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Influence of Spinach Supplementation on Exercise-Induced Muscle Damage

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ABSTRACT

This study was conducted to assess the effects of chronic daily spinach supplementation on known markers of muscle damage following half-marathon in well-trained healthy young men. Twenty well-trained men volunteered for this study. Participants were randomized in an open study placebo-controlled fashion into two groups, Spinach (S) (n = 10) and placebo (P) (n = 10). The participants took supplementation or placebo daily for 14 days before running. Participants ran 21 km. The spinach supplementation was prepared at 1 mg/kg body weight. Total Antioxidant Capacity (TAC) was determined as marker of plasma antioxidant capacity. LDH and bilirubin were measured as markers of muscle damage. TAC significantly elevated after supplementation in S group (P<0.05). The plasma level of LDH and bilirubin were significantly higher in P group after exercise (P<0.05). These results suggest that chronic daily oral supplementation of spinach has alleviating effects on known markers of muscle damage following a half-marathon in well-trained healthy young men.

Keywords, Spinach, TAC, LDH, Bilirubin.

INTRODUCTION

Exercise is beneficial for the maintenance of a good health but it generates reactive oxygen species (ROS) that may result in oxidative stress [1]. During exhaustive exercise, an insufficiency of endogenous antioxidants may cause antioxidant defense systems to be temporarily overwhelmed [2]. Considerable interest has been shown in finding ways to prevent heavy exercise-induced free-radical production and muscle injury.

An antioxidant is a compound that inhibits or delays the oxidation of substrates even if the compound is present in a significantly lower concentration than the oxidized substrate [3]. Antioxidant supplements, such as vitamin C (ascorbic acid) or vitamin E (alpha-tocopherol), have been very popular among athletes and individuals [4].

Spinach is an important dietary vegetable rich in antioxidants that is commonly consumed fresh in salads or after boiling fresh, frozen or canned leaves. Spinach contains several active antioxidant components, including flavonoids, *p*-coumaric acid derivatives, vitamin C and uridine, which are reported to act synergistically [5]. Spinach leaves contain about 1000–1200mg.kg^{-1} of total flavonoids [6, 7]. Flavonoids and other phenolic compounds act as antioxidants by the free radical scavenging properties of their hydroxyl groups, and are also effective metal chelators. Flavonoids also possess anti-allergic, anti-inflammatory, anti-thrombotic, anti-carcinogenic and antiviral actions, which in part may be related to their free radical scavenging properties [8]. The present study was therefore undertaken to evaluate the possible effects of chronic daily administration of spinach (1 mg/1kg body weight) for 14 days on markers of muscle damage following acute heavy exercise in trained healthy young men.

MATERIALS AND METHODS

Participants
Twenty well-trained healthy young men volunteered for this study. Each participant completed a pre-exercise health status questionnaire. They did not drink alcohol on a regular basis and did not take neither anti-inflammatory drugs nor antioxidant supplements and cigarettes or akin, throughout the study. All participants were verbally informed of the aim of the study and written informed consents were obtained. The protocol of the study was approved by the university ethics committee in accordance with the Helsinki Declaration.

Experimental design
All procedures were undertaken at the laboratory of the Ardabil Sports Medicine Committee. Three weeks prior to the main test, participants underwent a Bruce test on a treadmill to determine their VO_{2max} (maximal oxygen consumption). Participants were randomized in an open study placebo-controlled fashion into two groups, Spinach (S) (*n* = 10) and placebo (P) (*n* = 10). They arrived at the laboratory after overnight fasting.

A baseline blood sample was taken. Afterwards, subjects consumed either placebo (Half of Bread and 20g cheese) or spinach supplement (Half of Bread, 20g cheese plus spinach at 1mg/kg body weight) daily for 14 days. On day of the test, participants attended the athletics arena after overnight fasting. After a second blood sample was taken, participants had a 10-min warm-up involving running at almost 50%VO_{2max} for 5 min and stretching for 5 min. Then participants ran 21 km. They were allowed to consume water *ad libitum* throughout the trial. Blood samples were taken immediately, 2, 24 and 48 h after exercise.

