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The Effect of Anthocyanin Supplementation on Body Composition, Exercise Performance and Muscle Damage Indices in Athletes

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Original Article

ABSTRACT

Background: Flavonoids consider as a large group of plant metabolites that 6,000 types of them have been identified till now. In some studies, it has been shown that they can increase aerobic performance and maximal oxygen consumption (VO_2 max).The aim of this study was to evaluate the effect of anthocyanin (as one of the most important kind of flavonoids) supplementation on body composition, exercise performance and muscle damage indices in athletes.

Methods: This double-blinded clinical trial involved 54 female and male athletes at Isfahan University of Medical Sciences with athletic history of at least 3 years. Body composition, exercise performance, creatine kinase, and lactate dehydrogenase were assessed. Individuals were selected by simple sampling method, they divided into two groups using permuted block randomization method. First group received 100 mg anthocyanin pills, and the second group received 100 mg placebo pills, daily for 6 weeks. Participants asked to continue their routine diet and physical activity during the study period, and they were followed through phone calls or text messages.

Results: Soft lean mass, total body water and percent body fat were not changed significantly in the anthocyanin group after intervention but VO_2 max increased significantly in the anthocyanin group (48.65 ± 4.73 vs. 52.62 ± 5.04) ($P \leq 0.0001$), also a significant difference was observed between two studied groups (52.62 ± 5.04 for intervention group vs. 49.61 ± 5.33 for placebo) ($P = 0.003$).

Conclusions: Our findings suggested that the supplementation with anthocyanin in athletes may improve some indices of performance such as VO_2 max.

Keywords: Anthocyanin, athletes, body composition, exercise performance, muscle damage

INTRODUCTION

Flavonoids are among the large group of plant metabolites and 6,000 kinds of which have been identified till now.[1] Epidemiological studies reflected the relation between flavonoids intake and decrease of cardiovascular disease incidence.[2,3] In addition, some *in vitro* studies revealed antioxidant effects of flavonoids.[2] Anthocyanin is a natural flavonoid existing in many fruits and vegetables, such as cherry, strawberry, raspberry, barberry, blueberry and raisins so it can be found in many plant-derived foods and it is present in most people's diet.[4,5] Anthocyanin can decrease the risk of cancer and various chronic diseases because of having many antioxidant, anticarcinogenic, anti-inflammatory and heart protecting effects.[6,7] Despite the large number of studies investigating the effects of anthocyanin in relation to different diseases, there are few studies to examine its effects on athletes. Although some studies found that fruit juice can be rich of anthocyanin such as tart cherry and blueberry with the efficient antioxidant and anti-inflammatory capacity, which had beneficial effects on muscle damages reduction inducing eccentric exercise and improving recovery,[8-14] it has not been proved the effects of anthocyanin supplementation on muscle damage.

Several studies have shown that flavonoids are effective on the oxidation process of muscles. In some animal studies it has been described that they increase endurance performance and maximal oxygen consumption (VO_2 max), therefore they can increase body fitness in continuous flavonoid consumption as a result of elevated number of intra-cellular mitochondria number.[15-17] Natural antioxidants improve physical performance, and it might be attributed in lowering the negative and exhaustive effects of excessive oxygen and radical accumulation, during physical activities.[15] So, flavonoids would be able to slow muscular damage and its soreness, as well as neuromuscular dysfunction that may be associated with following of exercises. Some studies found that they could improve neuromuscular function and decrease soreness,[18,19] and others mentioned that this improving muscular strength can observe following a long-term consumption of flavonoid.[20] Several previous studies have investigated the relationship between body composition and antioxidant intake *in vitro* or in animals; however they cannot find significant effects on these indices in humans.[21,22] There are a lot of studies have suggested that flavonoids have beneficial effects on athletic performance, inflammatory indices and muscular damage, but fewer studies have examined the effect of anthocyanin intake in athletes performance.[9,11,23] Therefore, this study evaluated the supplementary effects of anthocyanin on athletic performance, muscular damage indices such as creatine kinase (CK) and lactate dehydrogenase (LDH) and body composition in male and female athletic students.

METHODS

Study design and participants

This double-blinded clinical trial has been conducted on 22 female and 32 male athletes at Isfahan University of Medical Science having an athletic history of at least 3 years. The subjects had not used antioxidant supplements during the past month. Simple sampling method was used to select the appropriate number of students with the power of 80% and 95%, probability.

Subjects excluded if they followed <70% of the study procedure, or they were unwilling to continue and persons with special diseases that needs especial treatment method. After explaining about the objectives and methods of the study for participants, written informed consents and demographics questionnaires were completed.

