

ORIGINAL RESEARCH

An investigation into the effect of eight weeks of aerobic exercise with orange juice consumption on antioxidant capacity and inflammatory factors in overweight middle-aged women

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Abstract

Objective: Overweight and obesity are among the most important public health concerns associated with several negative health outcomes. Several studies have found that the positive impact of orange juice may be due to the presence of its antioxidants, especially flavonoids and vitamin C. Vitamin C as an antioxidant is capable of neutralizing water-soluble ROS. Therefore, this study aimed to investigate the effect of eight weeks of aerobic exercise with orange juice extract on antioxidant capacity and inflammatory factors in overweight women.

Materials and Methods: A total of 60 overweight women participated in this study voluntarily. The research sample was selected using a random sampling method. They were then randomly divided into 4 groups. The treadmill endurance training protocol was carried out with a run-walk program for eight weeks, three sessions per week. All data were then entered into SPSS 24 to be analyzed.

Results: The results obtained by this study showed that eight weeks of aerobic exercise with orange juice consumption had no significant effect on high-sensitivity C-reactive protein (hs-CRP) levels and total antioxidant capacity in overweight middle-aged women, but eight weeks of aerobic exercise with orange juice consumption had a significant effect on malondialdehyde (MDA) levels and VO₂max amount in overweight middle-aged women ($P=0.001$).

Conclusion: Statistical studies showed that eight weeks of aerobic exercise with orange juice consumption causes a reduction in MDA levels, and orange juice consumption combined with aerobic exercise can have a synergistic effect compared to aerobic exercise alone and increase the effect of exercise on the oxidative stress suppression.

Keywords: Aerobic exercise, Orange juice, Antioxidant capacity, Inflammatory factors, Middle-aged women, Overweight

Introduction

Overweight and obesity are among the most important public health concerns associated with several negative health outcomes. Physical activity is an important lifestyle behavior that helps one regulate body weight (1). As obesity increases, fat is not only stored in adipose tissue but also accumulates around the limbs and abdominal cavity. Fat storage in the trunk, especially in the peritoneal cavity, is much more dangerous than fat storage in the limbs. Central obesity is also defined as the fat storage in the trunk. Central obesity causes an increase in the risk of developing metabolic syndrome through the concentration of free fatty acids in the portal vein. Additionally, it increasingly affects cortisol, growth, and sex hormones (2). Abdominal obesity is associated with metabolic abnormalities such as dyslipidemia, oxidative stress, and low levels of inflammation (3). If overweight is prevented and controlled, many metabolic diseases can be prevented. By having a balanced weight, we can have more health and longevity and improve our quality of life. Choosing a healthy diet along with regular exercise can significantly reduce the risk of getting many diseases and the risk of death. Antioxidants are materials whose presence in food or in the body, even in very small amounts, protect the body against different types of oxidative damage that may occur due to reactive oxygen species (ROS) (4-6). ROS, which are composed of oxygen free radicals and other chemical compounds, can develop oxidative stress in the body and lead to tissue damage (7, 8). An increase in the free radicals and simultaneously a decrease in the defense mechanisms against it can lead to damage to tissue and enzymes and increase lipid peroxidation and insulin resistance (9-11). Moreover, nitric oxide and ROS are involved in and interact with each other, and high blood glucose reduces the level of superoxide dismutase, which is an important antioxidant enzyme, and increases lipid peroxidation. Lipid peroxidation leads to the production of toxic aldehydes. One of the most toxic aldehydes is malondialdehyde (MDA), which is a colorless compound and the end product of lipid peroxide decomposition. MDA is currently regarded as

an indicator lipid peroxidation. Thus, the amount of oxidative damage to cellular lipids can be determined by measuring the level of MDA (12-16).

