ISSN: 0975-766X
CODEN: IJPTFI
Research Article

Available Online through
www.ijptonline.com

SERUM TOCOPHEROL, RETINOL AND ASCORBIC ACID STATUS IN THE HEALTHY LOCAL POPULATION OF TEHRAN

Afshin Rakhsha[a], Sima Sadrai[b], Maryam Jenabi[c], Zahra Kazemi Ashtiani[c], Farnaz Barati[c], Ghazaleh Moghaddam[c], Shokufeh Hassani[d], Naficeh Sadeghia[c], Mohammad Reza Oveisi[c], Behrooz Jannat[e], Mahboubeh Shaneshin[f], Mannan Hajimahmoodi[c*]

[a]Department of Radiotherapy, Shohada-e-Tajrish Hospital, Shaheed Beheshti Medical University, Tehran, Iran.
[b]Pharmaceutics Department, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
[c]Drug and Food Control Department, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran 14174, Iran.
[d]Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran.
[e] Ministry of Health and Medical Education, Research center. Tehran, Iran.
[f]Food and Drug Administration, Tehran University of Medical Sciences, Tehran, Iran.
Email: hajimah@sina.tums.ac.ir

Received on 13-02-2013 *Accepted on 28-02-2013*

Abstract

Vitamins are a class of nutrients which are essential for the body's various biochemical and physiological processes. The aim of this paper was to establish a reference range for serum concentration of vitamins E, A and C in healthy local population of Tehran.

Serum concentration of these vitamins in 173 subjects, aged between 16 and 59 years analyzed by chromatography and spectrophotometric methods. The mean serum tocopherol, retinol and ascorbic acid were higher than the cut-off point concentration for nutritional adequacy. The tocopherol/cholesterol ratio, tocopherol and retinol concentration of serum were similar in men and women but serum concentration of ascorbic acid in women was significantly higher than in men. A significant correlation between serum tocopherol concentrations with age, triglyceride, LDL and HDL were observed. Serum retinol concentration with BMI, were also correlated. Sex was found to be the most important factor affecting serum ascorbic acid concentration and was significantly higher in females than in males.

Key words: Tocopherol; Retinol; Ascorbic acid; Serum; Healthy subjects.

Introduction

Vitamins are a class of nutrients which are essential for the bodies various biochemical and physiological processes. Vitamins cannot be synthesized in the bodies which demonstrate the importance of vitamin rich diet for maintaining health (1). Vitamin E, A and C are dietary antioxidant and frequency counteracts oxidative damage or being involved in the oxidative process and may protect against chronic disease.

Epidemiologic studies have consistently shown that higher intakes of vitamin A and E were associated with low incidences of cardiovascular disease and several cancers (2).

Vitamin C also is effective in the prevention of many oxidant stress-based diseases such as cancer, atherosclerosis, ischemic heart disease, hypertension, age-related eye disorders, and Alzheimer's disease (3,4). The level of serum vitamins is influenced by life style, dietary habit, race and certain disease statue and varied considerably in different populations, therefore the definition of reference ranges obtained from a healthy general population living in each area is critical.

Published data showed that reference ranges for tocopherol, retinol and ascorbic acid shave been obtained for different populations (5-7). In Iran, data were not available from apparent healthy subjects and our literature survey, revealed no reference range for these vitamins in the healthy local population of Tehran. The objectives of the current research were to determine a reference ranges for serum tocopherol, retinol and ascorbic acid in randomly selected healthy local population of Tehran. In this study the relationship between serum vitamins concentration and definite variables were also determined.

Subjects and methods

Study population: 173 healthy subjects, 51 men and 122 women, aged between 16-59 years (mean age 27.54 years) were chosen based on a stratified random sampling method from Tehran residing people. Subjects with any clinical disease or those who were taking medications or vitamin supplement (cholestyramine, neomycin, cortisone, phenobarbitone), and pregnant women were excluded from the present study. None of the subjects were receiving or had received dietary counseling from a physician or dietitian and none were smoker.

Anthropometric and dietary assessment:

Trained certified dietitians conducted all anthropometric and dietary intake assessments. Body mass index (BMI) was calculated by dividing the weight in kg by square meter of height. Subjects were categorized based on their BMI according to the WHO guidelines (8) into low weight (BMI≤18.5), normal weight (18.5≤ BMI≤ 24.9), overweight (25≤ BMI≤ 29.9) and obese (BMI≥ 30).

