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Effects of Vitamin C and Vitamin E Supplementation on Anti-oxidative System of the Smokers and Non-smokers

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ABSTRACT

This study compared intake of vitamins and antioxidant nutritional status of smokers and nonsmokers, and the effect of supplementation of vitamin C and vitamin E on antioxidant system of smokers and nonsmokers. Subjects were 30 smokers and 30 non-smokers of male university students. They were divided into groups of 10 subjects each one with supplementation for 4 weeks, to investigate the effects of supplementation. Smokers were divided into vitamin C supplement group, vitamin E supplement group and vitamin C and vitamin E combination supplement group, and so were nonsmokers. The supplementation of vitamin C was 500mg and vitamin E was 400IU. There was no significant difference of antioxidant vitamin intakes between smokers and non-smokers, and plasma concentration of vitamin C in smokers was lower than non-smokers ($p < 0.05$). Blood pressure was higher in smokers ($p < 0.05$), with no difference in blood glucose levels, methemoglobin and TBARS, but SOD activity was significantly higher in non-smokers ($p < 0.001$). Vitamin C supplementation resulted in a significant decrease of diastolic blood pressure ($p < 0.01$), systolic blood pressure ($p < 0,001$) and methemoglobin ($p < 0.001$) in smokers. Also a significant decrease of diastolic blood pressure ($p < 0.05$), systolic blood pressure ($p < 0.05$), blood glucose ($p < 0.05$), methemoglobin ($p < 0.001$) and TBARS ($p < 0.05$), with significant increase of SOD activity ($p < 0.001$) were found in vitamin E supplement group. In vitamin C and vitamin E combination supplement group, there was a significant decrease of diastolic blood pressure ($p < 0.05$), methemoglobin ($p < 0.01$) and TBARS ($p < 0.05$). In non-smokers, methemoglobins ($p < 0.001$) of vitamin C supplement group and vitamin E supplement group decreased, and diastolic pressure ($p < 0.05$), methemoglobin ($p < 0.01$) and TBARS ($p < 0.05$) significantly decreased in vitamin C and vitamin E combination supplement group. These results indicated better efficacy of antioxidant supplementation in smokers than in nonsmokers, suggesting that the supplementation of vitamin C and vitamin E might decrease the oxidative stress and various risk factors of smoking-related diseases. (*J Community Nutrition* 6(3) : 146~154, 2004)

KEY WORDS : supplementation of vitamin C and vitamin E · antioxidant system · oxidative stress.

Introduction

Approximately 4,000 chemicals are known to cause not only chronic pulmonary diseases but also various cancers of lung, throat, esophagus, bladder, kidney, pancreas, uterine cervix and stomach. While the awareness of health damages of cigarette smoking has decreased the smoking rate in the developed countries since 1960, that of the men over the age of 20 in this country was reported to be 67.7% which is about twice of the number in the USA and France, respectively, according to the National Survey in 1998 (Nam et al. 1998). According to the recent report of the National Statistical Office, the death rates from lung cancer doubled, becoming the fastest growing as well as the second most popular cancer,

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while those from the cancers of stomach, liver and uterus tend to decrease. Although this rocketing rate of lung cancer is attri-buted to the pollution, it is also considered to result from the increase of the smoking rate (Choi et al. 2000).

Human body is well balanced between pro-oxidants and anti-oxidants. The reactive oxygen species such as peroxides and free radicals keep occurring during the cell respiration, which are effectively removed in the normal conditions. Oxidative stress takes place when this balance is not maintained any longer, harming the cells (Sies 1991). Cigarette smoke increases the oxidative stress, leading to the rise of free radicals, which also brings the acceleration of the peroxidation of lipids and cell membrane as well as denaturation of biomolecules such as proteins and DNA. Therefore, consistent smokers are considered to be continuously exposed to the risk of having cancer (Mayne 1994).

Antioxidants like vitamin C, α-tocopherol and β-carotene prevent free radicals from causing peroxidation of unsaturated fatty acids by reacting with the radicals. As a consequence, they take important roles in protecting the human body from the oxidative stress and recovering the damaged cells and tissues. Antioxidants, converted into active peroxides, are either excreted or reduced to the original forms. However, the level of antioxidants drops if they keep excreting due to the increase of oxidative stress. Oxidative stress brings a change of the activities of superoxide dismutase (SOD), catalase and GSH-peroxidase (GSH-px) along with antioxidant nutrients (Abou-Sief 1996). Antioxidant supplementation has been shown to increase the plasma level of antioxidant nutrients and enhance the activities of antioxidant enzymes. Consequently, this improves the general immune system by removing the reactive oxygen species increased by oxidative stress (Brown et al. 1996).

