Effect of Vitamin C Supplementation on Oxidative Stress Markers Following Thirty Minutes Moderate Intensity Exercise in Healthy Young Women

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Abstract: The aim of this double blind randomized controlled trial was to determine the effect of Vitamin C supplementation on oxidative stress following 30 min moderate intensity exercise. Forty-nine healthy young women randomly assigned into 500 mg day\(^{-1}\) vitamin C supplement (n = 25) or placebo (n = 24) groups for two weeks. Before supplementation and on the day after the intervention period, fasting blood samples were taken. Then all participants ran (1.4-1.7 m sec\(^{-1}\)) for 30 min. Third blood samples were taken at the end of exercise. Plasma malondialdehyde (MDA) and vitamin C were measured using HPLC method. Plasma total glutathione was measured with ELISA method. No significant differences were observed in demographic and vitamin C intakes before intervention between groups. Plasma MDA levels decreased and plasma total glutathione increased significantly (p<0.05) in both groups. No significant differences were observed between groups after exercise. There were significant differences in plasma vitamin C concentrations after intervention and exercise between groups. In conclusion, vitamin C supplementation (500 mg day\(^{-1}\)) for two weeks does not affect oxidative stress markers following moderate intensity exercise in healthy young women.

Key words: Vitamin C, malondialdehyde, glutathione

INTRODUCTION

Available evidence indicates that many chronic diseases, including cardiovascular diseases (CVD) and some forms of cancer are initiated by free radical oxidation of lipids, nucleic acids, or proteins (Timothy, 2004). Oxidative stress has been defined as a disturbance in the equilibrium status of prooxidant/antioxidant system. Many macromolecules are damaged with oxidative stress. There are some studies which indicate oxidative stress has a role in pathogenesis of more than one hundred diseases (Thomas, 2006).

Exercise can increase oxygen utilization up to 200-fold above resting levels in active muscle fibers and it has been suggested that superoxide production increases with this large increase in oxygen flux through muscle mitochondria during exercise (Khassef et al., 2003; Goldfarb et al., 2005a). An increase in oxidative stress markers during aerobic exercise has been reported. These findings suggest that the normal defense mechanisms in the body can be insufficient to adequately handle the increased production of Reactive Oxygen Species (ROS). Nutritional antioxidants have been proposed to help augment the normal antioxidant protection levels and help preventing damage to cellular components from ROS (Goldfarb et al., 2005a, b). At present, there is no perfect defined index for oxidative stress assessment, but various criteria have been suggested (Urso and Clarkson, 2003). Studies that examined the role of vitamin C in preventing exercise-induced oxidative stress in human are limited. Results of studies on the effects of vitamin C on lipid peroxidation and glutathione status have reported different results. In some of them, vitamin C supplementation has resulted in a decrease in lipid peroxidation after exercise (Goldfarb et al., 2005a, Mavroloudis et al., 2004; Schroder et al., 2000; Vasankari et al., 1997), while no changes have been shown in others (Goldfarb et al., 2005b, c; Duthie et al., 1990). The aim of this double-blind randomized controlled trial was to determine the effect of vitamin C supplementation on oxidative stress markers following 30 min moderate intensity exercise in healthy young women.

MATERIALS AND METHODS

Forty-nine healthy young women (aged 20-33 year) participated. All experimental procedures were explained.
and written consents were signed by the volunteers. The study was conducted during 2006 in Abwaz, located in south-west of Iran. The subjects had not performed professional exercise during 12 months prior the intervention. They had no history of chronic diseases. All subjects were nonsmokers. They had not consumed dietary supplements at least for 6 months before the intervention. Before supplementation, blood samples were collected under post absorptive condition (8-12 h). Anthropometric measurements were done between 08:00 to 10:00, according to the World Health Organization’s standard protocols (WHO, 1995). Subjects were randomly assigned to either vitamin C (500 mg day\(^{-1}\)) or placebo (500 mg day\(^{-1}\) lactose) for two weeks. Vitamin C and lactose were prepared in capsule forms. The subjects were advised to consume the capsules after dinner.