For collecting the vitamins E, A and C intake a single 24-hour recall and the USDA food composition tables (FCT) (9) were used. Trained interviewers asked participants to report their regularly-consumed foods and the reported dietary intake were then converted to average daily intake of vitamins for each participant.

Sex; years of education; Physical activity; serum lipid profile (triglyceride, total cholesterol, low-density lipoprotein; LDL-C, high-density lipoprotein; HDL) were other variables assessment.

Definition for vitamin E, A and C status:

Nutritional adequacy of vitamin E was represented by a serum tocopherol concentration equal to or greater than 7 µg/ml. Values less than 5µg/mL were considered as vitamin E deficient, values between 5 and 7µg/mL were regarded as marginal (10). Nutritional adequacy of vitamin A was represented by a serum retinol concentration equal to or greater than 0.2µg/mL 10. Values between 0.1 and 0.2 were considered as marginal, and values less than 0.1 were deficient. Subjects with concentrations ascorbic acid less than 6 µg/ml were deficient, and equal to or greater than 20 µg/ml were supernumerary (11).

Blood collection and biochemical analyses: Sera were separated from 10 ml fasting blood samples avoiding unnecessary exposure to light and then clinical laboratory test were performed for all volunteers. For ascorbic acid determination the serum was transferred into four different sample vials after centrifugation (6000 rpm, 10 min, 4°C), and then stabilized with 60 g/L meta-phosphoric acid 1:1 volume. All samples were stored at -70°C and were analyzed within three months (12).

Chemicals and standards:

All solvents were of analytical grade (Merck, Darmstadt, Germany) and were used without further purification. In tocopherol and retinol determination retinol acetate was used as internal standard and purchased from Sigma (St Louis, MO, USA).

Tocopherol and retinoldetermination:

Tocopherol and retinol were measured by high performance liquid chromatography. A detailed description of the laboratory methodology of these vitamins has been published by Siluk (13) and Nirungsan (14) previously.

Total serum ascorbic acid determination:

Total serum ascorbic acid was carried out by spectrophotometric and colorimetric method with the 2, 4-dinitrophenylhydrazine using 5% tri-chloroacetic acid (15).

Ethics:

The objectives and the procedures were fully described individually for the all subjects before taking written informed consent. The protocols and procedures of the present study were approved by the ethics committee of Tehran University of Medical Sciences (TUMS) and considered at the pharmaceutical sciences research center

(PSRC). Ethics committee meeting on March 12, 2009 approved from ethical point of view. The ethic reference numbers are: 87-724:10-14; 87-724:10-15; 87-1221:11-29 for Ascorbic acid, tocopherol and retinol determination respectively.

Statistical:

Data were analyzed using statistical package for social sciences, version 16 (SPSS Inc), Chicago, IL, USA). Data are expressed as mean ± SDs. Comparisons of mean differences were calculated by Paired t-test. One-way analysis of variances (ANOVA) was used for determining the differences between multiple means in continuous variables while chi-square test was used for categorical variables. Statistical significance was set at $P<0.05$.

Results

Descriptive characteristics of 173 healthy subjects are presented in **Table1**. The mean HDL-cholesterol of women was higher than men (48.8± 7.6 vs 42.5± 7.9, $p\leq0.01$). In contrast, men had higher triglyceride concentration and physical activity ($p\leq0.05$). The tocopherol, tocopherol/cholesterol ratio, retinol and ascorbic acid concentrations of serum in the studied samples based on gender, age and BMI are summarized in **Table 2**. The mean value of serum tocopherol (10.22±0.26μg/ml) and retinol concentration (0.35±0.02μg/ml) were higher than the cut-off point for nutritional adequacy of vitamin E and A (7μg/mL and 0.2 μg/mL, respectively) while the serum mean value of ascorbic acid (14.45±0.38 μg/ml) was in the normal range) The mean value of the serum tocopherol/cholesterol ratio in this study was 6.27 ± 0.13 and the prevalence of inadequate vitamin E statue using 2.8 μg/mg as the cut-off point was 0.71% (16).

Table-1: Baseline characteristics of the study population[1].

