Evaluation of Antioxidant Status and Correlation among Antioxidant Indices in Female College Students[*]

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ABSTRACT

This study was done to evaluate the antioxidant status of female college students by determining their intakes and plasma levels of antioxidnt vitamins (vitamin C, A and E) and total antioxidant status (TAS). Subjects were 46 healthy female college students aged 20 − 29 years. Body composition was determined by a multifrequency bioelectrical impedance analysis. Dietary intakes were examined by 24hr record method and nutrients intakes were analyzed by the Computer Aided Nutritional analysis program for professional (CAN-pro). Plasma vitamin C level were measured by spectrophotometric method and retinol, β-carotene, α-tocopherol were measured by HPLC. Plasma TAS was measured with a Randox kit using the trolox equivalent antioxidant capacity (TEAC) method. Daily energy and protein intakes of the female college students were 1670.5kcal (83% of RDA) and 63.3g (115.1% of RDA), respectively. However their intakes of Ca and Fe were below 75% of RDA. Their intakes of vitamin A and C were 596.6 μ gRE (85.2% of RDA) and 71.0mg (101.4% of RDA), respectively. Plasma levels of vitamin C, retinol, β-carotene and α-tocopherol were 14.7mg/L, 0.7mg/L, 0.2mg/L and 9.1mg/L, respectively which were within normal range. There was no subject with deficiency or marginal level in plasma vitamin A and C. However 1.6% of the subjects had below adequate level in vitamin E. Plasma TAS level was 1.2mmol/L. Correlation data showed that all plasma antioxidant vitamins were positively correlated with plasma TAS. Overall data indicate that the antioxidant status of female college students were pretty good. However it might be necessary to educate them to eat more fruits and vegetables for preventing many chronic diseases in a later life. (*J Community Nutrition* 5(1) : 13~20, 2003)

KEY WORDS : female college students · antioxidant vitamins · total antioxidant status.

Introduction

Oxidative stress is defined as a disturbance in the prooxidant-antioxidant balance in favor of the former. A prooxidant shift will promote damaging oxidative changes to important cellular constituents and this may, in turn, lead to dysfunction and chronic diseases such as aging, cancer, cardiovascular disease and diabetes (Buring & Hennekens 1997 ; Kaneto et al. 1999). Therefore body antioxidant capacity is an important factor for the prevention of many chronic diseases.

There are many naturally occuring substances, antioxidants to protect against the potentially harmful effects of prooxidants. Vitamin E is the major lipid-soluble antioxidant in cell membranes. It protects against lipid peroxidation by acting directly with a variety of oxygen radicals, including singlet oxygen, lipid peroxide products and the superoxide radical, to form a relatively innocuous tocopherol radical (Clarkson & Thompson 2000 ; Packer 1991). Vitamin C is water soluble and can directly react with superoxide, hydroxy radicals, and singlet oxygen in plasma (Frei et al. 1989). In addition, ascorbic acid has the ability to regenerate the activity of lipid-soluble antioxidants such as tocopherol and carotene (Jailal & Grundy 1991). β-carotene, the major carotenoid precursor of vitamin A, is the most efficient quencher of singlet oxygen (Krinsky 1993). It has been known that a combination of antioxidants is more effective than one individual antioxidant in isolation (Niki et al. 1995).

Vitamin C, A and E are the most abundant and effective antioxidants in human plasma and are believed to be of major importance in the protection against diseases caused by oxidative stress (Frei et al. 1989 ; Niki et al. 1995). Epide-

miological studies have demonstrated an association between increased intake of antioxidant vitamins such as vitamin E and vitamin C, and reduced morbidity and mortality from coronary artery disease (Diaz et al. 1997 ; Michael 1995). Therefore, increasing plasma levels of these antioxidant vitamins may lead to an increased antioxidant defense capability.

Some attempts have been made to determine a more global measure of oxidative stress. The measurement of the total antioxidant capacity (TAC) in human plasma has been applied in nutrition science. Particularly it has been used to evaluate the antioxidant contributions of dietary components and to study the bioavailability of dietary antioxidant nutrients. Several methods have been recently proposed for the measurement of the TAC in human plasma such as trolox equivalent antioxidant capacity (TEAC, Miller et al. 1993), total radical-trapping antioxidant capacity (FRAP ; Wayner et al. 1985) and oxygen radical absorbance capacity (ORAC, Cao et al. 1993), etc.