Procedures and variables assessment

Body composition indices were measured by clinical examination using Body Composition analyzer (model: Boca x1, South Korea). Height was measured without shoes by scale with an accuracy of 100 g. Then body mass index (BMI) was calculated. In order to evaluate body performance indices, exercise test was performed for all of the participants using Bruce treadmill test. The body composition was assessed by Bio impedance analyzer which measured percentage of body fat (PBF), soft lean mass (SLM), lean body mass and total body water (TBW). At the end of the test, VO_2 max and distance covered by each athlete were measured and registered.

Then individuals were divided into two groups using permuted block randomization method. First group received 100 mg supplemental anthocyanin pills, and the second one received 100 mg lactose pills (placebo), daily for 6 weeks. Placebo pills contained lactose and looked exactly the same as real pills. Participants were asked to continue their routine diet and physical activity during the study, and they were followed on study procedure, through phone calls or text messages. At the end of the intervention period, the remainder of pills evaluated to assess their supplement intake. The indices measured two times during the study at the beginning of the study as well as in the end.

Anthropometry

Height and body mass were measured with a wall stadiometer and a calibrated balance.

BMI was calculated from body mass (kg) and height (m): BMI (kg/m^2). Waist circumference was measured using a measuring tape with a spring handle in the standing position, midway between the lowest rib and the iliac crest (highest point of the ilium). The PBF was obtained from skinfold thickness measured at three sites (biceps, triceps, and calf) and calculated as described by body composition system.

Aerobic fitness test

In this study, maximal oxygen consumption (VO_2 max) of the participants was determined by an incremental graded Bruce protocol maximal exercise stress test. The test was deemed to be maximal if at least three of the following criteria were satisfied: An RER of ≥1.10, a leveling off in VO_2 with increasing workloads, volitional fatigue, or an HR greater than or equal to age -predicted maximum. Intensity for the exercise training program was calculated from the maximum HR achieved during the VO_2 max test.

Heart rate

Resting heart rate (HR) was measured in the morning immediately after the participants were awake. Measurement was taken from the radial artery with forefinger and the middle finger of the right hand placed horizontally across the subject's wrist while sitting on a chair. After that, the number of pulse beats multiplied by two to give the 1-min HR.

Exercise training program

Subjects in the exercise group were performed aerobic exercises at an intensity of 60–80% of their target HRs. Training was performed 3 days in a week during 6-week each 60–90 min period. All exercise training sessions were supervised by an exercise physiologist and conducted in the Isfahan University of Medical Science. Intensity for the exercise training program was calculated from the maximum HR achieved during the VO_2 max test. Exercise training intensity commenced at a level prescribed between 60% and 65% of the HR maximum (HR_{max}), and gradually increased so that, by week 6, the subjects were exercising at 75–80% HR_{max} (~65% VO_2 max). The target HR ranges were monitored using a heart rate monitor (Polar Electro S810, Finland) during each exercise session. For the duration of the 6-week period, subjects exercised 3 day/week for 60–90 min with a 10-min warm-up and cool-down. The subjects were permitted to perform both cycling and treadmill exercises during the 60-min exercise bout; however, it was required that 45-min of their total time were spent on the treadmill. They also performed stretching exercises for the quadriceps, hamstrings, and gastrocnemius before and after each session. Intensity, duration, resting, and exercise HR, and blood pressures were recorded for each session. VO_2 max tests were performed at baseline and repeated at 6 weeks of exercise to monitor progress. The exercise intensity was adjusted accordingly.

Subjects in the control group were instructed to continue their normal routine and not participate in any formal exercise program for the duration of the 6-week portion of the study.

Statistical analysis

All quantitative variables were first tested for normality using Kolmogorov–Smirnov statistical quantitative data were expressed as means test for within group comparisons was conducted using paired samples *t*-test while between group differences were evaluated using independent samples *t*-test or analysis of covariance. Statistical analysis was performed using the Statistical Program for the Social Sciences (SPSS Inc., Chicago, IL), version 19.0, was used for all statistical analysis. Statistical significance was set at $P < 0.05$.

RESULTS

During the study period, 10 subjects in control group were discontinued due to gastrointestinal problems in four participants and others because of unwillingness or other reasons, a total number of 44 participants (19 female and 25 male) were studied. Mean age of subjects in intervention and control groups was 24.96 ± 7.37 and 22.82 ± 6.84 years, respectively. There was no significant difference in the age between both groups ($P>0.05$).