	Men(n-51)	Women(n=122)	p value
Age (years)	28.0±8.9	27.3±9.0	0.67
BMI (kg/m^2)	24.0±3.8	22.8±4.1	0.09
Total cholesterol (mg/dl)	163.8±30.1	161.8±25.5	0.69
High-density lipoprotein-cholesterol (mg/dl)	42.5±7.9	48.8±7.6	<0.01
Low-density lipoprotein-cholesterol (mg/dl)	83.2±20.9	76.9±16.9	0.06
Triglyceride (mg/dl)	102.6±30. 8	85.8±23.2	<0.01
Education (years)	14.0±4.2	14. 8±2.5	0.23
Leisure-time physical activity (min)	34.6±47.2	19.7±36.0	0.04
Dietary intake of vitamin E (mg)	5.9±1.4	5.7±1.1	0.33
Dietary intake of preformed vitamin A(μg)	470.2±274.0	456.3±272.9	0.78
Dietary intake of vitamin C (mg)	69.1±27.9	76.6±28.3	0.11

[1]Values are expressed as mean ± standard deviation.

Table-2: Serum tocopherol, tocopherol/ cholesterol, retinol, ascorbic acid concentrations in Iranian subjects by age, gender and BMI.

		Tocopherol concentrations (µg/ml)		**Tocopherol/ cholesterol concentrations (µg/mg)**		**Retinol concentrations (µg/ml)**		**Ascorbic acid concentrations (µg/ml)**	
	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD
All									
BMI[1] (kg/m^2)[1]	173	10.22	0.26	6.27	0.13	0.35	0.02	14.45	0.38
Gender									
Male	51	10.12	0.54	6.11	0.23	0.38	0.04	11.75	0.96
Female	122	10.25	0.29	6.34	0.16	0.34	0.02	15.58	0.61
Age (y)									
<30	124	9.96	0.31	6.27	0.13	0.34	0.02	13.93	0.63
≥30	49	11.06	0.44	6.30	0.35	0.38	0.04	15.75	0.07
<18.5	17	10.12	0.59	7.24	0.32	0.28	0.04	13.70	1.96
18.5–24.9	104	10.07	0.34	6.15	0.20	0.34	0.02	14.70	0.68
25–29.9	40	10.59	0.56	6.17	0.30	0.39	0.04	13.90	1.15
≥30	12	10.63	1.17	6.47	0.4	0.50	0.17	15.14	0.53

Table-3: The deficiency prevalence in Iranian subjects by age, gender and BMI.

	Tocopherol			**Retinol**			**Ascorbic acid**		
	Normal N (%)	**Marginal N (%)**	**Deficiency N (%)**	**Normal N (%)**	**Marginal N (%)**	**Deficiency N (%)**	**Normal N (%)**	**Marginal N (%)**	**Deficiency N (%)**
All	117(83.6)	20(14.3)	3(2.1)	113 (80.7)	25 (17.9)	2 (1.4)	28(16.2)	107(61.8)	38(22)
Gender									
Male	30(76.9)	8(20.5)	1(2.6)	32 (82.1)	7 (17.9)	0 (0)	16 (31.4)	27 (52.9)	8 (15.7)
Female	87(86.1)	12(11.9)	2(2)	81 (80.2)	18 (17.8)	2 (2)	12 (9.8)	80 (65.6)	30 (24.6)
Age									
<30y	87(80.6)	18(16.7)	3(2.8)	86 (79.6)	20 (18.5)	2 (1.9)	23 (18.5)	77 (62.1)	24 (19.4)
≥30	30(93.8)	2(6.2)	0	27 (84.4)	5 (15.6)	0 (0)	5 (10.2)	30 (61.2)	14 (28.6)
BMI							3 (17.6)	11 (64.7)	3 (17.6)
<18.5	10(71.4)	2(14.3)	2(14.3)	10 (71.4)	3 (21.4)	1 (7.1)	18 (17.3)	60 (57.7)	26 (25)
18.5–24.9	76(85.4)	12(13.5)	1(1.1)	69 (77.5)	19 (21.3)	1 (1.1)	5 (12.5)	29 (72.5)	61 (15)
25–29.9	26(83.9)	5(16.1)	0	28 (90.3)	3 (9.7)	0 (0)	2 (16.7)	7 (58.3)	3 (25)
≥30	5(83.3)	1(16.7)	0	6 (100)	0 (0)	0 (0)	28(16.2)	107(61.8)	38(22)

