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Effect of Supplementation of Antioxidant Nutrients Against Oxidant Stress during Exercise*

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ABSTRACT

This study was undertaken to evaluate the effect of 4 weeks of α-tocopherol(800 I.U./d) supplementation on oxidant stress of eleven female aerobic-majoring students during rest and exercise. Changes in the activity of the antioxidant enzyme glutathione peroxidase were also studied. Serum α-tocopherol concentration was significantly increased with vitamin E supplementation(710.1±113.8μg/dl vs. 1,485.8±105.2μg/dl). In addition, serum MDA concentration, an index of lipid peroxidation, significantly decreased after vitamin E supplementation. However, MDA values after exercise increased to pre-supplementation levels. Serum glutathione peroxidase activity significantly increased with vitamin E supplementation. The enzyme activity showed a trend toward decrease after exercise. Serum cholesterol values were not significantly affected by vitamin E supplementation. However, serum triglycerides significantly increased after supplementation. The values diminished slightly after exercise, but remained higher than pre-supplementation levels. The findings in this study indicate a protective effect of α-tocopherol supplementation against oxidative stress during resting periods. These supplements apparently work by decreasing lipid peroxidation and increasing glutathione peroxidase activity. However, vitamin E supplementation did not prevent exercise-induced increases in lipid peroxidation. (*Korean J Nutrition* 30(9) : 1061~1066, 1997)

KEY WORDS : antioxidant · vitamin E · oxidant stress · exercise · glutathione peroxidase.

Introduction

Good nutrition is related to prevention and treatment of several diseases. Lack of physical activity is also linked to a broad spectrum of chronic diseases such as coronary heart disease, obesity, osteoporosis, and diabetes[1]. Therefore, both adequate nutrition and regular exercise are important factors to enhance health.

Free radicals are any species capable of independent existence that contains one or more unpaired electrons that are very reactive[2]. The oxygen free radicals can be produced in oxidation reactions of normal metabolism in the body. Approximately 2-4% of the oxygen that is consumed by mitochondria results in the generation of oxygen free radicals, due to electron leakage in the electron transport chain[3][4]. Activated oxygen species appear to play a key role in peroxidation injury. The products of peroxidation are involved in cell membrane alterations, protein damage, and DNA damage[5]. The generation of oxygen free radicals has been reported to be increased during exercise as a result of increases in mitochondrial oxygen consumption and electron transport flux [6][7]. The magnitude of oxidative damage occurring after exercise is dependent on the rate of oxygen consumption and the balance of antioxidants and pro-oxidants in the body[8].

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Our bodies have several defense mechanisms against attack by oxygen free radical[9]. The well known antioxidant nutrients are vitamin E, vitamin C, β-carotene, and selenium. A reduction in vitamin E concentration is thought to increase the susceptibility of tissues to oxidative stress and injury. Vitamin E is concentrated in membranes and blood lipoproteins[2]. Increase in expired pentane, loss of membrane integrity, reduced oxidative capacity, and increase in lipid peroxidation have been reported in vitamin E deficient-animals[10][11]. In humans, the production of expired pentane, an index of peroxidation, was lowered by vitamin E supplementation[12]. There are also antioxidant enzymes in the body involved in protecting cells from oxidative damage[13][14]. The concentration of these enzymes may be affected by several nutrients. The changes in the antioxidant enzyme glutathione peroxidase with vitamin E supplementation and exercise have not been examined so far.

Few studies have examined the effects of vitamin E supplementation on exercise-induced lipid peroxidation and antioxidant enzyme activities. Therefore, this study has been designed to investigate the effect of α-tocopherol supplementation on oxidative stress in female physical education students and the effect of α-tocopherol on limiting exercise-induced oxidative stress.

