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EFFECT OF TWO DIFFERENT DOSES OF VITAMIN C SUPPLEMENTATION ON EXERCISE-INDUCED LIPID PEROXIDATION AND MUSCLE DAMAGE

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SUMMARY

Oxygen free radicals are highly reactive species that are produced in increased quantities during strenuous exercise and can damage critical biological targets such as membrane phospholipids. The purpose of this study was to compare effects of high or moderate doses of vitamin C (vitC) supplementation on exercise-induced oxidative stress and muscle damage. Twenty-four healthy untrained males performed a 30-min exercise test at 75% VO_2max. Subjects were randomly assigned to one of three groups: placebo (P, lactose), moderate dose (MD, 500 mg vitC) and high dose (HD, 1000 mg vitC). Blood samples were obtained prior to and 2h after supplementing; immediately, 2h and 24h following exercise. Analysis of covariance for repeated measures was used to detect statistical significance of between- and within-subject differences. Plasma levels of vitC and malondialdehyde (MDA), total antioxidant capacity (TAC) and creatine kinase (CK) activity were measured. Plasma vitC levels increased 2h after supplementation, and continued even 2h after exercise in both supplemented groups ($p<0.05$). TAC decreased significantly only in the P group, 24 h after exercise ($p<0.05$). CK increased immediately and 2h after exercise in all groups, and 24h after exercise only in the placebo group, compared with the pre-exercise state ($p<0.05$). Although MDA levels were similar among groups at baseline, it increased significantly 2h after exercise only in the P group ($p<0.05$). Both types of vitC supplementation are likely effective in preventing muscle damage and exercise-induced lipid peroxidation. There is no apparent need to supplement vitamin C in high doses.

Key words: Free radicals, exercise, supplementation, vitamin C

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ÖZET

FARKLI İKİ DOZDA C VİTAMİNİ DESTEĞİNİN EGZERSİZE BAĞLI LİPİD PEROKSİDASYONU VE KAS HASARINA ETKİSİ

Şiddetli egzersizde artan miktarlarda üretilen serbest oksijen radikalleri yüksek derecede reaktif türler olup, membran fosfolipidleri gibi kritik biyolojik hedeflere hasar verebilirler. Bu çalışmanın amacı yüksek ve orta dozlardaki C vitamini (vitC) desteğinin egzersizle uyarılmış oksidatif stres ve kas hasarına etkilerini karşılaştırmaktı. Sağlıklı, ancak antrene olmayan 24 erkek % 75 VO_2max'da 30 dk'lık bir egzersize katıldılar. Denekler rastgele ve eşit olarak plasebo (P, laktoz), orta doz (MD, 500 mg vitC) ve yüksek doz (HD, 1000 mg vitC) gruplarına ayrıldılar. Destek alımının hemen öncesinde ve 2 saat sonrasında; egzersizin akabinde ve 2 ve 24 saat sonrasında kan örnekleri alındı. Tekrarlayan ölçümler için kovaryans analizi ile grup içi ve gruplar arası değişimlerin istatistiksel anlamlılıkları incelendi. Plazma vitC ve malondialdehit (MDA), total antioksidan kapasite (TAC) ve kreatin kinaz (CK) düzeyleri ölçüldü. Plazma vitC düzeyleri her iki (MD ve HD) destek grubunda destekten 2 saat sonra yükseldi ve egzersizden 2 saat sonrasına kadar yükselmeye devam etti. ($p<0.05$). TAC sadece P grubunda egzersizden 24 saat sonra anlamlı olarak düştü ($p<0.05$). CK aktiviteleri tüm gruplarda derhal yükselip egzersizden 2 saat sonra da yüksek kalırken, egzersiz öncesi duruma kıyasla sadece plasebo grubunda egzersizden 24 saat sonra da yüksek kaldı ($p<0.05$). MDA düzeyleri bazal durumda benzerken, sadece P grubunda egzersizden 2 saat sonra anlamlı düzeyde arttı ($p<0.05$). Her iki düzeydeki vitC desteğinin egzersizle uyarılmış lipid peroksidasyonunu ve kas hasarını engellemede benzer etkide oldukları saptandı. Yüksek dozlarda C vitamini desteğine gerek olmadığı sonucuna varıldı.