The deficient and marginal prevalence of serum retinol were 1.4% and17.9%, respectively. However, 2.1% and 14.3% of the population had serum tocopherol that fell within the deficient and marginal respectively ranges (Table 3). These results indicate that the most studied subjects were in the normal serum tocopherol and retinol concentrations, but as it can be seen in Table 3 the prevalence of marginal serum tocopherol concentrations was higher in men than in women ($p \leq 0.01$). 61.8% of samples had serum ascorbic acid concentration which fell within the marginal range and 15.7% of men and 24.6% of women fell in the deficient range. A significant correlation between serum tocopherol concentration with age ($r^2=0.070$; $p<0.01$), concentrations of triglyceride ($r^2=0.058$; $p<0.01$), LDL-cholesterol ($r^2=0.285$; $p<0.01$) and HDL-cholesterol ($r^2=0.077$; $p<0.01$) were observed. Serum retinol concentration ($r^2=0.042$; $p<0.05$) with BMI, were also correlated, while cholesterol and HDL-cholesterol were not significant predictors for retinol analysis. Among the studied variables sex is the most important factor effecting the vitamin C concentration.

Discussion

In this study, which is the first of its kind in the healthy local population of Tehran, the serum vitamin E, A and C concentrations are reported as well as the effective factors such as sex, age and BMI.

Serum vitamins- lipid relation

It is known that serum tocopherol is highly associated with blood lipids and this association was also observed in this study. The most positive correlation was observed between serum tocopherol concentration and cholesterol. Winklhofer-Roob et al (17) suggested that some to the increase in α-tocopherol level was dependent of the increase in serum lipids that occurs during adulthood. Also it can be seen that tocopherol was highly correlated to the serum lipids. Tocopherol is transported in lipophilic fractions in the blood and it is one of the major factors affecting the lipophilic association between vitamin E and serum lipids (18). Therefore it is suggested that the serum tocopherol/cholesterol ratio was calculated as an alternative serum vitamin E status indicator.

In regard to the retinol lipophilic property, it is expected that serum retinol level was positively correlated with total lipid factors but in this report the lipophilic association was only observed for triglycerides and LDL-cholesterol.

Serum vitamins- age relation: One of the significant reported factors on the serum vitamin concentration was age. In this study a positive correlation between age and serum tocopherol was found. However, it seems that age was not correlated with serum tocopherol after adjusting lipid factors an observation which confirmed by this study. In

addition no differences were observed between age, serum retinol and ascorbic acid concentration that agree well with other studies (5, 19) but contrasts with those reported positive or negative correlation (7, 17). It should be noted that the most selected samples in this study were young (Mean= 27.5) and the data cannot be extended to the other age groups.

Serum vitamins- sex relation

Sex-related differences in serum tocopherol, tocopherol/ total cholestrol and retinol concentrations were not observed in the present study which agree well with the other studies (18, 20) and against with those reported higher serum tocopherol, and retinol concentrations in males (21, 22) or in females (5, 16, 23). In contrast gender was found to be the most important factor affecting serum vitamin C concentration and mean serum ascorbic acid levels were significantly higher in females than in males $p \leq 0.001$. Many studies on serum vitamin C concentration have shown that subjects were established according to gender (24-26). Miratashi et al supposed that women stayed at home more than men, and so foods with higher vitamin C content were available to them (27, 28).

Dietary intakes of vitamins

The retinol recommended dietary allowance (RDA) was 900 and 700 µg /day whereas RDA for ascorbic acid was 90 and 75 mg/day for men and women respectively (28). The tocopherol RDA was revised by Food and Nutrition Board of the Institute of Medicine in 2000 to the 15 mg/day for both men and women (29).

The mean dietary intakes of vitamin E, A and C for men and women are shown in Table 1 separately. These intakes are well below the current tocopherol, retinol and ascorbic acid RDA. It has been estimated that more than 90% of subjects do not meet daily dietary recommendations for vitamin E and A. However in comparison of serum vitamin E concentration of samples with other countries (Table 4), low daily dietary is not very effective factor for serum concentration. Therefore in this study dietary intake of vitamin E was poor predictor for serum vitamin E which agrees well with some other studies (3, 30). It seems that low daily dietary intake of vitamin A may explain the lower serum vitamin A concentration in samples. A weak dietary vitamin A-serum association was observed in this study which is in agreement with some other reports (31). A significant correlation between diet and serum concentration of vitamin E and A were only reported when supplement use was included in the analysis (32).