Table 1 shows body composition indices among groups before and after the study. As can be seen, both within and between group differences for BMI, SLM, TBW, and PBF were not statistically significant in two groups.

As Table 2 shows summarizing body performance indices, VO_2 max in the anthocyanin group significantly increased after intervention ($P < 0.001$) and its changes were significantly different between two studied groups ($P = 0.003$). Although CPK decreased in the anthocyanin group after intervention but it was not significant, also the between group differences were not statistically significant. In addition, LDH decreased significantly in the anthocyanin group; however, the between group difference was not statistically significant.

DISCUSSION

In this clinical trial, the effects of anthocyanin supplements on physical and body performance

Table 1: The results of between and within group comparisons of body composition indices in both group

	Total	Anthocyanin group	Placebo group	*P*
BMI (kg/m^2)				
Before	22.96±3.40	22.37±3.62	23.55±3.14	0.563
After	22.91±3.26	22.35±3.6	23.48±2.86	
P	0.501	0.793	0.523	
TBW (kg)				
Before	38.29±7.81	37.51±8.66	39.06±6.97	0.244
After	38.31±7.69	37.66±8.63	38.96±6.77	
P	0.774	0.334	0.394	
SLM (kg)				
Before	49.09±9.89	48.00±10.79	50.17±9.02	0.576
After	49.22±9.84	48.22±10.85	50.22±8.86	
P	0.330	0.378	0.704	
PBF(%)				
Before	22.66±4.66	22.10±4.36	23.20±4.98	0.273
After	22.52±4.30	21.84±3.95	23.21±4.61	
P	0.493	0.388	0.986	

BMI=Body mass index, TBW=Total body water, SLM=Soft lean body mass, PBF=Percent body fat

Table 2: The comparison of body performance and muscle damage indices between 2 groups

	Total	Anthocyanin group	Placebo group	*P*
Vo2 max (L/min)				
Before	49.26±4.97	48.65±4.73	49.88±5.23	*0.003
After	51.11±5.34	52.62±5.04	49.61±5.33	
P	0.010	<0.001	0.779	
LDH (mg/dl)				
Before	416.55±308.45	378.59±87.14	454.50±429.19	0.710
After	306.36±38.72	304.82±29.71	307.91±46.71	
P	0.026	0.001	0.134	
CK (u/ml)				
Before	146.23±80.26	144.32±105.35	148.14±45.66	0.211
After	128.43±97.54	107.73±49.76	149.14±126.91	
P	0.255	0.054	0.968	

CK=Cearatin kinaes, LDH=Lactate dehydrogenase $^*P < .01$

and muscle injury was assessed. Results revealed that there was no significant difference in LDH, CK, TBW, LBM, and PBF among individuals with anthocyanin supplementation and placebo group; however, CK and LDH have showed no significant decrease in anthocyanin group but VO_2 max significantly improved in anthocyanin group after intervention period.

In the studies that examined the effects of fruit-rich anthocyanin on physical performance in athletes it has seen conflicting results. In the 2008 London marathon, it has done a placebo-controlled trial group on 20 runners divided into two groups. Subjects drank 8 oz of cherry juice blend or a placebo for 8 days. Similar to our study there were no significant differences in markers of postexercise muscle damage (CK, LDH, muscle soreness and isometric strength), inflammation (interleukin-6 [IL-6], C-reactive protein [CRP] and uric acid), total antioxidant status (TAS) and oxidative stress (thiobarbituric acid reactive species [TBARS] and protein carbonyls) were assessed and the authors suggested that muscle recovery was faster with cherry juice intake.[9]

This was the first study that examined anthocyanin supplementation on body composition, exercise performance and muscle damage indices of athletes. Some other studies that evaluated the effect of other antioxidants such as Vitamin C, Vitamin E and quercetin showed inconsistent findings. In a clinical trial, Davis *et al*. evaluated the quercetin effects on exercise performance among 12 volunteers. The participants were divided into two groups of the intervention group (500 mg daily quercetin) and control group (500 mg daily placebo). They found a significant improvement in time to fatigue; VO_2 max and endurance values among nonathletic individuals during a 30-km bicycle ride.[24] Utter *et al*. also compared the effects of 250 mg quercetin daily for 3 weeks with placebo among marathon runners and cyclists. They did not find any significant difference in the perceived exertion ratings between the two groups.[25] In a crossover clinical trial by MacRae and Mefferd the effects of quercetin and a combination of quercetin and vitamins were compared on 11 male cyclists during a 6-week period. Although in their results did not find any significant difference in the total time of a 30 km ride and VO_2 max after the intervention, there was a significant increase in peak power among the second group of cyclists.[15] Ganio *et al*. performed a double-blind clinical trial on 11 nonathletic inactive men and women participants. Although their intervention and control groups received 1,000 mg of daily quercetin and placebo for 22 weeks, respectively, it had no significant differences in terms of VO_2 max between the two groups.[26]