Anahtar sözcükler: *Serbest radikaller, egzersiz, C vitamini desteği*

INTRODUCTION

Physical exercise may increase accumulation of free radicals and induce oxidative stress as a response to increased oxygen consumption (21). Oxidative stress is a condition in which the existing balance between free radicals production and their subsequent amelioration via the antioxidant defense system becomes skewed in favor of free radical expression (10). Free radicals, or more generally reactive oxygen and nitrogen species (RONS) are products of normal cellular metabolism. RONS are well known for playing a dual role as both deleterious and

beneficial species, since they can be either harmful or beneficial to living systems. The excess RONS can damage cellular lipids, proteins, or DNA; impairing their normal function (27) Evidence for increased RONS production during and following exercise is provided by numerous investigations noting an increase in various oxidative stress biomarkers following both acute aerobic and anaerobic exercise (10).

Postulating that the antioxidant defense system may be temporarily overwhelmed insufficiency of endogenous antioxidants during strenuous exercise, supplementation with antioxidants may be an effective intervention to reduce oxidative stress (28). Available evidence suggests that the ingestion of large amounts of vitamin C (vitC) offers some protection against lipid peroxidation (7). Because vitC is water-soluble, availability may be increased after a single dose, and there may be no need for prolonged supplementation. In a previous study, single high dose vitamin supplementation 2h before one bout of exercise was found to influence lipid peroxidation and muscle damage (19). However, high dose vitC supplementation has some side effects such as iron poisoning (11), and kidney stone prduction (17). The aim of this study was to compare effects of moderate and high doses of vitC supplementation on lipid peroxidation and muscle damage indices.

MATERIAL and METHODS

Subjects: Twenty-four untrained male students volunteered to take part in this study, which received approval from Guilan University Ethical Advisory Committee. All subjects were informed verbally and in writing about the nature and demands of study, and subsequently completed a health history questionnaire and gave their written informed consent. Subjects who smoked or took vitamin supplements were excluded from the study. Subjects were allocated to three groups of eight in a double blind design: high dose vitC (HD), moderate dose vitC (MD) or placebo (P). Physical characteristics and, biceps, triceps, subscapular and suprailiac skinfold measurements were similar in the three groups (Table 1).

Table 1. Subjects characteristics in placebo (P), moderate (MD), and high dose (HD) vitamin C groups. Values for each group represent means (SEM).

Groups (n=8)	Age (yrs)	Height (cm)	Body mass (kg)	BMI (kg m^{-2})	Skinfolds (mm)	VO_2max (ml min^{-1} kg^{-1})
P	22.1 ± 0.6	174.1 ± 1.8	72.4 ± 3.1	23.8 ± 0.8	49.6 ± 5.8	39.1 ± 1.6
MD	21.5 ± 0.8	173.1 ± 5.8	68.4 ± 10.3	21.5 ± 1.0	43.9 ± 5.7	40.6 ± 4.7
HD	20.9 ± 0.7	176.4 ± 2.3	67.4 ± 4.4	21.5 ± 1.0	37.4 ± 3.6	39.3 ± 1.9

Preliminary measurements: The Bruce protocol was used for the VO_2max test. Subjects ran on a treadmill beginning at a moderate pace; every 3 min both the grade and intensity were increased until exhaustion. This was performed at least two weeks before the main trial (16).

Experimental design and procedure: On the day of the test, subjects arrived at the laboratory after an overnight fast of at least 10h. A venous blood sample was taken after subjects had been resting for at least 15 min, after which they consumed a light standardized meal (two boiled eggs) and two capsules of 500 mg vitC for the HD group, one capsule of 500 mg vitC and one capsule of 500 mg lactose for the MD group, and two capsules of 500 mg lactose for the placebo group. After 2h resting, venous blood samples were again taken. Following a warm-up consisting of 5-min running at 50% VO_2max, and 5-min stretching, subjects ran on the treadmill for 30-min at 75% VO_2max. Blood samples were taken immediately, 2h and 24h following the exercise.

Blood sampling and analysis: Approximately 7.0 ml of whole blood was withdrawn in each sampling. About 3.0 ml of whole-blood was added into tubes containing EDTA as anticoagulant. An aliquot of EDTA-treated blood (1.5 ml) was subsequently centrifuged at 3000 g for 15 min (4ºC) to obtain plasma. For vitC analysis, to 0.03 ml of plasma, 0.03 ml distilled water and 0.06 ml of 10% metaphosphoric acid (Merck, Germany) were added and vortexed in a 1.5-ml centrifuge tube for about 10s. The mixture was placed over ice for at least 10 min and sheltered from strong light. It was then centrifuged at 23000 g for 10 min at 4ºC. A 0.05 ml sample of supernatant was immediately injected into an HPLC column (Jasco, Japan) to determine plasma vitC concentration (4).