There are conflicting reports regarding the effect of smoking on oxidative stress, depending on the many factors such as age and gender of the subjects as well as analyzing method. Teenagers with a short term smoking history can have evidence of oxidative stress (high serum TBARS and low serum vitamin C and folate levels) and impaired oxidative defense system (Kim et al. 2003). In contrast, another study (Kim et al. 2004) reported that serum TBARS production were not influenced by smoking. There has even been a study that demonstrated a higher lipid peroxide levels in nonsmokers (Yoon 1997). Therefore, this study compared the difference in antioxidant nutrient status between smokers and nonsmokers and determined the effects of vitamin C and vitamin E supplementation on their plasma levels and the activities of anti-oxidative enzymes as an attempt to provide a strategy to reduce the smoking-related diseases.

Sampling and Methods

1. Subjects and period of study

This study was performed in a university located in Gyeonggi Province, with male university students, thirty smokers and thirty non-smokers, without specific diseases or intake of nutrient supplements. Subjects were divided into 6 groups of 10 persons each according to the supplementation experiment. Smokers and nonsmokers were divided into three groups each as vitamin C supplement group, vitamin E supplement group and both vitamin group (vitamin C and vitamin E combination). All subjects were alerted to take the supplementation in conditions not different from the usual for 4 weeks from November 2 to November 30, 2000.

The doses of supplementation were 500mg of vitamin C, 400IU vitamin E , and 500mg of vitamin C + 400IU vitamin E, respectively in vitamin C, vitamin E and combination supplement group.

2. Assessment of antioxidant vitamin intake

Food frequency method (Pryor Stone 1993) by direct interview was used to determine the intake of antioxidant vitamins in 92 types of foods, with the frequency classified in 5 categories. Food portion models and pictures of estimated portion sizes were utilized to facilitate the assessments, and seasonal fruits and vegetables were calculated to average intake in a year.

3. Anthropometric measurement and blood pressure

The BMI [Body Mass Index : Weight (kg) /height (m^2)] was calculated using height and weight measured by trained investigator. The blood pressure was measured after at least 10 minutes of rest, utilizing Digital blood pressure instrument (OMROM HEM-705C, Japan), and blood glucose was measured 2 hours after meal, utilizing a stripe (Accutrend GC, Roche). Body fat weight was measured in fasting by B.I.A. method (Bioelectrical Impedance Analysis, Bioelectrical Impedance Fatness analyzer GIF-891, Gilwoo Trading).

4. Blood collection and plasma separation

Fasting blood was taken using disposable syringes pricked

into upper arm vein, and 10ml collected in heparin treated bottles. Part of the whole blood was kept to methemoglobin analysis, and the rest was stabilized for 20 minutes, centrifuged for 15 minutes at 4℃, with 3000rpm rotation, and than stored in deep freezer(−80℃) until analysis. Blood was collected twice, once before supplementation and once after.

5. Measurement of markers of oxidative stress in blood

Methemoglobin was measured by Thomas et al. method (1960). TBARS as an index of lipid peroxidation, and it was measured by Ohkawa et al. (1979) method, with the substance extracted from the reaction with thiobarbituric acid(TBA). An aliquot of 0.2ml plasma was mixed with 1.5ml of 8.1% sodium dodecyl sulfate and 1.5ml of 20% acetic acid, and heated for 1 hour at 95℃ water bath after adding 1.5ml of 0.8% TBA and 0.6ml of distillated water. After cooling in a refrigerator for 5 minutes, it was shaken with 1ml of distillated water and 5.0ml of n-butanol : pyridine(15 : 1, v/v) for 30 seconds. The upper phase(n-butanol : pyridine phase) was collected after centrifugation for 10 minutes at 3000rpm and stabilized for 10 minutes at room temperature, before the absorbance was measured at 532nm with 1,1,3,3-tetraethoxypropane(TEP) as the standard substance.

The SOD activity followed Crapo et al. method(1978) and detected by reaction of superoxide produced by xanthine and xanthine oxidase with cytochrome C.