Most previous studies did not report significant differences in body composition among antioxidant consumers. Knab *et al*. designed a clinical trial in which quercetin and Vitamin C were used in both groups. They performed a 12 weeks treatment with two doses of quercetin along with Vitamin C and niacin on 941 male and female subjects with the age range of 18–85 years. The participants were randomly divided into three groups of 500 mg daily quercetin, 1,000 mg daily quercetin, and placebo. Similar to the present study their results did not show any significant differences between the intervention and placebo groups in terms of BMI or any other body composition indices.[21] Egert *et al*. investigated the effects of quercetin consumption on 93 obese subjects aged 25–65 years in a crossover study. The participants were received 6 weeks of 150 mg daily quercetin followed by a 5 weeks washout and a course of placebo. However, CRP and body composition indices, including weight, waist circumference, body fat mass, and fat-free mass, had no significant change after the intervention period.[22] Similar to previous studies, the present study did not show any significant differences between the applied anthocyanin and placebo groups.

In an investigation to determine the efficacy of a tart cherry juice in aiding recovery and reducing muscle damage, inflammation and oxidative stress on twenty recreational Marathon runners assigned to either consume cherry juice or placebo for 5 days before, the day of and for 48 h after a Marathon run. Markers of muscle damage (CK, LDH, muscle soreness and isometric strength), inflammation (IL-6, CRP and uric acid), TAS and oxidative stress (TBARS and protein carbonyls) were examined before and following the race. Isometric strength recovered significantly faster in the cherry juice group. No other damage indices were significantly different. Inflammation was reduced in the cherry juice group (IL-6, CRP, uric acid) and TAS was "<10%" greater. Protein carbonyls were not different; however, TBARS was lower in the cherry juice than the placebo at 48 h. They were concluded that cherry juice appears to provide a viable means to aid recovery following strenuous exercise by increasing total antioxidative capacity, reducing inflammation, lipid peroxidation and it may aid to the recovery of muscle function.[9]

The variation across studies in the oxidative damage response to resistance exercise may be attributable to differences in the exercise mode, duration and intensity, training status of participants as well as the use of a variety of indirect measures of oxidative damage to muscle.

The efficacy of dietary antioxidant supplementation in recovery acceleration following muscle damaging exercise is under debate while it is well approved that antioxidants play a vital role in countering free radical activity but within the body research investigating classical antioxidant supplementation on the recovery rate it has consistently shown little or no benefit from supplementation.[27-30] Howatson *et al.*[9] found that the magnitude of the immediate reduction in maximum isometric force production after completing the marathon was not different between trials. This was suggested to indicate that the cherry juice supplement did not prevent the initial muscle injury, which was presumably induced by a combination of mechanical disruption of the myofibrils and increased generation of *reactive oxygen species* and *nitric oxide* species during exercise. Instead the cherry juice was suggested to blunt the secondary muscle damage response associated with the local inflammatory response in the damaged muscle, and this was corroborated by the finding of reduced IL-6, CRP and uric acid response to the marathon race.

Askari *et al*. in a study evaluated the effects of supplementary quercetin on athletic performance, muscular damage and body composition in male athlete students. The results of this study showed that in the quercetin group Lean body mass, TBW, basal metabolic rate, and total energy expenditure increased significantly after intervention. On the other hand, VO_2 max increased, nonsignificantly in the "quercetin" and "quercetin + Vitamin C" groups following the intervention. In this randomized clinical trial, there were four groups who were evaluated to determine the effects of quercetin (a kind of flavonoid) intake on physical and body performance and muscle injury by measuring the LDH, VO_2 max, TEE, TBW, and LBM among the quercetin and Vitamin C groups.

Although the measured indices in this study have been similar to our study, the results are not in concordance[31] as the latter showed that supplementation with quercetin in athletes may improve some indices of performance while the former concluded that anthocyanin supplementation does not affect body composition and body performance indices. It may be due to the difference in the age of participants or even resulted from different follow-up duration.

CONCLUSIONS

We conclude that anthocyanin supplementation does not affect body composition and body performance indices. Longitudinal research with long follow-up periods is suggested to evaluate the effects of anthocyanin intake for future studies. Clinical trials with larger sample sizes of athletes and nonathletes are also recommended.

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