It is important for college students to develop desirable eating behaviors and good lifestyles because they will continue in a later life. However Korean college students have shown poor eating behaviors such as skipping meals, irregular meal time, frequent snacking, frequent eating-out, and excessive drinking (Kim & Lee 1996 ; Kim et al. 1997). Particularly, college women are more vulnerable to poor health and nutrition because they have a great desire for thinness and show a great interest for body image than any other age groups (Kim et al. 1998). A lot of studies revealed that college women are deficient in many nutrients such as calories, calcium, iron, vitamin A (Kim et al. 2002 ; Oh et al. 1996 ; Son & Sung 1998). Therefore it is important to evaluate the health and nutritional status of college young women because they will be a mother in the future. Their health status is very important for the health of next generation.

The objective of this study was to evaluate the antioxidant status of female college students by determining their intakes and plasma levels of antioxidnt vitamins (vitamin C, A and E) and total antioxidant status (TAS). In addition, it was aimed to investigate the relationship between TAS and antioxidant vitamins

Subjects and Methods

1. Subjects

Subjects were 63 healthy female college students attending nutrition-related classes at a university located in Seoul. Height and weight were measured. Body composition was determined by a multifreqency bioelectrical impedance analysis (Inbody 3.0, Biospace Co Ltd., Seoul, Korea). Students who took medication and a multivitamin supplement, suffered from any chronic disease, or had a problem with blood collection which resulted in insufficient samples for carrying out the analyses were excluded from dietary and biochemical analysis.

Therefore, the remaining 46 subjects were used for dietary and biochemical analysis.

2. Dietary assessment

Dietary data were obtained through questionnaires. Dietary intakes were examined by three-days record method (2 weekdays and 1 weekend day) and nutrients intakes were analyzed by Computer Aided Nutritional analysis program for professional (CAN-Pro, The Korean Nutrition Society, 1997).

3. Biochemical Analysis

1) Plasma antioxidant vitamins

Vitamin C was assayed immediately after plasma separation The plasma was deproteinized with 0.75M meta-phosphoric acid and measured by 2, 4-dinitrophenylhydrazine method using a UV pectrophotometer (Pesce & Kaplan 1987).

Vitamin A and E was assessed by measuring retinol and α-tocopherol, repectively. Plasma vitamin A and E were extracted with ethyl alcohol and hexane. Retinol and α-tocopherol were separated by HPLC on Nova-Pak C 18 (3.9 $\times$ 150mm) column using methanol-water (95 : 5, v/v) as the mobile phase. Elution was detected spectrophotometrically at 292nm (Bieri et al. 1979).

Plasma β-carotene was extracted with alsolute alcohol : distilled water : hexane (1 : 1 : 2) and separated by HPLC on Nova-Pak C18 (3.9 $\times$ 150mm) column using acetonitrile : dichrolomethane : methanol (7 : 2 : 1) as mobile phase. Elution was detected spectrophotometrically at 452nm (Bieri et al. 1985)

2) Total antioxidant status

Total antioxidant capacity was determined by Randox TAS kit using trolox equivalent antioxidant capacity (TEAC) method (Rice-Evans & Miller 1994).

4. Statistical Analysis

All data were expressed as mean $\pm$ SE and statistically an-

alyzed by SAS 6. 12 PC package program (SAS Institute, Inc). The relationship between intakes and plasma levels of antioxidant vitamins was analyzed by Pearson's correlation test.

Results and Discussion

1. Anthropometric indices of subjects (Table 1)

The mean age of the students was 21.4. The average height and weight were 162.2 ± 0.6cm and 52.4 ± 0.8kg, respectively. These values were similar to the Korean standard (162cm, 52kg) for females aged 20 – 24 years. The average BMI and body fat (%) was 20.1 ± 0.2 and 27.2 ± 0.5, res-

pectively. These values were also similar to those of other studies for female college students (Kim et al. 1998 ; Son & Sung 1998). In '98NHANS (Ministry of Health and Welfare 1999), the average BMI of females aged 20 – 29 was 21.6 and 75th perentile value of them was 23.2. When we examined the prevalence of underweight or overweight of the subjects by BMI, the percentage of underweight, normal and overweight subjects were 17.5%, 73% and 9.5%, respectively. However, when we used the criteria of obesity as over body fat 30%, about 28.5% of the subjects could be categorized into the overweight. These data might imply that some students with normal BMI could be overweight by body fat %.