6. Measurement of vitamin C concentration in blood plasma

Vitamin C concentration in blood plasma was measured utilizing Tsan et al.(1982). An aliquot of 0.2ml of blood plasma was added with 0.1ml of ascorbate oxidase working solution in 20mg/L and mixed well. After incubation for 15 minutes at 37℃, it was added with 2.5ml of pH 6.3, 0.3mol/L Acetate buffer, 0.3ml of 8.0mmol/L TPTZ and 0.2ml of 20mmol/L ferric chloride solution, in sequence. After stabilization at room temperature for exactly 5 minutes, the absorbance was measured utilizing spectrophotometer Pharmacia LKB, Utrospec III with 593nm wave length. Another measurement was conducted with 0.2ml of blood plasma added with 0.1ml of distillated water substituting the Ascorbate oxidase working solution, and after the same procedure, it was measured at 593nm wavelength.

Table 1. The operating condition of HPLC for analysis of vitamin E in plasma

Items	Vitamin E
Pump	Waters 515 pump
Detector	Waters 996 Photodiode Array Detector UV 292nm
Injector	Waters
Column	Nucleosil C18 4.6 − 250mm
Mobile Phase	Methanol
Flow rate(ml/min)	1.0

7. Measurement of vitamin E concentration in blood plasma

Vitamin E was measured by Craft et al. method(1988), utilizing HPLC. Into a test tube, 150 μl of 20mg/L tocopherol acetate was added with 150 μl of blood plasma and before mixing with vortex mixer for 30 seconds, and added with 300 μl of hexane to be centrifuged at 1040g for 5 minutes. After collection and concentration of 100 μl of upper phase, it was diluted with 50 μl of ether and 50 μl of methanol for 30 seconds, and 20 μl was injected to HPLC. The operation condition of device for analysis of vitamin E is shown in Table 1.

8. Analysis of data and statistical methodology

Statistical analysis of data was performed utilizing SAS (Statistical Analysis System) package. Means and standard deviation of factors were calculated by each group, and Student's t-test was performed to validate the significance between the two groups. Significance validation was performed with paired t-test between results of before and after experiments. Correlations between anthropometry and biochemical substances and among the substances were performed by Pearson's correlation coefficient. All methods were validated at $p < 0.05$ level of significance.

Results and Discussion

1. Anthropometric and general information

Comparison of anthropometric measurements between smokers and non-smokers is shown in Table 2. Ages of smokers and nonsmokers were 24.4 ± 1.8 and 22.4 ± 2.5 years, respectively, demonstrating that smokers were older than non-smokers. There was no significant difference in heights and weights between smokers and nonsmokers. Smokers and nonsmokers had heights of 173.3 ± 2.9cm 172.6 ± 6.4cm, respectively, and weights of 69.8 ± 10.5kg and 67.9 ± 10.7kg, respectively. In comparison with physical standards of 20 − 29 years old men, which is 173.6cm and 66.6kg,

Table 2. Physical characteristics of smokers and non-smokers

	Smokers (n = 30)	Non-smokers (n = 30)
Age (year)	24.4 ± 1.8[1]	22.4 ± 2.5
Height (cm)	173.4 ± 4.9	172.6 ± 6.4
Weight (kg)	69.8 ± 10.5	67.9 ± 10.7
BMI[2] (kg/m^2)	23.1 ± 3.2	22.7 ± 2.9
Body fat (%)	16.4 ± 4.4	16.1 ± 3.3
Fat weight (kg)	11.9 ± 5.1	10.9 ± 3.9
LBM[3] (kg)	57.9 ± 7.1	57.1 ± 8.0
TBW[4] (L)	42.4 ± 5.2	41.7 ± 5.8

1) Mean ± S.D.
2) BMI: Body Mass Index [Weight (kg) /Height (m^2)]
3) LBM: Lean Body Mass
4) TBW: Total Body Water
n: number of subjects
All data were not significantly different between smokers and non-smokers at $p < 0.05$ by t-test

these subjects had similar height, but heavier weight (Kor Ntr Soc 2000).