Blood sampling and analysis
Approximately 10 ml of blood was withdrawn at each time point. Three milliliters of blood was placed in heparinized tubes and centrifuged at 3000 rpm for 10 min at 4°C. Plasma was transferred to microtubes and stored at -80°C for subsequent analysis. The rest of the blood was allowed to clot and centrifuged at 5000 rpm for 10 min. Serum was removed and aliquoted in 0.2 ml volumes and stored at -80°C until analysis. Serum LDH and bilirubin were measured using commercial available kits (DGKC, UV) and (DSA) respectively. TAC was analyzed by Varga et al. method [9].

Statistical analysis
Results are expressed as means ± SEM, and $P<0.05$ was considered statistically significant. Data were analyzed for time and group inter-variability using repeated-measures analysis of variance. Where no group effect was found, data was pooled. If the data showed significance, a t-test was performed determining time points of significance.

RESULTS AND DISCUSSION

Participants' characteristics
The characteristics of participants, including age, height, BMI and preliminary VO_{2max}, are summarized in Table 1.

Antioxidant index
Baseline resting serum TAC was not different between groups. TAC increased after supplementation and immediately after exercise compared with baseline just in S group ($P<0.05$). There was significant increase in S immediately after exercise compared to P group ($P=0.016$) (Figure1).

Muscle injury indices
LDH significantly increased immediately post, 2, 24h and 48h after exercise in both groups ($P<0.05$). There was significant difference between groups immediately after exercise ($P<0.001$) (Table2).

Bilirubin was significantly increased immediately and 24h after exercise in P group compared to pre-exercise and 48h after exercise in S group ($P<0.05$). There was significant differences between groups immediately ($P=0.027$), 2h ($P=0.033$) and 24h ($P=0.004$) after exercise (Table2).

To our best knowledge, the present study is first report of the effects of chronic administration of spinach on exercise-induced muscle damage in men. The purpose of this study was to evaluate the effect of 14days spinach supplementation on antioxidant capacity and muscle damage indices.

Pellegrini et al. (2003) showed among some antioxidant vegetables, spinach had the highest antioxidant capacity in the TEAC and FRAP assays [10]. According our data, TAC as a marker of antioxidant power is significantly higher in S group after 2weeks supplementation and immediately after exercise compared with P group showing the influence of this type of supplementation of spinach on antioxidant power.

Half marathon increased LDH immediately, 2, 24 and 48h after exercise compared with pre-exercise in both groups. Furthermore, the rate of enhancement immediately after exercise was significantly higher in P group compared with S group. There are two possible explanations for this difference. First, the anti-inflammatory effects of spinach might be responsible of this phenomenon [11]. Secondly, spinach is a strong antioxidant [12-14]. Elevation of LDH serum concentration shows promotion in leakage of this enzyme after exercise through cell membrane. Cell membrane damage may be results from lipid peroxidation. Decrease of between groups LDH may be owing to effect of spinach as an antioxidant preventing lipid peroxidation. According some researches, lipid peroxidation may lead to membrane permeability and the escape of muscle constituents [15-17]. Inhibitory effects of spinach on lipid peroxidation may have prevented leakage of LDH from cell membrane and consequently alleviation of LDH serum concentration.

It seems spinach supplementation has been able to decrease bilirubin serum concentration, one of the other markers of muscle damage. Some studies reported the elevation of bilirubin after exercise [18]. Bilirubin level seems to depend on the oxidative stress and the oxidative stress can induce hemeoxygenase and leads to heme degradation and bilirubin synthesis [19].

Table1. Subjects' Characteristics

	P group	S group
Age (years)	22.10 ± 1.85	22.60 ± 1.65
Height (cm)	176.80 ± 4.16	177.50 ± 8.38
Weight (kg)	71.00 ± 7.39	75.90 ± 10.73
VO_{2max} (ml.kg^{-1}.min^{-1})	49.70 ± 4.32	48.90 ± 4.91

Values are mean ±SD, VO_{2max}, maximal oxygen consumption

Table 2. LDH, Bilirubin.