Methods

1. Subjects

The subjects were eleven female students who were majoring in aerobics. The subjects were regularly trained for 1.5 hour/day, 6 times per week. The two day dietary records were obtained from each subject before commencement of the study. Maintenance of established dietary patterns was encouraged throughout the study. Subjects were supplemented with 800 IU vitamin E(Longs Drug Stores, CA, USA) taken as two capsules(400 IU twice daily) for 4 weeks. Compliance with the supplement was confirmed by measurement of subject's serum α-tocopherol before and after the treatment period(Fig. 1). Fasting blood samples were collected before and after exercise on the starting day(day 0) of vitamin E supplementation and the ending day(day 28) of supplementation. The exercises on the day of blood collection consisted of 10 minutes of preparation, followed by 30 minutes of main aerobic exercise. The intensity of aerobic exercise was set at 75－80% of maximum heart rate of the subjects(150－160 beats/min). The blood was collected from an anticubital vein just before exercise(in 5 minutes) and right after exercise(in 5 minutes). Blood samples were centrifuged at 2,000 rpm for 30 minutes. The serum was divided into aliquots and stored at －32℃ until further analysis. Samples collected at different time points from each subject were analyzed at the same time for specific biochemical measurements to eliminate interassay variation.

2. Biochemical analysis

Serum was analyzed for vitamin E using high performance liquid chromatography(HPLC) by Bieri's method[15]. DL-α-tocopheryl acetate and dl-α-tocopherol were used as internal and external standards. Eluted peaks were detected at 292nm with a UV detector(Young Hwa Scientific Co., Korea). MDA was measured by a thiobarbituric acid assay procedure using tetramethoxypropane as a standard and results were expressed as nanomoles of MDA per mililiter of serum[16].

Serum glutathione peroxidase activity was determined by the coupled assay of Paglia and Valentine[17]. The reaction mixture contained 50mM potassium phosphate buffer at pH 7.0, 1mM EDTA, 1mM NaN_3, 0.2mM NADPH, 1U/ml glutathione, 0.1 mM H_2O_2, and 0.05－0.1ml of serum in a total volume of 1ml. One unit of glutathione peroxidase activity was defined as 1μmol NADPH oxidized per min.

Total serum protein, cholesterol, and triglyceride concentrations were analyzed by test kits(Yong Dong Co., Korea). HDL-cholesterol was analyzed by a test kit(Gook Je Co., Japan). LDL-cholesterol value was calculated by using Friedewald's formula[18].

3. Statistical analysis

Statistical analysis was carried out with SAS software[19]. Values reported are means±SEM. The data was subjected to the general linear model procedure test for repeated measurements and least significant

test to determine the statistical significance of vitamin E supplementation and exercise on the measured parameters.

Results and Discussion

1. General characteristics of the subjects

General characteristics of the subjects are presented in Table 1. Mean age of the subjects was 21.2 years old. Mean height was 159.9cm and body weight was 50.5kg. Mean body fat of the subjects measured by electrical impedence method(Gilwoo Co., Korea) was 22.3%. The nutrient intake of each subject is shown in Table 2. The mean energy intake of the subjects was 1,487.1kcal. The mean vitamin A and vitamin C intakes were 331.3RE(47.3% of RDA) and 134.4mg(244.4% of RDA), respectively.

2. Serum vitamin E status of the subjects

Serum vitamin E concentrations of the subjects are shown in Fig. 1. Serum vitamin E concentration before vitamin E supplementation was 710.1μg/dl. Acceptable values of vitamin E in serum are known to be above 800μg/dl[20]. Therefore, subjects in this study had slightly lower serum vitamin E concentrations in the beginning of the experiment. Vitamin E supplementation(800 I.U./day) for 4 weeks resulted in a significant increase in serum α-tocopherol concentration. The mean value was 1,485.8μg/dl. Meydani et al.[8] has reported similar increases in serum vitamin E concentration from 905μg/dl to 1,416μg/dl after 48 days of α-tocopherol(800 I.U./day) supplementation.