Smokers are known to have lower weight than non-smokers due to inefficacy and changes of metabolism by nicotine that increases energy expenditure (Albanes et al. 1987 ; Glauser et al. 1970). On the contrary, Kim (1997) reported that smoking female university students had a significantly higher weight than nonsmoking counterpart. However, our study showed similar results with Park et al. (1996), with no significant differences in weight. Discrepancy might be attributed to the fact that the smokers were younger than their counterpart in this study, suggesting they might have been smoking for shorter period of time. The BMI (Body Mass Index) showed lower BMI in smokers than in non-smokers, was 23.1 ± 3.2kg/m^2 in smokers and 22.7 ± 2.9kg/m^2 in non-smokers, with no differences.

There were no differences in body fat, namely, 16.4 ± 4.4% in smokers and 16.1 ± 3.3% in non-smokers, with results similar to studies performed in female university smokers, which resulted 22.9% in smokers and 21.3% in non-smokers (Moon 1996). On the other hand, Kim et al. (1998) reported that smokers had significantly lower body fat than non-smokers. Smokers and nonsmokers had weights of 11.9 ± 5.1kg and 10.9 ± 3.9kg, respectively and lean body mass (LBM) of 57.9 ± 7.1kg and 57.1 ± 8.0kg, respectively and total body water (TBW) of 42.4 ± 5.2L and 41.7 ± 5.8L, respectively, with no differences.

2. Intake of antioxidant vitamins

The intake of antioxidant vitamins in smokers and non-smokers was investigated by food frequency questionnaire and is shown in Table 3. Vitamin A intake was 505.15 ± 123.32 μgR.E. in smokers and higher in non-smokers, 519.48 ± 111.25 μgR.E., but there was no significant differences, which is consistent with others (Fieding 1985).

Table 3. Comparison of antioxidant vitamins intakes between smokers and non-smokers

	Smokers (n = 30)	Non-smokers (n = 30)	p-value
Vitamin A (μg)	505.15 ± 123.32[1]	519.48 ± 111.25	NS
Vitamin C (mg)	133.13 ± 37.76	124.68 ± 27.12	NS
Vitamin E (mg)	6.91 ± 2.39	7.09 ± 1.73	NS

1) Mean ± S.D.
NS: Not significantly different at $p < 0.05$ by t-test
n: number of subjects

There were no significant differences in vitamin C intake between smokers, 133.13 ± 37.76mg and non-smokers, 124.68 ± 27.12mg. Smoking lead to an influx of tobacco alkaloids, as nicotine and carbon monoxide in the body, and antioxidants like vitamin C are necessary to excrete these substances by respiration or urine. Smokers have faster metabolism than non-smokers (Kallner et al. 1981), and they need an increase of vitamin C intake because nicotine stimulates the separation of catecholamines of adrenal medulla, and to produce catecholamines, they need vitamin C (Hoeffl 1977). Therefore, smokers need 53 − 79mg (average 65mg) more vitamin C daily, to maintain the same level of vitamin C as non-smokers (Smith Hodges 1987). RDA of USA regulates an addition of 50% (+40mg) of vitamin C in intake recommendation for smokers (Choi et al. 2000).

Vitamin E intake was 6.91 ± 2.39mg in smokers and 7.09 ± 1.73mg in non-smokers, with no significant difference. Moreover, either group did not achieve the recommendation of 10mg for 20 − 29 year-old men. These differences between studies might have occurred due to not only differences in subjects.

Antioxidant vitamins have independent antioxidant capability, and they also have reciprocal correlations with each other, which increases their ability and economizes the antioxidants that are destroyed by oxidative stress. However, an antioxidant cannot function as onother antioxidant, so the intakes of all nutrients are necessary (Pelletier 1977).

3. Blood pressure, blood glucose, methemoglobin, lipid peroxides, SOD activity

Table 4 shows blood pressure, blood glucose, methemoglobin, TBARS and SOD of smokers and non-smokers. Systolic blood pressure was 128.9 ± 10.8mmHg in smokers and

Table 4. Comparison of blood pressure, blood glucose, methemoglobin, TBARS, SOD, plasma vitamin C and vitamin E concentration activity between smokers and non-smokers

	Smokers (n = 30)	Non-smokers (n = 30)	p-value
SBP[2] (mmHg)	128.9 ± 10.8[1]	122.2 ± 11.3	*
DBP[3] (mmHg)	81.0 ± 7.6	75.9 ± 9.4	*
Glucose (mg/dl)	95.0 ± 18.5	88.1 ± 14.5	NS
Methemoglobin (%)	22.9 ± 3.7	23.2 ± 2.6	NS
Plasma TBARS[4] (μmol/L)	2.8 ± 0.8	2.5 ± 0.6	NS
Plasma SOD[5] (unit/ml)	0.29 ± 0.09	0.50 ± 0.27	***
Plasma vitamin C (mg/dl)	80.59 ± 14.61	88.32 ± 13.98	
Plasma vitamin E (mg/L)	9.82 ± 2.35	9.81 ± 2.12	NS