Table-4: Blood tocopherol, retinol, and ascorbic acid concentration in different continents.

	Tocopherol	Retinol	Ascorbic acid
Asia	10.145±1.288	0.436±0.070	11.194±6.190
Europe	11.302±1.532	0.436±0.050	10.298±2.877
America	11.540±0.00	0.5±0.00	10.160±0.197
Africa	-	-	15.400±0.000

Asia consists of: Japanese, Taiwan, Saudi Arabia, Kuwait, Korean, Chinese, Singapore, Malaysia, Nepal, and India
Europe consists of: Swiss, Italia, Spain, Finland, Greece, Czech, France, Mexico, Netherland, UK, and Poland
America consists of: America, Maryland State in USA
Africa consists of: Nigeria

Vogel et al (19) found that a positive association was observed when vitamin E intake exceeded the third quartile of total vitamin E consumption and this intake level was achieved only with supplement use.

The dietary vitamin C intake in normal, deficient or supernumerary groups was also compared, which showed that people who were deficient, had a significant lower dietary intake $p<0.05$. However no significant relationship between dietary vitamin C intake and serum concentration was observed, which was confirmed by other reports (15, 33). Some studies found a significant positive relationship between dietary vitamin C intake and serum concentration (7).

Serum vitamins- BMI relation

There was a positive correlation between serum retinol concentrations and BMI, so that increasing BMI values led to increased serum retinol concentrations. This is in agreement with Hu et al (32) study in which they suggested weight or BMI and serum retinol to be directly related to each other.

Previous studies have showed a reverse relationship between BMI and serum vitamin C concentration (34), but in this study, no relationships between BMI and vitamin C concentration was found which is similar to results of Ness et al (35). In addition, there were not any relationships between BMI, tocopherol and tocopherol/ total cholesterol concentration.

Serum vitamin-other factors relation

Some factor such as occupation, education level and welfare status and physical activity were also evaluated in this study. No significant correlation between these factors and tocopherol or retinol were observed but serum ascorbic acid concentration showed a significant relation with occupation, education level and welfare status. It seems that welfare status was correlated with the ability to consume more natural vitamin C sources such as orangery. Education level was another factor related to serum vitamin C concentration in this study, which was confirmed by

other reports (36). This difference may be the result of subjects with a higher education level paying more attention to their nutritional status.

Serum vitamins compression between this study and other countries

The mean values of tocopherol, retinol and ascorbic acid in different countries were calculated from other studies to obtain comprehensive table describes continentals' healthy population status. The serum tocopherol value for healthy local population of Tehran was lower than Europe (12,23-25,37-41) and America (6,42) but similar to the Asia (5,11,16,20,21,43-47) other continental reported in this study had higher blood retinol concentration than our subjects but all continental had lower ascorbic acid value except of Africa (41) (Table 4). Finally, this study was performed in a small sample, therefore it is suggested that, other studies with more subjects and all age groups should be perform to obtain more accurate results.

Acknowledgements

Authors would like to thank the participants for their enthusiastic support. This work was supported by a grant from Tehran University of Medical Sciences. None of the authors had any personal or financial conflicts of interest.

References

1. Mahan LK, Escott-Stump S. *Krause's Food, Nutrition and diet therapy*. 10th ed. WB Saunders, Philadelphia. (2008) p 68-100.
2. Gaziano JM. Vitamin E and cardiovascular disease: observational studies. *Ann N Y Acad Sci*. (2004) 1031: 280-91.
3. Sasazuki S, Hayashi T, Nakachi K, Sasaki S, Tsubono Y, Okubo S et al. Protective effect of vitamin C on oxidative stress: a randomized controlled trial. *Int J Vitam Nutr Res*. (2008) 78: 121-8.
4. Patterson C, Feightner JW, Garcia A, Hsiung GY, MacKnight C, Sadovnick AD. Diagnosis and treatment of dementia: 1. Risk assessment and primary prevention of Alzheimer disease. *CMAJ*. (2008) 178: 548-56.
5. Adhikari D, Baxi J, Nagamma T, Azhari S, Singh PP. Vitamin C status in cancer patients and healthy subjects from the local population of Pokhara. *Kathmandu Univ Med J*. (2006) 4: 302-6.
6. Ford ES, Sowell A. Serum alpha-tocopherol status in the United States population: findings from the Third National Health and Nutrition Examination Survey. *Am J Epidemiol*. (1999) 150: 290-300.

