the indexes related to oxidative stress in non-athlete subjects that 16 weeks of physical activity was associated with an increase in antioxidant activity and a decrease in MDA levels (24).

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Table 2. Comparison of within-group and between-group variables of superoxide dismutase, catalase malondialdehyde, glutathione and glutathione peroxidase

Variables	Groups	Mean (±SD)		*P*-value	*P*-value
		Pre-test	Post-test	Within-group	Between-group
Glutathioneperoxidase (µmol / ml / min)	training	11.56 (±1.11)	11.68 (±1.97)	0.94	0.13
	Supplement + training	10.69 (±1.28)	12.82 (±1.13)	0.05*	
	Supplement	12.18 (±1.03)	15.65 (±1.09)	0.09	
	Control	11.29 (±0.78)	10.95 (±1.24)	0.72	
Superoxidedismutase (µmol / ml / min)	training	0.14 (±0.005)	0.10 (±0.011)	0.16	0.02* a
	Supplement + training	0.14 (±0.008)	0.17 (±0.004)	0.015*	
	Supplement	0.14 (±0.005)	0.16 (±0.005)	0.006*	
	Control	0.17 (±0.014)	0.17 (±0.01)	0.76	
Catalase (µmol / ml / min)	training	0.21 (±0.01	0.32 (±0.04	0.14	0.02* d
	Supplement + training	0.22 (±0.006)	0.41 (±0.02)	0.001*	
	Supplement	0.17 (±0.004)	0.32 (±0.04)	0.019*	
	Control	0.2 (±0.01)	(±0.21)	0.81	
Malondialdehyde (µmol / l)	training	3.02 (±0.21)	3.7 (±0.054)	0.004*	0.001* a, b, c, d
	Supplement + training	3.49 (±0.14)	2.63 (±.0.08)	0.009*	
	Supplement	3.13 (±0.32)	2.93 (±0.3)	0.18	
	Control	3.09 (±0.15)	3.07 (±0.17)	0.45	
Glutathione (µmol / l)	training	46.85 (±2.04)	41.78 (±0.66)	0.15	0.002* a, b
	Supplement + training	42.19 (±0.77)	45.67 (±0.73)	0.006*	
	Supplement	42.75 (±0.98)	43.36 (±1.06)	0.001*	
	Control	43.73 (±1.76)	43.01 (±1.29)	0.74	

- Results of P within group based on dependent T-test
- Results of P Between-group based on one way ANOVA test (Bonferroni post hoc test)
- A significant level was considered ($P \leq 0.05$).
- Results of Post- hoc test based on the Bonferroni test

1. a is Significant between the training group and the training-supplement
2. b is Significant between the training group and the supplement
3. c is Significant between the training group and control
4. d is Significant between the training-supplement group and control

It can be concluded from the available reports that, depending on the type and intensity of physical activity, and the level of fitness of individuals and their compatibility with exercise, it is possible to increase, decrease or not change the MDA after training (29). With physical activity, especially high-intensity interval training, which is associated with a massive increase in metabolism, the antioxidant system in the body could not neutralize the production of reactive oxygen species and nitrogen (RONS) production (30). Research has shown that severe periodic exercises may result in the production of RONS from xanthine, NADPH oxidase, ischemic reperfusion, calcium homeostasis changes and muscle damage due to excessive oxygen intake as well as high anaerobic metabolism (31). NADPH oxidase has been reported to be an enzyme responsible for producing ROS in the arteries, which is suppressed by polyphenols (32). Research has shown that curcumin, as a polyphenol, prevents free radical production and oxidative stress, and has a higher antioxidant capacity than vitamins E (33). Curcumin can interfere with the activity of GSH, CAT and SOD enzymes in neutralizing free radicals (34), and also inhibit the activity of ROS-producing enzymes such as lipoxygenase/cyclooxygenase and xanthine hydrogenase/oxidase (35).

In the present study, levels of GSH, SOD, and CAT in two groups of curcumin users showed a significant increase compared to pre-test. GPX and MDA showed a significant increase and decrease respectively in supplement-training group, this is consistent with results of several studies (36-39). In contrast, Heusser et al. (2009) showed that 4 weeks of intense aerobic exercise with vitamin C supplements on men aged 25 to 35 years did not significantly alter the antioxidant (superoxide dismutase and glutathione peroxidase) indices compared to the control group (40). Since the present study showed a significant increase in GPX and SOD in the supplement-training

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group, the main reason for differences in results of these two studies may be in the discrepancy in the supplement type and the duration exercise (6 weeks vs. 4 weeks) (41). Also, Padround et al. (2014) examined the effect of 6 weeks of endurance training with daily intake of 1 g of ginger supplement on the inactive men's lipid peroxidation. The results of their study showed that daily intake of 1 g of ginger supplement, have no effect on reduction of exercise-induced MDA (41). The reasons for the inconsistency of this study with the present study are the Supplement type and gender of the subjects. Research also showed women's mitochondria produce free radical half of men (42). Women are more resistant to oxidative stress than men from exercises to exhaustion. Also, women's better protection against exercise-induced oxidative stress and skeletal muscle destruction may be attributed to the antioxidant activity of female sex hormones (17 beta-estradiol E2). Levels of 17 beta-estradiol E2 as antioxidants in girls are higher than males. It has also been reported that 17 beta-estradiol E2 has the property of stabilizing the cell membrane and counteracting oxidative stress by donating the hydrogen atom to the proxy radical. (43).

Finally, the present study had strengths and limitations. One of the strengths of the present study is the use of nanocurcumin supplement which has high bioavailability. This study focused only on Overweight healthy girls. Thus, the findings of this study cannot be generalized to other populations, such as men and postmenopausal women. Also, another limitation of the present study was the assessment of serum levels antioxidative indices and malondialdehyde. Therefore, it is recommended to evaluate the levels of these markers in different tissues including heart, kidney and liver and other tissues.

Conclusions

The results of this study showed that 6 weeks of high-intensity interval training caused a significant increase in MDA as an indicator of lipid degradation and a lack of change in serum antioxidant levels of SOD, GSH, CAT, GPX. Since HIIT and supplementation with curcumin caused a significant reduction of MDA serum levels and a significant increase in CAT, GPX, GSH, and SOD. Therefore, exercise combined with taking curcumin supplement has unique antioxidant properties, so it is recommended that overweight people, take curcumin supplement along with intensive interval exercises.

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Conflict of Interest

The authors state that they have no conflict of interest.

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