1) Mean ± S.D.
2) SBP: Systolic Blood Pressure
3) DBP: Diastolic Blood Pressure
4) TBARS: Thiobarbituric Acid Reactive Substances
5) SOD: Superoxide Dismutase
*, ***: Significantly different between smokers and non-smokers at $p < 0.05$, $p < 0.001$ by t-test
NS: Not significantly different at $p < 0.05$ by t-test

122.2 ± 11.3mmHg in non-smokers, with significant differences between two groups ($p < 0.05$). Diastolic blood pressure was 81.0 ± 7.6mmHg in smokers and 75.9 ± 9.4mmHg in non-smokers (significantly higher in smokers) ($p < 0.05$). These results are similar to studies that described high blood pressure in smokers (Gilson 1990). The heart rate of smokers at rest was higher than non-smokers, due to nicotine from cigarettes, and it is known that the blood pressure also increases. Our study also indicated that blood glucose tended to be higher in smokers (95.0 ± 18.5mg/dl) than nonsmokers (88.1 ± 14.5mg/dl).

Methemoglobin levels indicate the damage to hemeproteins by oxidative stress. In the present study, the methemoglobin level was 22.9 ± 3.7% and 23.2 ± 2.6% in smokers and non-smokers, respectively, without significant differences. Lipid peroxides in the body are biomarkers of irreversible oxidative stress damages, and the increase of this level is considered as one of the factors that lead to aging and degenerative diseases. There are many ways to quantify these alterations in the body, but the radicals originate and disappear very fast, so nowadays, the biomarkers produced by oxidative stress have been analyzed instead of the radicals themselves. The levels of TBARS, a type of lipid peroxides, have been widely utilized.

As results of this study, the levels of TBARS in smokers were 2.8 ± 0.8 μmol/L, and 2.5 ± 0.6 μmol/L in non-smokers, with no significant differences between groups. While many studies indicated significantly higher levels of lipid peroxides in smokers (Ha Natholyn 1997), Kim (1998) indicated no significant differences in the study with high school female smokers.

The levels of blood SOD according to smoking status were 0.29 ± 0.09unit/ml for smokers and 0.50 ± 0.27unit/ml for non-smokers, resulting in higher SOD activity in non-smokers ($p < 0.001$). These results were different from studies (Kim 1998) that indicated high SOD activity in smokers, but similar with studies (Lim 2000) that indicated significantly lower results in smokers, with 1.57mg/gHb and 2.00 mg/gHb for non-smokers. Kim et al. (1999) described that heavy smokers had significant low SOD activity than light smokers, indicating that the period of time or quantity of smoking led to a deterioration of antioxidant ability due to decrease of activity in enzymes correlated with antioxidation.

4. Concentration of plasma vitamin C and vitamin E

The concentration of plasma antioxidant vitamins in smokers and non-smokers is described in Table 4. The concentration of plasma vitamin C was 80.59 ± 14.61mg/dl in smokers and 88.32 ± 13.98mg/dl in nonsmokers, indicating significantly lower levels in smokers. Another study (Beniwitz 1988) indicated that the concentration of vitamin C in smo-kers was significant lower than non-smokers, but there are other studies that reported no effect of smoking status in plasma vitamin C concentration of smokers and non-smokers (Ma et al. 2000).

It has been reported that the decrease of vitamin C concentration in the body is caused due to increase of expenditure of antioxidant substances against oxidative stress caused by smoking. Complying with Handelman et al. (1996), some substances in cigarette smoke directly decompose the antioxidant nutrients, and especially, are considered one of the ma-jor causes of exhaustion of vitamin C inside the body, due to the direct reactions with this vitamin. Another reason is the sti-mulation of catecholamine release from adrenal by nicotine of cigarettes, and the participation of vitamin C in catecho-lamine synthesis (Abby et al. 1995).