2. Dietary intakes of female college students

Data for all nutrient intakes are presented in Table 2. Average daily intakes of energy and protein in the subjects were 1670.5kcal and 63.3g. These intakes of the subjects seemed to be slightly lower than those (1877.2kcal, 67.5g) of the Korean females aged 20 – 29 in '98NHANS (Ministry of Health and Welfare 1999). When compared to 7th Korean RDA (Korean Nutrition Society 2000), the intakes for energy and protein were 83.5% and 115.1% of RDA, respectively. In addition, 26.6% of the subjects consumed below 75% of RDA in their energy intake. Daily fat intake was 46.9g, comprising 25.3% of total calorie intakes. These value were slightly higher than the recommended 20% and the previous studies of female college students (Kim et al. 1999) or Korean average intake (43.5g) of females aged 20 – 29 years in

Table 1. Anthropometric indices of female college students

Variable	Female college students (n = 63)	Range
Age	21.4 ± 0.6[1]	20 – 29
Height (cm)	162.2 ± 0.6	151.0 – 173.0
Weight (kg)	52.4 ± 0.8	40.8 – 69.3
BMI (kg/m²)	20.1 ± 0.2	
Underweight (<18.5)	11 (17.5)[2]	14.9 – 24.9
Normal weight (18.5 ≤ BMI < 23)	46 (73.0)	
Overweight (23.0 ≤ BMI < 25)	6 (9.5)	
Body fat (%)	27.2 ± 0.5	18.5 – 38.4
> 30	18 (28.5)	

1) Mean ± SE
2) number of subjects (percentage of subjects)
BMI: Body Mass Index (kg/m²) = Body weight (kg) /height² (m²)
n: number of subjects

Table 2. Dietary intakes of nutrients in female college students

Nutrients	Female college students (n = 46)	Range	%RDA	<75% RDA
Calories (kcal)	1670.5 ± 53.3[1]	986.2 – 2946.7	83.5[2]	26.6[3]
Protein (g)	63.3 ± 3.3	36.6 – 178.5	115.1	6.5
Fat (g)	46.9 ± 1.9	22.5 – 76.9		
Carbohydrate (mg)	248.0 ± 9.8	96.7 – 493.8		
Calcium (mg)	443.4 ± 19.6	244.8 – 862.9	63.3	68.8
Phosphorous (mg)	891.0 ± 31.1	472.3 – 1539.6	127.3	0.0
Iron (mg)	10.4 ± 0.12	4.87 – 16.8	65.0	47.8
Vit A (μgRE)	596.6 ± 33.9	164.8 – 1198.1	85.2	42.2
Retinol (μg)	131.2 ± 12.7	1.8 – 477.5		
β-carotene (μg)	2427.4 ± 193.0	311.3 – 6317.5		
Vit B₁ (mg)	1.2 ± 0.1	0.5 – 2.1	115.0	4.4
Vit B₂ (mg)	1.1 ± 0.1	0.4 – 3.8	89.2	44.4
Niacin (mg)	12.7 ± 0.5	6.0 – 19.8	95.2	15.6
Vit C (mg)	71.0 ± 5.3	24.9 – 170.7	101.4	19.6

1) Mean ± SE
2) Percentage of Korean RDA (2000)
3) Percentage of subjects consumed below 75% of RDA

'98NHANS (Ministry of Health and Welfare 1999).

Similar to other studies (Kim et al. 1997 ; Kim et al. 2002), most nutrients except vitamin B₁ and vitamin C did not reach the Korean RDA (Fig. 1). The average consumption of Ca and Fe was 63.3% and 65.0% of RDA, respectively. The percentage of the subjects consumed below 75% of RDA was 68.8% in Ca and 47.8% in Fe. Furthermore, students with weight-control experience or vegetarian were more vulnerable to poor nutritional status (Lee et al. 2001 ; Sung et al. 1998). Calcium and iron are important nutrients in young women of child-bearing age. Therefore it is necessary to educate on the proper intake of calcium and iron for college students.

The average intake of vitamin C was 71.0mg/day in the female college students (Table 2). The range of vitamin C intake was 24.9 − 170.7mg/day. When vitamin C intake was compared to Korean RDA, average vitamin C intake of female college students was about 101.4%. But 19.6% of the subjects consumed less than 75% of Korean RDA.

According to the '98 National Health and Nutrition Survey ('98NHANS), daily average intake of vitamin C of Korean females aged 20 − 29 years was 131.5mg/day (Ministry of Health and Welfare 1999). In other studies (Kim et al. 1999 ; Kwon et al. 1999), the daily intake of vitamin C in university students was 44.7 in the Andong area and 84.7mg/day in the Seoul area, respectively. Such a difference in vitamin C intake between studies might be mainly due to dietary assessment methods. In addition to method, vitamin C intake depend on which season was examined and the living area of subjects.