Several antioxidants, such as vitamin C, vitamin E, and beta-carotene, are known to protect cells from free radicals. As one of the most famous essential micronutrients in the body, vitamin C is capable of neutralizing water-soluble ROS. High concentrations of blood glucose compete with vitamin C to enter the cell in diabetic people. As a result of this competition, cell levels of ascorbic acid decrease and make the cell more vulnerable to ROS (17-19). In establishing antioxidant defense, the function of the defense system is dependent on many essential micronutrients received from food, including vitamin C, vitamin E, and beta-carotene (6,20). Previous epidemiological studies have revealed a relationship between the high consumption of fruits and vegetables and the reduced risk of cardiovascular disease. This positive effect can be related to the minor components, especially flavonoids, which are also present in orange juice and exert their action by inhibiting the oxidation of LDL and the accumulation of platelets, and vitamins C, E and β -carotene, which mainly act as anti-inflammatory agents. Folic acid and natural folate existing in large amounts in citrus fruits and green leafy vegetables have also been reported to reduce total plasma homocysteine. Moreover, methionine metabolism is regarded as a risk factor for cardiovascular diseases. Citrus juices, especially orange and grapefruit juice, are rich sources of flavonoids, folate, and vitamin C, but their role in cardiovascular health has not been completely investigated (21). Generally, total antioxidant capacity with a comparative advantage over other antioxidants, which shows all the factors related to antioxidant defense, is a very reliable and important indicator in measuring the total antioxidant capacity of humans (13, 22,23). One of the problems related to the disruption of the body's antioxidant defense mechanisms is the lack of vitamin C as one of the most well-known essential micronutrients within the cells. As one of the thirteen essential vitamins for the human

body, vitamin C is capable of neutralizing water-soluble ROS. Cellular levels of ascorbic acid decrease in people deficient in this vitamin, making the cell more vulnerable to ROS (11,14,15). Total antioxidant capacity is also associated with a set of compounds. Antioxidants are compounds which are capable of protecting biological systems against the harmful effects of ROS and reactive nitrogen species (RNS). In fact, antioxidants play an important role in inhibiting ROS and RON and preventing their formation. In addition, MDA is one of the end products of unsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. MDA levels are commonly known as markers of oxidative stress and antioxidant status in patients with cancer (24). In addition, reactive protein C (CRP) is a chronic low-grade inflammatory marker, which is capable of providing a higher predictive value beyond traditional risk factors and may pose an increased risk of heart attack for people, while the high level of hs-CRP is accompanied by an adverse risk of cardiovascular disease (25). Therefore, the purpose of this study is to investigate the effect of eight weeks of aerobic exercise with orange juice consumption on antioxidant capacity and inflammatory factors in overweight middle-aged women.

Materials and Methods

This is an applied research conducted using quasi-experimental research with pretest-posttest and control group design. This clinical trial, double blind study was carried out on 60 cases of the 30-59-year-old overweight women with BMI greater than 28 kg/m² referring to Kan Health Center of Tehran who voluntarily participated in training sessions of Shahid Haj Shaban Ali Sports Complex in Kan region after getting the informed consent. Samples were randomly assigned into four groups: control group (15), supplement group (15), exercise group (15), and exercise + orange juice supplement (15) group. Free radical scavenging ability of juice was examined based on the radical ablation effect on DPPH free radical. Juices (12.5 µL to 100 µL/ml) were prepared in methanol. A total volume of 2 ml of DPPH solution (0.002% in methanol) was separately mixed with 2 ml of juice at a different

concentration in a clean labeled test tube. The tubes were then incubated at room temperature in the dark for 30 minutes and measured with a UV-Vis spectrophotometer at a light density of 517 nm. DPPH control adsorption was also mentioned. Juices scavenge activity was calculated using the following formula:

Scavenging activity (%) = $[(A - B) / A] \times 100$, where A is DPPH adsorption and B is DPPH adsorption and juice composition. Additionally, juices (12.5 µL to 100 µL / ml) were mixed in 1 ml of methanol in separate tubes containing 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The tubes were put in a boiling water bath at 50 °C for 20 min, washed quickly, and replaced with 2.5 ml of 10% trichloroacetic acid (TCAA) and 0.5 ml of 0.1% ferric chloride. The amount of the formed iron (II) -ferricyanide compounds was determined by measuring the formation of Perls' Prussian blue at 700 nm after 10 minutes. The increased absorbance of reaction mixtures indicates an increase in reducing power (26).