7. Olmedilla B, Granado F, Gil-Martinez E, Blanco I, Rojas-Hidalgo E. Reference values for retinol, tocopherol, and main carotenoids in serum of control and insulin-dependent diabetic Spanish subjects. *Clin Chem*. (1997) 43: 1066-71.
8. World Health Organization. Obesity-preventing and managing the global epidemic. Report of WHO consultation on obesity. *Geneva*. (1997) 5.
9. Ars.usda.gov [homepage on internet. Washington DC: United States department of agriculture, agriculture of research service.
10. Sauberlich HE, Dowdy RP, Skala JH. *Laboratory tests for the assessment of nutritional status*. 1st ed. CRC Press: Cleveland, Ohio. (1974) p. 7.
11. Chernecky CA, Berger BJ. *Laboratory test and diagnostic procedure*. 5th ed. WB Saunders Company, Philadelphia. (1997) 1034.
12. Talwar DK, Azharuddin MK, Williamson C, Teoh YP, McMillan DC, St J O'Reilly D. Biological variation of vitamins in blood of healthy individuals. *Clin Chem*. (2005) 51: 2145-50.
13. Siluk D, Oliveira RV, Esther-Rodriguez-Rosas M, Ling S, Bos A, Ferrucci L et al. A validated liquid chromatography method for the simultaneous determination of vitamins A and E in human plasma. *J Pharm Biomed Anal*. (2007) 44: 1001-7.
14. Nirungsan K, Thongnopnua P. Simple and rapid high-performance liquid chromatographic method for endogenous alpha-tocopherol determination in human plasma. *Biomed Chromatogr*. (2006) 20: 774-81.
15. McCormick DB, Wright LD. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. In: Omay ST, Turnbull JD, editors. Methods in enzymology. 1st ed. Academic Press, New York. (1979) p.7.
16. Cheng WY, Fu ML, Wen LJ, Chen C, Pan WH, Huang CJ. Plasma retinol and a-tocopherol status of the Taiwanese elderly population. *Asia Pac J Clin Nutr*. (2005) 14: 256-62.
17. Winklhofer-Roob BM, Van't Hof MA, Shmerling DH. Reference values for plasma concentrations of vitamin E and A and carotenoids in a Swiss population from infancy to adulthood, adjusted for seasonal influences. *Clin Chem*. (1997) 4: 146-53.

18. Looker AC, Underwood BA, Wiley J, Fulwood R, Sempos CT. Serum alpha-tocopherol levels of Mexican Americans, Cubans, and Puerto Ricans aged 4-74 y. *Am J Clin Nutr*. (1989) 50: 491-6.
19. Vogel S, Contois JH, Tucker KL, Wilson PW, Schaefer EJ, Lammi-Keefe CJ. Plasma retinol and lipoprotein tocopherol and carotenoid concentrations in healthy elderly participants of the Framingham Heart Study. *Am J Clin Nutr*. (1997) 66: 950-8.
20. Ito Y, Ochiai J, Sasaki R, Suzuki S, Kusuhara Y, Morimitsu Y et al. Serum concentrations of carotenoids, retinol, and alpha-tocopherol in healthy persons determined by high-performance liquid chromatography. *Clin Chim Acta*. (1990) 194: 131-44.
21. Abiaka C, Olusi S, Simbeye A. Serum concentrations of micronutrient antioxidants in an adult Arab population. *Asia Pac J Clin Nutr*. (2002) 11: 22-7.
22. Boonsiri P, Pooart J, Tangrassameeprasert R, Hongsprabhas P. Serum beta-carotene, lycopene and alpha-tocopherol levels of healthy people in northeast Thailand. *Asia Pac J Clin Nutr*. (2007) 16: S47-51.
23. Knekt P, Seppänen R, Aaran RK. Determinants of serum alpha-tocopherol in Finnish adults. *Prev Med*. (1988) 17: 725-35.
24. Millet P, Guilland JC, Fuchs F, Klepping J. Nutrient intake and vitamin status of healthy French vegetarians and nonvegetarians. *Am J Clin Nutr*. (1989) 50: 718-27.
25. Garry PJ, Goodwin JS, Hunt WC, Gilbert BA. Nutritional status in a healthy elderly population: vitamin C. *Am J Clin Nutr*. (1982) 36: 332-9.
26. Loria CM, Klag MJ, Caulfield LE, Whelton PK. Vitamin C status and mortality in US adults. *Am J Clin Nutr*. (2000) 72: 139-45.
27. Miratashi SA, Besharati MR, Shoja MR, Rastegar A, Manaviat MR. Evaluation of vitamin C concentration of aqueous humor in senile cataract. *Med J IAS*. (2001) 14: 35-40.
28. SmolinLA, Grosvenor MB. *Nutrition Science and Applications*. 1st ed. John Wiley & Sons, Inc., New York. (2003) :50.
29. Food and Nutrition Board, Institute of Medicine: *Vitamin E. In: Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*. National Academy Press, Washington, DC; (2000).