In this study, the average intake of vitamin A was 596.6 μgRE 85.2% of RDA). The percentage of subjects consumed less than 75% of Korean RDA was 42.2%. The

'98NHANES showed that the average intake of vitamin A in Korean females aged 20 − 29 years was 692 μgRE. One study (Kim et al. 1999) showed 545.3 μgRE/day of vitamin A intake in college female students. But a more recent study (Kim et al. 2002) showed that vitamin A intake of female college students was 635.6 μgRE/day. It might be associated with a difference of dietary assessment method and/or higher concerns for antioxidant vitamins these days.

3. Plasma levels of antioxidant vitamins and total antioxidant status in female college students

Plasma levels of antioxidant vitamins are presented in Table 3. The average level of plasma vitamin C was 14.7 mg/L. The range of plasma vitamin C level seen in persons on a normal diet is from 6 to 20mg/L. Criteria from the Nutrition Canada Survey were used to classify the risk of clinical survey. Respondents with serum vitamin C levels of 2mg/L or less were considered to be at high risk for clinical vitamin C deficiency while those with serum levels of 2 − 4mg/L were considered to be at marginal risk (Schectman et al. 1989). Of all subjects, there were no students who had plasma levels of 2mg/L or less. Furthermore there were no subjects with marginal level (2 − 4mg/L) of vitamin C (Fig. 2). The percentages of subjects in the range of 4 − 10mg/L

Table 3. Plasma antioxidant vitamin levels and plasma total antioxidant capacity of female college students

Variables	Female college students (n = 46)	Range
Vit C (mg/L)	14.7 ± 1.3[1]	7.2 − 23.5
Retinol (mg/L)	0.7 ± 0.2	0.3 − 1.4
β-carptene (mg/L)	0.2 ± 0.0	0.1 − 0.4
α-tocopherol (mg/L)	9.1 ± 0.8	3.7 − 15.7
Total antioxidant capacity (mmol/L)	1.2 ± 0.1	1.0 − 1.8

1) Mean ± SE
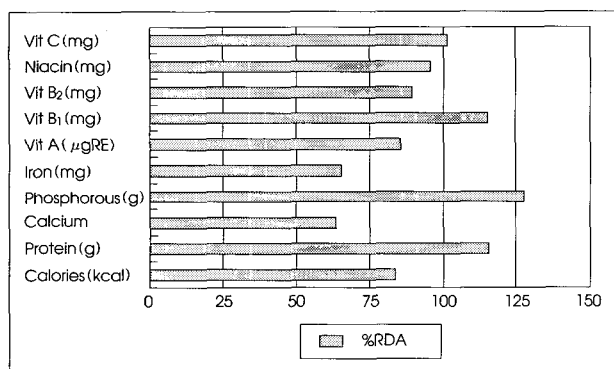
Fig. 1. Percentage of 2000 Korean RDA for nutrient intakes in female college students.
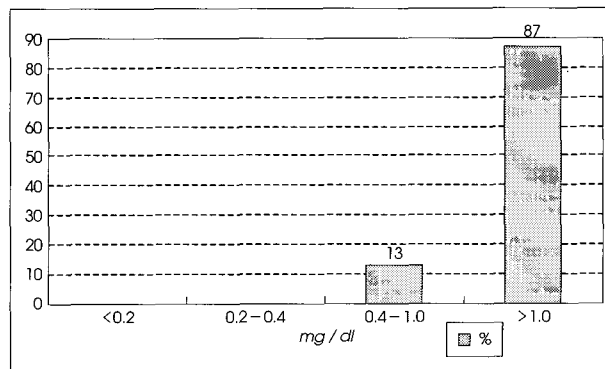
Fig. 2. Distribution of female college students by plasma vitamin C level.

and above 10mg/L was 13% and 87%, respectively. These data were similar to the results of the previous study with college women smokers (Kim & Moon 1997). Therefore the vitamin C status of female college students seemed to be pretty good. However recently it has been suggested that 200mg of vitamin C intake may be ideal to consider beneficial effects on functional and clinical outcomes (Kwon & Levine 2002). National Cancer Institute of NIH recommended the consumption of five servings of fruits and vegetables daily to protect against cancers of the GI tract and respiratory tracts with potential benefit in preventing heart disease. Five servings of fruits and vegetables provide 210 − 280mg of vitamin C daily.