One week before the start of the study, the volunteers' weight and height were measured (using the Seca digital height and weight Scale made in German) to determine homogeneous samples. In addition, subjects began dietary supplementation and training exercises the day after blood sampling. The supplement groups received 500 cc of orange juice on the training day for 8 weeks and three times a week. The control group did not participate in any exercise program.

The treadmill endurance training protocol was performed as a run/walk program for 8 weeks 3 sessions per week with intensity exercise of about 65-80% of the maximum heart rate. The duration of the training sessions lasted 20 -30 minutes and before and after each training session, warm-up and cool- down was performed for 10 and 7 minutes, respectively. During the initial treadmill exercises, participants were allowed to choose to walk at either slow or fast speeds while maintaining gait. The treadmill endurance training program was implemented as a run/walk program for 8 weeks 3 sessions per week. The exercise intensity in the first two weeks, second two weeks, third two weeks, and last two weeks was about 65, 70, 75, and 80% of the maximum heart rate, respectively, which was controlled

with the Polar heart rate monitor. The duration of training sessions increased from 20 minutes in the first week to 40 minutes in the last week, and before and after each training session, warm-up and cool-down was performed for 10 and 7 minutes, respectively (27). During each training session, the participants' heart rate was monitored using the Polar heart rate monitor and recorded every 10 minutes so that they can keep the target intensity of their training. All exercises were supervised by a research assistant. Participants in the control group did not engage in any physical activity during the study period (28). Additionally, the maximum oxygen consumption (VO₂max) of the subjects was estimated using the shuttle run test as follows:

In this test, the speed is set by a programmed timer with a beep to travel each 20-meter shuttle in proportion to the increase in speed at each step. Subjects should begin to run as they hear the beep sound and reach the end of the track, and then continue in the opposite direction. The beginning and end of each shuttle is marked with an obstacle and a line. The shuttle run has 21 levels and each level includes a certain number of shuttles with a specific timing, so that the time reduces from level 1 to level 21 and accordingly the speed of the runner should increase at each level. The time of each shuttle is 9 seconds and 3.89 seconds at level 1 and level 21, respectively. At each level and shuttle that the subject fail twice to reach the finish line of the shuttle simultaneously with the beep sound, the test can no longer be continued and the level and shuttles traveled are recorded for him and the oxygen consumed by the subject is calculated based on it. The speed of each shuttle is 8 km/h at level 1, 13 km/h, at level 10, and 10 18.5 km/h at level 21 (29).

Blood sampling was done in two stages of pre-test and post-test by an experienced laboratory professional. To this purpose, the subjects were asked to refer to the laboratory after 12 hours of night fasting. The subjects were also asked to refrain from strenuous exercise 2 days prior to the pre-test blood sampling and get enough sleep the night before the blood sampling. After attending the laboratory, the subjects rested for 30 minutes. 5 cc of blood was then taken from the brachial vein while sitting up in special blood-drawing chairs. Blood sampling was done between 9 and 10 am.

All measurements were performed at the same temperature, moisture, ventilation and ambient light. After blood samples were taken, they were centrifuged at 3000 rpm for 10 minutes and the plasma was separated from the red blood cells and kept at a temperature of -70°C until the experiment was done. The Padgin Teb Co. kit with an accuracy of 3 micro/liter was used to measure hs-CRP. Moreover, the Padgin Teb Co. kit with an accuracy of 0.3 mmol per liter was employed to measure MDA and TAC. After laboratory analysis, quantitative description of the data was performed using central scattering indices such as mean and standard deviation. First, Shapiro-Wilk test was used to ensure the normal distribution of data. As the results of this test indicated the normal distribution of data, parametric tests were used. To compare the between-group changes (between control, orange juice, exercise, and exercise + orange juice groups), analysis of covariance (ANCOVA) was used. Bonferroni Post Hoc test was used in case of the significant difference between groups. Moreover, the significance level of $P < 0.05$ was considered to test the hypotheses and decide whether to support or reject the hypotheses. For $P < 0.05$, the changes were considered significant. All statistical methods were performed using SPSS software version 24.

Results

The levels of hs-CRP, MDA, total antioxidant capacity, body weight, BMI of subjects in the control, orange juice, and exercise + orange juice groups in two stages of pre-test and post-test are shown in table 1 as mean $\pm$ standard deviation.