Serum was obtained by allowing about 4.0 ml of whole blood to clot for 20 min, followed by chilled centrifugation (4ºC) at 3000 g. For MDA analysis, to an aliquot of 0.05 ml serum, 0.25 ml 0.1M trichloroacetic acid (TCA) and 0.7 ml of distilled water were added. Then, the samples were centrifuged at 4500 g for 5 min and used for HPLC analysis (14). Serum creatine kinase (CK) activity was determined at 37ºC using commercially available kit (Roche Hitachi-911, Germany and Japan).

Statistical analysis: All results are expressed as means ± SEM, and $p<0.05$ was considered to be statistically significant. An independent two-way analysis of variance with repeated measures was used to compare results between treatments and over time. Where significant F ratios were found, the Tukey honest significant difference test was used to determine location of variance.

RESULTS

Plasma vitC and TAC: Baseline resting plasma vitC levels were not different among groups (Fig. 1). Two hours after supplementation, plasma vitC was significantly elevated in HD and MD groups ($p<0.05$). VitC concentrations decreased over the course of exercise in the HD and MD groups but were still significantly higher ($p<0.05$) immediately and 2h after the exercise. Twenty-four hour after exercise, plasma vitC concentrations in the MD and P groups were almost similar to their baseline values, but it was still higher than baseline in the HD group. Baseline resting serum TAC was not different between groups (Fig. 2).

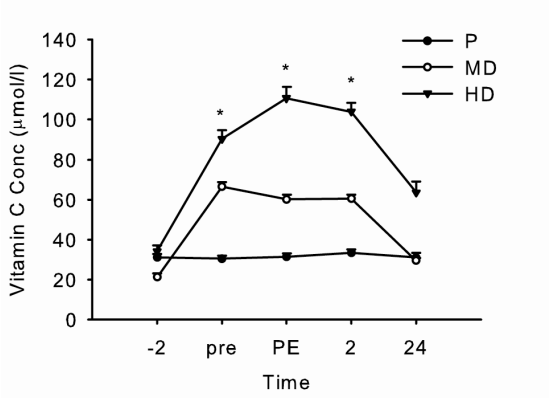

Fig. 1. Vitamin C concentrations in plasma.
*: above baseline and placebo values ($p<0.05$); -2: baseline, PE: post-exercise.

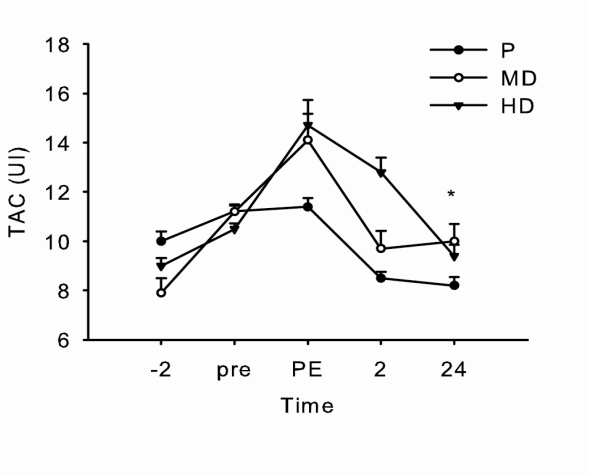

Fig. 2. TAC concentrations in plasma.
*: decline wrt pre-exercise ($p<0.05$).

Although TAC increased after exercise in all groups, it was not statistically significant ($p>0.05$). In the placebo group, TAC declined significantly ($p<0.05$) 24h after the exercise compared with pre-exercise, unlike the supplemented groups. There were no significant differences between groups ($p>0.05$).

Markers of lipid peroxidation and muscle damage: Serum MDA levels are shown in Fig. 3. MDA increased 2h after exercise only in the P group ($p<0.05$). There were no significant differences among groups for MDA over the course of exercise ($p>0.05$). Serum CK activities are displayed in Fig. 4. CK increased above baseline levels after the exercise in all groups. Increases in CK were statistically significant immediately and 2h after exercise in all groups, and 24h after exercise only in the P group ($p<0.05$). There were no differences among groups for CK levels over the course of the experiment ($p>0.05$).