After completing supplementation, all subjects rested at least 10 min before starting the exercise and 7 mL blood sample was taken. Then they ran with a 5-6 km h\(^{-1}\) intensity for 30 min (Pate et al., 1995). Two subjects together (one person from supplement group and one person from placebo group) initiated to run. Third blood samples were taken immediately (2-3 min) after running. After plasma preparation (15 mg EDTA per 7 mL blood, centrifuged at 3000 g for 10 min), plasma samples were stored in -70°C freezer until biochemical analysis.

Malondialdehyde (MDA) as a lipid peroxidation marker was determined using HPLC (High Performance Liquid Chromatography) method as described by Wong et al. (1987) and Seljeskog et al. (2006). MDA is reacted with thiobarbituric acid (TBA) when heated under acidic condition and MDA-TBA, pink complex is produced. This complex can be measured using florescence detector with wavelengths of 525 nm (excitation) and 560 nm (emission). HPLC analysis was performed using a Jasco HPLC (Jasco Corporation, Japan).

Total glutathione (TGS) was measured using ELISA method (Goldfär et al., 2005b) with glutathione assay kit (cat. No. 703002, USA, Cayman chemical company). According to the protocol of kit, plasma samples were deproteinized with metaphosphoric acid before assay and then concentrated by lyophilization. Finally, TGS was measured using ELISA method.

Vitamin C status was assessed using HPLC method as described by Fidanza (1991). After primary preparation, ascorbic acid was converted to dehydroascorbic acid with ascorbate oxidase spesula (Cat. No. 1073661001, Roche, Germany) and then OPDA (O-phenylenediamine)-dehydroascorbic acid yellow complex was produced. This component can be measured using florescence detector wavelengths of 355 nm (excitation) and 425 nm (emission). HPLC analysis was performed using a Jasco HPLC (Jasco Corporation, Japan).

**Statistical analysis:** All analysis were performed using SPSS statistical software (version 11.5). Statistical significance level was p<0.05.

Differences between groups were tested by independent samples t-test. Differences within groups (before and after exercise) were tested by paired samples t-test. Plasma vitamin C concentrations during each session (before and after intervention) and after exercise were tested by repeated measures analysis of variance.

**RESULTS AND DISCUSSION**

All subjects successfully completed the study. There were no statistical differences with regard to age, weight, height and BMI between supplement and placebo groups (Table 1). Vitamin C and energy intakes were not statistically different between groups.

Plasma MDA and TGS concentrations were not statistically significant between the study groups after exercise (p = 0.43 and 0.57, respectively). Before and after exercise comparison indicated that there is significant decrease in MDA and increase in TGS in S and P groups, respectively (Table 2, 3).

There were significant differences in relation to plasma vitamin C concentration after intervention and after exercise between groups (p = 0.04 and 0.02, respectively). No significant difference was seen in supplement group after exercise and after intervention, but there was a significant (p<0.05) difference in placebo group (Fig. 1).

The present investigation indicated that 500 mg vitamin C supplementation after a short period did not

<table>
<thead>
<tr>
<th>Variables</th>
<th>Supplement group (n = 25)</th>
<th>Placebo group (n = 24)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>24.00±0.3</td>
<td>23.00±2</td>
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<tr>
<td>Weight (kg)</td>
<td>56.00±8</td>
<td>57.00±7</td>
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<td>Height (cm)</td>
<td>161.00±4</td>
<td>159.00±5</td>
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<tr>
<td>BMI (kg m(^{-2}))</td>
<td>21.00±2</td>
<td>22.00±2</td>
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<tr>
<td>Plasma vitamin C concentration (μM L(^{-1}))</td>
<td>11.36±0.13</td>
<td>9.00±1.66</td>
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</table>

<table>
<thead>
<tr>
<th>Plasma MDA concentration (Mean±SD) in supplement and placebo groups before and after exercise</th>
<th>Before exercise</th>
<th>After exercise</th>
<th>Difference</th>
<th>p-value</th>
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</thead>
<tbody>
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<td>Supplement group</td>
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<td>1.09±0.14</td>
<td>0.20</td>
<td>0.002</td>
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<td>Placebo group</td>
<td>1.29±0.24</td>
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<td>0.15</td>
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<table>
<thead>
<tr>
<th>Plasma TGS concentration (Mean±SD) in supplement and placebo groups before and after exercise</th>
<th>Before exercise</th>
<th>After exercise</th>
<th>Difference</th>
<th>p-value</th>
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<tr>
<td>Supplement group</td>
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<td>4.53</td>
<td>0.001</td>
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</tbody>
</table>
Fig. 1: Comparison of plasma vitamin C concentration in supplement and placebo groups (*p<0.05)

significantly influence the oxidative stress markers (MDA and TGS) after moderate exercise. But a significant effect was observed in plasma vitamin C concentration.