Table 1- Levels and values of research variables as mean $\pm$ standard deviation in pre-test and post-test

Group	Variables	Control group	Training group	Orange juice group	Training + Orange juice group
(mg/dl) hs-CRP	Before	2.76 $\pm$ 2.34	1.48 $\pm$ 1.87	1.99 $\pm$ 2.49	1.53 $\pm$ 1.96
	After	2.72 $\pm$ 2.65	1.53 $\pm$ 1.50	2.09 $\pm$ 1.87	1.45 $\pm$ 1.41
(μ m/L) MDA	Before	0.60 $\pm$ 0.21	0.47 $\pm$ 0.24	0.55 $\pm$ 0.23	0.51 $\pm$ 0.18
	After	0.59 $\pm$ 0.20	0.35 $\pm$ 0.24	0.42 $\pm$ 0.22	0.28 $\pm$ 0.18
Total antioxidant capacity (TAC) (μ m/L)	Before	0.56 $\pm$ 0.18	0.17 $\pm$ 0.03	0.42 $\pm$ 0.09	0.20 $\pm$ 0.07
	After	0.54 $\pm$ 0.20	0.47 $\pm$ 0.24	0.56 $\pm$ 0.24	0.54 $\pm$ 0.17
(kg/m ²) BMI	Before	28.26 $\pm$ 2.02	29.39 $\pm$ 2.45	29.00 $\pm$ 2.59	28.18 $\pm$ 1.86
	After	28.54 $\pm$ 1.77	28.38 $\pm$ 2.34	29.10 $\pm$ 2.61	26.98 $\pm$ 1.83
Weight (kg)	Before	72.08 $\pm$ 5.68	74.56 $\pm$ 6.12	72.55 $\pm$ 7.64	70.73 $\pm$ 5.58
	After	72.79 $\pm$ 6.09	72.02 $\pm$ 5.46	72.81 $\pm$ 7.73	67.83 $\pm$ 5.61
(ml.kg.m ⁻²) VO ₂ max	Before	31.92 $\pm$ 7.81	33.36 $\pm$ 9.31	34.78 $\pm$ 8.21	34.65 $\pm$ 8.19
	After	31.54 $\pm$ 7.75	36.18 $\pm$ 8.05	35.12 $\pm$ 8.49	37.04 $\pm$ 7.63

The results of ANCOVA test regarding changes in hs-CRP levels showed that eight weeks of aerobic exercise with orange juice consumption

had no significant effect on hs-CRP levels and total antioxidant capacity in overweight middle-aged women (Table 2). Additionally, the statistical studies showed that eight weeks of aerobic exercise with orange juice consumption had a significant effect on MDA levels and the amount of VO₂max in overweight middle-aged women ($P=0.001$) (Table 2).

Table2- Results of covariance analysis of hs-CRP, malondialdehyde, TAC and VO₂max levels

		Degrees of freedom	average of squares	F	P value	Effect size
hs-CRP	Corrected model	4	11.481	4.562	0.005	0.343
	Initial values	1	35.533	14.120	0.001	0.287
	group	3	0.992	2.179	0.108	0.033
	Error	55	2.517			
MDA	Corrected model	4	0.559	831.401	0.000	0.990
	Initial values	1	1.693	2515.881	0.000	0.986
	group	3	0.087	128.567	0.000	0.917
	Error	55	0.001			
TAC	Corrected model	4	0.026	0.542	0.706	0.058
	Initial values	1	1.468	30.507	0.000	0.032
	group	3	0.035	0.720	0.547	0.058
	Error	55	0.048			
VO ₂ max	Corrected model	4	86.276	101.634	0.000	0.891
	Initial values	1	170.415	196.154	0.000	0.916
	group	3	31.862	39.211	0.001	0.426
	Error	55	0.924			

Also, the results of Bonferroni post hoc test showed a significant decrease in the MDA levels in exercise groups ($P=0.001$), orange juice ($P=0.001$), and exercise + orange juice ($P=0.001$) compared to the control group. Furthermore, a significant reduction was observed in the levels of MDA in the exercise + orange juice group ($P=0.001$) compared to the exercise and orange juice groups. The results of Bonferroni post hoc test also showed that the increased amount of VO₂max in the exercise group ($P=0.001$) and exercise + orange juice group ($P=0.001$) was significant compared to the control group and orange juice group (Table 3).