In addition, it has been known that smoking increase the risk of hypovitaminosis C. Kallner et al (1981) measured vitamin C kinetics using radio-labeled ascorbic acid and demonstrated an increased turnover of vitamin C in smokers but only small differences in absorption when compared to non-smokers. Recently it has been suggested that the recommended intake for smokers has to be increased by 35mg/day (Food and Nutrition Board 2000).

The nutritional status of vitamin A can be assessed by the measurement of plasma retinol. The plasma retinol level was 0.7mg/L (Table 3). The average plasma retinol level of the subjects was within normal range, 0.32 − 0.90mg/L (Pesce & Kaplan 1987). Signs of vitamin A deficiency are usually seen at plasma retinol levels less than 0.1mg/L, while those with plasma levels between 0.1 and 0.3mg/L were considered to be at marginal risk (Wahed et al. 1995). Of all subjects, none had plasma levels of deficiency or marginal Subjects with plasma levels between 0.3 and 1.0mg/L and above 1.0mg/L were 82.5% and 17.5%, respectively (Fig. 3).
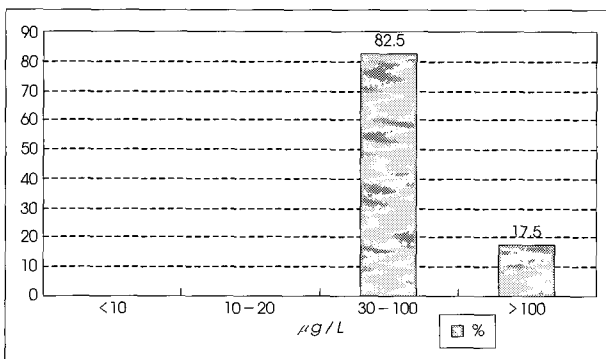
Plasma α-tocopherol level of the college females was

9.1mg/L, which was within normal range (5 − 12mg/L). Total tocopherol level above 5mg/L are generally considered nutritionally adequate (Machlin 1991). An adequate α-tocopherol level can be defined as 0.8mg/g total lipid in adults (Pesce & Kaplan 1987). Plasma α-tocopherol level itself might not be a good indicator for vitamin status. Overall vitamin E status of female college students seemed to be generally good even though the intakes of vitamin E were not estimated. However 1.6% of subjects were below adequate level in plasma vitamin E (Fig. 4). In our previous study (Kim et al. 1997), 12.12% of college women smokers were below 5mg/L, but only 2.38% were below the value in non-smokers.

Plasma TAS level was 1.2mmol/l, which is a little lower than reference value, 1.30 − 1.77mmol/l estimated for European worker (Table 3). To estimate total antioxidant capacity of human plasma, there are several methods such as TEAC (Miller et al. 1993), FRAP (Wayner et al. 1985) and ORAC (Cao et al. 1993) etc. In this study, we used TEAC method because it can be easily measured by the Randox TAS kit. However most methods were based on the measurement of the ability of plasma to withstand the oxidative damage induced by aqueous radical initiator. Therefore there are some limitation for these methods to measure total antioxidant capacity in both the aqueous and lipid compartments of plasma. Now there is being developed a selective measurement of antioxidant capacity in both the aqueous and lipid compartments of plasma (Aldini & Yeum 2002).

4. Correlation among antioxidant indices

Table 4 shows correlation data among antioxidant indices. Dietary vitamin C intake was positively correlated with vitamin A intake and retinol intake. Dietary vitamin A intake
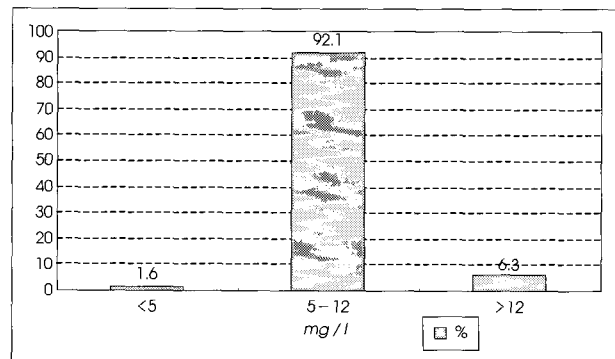

Fig. 3. Distribution of female college students by plasma retinol level.

Fig. 4. Distribution of female college students by plasma α-tocopherol level.