The mean vitamin E value after exercise changed from 859.9μg/dl to 1509.6μg/dl with vitamin supplementation. There was a tendency toward increase in vitamin E concentration after exercise, but the values were not significantly different from resting values. Meanwhile, Duthie et al.[21] reported a significant increase in α-tocopherol serum concentration in a vitamin E-supplemented group after completion of a marathon race. The α-tocopherol mobilization in that study was interpreted as a coreaction with increased fatty acid utilization due to exercise. Pincemail et al.[22] proposed that mobilization of tocophe-

Table 1. General characteristics of the subjects

Variables	Mean±SEM	Range
Age(year)	21.2±0.6	18 – 24
Height(cm)	159.9±1.0	154 – 163
Weight(kg)	50.6±1.2	45 – 58
Body fat(%)	22.3±1.0	18.9 – 26.3

Table 2. Mean daily intake of nutrients

	Mean intake	% RDA[1]
Energy(Kcal)	1487.1 ±90.4[2]	74.4
Protein(g)	52.5 ± 2.4	87.5
Fat(g)	35.9 ± 4.5	
CHO(g)	238.6 ±17.1	
Ca(mg)	351.7 ±42.9	50.2
Fe(mg)	12.3 ± 0.8	68.3
Vit A(RE)	331.3 ±22.8	47.3
Vit B_1(mg)	0.95± 0.06	95.0
Vit B_2(mg)	0.92± 0.07	76.7
Niacin(mg)	14.8 ± 1.0	113.8
Vit C(mg)	134.4 ±26.6	244.4

1) % RDA : % of Korean Recommended Daily Allowances for 20 – 29year old women
2) mean±S.E.

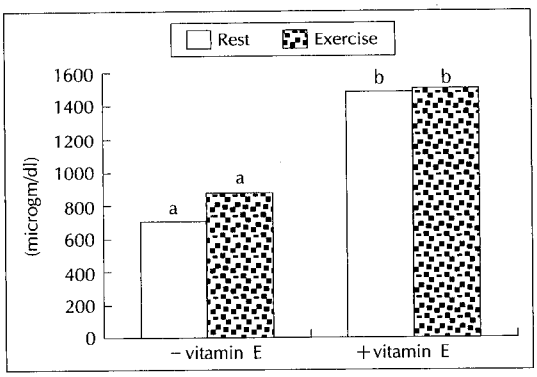

Fig. 1. Serum vitamin E values(μg/dl) of the subjects. Groups with different letters are significantly different($p<0.05$).

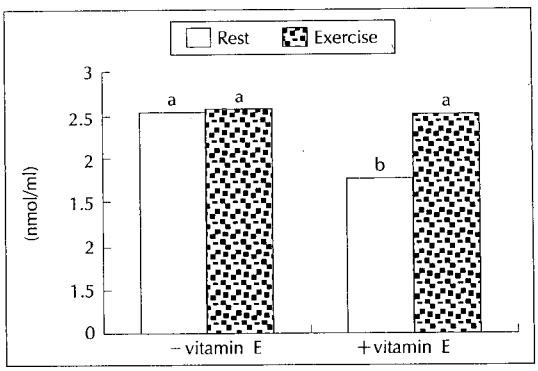

Fig. 2. Malondialdehyde contents of serum(nmol/ml). Groups with different letters are significantly different($p<0.05$).

rol with long-duration exercise could help to prevent severe lipoperoxidation phenomena from occurring in skeletal muscle.

3. Lipid peroxidation and glutathione peroxidase activities in the serum

Serum malondialdehyde(MDA) contents of the subjects are shown in Fig. 2. In the resting period, serum MDA production was significantly decreased from 2.57nmol/ml to 1.83nmol/ml with vitamin E supplementation. However, exercise diminished the beneficial effect of vitamin E supplementation by increasing MDA production near to the previous value (2.59nmol/ml before supplementation vs. 2.47nmol/ml after supplementation). Kanter et al.[23] showed a similar result after supplementing subjects with vitamin E, vitamin C, and β-carotene mixed antioxidants. In their study, ingestion of antioxidant vitamins resulted in a significantly lower resting level of serum MDA. However, antioxidant supplementation did not prevent exercise-induced increases in lipid peroxidation. Meanwhile, Sumida et al.[24] reported that supplementation of 300mg vitamin E for 4 weeks reduced exercise-induced lipid peroxidation. In that study, subjects cycled on an ergometer to exhaustion. Therefore, it is suggested that the effect of supplemental vitamin E against MDA production may be amplified under extreme exercise conditions.