Table3- Bonferroni post hoc test results for Malondialdehyde (MDA) and VO₂max levels

	Group	Control group	Training group	Orange juice group	Training + Orange juice group
MDA	Control group	-	0.001	0.001	0.001
	Training group	-	-	1.000	0.001
	Orange juice group	-	-	-	0.001
	Training + Orange juice group	-	-	-	-
VO ₂ max	Control group	-	0.001	1.000	0.001
	Training group	-	-	0.001	1.000
	Orange juice group	-	-	-	0.001
	Training + Orange juice group	-	-	-	-

Discussion

This study aimed to investigate the effect of eight weeks of aerobic exercise with orange juice consumption on body composition, lipid profile, and antioxidant capacity, and inflammatory factors in the overweight middle-aged women. The results obtained by this study indicated that eight weeks of aerobic exercise with or without orange juice consumption had no significant effect on hs-CRP levels in the overweight middle-aged women.

Systemic inflammation whose one of the indicators is the increased levels of hs-CRP precedes hyperglycemia or oxidative stress, and hs-CRP is regarded as a predictor of cardiovascular diseases (30). Accordingly, diabetic patients can be divided into low-risk, moderate, and high-risk effects due to CRP levels (31). In contrast, sport exercises have anti-inflammatory effects due to modulation in the levels of inflammatory cytokines. Therefore, regular exercise is regarded as an anti-inflammatory therapeutic factor (32). Regular exercise has been reported to reduce the inflammatory factors such as CRP and IL-6 levels at the same time with increasing the anti-inflammatory agents such as IL-4 and IL-10 (33).

C-reactive protein (CRP), is an example of acute phase proteins, which is also known as a sensitive marker of inflammation (34). There are contradictory results regarding the impact of different exercises on the levels of CRP and have reported a reduction (35) or no change (36) in CRP levels after exercise. The results from this study also showed a 5.2% reduction in hs-CRP levels after eight weeks of aerobic exercise with orange juice consumption, which were not statistically significant. Additionally, no significant change in hs-CRP levels was observed in the aerobic exercise group. Abolfathi et al. (2015) reported that eight weeks of intermittent aerobic exercise has no significant effect on adiponectin and hs-CRP levels in women with type 2 diabetes, which is consistent with the findings of the present study. They stated that despite the increase in physical fitness, the lack of changes in the levels of hs-CRP may be associated with the lack of changes in the levels of body fat percentage and body composition. Additionally, they proposed the small sample

size as one of the reasons for constant levels of hs-CRP, which also holds true for the present study. Unlike the present study that was conducted on the overweight middle-aged women, the subjects in the study by Abolfathi et al. (2015) were type 2 diabetic women and the type of exercise program was intermittent aerobic exercise (compared to endurance in the present study) (37). In consistent with our findings, Nassis et al. (2005) showed that 12 weeks of endurance exercise have no significant effect on serum levels of inflammatory factors, including IL-6 and CRP, but they caused a significant decrease in insulin resistance. The researchers stated that a significant reduction in the body fat percentage is probably needed to observe significant changes in CRP levels. Of course, the results obtained by Nassis et al. (2005) also revealed a reduction in CRP levels, but the reduction was not statistically significant. Though the subjects (mice compared to humans) and their status (diabetic versus obese) were different, the results similar to those obtained by Nassis et al. (2005) were found in the present study (36). In consistent with the present findings, Zoppini et al. (2006) showed that six months of moderate-intensity aerobic exercise has no significant effect on CRP levels in the elderly men with type 2 diabetes. This was accompanied by the lack of any significant change in anthropometric factors, such as the fat percentage, body weight, and BMI. They stated that the lack of significant changes in anthropometric and inflammatory factors is associated with the type, intensity, frequency, and duration of training sessions. In their study, the training program was implemented 2 sessions a week. Accordingly, it seems that the exercise frequency (number of sessions per week), intensity, and duration are of paramount importance in creating compatibility following sport exercises. Thus, to observe the anti-inflammatory effects of exercise, especially in obese people or people with metabolic disorders, the exercise program should be of sufficient frequency, intensity and duration (38). But unlike the findings of the present paper, Heidarianpour et al. (2016) compared the effects of three types of exercises (endurance, resistance, and combined endurance and resistance) in people with type 2 diabetes and showed that the endurance exercise and combined exercise are accompanied by a