Parameters	Groups	Baseline (mean ± SD)	Pre (mean ± SD)	Post (mean ± SD)	2h (mean ± SD)	24h (mean ± SD)	48h (mean ± SD)
LDH (U/L)	P	269.35 ± 27.53	250.24 ± 26.27	504.46 ± 79.23†	502.16 ± 152.66*	433.02 ± 262.47*	392.96 ± 156.90*
	T	251.12 ± 19.39	234.89 ± 23.09	341.67 ± 51.76†	428.68 ± 112.47*	352.38 ± 59.21*	307.08 ± 62.53*
Bilirubin (mg/dl)	P	1.05 ± 0.38	0.99 ± 0.44	2.00 ± 1.20†	1.76 ± 1.03	1.61 ± 0.55†	1.33 ± 1.17
	T	0.93 ± 0.41	0.80 ± 0.37	0.98 ± 0.36	0.94 ± 0.22‡	0.94 ± 0.27	1.23 ± 1.00*

* indicates Significant increase compared with pre-exercise ($P<0.05$). † indicates Significant differences compared with pre-exercise and between groups ($P<0.05$). ‡ indicates Significant differences just between groups ($P<0.05$). Values are mean ±SD. Pre pre-exercise, Post post-exercise.

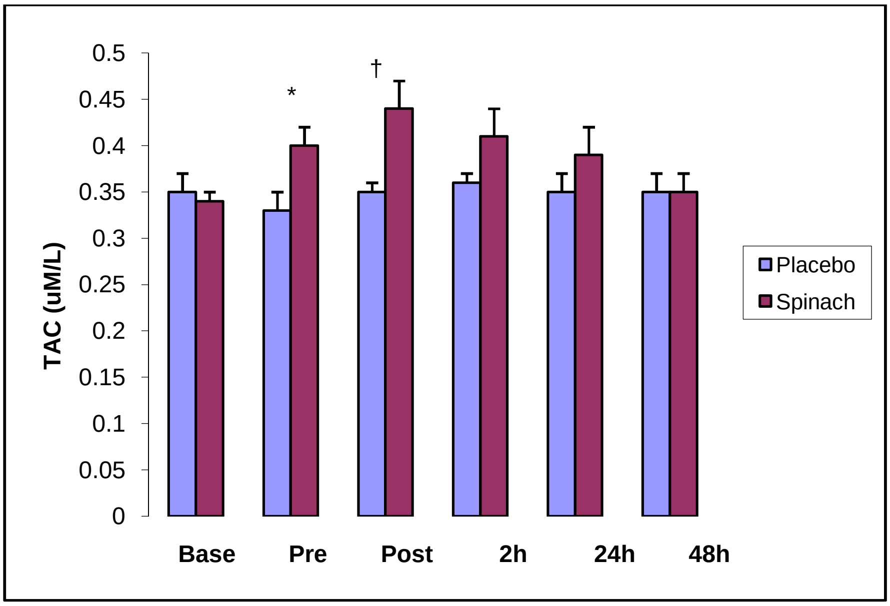

Figure1. The effect of spinach supplementation on plasma totals anti-oxidant capacity (TAC) in trained healthy young men. Values represent mean ± SEM (n=10). * P < 0.05 vs pre-exercise values. † P < 0.05 vs placebo and pre exercise. Pre pre-exercise, Post post-exercise

CONCLUSION

The present study demonstrates that an acute bout of exercise can induce oxidative stress in well-trained healthy young men. Chronic daily oral supplementation with spinach for 14 days has some alleviating effects on muscle damage markers such as LDH and bilirubin and increased antioxidant capacity. It seems antioxidant contents of spinach such as flaveniods, vitamin C and others of that ilk have been able to attenuate the markers of muscle damage.

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