Glutathione peroxidase is one of the most important antioxidant enzymes in the body. Selenium is an integral component of this antioxidant enzyme. The relationships between selenium and vitamin E in preventing diseases related to oxidative injury like liver necrosis and exudative diathesis in animals are well known. However, changes in glutathione peroxidase activity with vitamin E supplementation and exercise have not been extensively examined so far. Serum glutathione peroxidase activities in the subjects are listed in Table 3. Selenium dependent glutathione peroxidase activity(U/mg protein) significantly increased from 1.51U/mg protein to 1.85U/mg protein with vitamin E supplementation. Therefore, it is suggested that increases in serum vitamin E concentration have a synergistic effect on serum glutathione peroxidase activity. With exercise, the enzyme activity tended to decrease, but the value was kept high with vitamin E supplementation.

4. Serum lipid status of the subjects

Serum lipid status of the subjects is listed in Table 4. Serum cholesterol and triglyceride values were within normal ranges throughout the experimental period. Serum cholesterol, HDL-cholesterol, and LDL-cholesterol values were not changed much either by vitamin E or by exercise. Similar results have been reported by several other authors[25-27]. Studies on the effects of vitamin E supplementation on serum HDL-cholesterol have shown conflicting results. Some studies showed no changes in HDL-cholesterol[25)28)], but others showed increases with vitamin E supplements [26)29)]. Kalbfleisch et al.[30] reported that only subjects with very low HDL-cholesterol could increase it

Table 3. Glutathione peroxidase activities of serum

	−Vitamin E		+Vitamin E	
	Rest	Exercise	Rest	Exercise
GSHPx(U/ml)	$1.04\pm0.09^{1,a}$	0.88 ± 0.06^{a}	1.27 ± 0.09^{b}	1.23 ± 0.04^{b}
GSHPx(U/mg pro.)	1.51 ± 0.12^{a}	1.28 ± 0.11^{a}	1.85 ± 0.13^{c}	1.76 ± 0.06^{bc}
Protein(mg/dl)	6.85±0.13	6.95±0.14	6.86±0.08	6.98±0.09

1. Values are means±SEM
2. Values with different superscripts within a row are significantly different($p<0.05$)

Table 4. Serum lipid status of the subjects

	−Vitamin E		+Vitamin E	
	Rest	Exercise	Rest	Exercise
Cholesterol(mg/dl)	155.7 ± 8.9^{1}	156.8± 9.1	154.5± 7.2	153.9±4.8
HDL-chol(mg/dl)	53.0± 2.9	50.4± 5.2	49.2± 3.3	52.1±3.5
LDL-chol(mg/dl)	84.0±10.3	88.6±18.2	76.4± 5.4	81.5±5.8
TG(mg/dl)	79.0 ± 3.5^{a}	83.0 ± 4.3^{ab}	100.7 ± 10.0^{c}	91.5 ± 5.6^{bc}

1. Values are means±SEM
2. Values with different superscripts within a row are significantly different($p<0.05$)

with vitamin E supplements.

Serum triglyceride concentration of the subjects was significantly increased from 79.0mg/dl to 100.7mg/dl by vitamin E supplementation. It may be due to an increased intake of fat(1.5g/day) contained in vitamin E capsules. Others[31)32)] also found increases in triglycerides by supplementing with vitamin E. However, in some reports[25)26)], no changes in serum triglycerides were found.

Conclusion

Proper exercise is beneficial to enhance health. However, exercise can temporarily increase oxidant stress by increasing oxidation reactions in the muscle. Therefore, it is recommended to acquire enough antioxidants, especially for athletes. In this study, female university aerobic-majors were supplemented with vitamin E(800 I.U.) for 4 weeks to evaluate the protective effect of vitamin E against oxidant stress. Vitamin supplementation significantly increased serum α-tocopherol concentration in the subjects and decreased lipid peroxidation by decreasing serum malondialdehyde production. Glutathione peroxidase, another well known antioxidant enzyme, showed significantly increased activity with vitamin E supplementation. Therefore, it was concluded that four weeks of vitamin E(800I.U.) supplementation has protective effects against oxidant stress in athletic students. However, the protective effects were not strong enough to prevent exercise-induced increases in lipid peroxidation.

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