significant reduction in the CRP levels, while no significant change in hs-CRP was observed in the endurance exercise group. Accordingly, researchers stated that aerobic and combined exercises can be used as an effective solution in the suppression of secondary complications of inflammation-induced diabetes. They attributed the lack of reduction in CRP levels in the resistance group to the nature and different effect pathways of resistance exercise (39). As aerobic exercise is regarded as the main production site of inflammatory factors, such as CRP, due to its effective role in fat metabolism and the reduction of the body fat percentage (40), it can also be effective in reducing inflammation.

The anti-inflammatory effects of sports activities seem to be closely related to the conditions of sports activities, including the volume, intensity and type of sports activities. The results of research studies have shown that higher intensity exercises are more effective in reducing CRP levels and the exercise duration can also play an important role in reducing CRP levels. In addition, combined exercise (endurance-resistance) is more effective in reducing CRP levels than endurance exercise and resistance exercise (41). As the intensity of exercise in the present study was moderate, the lack of changes in hs-CRP levels following exercise may be due to the low intensity of aerobic exercise in the present study, and by increasing the intensity of aerobic exercise more changes may be observed in the inflammatory status in the subjects.

One of the other findings of the present research indicates that orange juice consumption alone or in combination with aerobic exercise is not accompanied by a significant change in the levels of hs-CRP. In consistent with the findings of this study, Davison and Gleeson (2005) reported that acute vitamin C supplementation has no significant effect on the production of inflammatory mediators including interleukin 6 after 2 and a half hours of cycling in the relatively practiced men (42). These researchers in 2006 reported that even 2 weeks of vitamin C supplementation has no significant effect on the interleukin 6 concentration post-sport activity (43). But in contrast to the present findings, some researchers have shown that three months of antioxidant supplementation causes a significant reduction in inflammatory factors

such as interleukin6 and hs-CRP levels (44). However, unlike this study, Devaraj et al. (2000) conducted research on subjects with type 2 diabetes that is known as an inflammatory condition. Moreover, some of the inconsistencies with the present findings are associated with the type of the antioxidant supplement used (vitamin E) and its dosage (1200 IU / d) in Devaraj et al.'s (2000) study compared to the present study. It seems that the anti-inflammatory effects of exercise alone or in combination with antioxidant supplements, such as orange juice are more pronounced when subjects have inflammatory disorders and high baseline levels of inflammatory markers, like for example the situation observed in severe obesity or some inflammatory diseases such as type 2 diabetes. In healthy people, long periods or exercises with higher intensities may be needed to observe significant effects on inflammatory markers. However, the supplement type and dosage can also be effective (44).

The results from this study showed that the reduced level of MDA in the orange juice group, exercise group, and exercise + orange juice group was significant compared to the control group. Additionally, MDA levels in the post-test phase significantly reduced in the orange juice group, exercise group, and exercise + orange juice group in comparison to the pre-test. The results of the ANCOVA test for total antioxidant capacity showed no significant difference between different groups. However, the results of the paired t-test showed that the total antioxidant capacity in the exercise group and exercise + orange juice group significantly increased in the post-test phase compared to the pre-test. Several studies have confirmed the positive role of exercise in increasing the antioxidant capacity and reducing MDA levels. In consistent with these findings, Metin et al. reported that regular exercise is accompanied by the increased levels of thiol as an indicator of total antioxidant capacity and decreased levels of MDA (45).