The concentration of vitamin E in blood was 9.82 ± 2.35mg/L in smokers and 9.81 ± 2.12mg/L in non-smokers, and these results were similar with reports (Abby et al. 1995) that indicated no significant differences of vitamin E concentration according to smoking status. On the other hand,

Table 5. Blood pressure, blood glucose, methemoglobin, TBARS, SOD, plasma vit C and vit E activity of smokers before and after antioxidant vitamin supplemented for 4 weeks

	Vit. C		Vit. E		Vit. C + Vit. E	
	Pre	Post	Pre	Post	Pre	Post
SBP[2] (mmHg)	133.7 ± 10.3[1]	120.8 ± 7.3**	129.2 ± 8.8	119.2 ± 6.7*	123.7 ± 11.5	117.7 ± 6.1
DBP[3] (mmHg)	84.4 ± 6.7	74.6 ± 8.7***	80.5 ± 8.0	75.2 ± 9.9*	78.2 ± 7.5	71.4 ± 7.1*
Glucose (mg/dl)	86.1 ± 13.9	85.3 ± 16.2	102.2 ± 11.2	89.3 ± 15.8*	96.6 ± 25.2	86.1 ± 10.4
Methemoglobin (%)	24.9 ± 3.1	18.8 ± 2.7***	22.8 ± 2.8	15.0 ± 2.1***	21.0 ± 4.1	15.3 ± 2.9**
Plasma TBARS[4] (μmol/L)	2.7 ± 0.9	2.1 ± 0.5	2.7 ± 0.7	2.3 ± 0.5*	3.0 ± 0.7	2.3 ± 0.6*
Plasma SOD[5] (unit/ml)	0.31 ± 0.08	0.30 ± 0.09	0.23 ± 0.07	0.4 ± 0.10***	0.32 ± 0.10	0.50 ± 0.47
Plasma vitamin C (mg/dl)	77.29 ± 13.25[1]	88.20 ± 8.57*	81.71 ± 15.63	80.22 ± 12.88	82.76 ± 15.78	89.85 ± 13.04*
Plasma vitamin E (mg/L)	9.53 ± 2.74	10.73 ± 3.90	9.61 ± 1.64	13.29 ± 3.02**	10.31 ± 2.68	14.82 ± 4.00**

1) Mean ± S.D.
2) SBP: Systolic Blood Pressure
3) DBP: Diastolic Blood Pressure
4) TBARS: Thiobarbituric Acid Reactive Substances
5) SOD: Superoxide Dismutase
*, **, ***: Significantly different between pre- and post- intervention at $p < 0.05$, $p < 0.01$, $p < 0.001$ by paired t-test

Table 6. Blood pressure, blood glucose, methemoglobin, TBARS, SOD, plasma vit C and vit E activity of non-smokers before and after antioxidant vitamin supplemented for 4 weeks

	Vit. C		Vit. E		Vit. C + Vit. E	
	Pre	Post	Pre	Post	Pre	Post
SBP[2] (mmHg)	116.4 ± 10.2[1]	116.5 ± 11.9	123.6 ± 12.3	120.4 ± 10.9	126.6 ± 9.9	119.2 ± 6.9*
DBP[3] (mmHg)	76.0 ± 9.8	70.1 ± 6.3	76.6 ± 10.3	72.8 ± 12.3	75.0 ± 8.8	73.6 ± 7.6
Glucose (mg/dl)	83.2 ± 9.5	80.1 ± 17.2	91.5 ± 18.6	84.6 ± 11.1	89.7 ± 14.1	85.7 ± 15.6
Methemoglobin (%)	23.1 ± 2.2	16.2 ± 2.8***	23.1 ± 2.8	17.8 ± 3.2***	23.3 ± 3.0	18.7 ± 2.3**
Plasma TBARS[4] (μmol/L)	2.4 ± 0.6	2.0 ± 0.5	2.6 ± 0.7	1.9 ± 0.7	2.5 ± 0.6	1.9 ± 0.5*
Plasma SOD[5] (unit/ml)	0.37 ± 0.10	0.45 ± 0.12	0.68 ± 0.29	0.45 ± 0.14	0.46 ± 0.28	0.52 ± 0.15
Plasma vitamin C (mg/dl)	88.87 ± 14.83[1]	95.32 ± 18.26*	88.34 ± 14.91	90.21 ± 14.54	87.75 ± 13.66	94.67 ± 12.80*
Plasma vitamin E (mg/L)	9.90 ± 2.51	9.93 ± 2.78	9.88 ± 1.44	13.81 ± 4.46**	9.63 ± 2.45	14.80 ± 3.72***