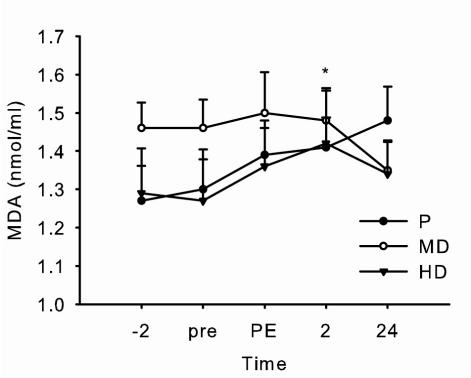

Fig. 3. Serum MDA concentrations.
*: above pre-exercise and baseline in the placebo group ($p<0.05$).

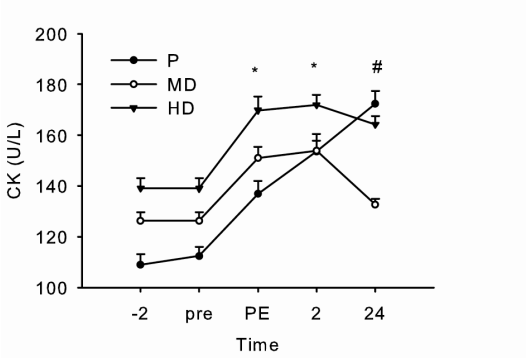

Fig. 4. Serum CK before and after exercise.
*: all groups above pre-exercise ($p<0.05$); #: above pre-exercise in P ($p<0.05$).

DISCUSSION

The main purpose of this study was to investigate whether there are any differences between acute high and moderate doses of vitC supplementation 2h prior to exercise, with respect to lipid peroxidation and muscle damage parameters following a 30-min run at 75% VO_2max. The moderate dose intake has been able to escalate significantly plasma vitC concentration 2h after supplementation, immediately and 2h following exercise, although the quantity of increase was less than the high dose intake (Fig. 1).

TAC significantly declined 24h after exercise in comparison with before exercise levels in the P group, unlike MD and HD groups (Fig. 2). It seems that vitC supplementation in both groups was associated with TAC decrease prevention until 24h after exercise. Change patterns of TAC after supplementation were nearly the same in both groups.

MDA was significantly blunted after exercise in both vitC supplemented groups, whereas it increased significantly 2h after exercise in the placebo group. The result of the study is in agreement with that of Ashton et al. (2), but not Thompson et al. (22,23,24) and Davidson et al. (5,6). The effect of vitC on the lipid peroxidation marker MDA is possibly due to the fitness level or training status of the participants (12). It is possible that untrained individuals may be more responsive to antioxidant supplementation than endurance-trained athletes. Some (8,18), but not all studies (25,26) indicate that endurance training improves endogenous antioxidant defenses. According to these results, effects of both doses of vitC are similar, and moderate dose supplementation has been able to prevent MDA increase after exercise, similar to the high dose intake.

In this study, serum CK activity was increased immediately and 2h after exercise in all groups. Increase in CK may be due to disruption of the sarcomeric Z disk, accompanied by the leakage of this protein out of the cell and into the circulation (9). Furthermore, the efflux of this protein from muscle may occur as a result of increase in the permeability of the myocellular and/or intramuscular vasculature (3). According to some researches; exercise-induced ROS may lead to membrane permeability, and the escape of muscle constituents such as CK (1). In most researches, it is reported that the peak of serum CK activity was observed 24h or 48h following exercise (15,20).

In the present study, CK returned to pre-exercise levels after 24h in the vitC supplemented groups. It seems that vitC supplementation in

both doses has been able to blunt serum CK, similar to serum MDA response. The cause of the effect of vitC on CK is probably due to inhibition of lipid peroxidation in vitC supplemented groups. According to some researches, lipid peroxidation may lead to membrane permeability, and the escape of muscle constituents such as CK (1,13).

In summary, acute supplementation 2h prior to exercise with both doses of vitamin C, increased plasma concentrations of this vitamin before and after exercise, and blunted MDA and CK levels after exercise compared to the placebo group. As a result, acute supplementation with vitamin C in both doses could alleviate lipid peroxidation and muscle damage after 30-min running at 75% VO_2max in untrained males, and probably for prevention of exercise-induced lipid peroxidation. It seems likely that there is no need to intake high doses of vitamin C prior to exercise.

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