Yahya Zadeh et al. (2015) showed a significant increase in the antioxidant capacity after eight weeks of moderate intensity aerobic exercise and exercise + omega 3 supplement, which is in agreement with the findings of the present research. These findings put emphasis on the

fact that sport exercises alone or in combination with food supplements can play an effective role in improving the antioxidant capacity (46). Though the supplement used in the above mentioned research was different from the present study, the type of training program was similar to the present study. In another study, Naqizadeh et al. (2009) reported that eight weeks of endurance exercise combined with antioxidant supplementation (vitamin E) cause a significant increase in the total antioxidant capacity and lead to a reduction in MDA levels in young active men. However, changes in antioxidant capacity and MDA levels were not significant in the group that performed only endurance exercise without supplementation. This inconsistency in the findings can be attributed to the physical condition of the subjects in the present study (obese subjects) (47). In support of this hypothesis, it is stated that the antioxidant capacity in obese subjects is 6% lower than normal weight individuals (48). Considering the lower initial levels of the antioxidant capacity in obese people, changes in its levels following exercise are more significant. Accordingly, the present findings support the positive role of endurance exercise with or without the orange juice consumption in increasing total antioxidant capacity.

Additionally, Azamian et al. (2016) showed a significant increase in the antioxidant capacity and a significant reduction in MDA levels in overweight women with type 2 diabetes after eight weeks of combined exercise. Considering the effect of obesity and adipose tissue on reducing antioxidant capacity and increasing MDA levels, part of the positive effect of exercise in improving the antioxidant status and modulating MDA levels seems to be associated with the role of exercise in reducing fat percentage. The results from Azamian et al.'s (2016) study and findings of the present study support the improvement of antioxidant status and reduction of MDA levels following a reduction in the body fat percentage and BMI caused by regular exercise (49). In confirmation of the present findings with regard to changes in the levels of MDA, Yasrebi et al. (2009) stated that eight weeks of speed training alone or in combination with antioxidant supplement in the inactive male students lead to a significant reduction in MDA levels. Results of

the present study showed a significant reduction in MDA levels in the exercise + orange juice group compared to the exercise group, indicating the synergistic effect of exercise + orange juice compared to aerobic exercise alone in modulating the levels of MDA. In addition, the present results showed that the reduction of MDA levels was also significant in the orange juice group. The discrepancy with the findings of Yasrebi et al. (2009) can be attributed to the different type (alpha-tocopherol and ascorbate in comparison with natural orange juice) and dosage (400 international units of alpha-tocopherol and 500 mg of ascorbate in comparison to 500 ml of orange juice) of the supplement used compared to the present study. Of course, the type of the training program and the frequency of exercise (three sessions per week compared to five sessions per week in the present study) in the above mentioned research were different from the present study. Perhaps some causes of the discrepancy in relation to changes in MDA levels can be related to the larger volume of exercise in the present study (50). Naziroğlu et al. (2005) also showed a significant increase in the blood antioxidant levels in diabetic rats after eight weeks of moderate-intensity exercise with vitamin C and vitamin E supplementation (51). However, contrary to the findings of the present study, Riso et al. (2005) reported that the daily consumption of 600 ml orange juice for 21 days has no significant effect on the MDA levels in healthy women. The reason for the

inconsistency with the present findings can be associated with the shorter period of orange juice consumption in the subjects of Riso et al.'s (2005) research. Moreover, As significant changes in subjects' body composition was reported after 21 days of orange juice consumption, the lack of changes in the levels of adipose tissue as a tissue affecting oxidative stress-related factors can be another reason for the inconsistency of the results with those obtained by Riso et al. (2005) (52). In the present study, the subjects consumed orange juice for eight weeks, while the subjects of the above mentioned research consumed orange juice supplement for 21 days.

The findings from this research showed that eight weeks of aerobic exercise with orange juice is accompanied by useful metabolic effects in obese women. Orange juice consumption alone can cause a decrease in MDA levels and orange juice consumption in combination with aerobic exercises can have a synergic effect in comparison to aerobic exercises alone and increase the effectiveness of exercise in suppressing oxidative stress. Therefore, to increase the effectiveness of exercise, especially to reduce oxidative stress, the consumption of natural antioxidants, such as orange juice seems to be of paramount importance.

Conflict of interest

Author declares no conflict of interest.

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