1) Mean ± S.D.
2) SBP: Systolic Blood Pressure
3) DBP: Diastolic Blood Pressure
4) TBARS: Thiobarbituric Acid Reactive Substances
5) SOD: Superoxide Dismutase
*, **, ***: Significantly different between pre- and post- intervention at $p < 0.05$, $p < 0.01$, $p < 0.001$ by paired t-test

Munro et al. (1997) showed that smokers had significantly lowered plasma vitamin E level (7.85mg/dl in smokers vs 9.2mg/dl in non-smokers) and described that it occurred because smokers had weak absorption of vitamin E in the digestive tract or because they cannot utilize and easily decompose the vitamin E absorbed. Fukuzawa et al. (1985) described another reason, reporting that it occurred because the vitamin E, present in membrane cells, is oxidized first in substitution of many unsaturated lipids by oxygen or substances with oxygen, to protect the membrane intact against oxidation.

5. Effects of antioxidant vitamins supplementation on blood pressure, blood glucose, methemoglobin, TBARS and SOD activity

Table 5 shows blood pressure, blood glucose, methemoglobin, TBARS and SOD activity before and after supplementation of vitamin C and vitamin E to smokers, and Table 6 shows results of nonsmokers. Systolic blood pressure of smokers with vitamin C supplementation decreased significantly from 133.7 ± 10.3mmHg to 120.8 ± 7.3mmHg, and that of the vitamin E supplement group was 129.2 ± 8.8 mmHg, decreased significantly from 119.2 ± 6.7mmHg. Vitamin C and vitamin E combination supplement group resulted in 123.8 ± 11.5mmHg, decreased from 117.7 ± 6.1mmHg, without significant differences. Diastolic pressure significantly decreased from 84.4 ± 6.7mmHg to 74.6 ± 8.7mmHg in vitamin C supplement group, from 80.8mmHg to 75.2 ± 9.9mmHg in vitamin E supplement group, and from 78.2 ± 7.5mmHg to 71.4 ± 7.1mmHg in vitamin C and vitamin E combination supplement group. In nonsmokers,

the systolic and diastolic blood pressure did not significantly change by the supplementation of vitamin C and vitamin E except systolic pressure in vitamin C and vitamin E combination supplementation group (significantly decreased from 126.6 ± 9.9mmHg to 119.4 ± 6.9mmHg).

The blood glucose in smokers tended to decrease from 86.1 ± 13.9mg/dl to 85.3 ± 16.2mg/dl in vitamin C supplement group and from 96.6 ± 25.2mg/dl to 86.1 ± 10.4mg/dl in vitamin C and vitamin E combination supplement group. Smokers had a significantly lowered blood glucose (102.2 ± 11.2mg/dl to 89.3 ± 15.3mg/dl) by vitamin E supplement. According to Morel (1989), the vitamin does not directly change the high blood glucose, but it inhibits the toxicity of cells caused by lipid peroxides. In nonsmokers, the blood glucose tended to decrease from 83.2 ± 9.5mg/dl to 80.1 ± 17.2mg/dl by supplementation of vitamin C, from 91.5 ± 18.6mg/dl to 84.6 ± 11.1mg/dl by supplementation of vitamin E, and from 89.7 ± 14.1mg/dl to 85.7 ± 15.6mg/dl by supplementation of combined vitamin supplementation.

In this study, methemoglobins of smokers were significantly decreased by supplementation of vitamin C, vitamin E and combined vitamins (25.9 ± 3.1% to 18.8 ± 2.7%, 22.8 ± 2.8% to 15.0 ± 2.1%, and 21.0 ± 4.1% to 15.3 ± 2.9%, respectively). Methemoglobin in nonsmokers also decreased significantly by vitamin supplementations (23.1 ± 2.2% to 16.2 ± 2.8%, 23.1 ± 2.8% to 17.8 ± 3.2% and 23.3 ± 3.0% to 18.7 ± 2.3%, respectively in vitamin C supplementation, vitamin E supplementation and vitamin C and vitamin E combination supplement group). Plasma TBARS of smokers tended to decrease by vitamin C supplementation, namely, 2.7 ± 0.9 μmol/L to 2.1 ± 0.5 μmol/L while it significantly decreased by vitamin E supplementation (2.7 ± 0.7 μmol/L to 2.3 ± 0.5 μmol/L) and combined vitamin supplementation (3.0 ± 0.7 μmol/L to 2.3 ± 0.6 μmol/L).