Table 4. Correlation coefficient between antioxidant vitamin intakes and/among plasma antioxidant indices

		Intake				Plasma level				
		Vit C	Vit A	β-carotene	Retinol	Vit C	Vit E	Retinol	β-carotene	TAS
Intake	Vit C									
	Vit A	0.302*								
	β-carotene	0.282	0.895*							
	retinol	0.358*	0.219	−0.003						
Plasma level	Vit C	0.374*	−0.066	−0.064	0.244					
	Vit E	−0.185	−0.122	−0.007	−0.240	0.404**				
	retinol	0.095	−0.006	−0.033	0.359	−0.329*	0.845**			
	β-carotene	−0.293	−0.234	−0.306	0.144	−0.480**	−0.864**	0.755**		
	TAS	−0.177	0.197	−0.139	−0.277	0.380*	0.818**	0.900**	0.708**	

*, **: significantly different at $p < 0.05$, $p < 0.01$ by Pearson's correlation

was positively correlated with β-carotene intake since a major part of dietary vitamin A intake was β-carotene in Table 2. When we examined the relationship between antioxidant intakes and their plasma levels, only a significant positive correlation between vitamin C intake and its plasma level was observed. These data imply that plasma vitamin C level is reflected by its intake. In general, plasma vitamin C level can also be influenced by smoking or vitamin supplementation and medication, etc (Ausman 1999 ; Carr & Frei 1999 ; Park et al. 1998). Particularly plasma vitamin C level was negatively correlated with cigarette smoking (Hornig & Glatthaar, 1985 ; Kim & Moon, 1997).

Plasma retinol level was positively correlated with plasma β-carotene level. There were significant positive correlations ($r = 0.404$, $p < 0.001$) between plasma vitamin C and vitamin E levels. These data might suggest that vitamin C regenerates lipid soluble antioxidant, α-tocopherol and thereby saves it.

All plasma antioxidant vitamins were positively correlated with plasma total antioxidant capacity. The correlation coefficient of TAS was the highest with plasma retinol level and the lowest with plasma vitamin C level. On the other hand, there are a lot of other antioxidants in plasma such as albumin, uric acid and bilirubin. When Cao & Prior (1998) examined their estimated contribution of individual serum antioxidants to serum TEAC, major contributors were serum albumin (28.0%), uric acid (19.3%) and others (46.8%). The contribution of serum ascorbic acid and α-tocopherol to TEAC were only 3.08% and 1.74%, respectively. Therefore plasma TAS might not be an appropriate indicator to examine the association between antioxidant vitamins and total antioxidant capacity.

Summary and Conclusions

The purpose of this study was to evaluate the antioxidant status of female college students by determining their intakes and plasma levels of antioxidant vitamins (vitamin C, A, E) and total antioxidant status. Subjects were 46 female college students attending nutrition-related classes at a university located in Seoul. The results of this study were summarized as follows :

1) Daily energy and protein intakes of female college students were 1670.5kcal (83.5% of RDA) and 63.6g (106.1% of RDA), respectively. The average intakes of Ca and Fe were below 75% of RDA while those of other vitamins were above 75% of RDA.

2) The average intakes of vitamin A and C were 596.6 μgRE (85.2% of RDA) and 71.0mg (101.4% of RDA) in female college students, respectively.

3) The average level of plasma vitamin C was 14.7mg/L in female college students, which was within normal range (6 − 20mg/L). There was no subject with a deficiency level (< 2mg/L) nor a marginal level (2 − 4mg/L) of vitamin C.

4) The average levels of plasma retinol and β-carotene were 0.7mg/L and 0.2mg/L for the female college students, respectively, which were within normal range. There was no subject with a deficiency level (0.1mg/L) nor a marginal level (0.1 − 0.3mg/L)

5) The average level of plasma α-tocopherol of the female college students was 9.1mg/L for female college students, which was within normal range (5 − 12mg/L). However 1.6% of subjects had below adequate level.

6) Plasma total antioxidant capacity was 1.2mmol/l, which

was a little lower than reference value (1.30 − 1.77mmol/l).

7) Correlation data showed that all plasma antioxidant vitamins were positively correlated with plasma total antioxidant capacity.

Overall data suggest that nutritional status of antioxidant vitamins was pretty good in college female students. However they consumed Ca and Fe below 75% of RDA. Therefore it is necessary to educate them to eat more balanced foods as well as to eat more fruits and vegetables for preventing many chronic diseases in a later life

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