MDA, lipid peroxidation marker, was not affected by vitamin C supplementation, but a significant decrease was observed under exercise. Goldfarb et al. (2005b) demonstrated no significant changes in MDA with 500 and 1000 mg vitamin C per day for 2 weeks after running at 75% \( V_{O_{2 max}} \). The result of the present investigation is similar to findings of other studies (Goldfarb et al., 2005a; Wen et al., 1997). Alessio et al. (1997) reported a significant increase in TBARS (Thiobarbituric Acid Reactive Substances) in both groups (supplement and placebo) after exercise at 80% \( V_{O_{2 max}} \), but this increase was attenuated in supplement group with 1 g day\(^{-1}\) vitamin C for 2 weeks (33 and 46% in supplement and placebo groups, respectively). The present study does not confirm their findings with regard to MDA. The decreased MDA in Rokitzki et al. (1994) study with vitamins C and E supplement after marathon run was similar to the results of present study. Influence of vitamin C supplementation on oxidative stress may have potential beneficial effects but this is still controversial and appears to be related to dose of vitamin C and intensity or duration of exercise.

Another potential confounding factor that could influence the result of vitamin C supplementation was the dietary consumption of vitamin C by the subjects. Controlling this variable was done by advising the subjects to maintain their usual diets and estimating their intake by dietary recalls for 3 days. There were no significant differences between groups with regard to vitamin C consumption.

In Schroder et al. (2000) study, lipid peroxidation decreased in supplements group (vitamins C, E and ß-carotene) after exercise. Based on Bloomer et al. (2005) research, the plasma MDA levels were significantly elevated in the subjects after aerobic exercise with high intensity (>80% \( V_{O_{2 max}} \)). These studies indicated that intensity of the exercise affects lipid peroxidation. Probably, diminished MDA in this study can be contributed by moderate intensity exercise.

According to Johnston and Cox (2001) study, MDA were lowered significantly once mean plasma vitamin C concentration approached 50 \( \mu M \), a level that corresponded to supplemental intake over 500 mg day\(^{-1}\) for 2 weeks. In this study, because more than 90% of participants lived in dormitory, their vitamin C intakes were probably low. Consequently, their usual plasma vitamin C concentrations are low, so optimum level of vitamin C was not obtained with 500 mg day\(^{-1}\) vitamin C supplement for 2 weeks.

Some investigators such as Goldfarb et al. (2005a and b) have reported no significant changes in TGS following exercise. In numerous studies glutathione has been measured in reduced and oxidized forms separately, while in this study only total glutathione was assessed. It is unclear why vitamin C supplementation has no effect on the glutathione system. Thiol moieties in protein structure and other substances were swiftly oxidized in the absence of vitamin C in the *in vitro* study, however, when vitamin C was added, these substances were protected from ROS. The absorption and distribution of vitamin C *in vivo* might be a factor to be considered in order to understand why proteins were protected but the glutathione system was not protected. The reason for the protection of protein by vitamin C against ROS as opposed to the glutathione system in blood remains to be elucidated. Sahlin et al. (1992) reported that TGS increases significantly following 80 min exercise at 60% \( V_{O_{2 max}} \), but in that study effect of exercise without supplementation was considered.

Plasma vitamin C concentration increased significantly in placebo group after exercise, while no significant change was observed in supplement group. Plasma ascorbate is the first compound that becomes oxidized in oxidative stress condition (Thomas, 2006), therefore it is expected that plasma vitamin C concentration decreases after exercise. This decline was not significant in supplement group. An increase in plasma vitamin C levels following exercise has been reported in some studies (Rokitzki et al., 1994; Nieman et al., 2002; Petersen et al., 2001). Proposed
mechanisms include increased cortisol secretion during exercise that results in vitamin C flux from adrenal glands which finally increases vitamin C levels. In this study such increase took place only in placebo group.

In summary, findings of this double blind randomized controlled trial showed that vitamin C supplementation (500 mg day⁻¹) for two weeks does not affect plasma MDA and TGS concentrations.

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