Plasma TBARS of nonsmokers significantly decreased by combined vitamin supplementation (2.5 ± 0.6 μmol/L to 1.9 ± 0.5 μmol/L) while that tended to decrease (2.4 ± 0.6 μmol/L to 2.0 ± 0.5 μmol/L by vitamin C supplementation and 2.6 ± 0.7 μmol/L to 1.9 ± 0.7 μmol/L by vitamin E), indicating that the best effect was from vitamin C and vitamin E.

Plasma SOD activity of smokers significantly increased by vitamin E supplementation (0.23 ± 0.07unit/mL to 0.40 ± 0.10unit/mL) while there were no significant changes by other supplementation. Blood plasma SOD activities of nonsmokers did not show any significant changes by supplementations of vitamin C and vitamin E.

7. Levels of vitamin C and vitamin E in blood plasma

Levels of antioxidant vitamin between smokers and nonsmokers according to antioxidant supplementation are shown in Tables 5 and 6. In smokers and nonsmokers, plasma levels of vitamin C (77.29 ± 13.25mg/dL to 88.20 ± 8.57mg/dL in smokers and 88.87 ± 14.83mg/dL to 95.32 ± 18.26mg/dL in nonsmokers) and E (29.2 μmol/L to 49.3 μmol/L in smokers and 9.61 ± 1.64mg/L before to 13.29 ± 3.02mg/L in nonsmokers) were significantly increased respectively, by the supplementation of vitamin C and vitamin E. Plasma levels of both vitamin C and vitamin E were significantly increased in smokers and nonsmokers by the combined vitamin supplementation.

These results were consistent with other studies (Bolston-Smith 1993 ; Pryor 1993). Also, Jens et al. (2000) supplemented smokers and nonsmokers with multivitamins for 3 months, and reported the increase of 194% of blood plasma vitamin C level in smokers and 43% in nonsmokers, suggesting that vitamin C supplementation had more effect in smokers than in non-smokers.

Conclusions

This study investigated effects of smoking and supplementation of vitamin C and vitamin E on the intake of antioxidant vitamins, nutritional status of the vitamins and antioxidant systems of the healthy male university students. Through questionnaire and biochemical investigation, the results below were obtained.

1) There was no significant difference in the heights, weights and percent of body fat between smokers and nonsmokers. Vitamin C and vitamin E intakes were not significantly different between smokers and nonsmokers, although smokers tended to have a higher intake of vitamin C.

2) Smokers were shown to have adverse health aspects such as significantly higher systolic and diastolic blood pressures and lower plasma level of vitamin C, although there was no significant difference in plasma concentrations of methemoglobin and TBARS compared to nonsmokers. Also smokers had a significantly higher level of SOD. Considering that vitamin C intake of the smokers tended to be higher than nonsmokers, a significantly lower level of plasma vitamin C

indicated an increased demand of the antioxidant vitamins by smoking.

3) Supplementation of vitamin C and vitamin E effectively increased the plasma levels of corresponding vitamins.

4) Smokers supplemented with antioxidant vitamins showed various positive changes. Vitamin C supplementation resulted in a significant decrease of diastolic blood pressure, systolic blood pressure and methemoglobin in smokers. Also significant decreases of diastolic blood pressure, systolic blood pressure, plasma glucose, methemoglobin and TBARS were found in vitamin E supplement group. In vitamin C and vitamin E combination supplement group, there was a significant decrease of diastolic blood pressure, methemoglobin and TBARS.

5) Nonsmokers also had benefits from supplementation of vitamin C and vitamin E, namely significantly lower methemoglobins of vitamin C supplement group and vitamin E supplement group and systolic pressure, methemoglobin and TBARS in vitamin C and vitamin E combination supplement group.

These results indicated better efficacy of antioxidant supplementation in smokers than in nonsmokers, suggesting that the supplementation of vitamin C and vitamin E might decrease the oxidative stress and various risk factors of smoking-related diseases. Consequently, the necessity of correlation studies between antioxidant nutrients and smoking in the middle-aged or the elderly with longer period of smoking, or studies with other antioxidant nutrients as well as vitamin C and vitamin E and anti-oxidative enzymes have to be considered in the future.

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