30. Marshall JR, Lanza E, Bloch A, Caan B, Caggiula A, Quandt S et al. Indexes of food and nutrient intakes as predictors of serum concentrations of nutrients: the problem of inadequate is criminant validity. The Polyp Prevention Trial Study Group. *Am J Clin Nutr*. (1997); 65: 1269-74.
31. Neuhouser ML, Rock CL, Eldridge AL, Kristal AR, Patterson RE, Cooper DA et al. Serum concentrations of retinol, alpha-tocopherol and the carotenoids are influenced by diet, race and obesity in a sample of healthy adolescents. *J Nutr*. (2001) 131: 2184-91.
32. Hu W, Tong S, Oldenburg B, Feng X. Serum vitamin A concentrations and growth in children and adolescents in Gansu Province, China. *Asia Pac J Clin Nutr*. (2001) 10: 63-6.
33. Driskell JA, Giraud DW, Drewel BT, Davy SR. Dietary intakes and plasma concentrations of vitamin C, selenium and carotenoids of a group of preschool children. *Nutr Res*. (2006) 26: 23-6.
34. Wannamethee SG, Lowe GD, Rumley A, Bruckdorfer KR, Whincup PH. Associations of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis. *Am J Clin Nutr*. (2006) 83: 567-74.
35. Ness AR, Cappuccio FP, Atkinson RW, Khaw KT, Cook DG. Plasma vitamin C levels in men and women from different ethnic back grounds living in England. *Int J Epidemiol*. (1999) 28: 450-5.
36. Jacob RA, Otradovec CL, Russell RM, Munro HN, Hartz SC, McGandy RB et al. Vitamin C status and nutrient interactions in a healthy elderly population. *Am J Clin Nutr*. (1988) 48: 1436-42.
37. Bui MH, Sauty A, Collet F, Leuenberger P. Dietary vitamin C intake and concentrations in the body fluids and cells of male smokers and nonsmokers. *J Nutr*. (1992) 122: 312-6.
38. Caperle M, Maiani G, Azzini E, Conti EM, Raguzzini A, Ramazzotti V et al. Dietary profiles and anti-oxidants in a rural population of central Italy with a low frequency of cancer. *Eur J Cancer Prev*. (1996) 5: 197-206.
39. Muzáková V, Rousar T, Vojtísek P, Skalický J. Antioxidant vitamin pool in senior population. *Cent Eur J Public Health*. (2004) 12: S64-6.
40. Boekholdt SM, Meuwese MC, Day NE, Luben R, Welch A, Wareham NJ et al. Plasma concentrations of ascorbic acid and C-reactive protein, and risk of future coronary artery disease, in apparently healthy men and women: the EPIC-Norfolk prospective population study. *Br J Nutr*. (2006) 96: 516-22.
41. Goraca A, Skibska B. Plasma antioxidant status in healthy smoking and non-smoking men. *Bratisl Lek Listy*. (2005) 106: 301-6.

42. Sinha R, Block G, Taylor PR. Determinants of plasma ascorbic acid in a healthy male population. *Cancer Epidemiol Biomarkers Prev*. (1992) 1: 297-302.
43. Al-Saleh I, El-Doush I, Billedo G, Muammer AB, Mohamed GE et al. Selenium and vitamin status in the Al-kharj district, Saudi Arabia. *J Nutr Environ Med*. (2005) 15: 190-211.
44. Yeum KJ, Lee-Kim YC, ZhuS, Xiao S, Mason Joel, Russell MR. Serum concentration of antioxidant nutrients in healthy American, Chinese and Korean adults. *Asia Pacific J Clin Nutr*. (1999) 8: 4-8.
45. Khan RM, Iqbal MP. Deficiency of vitamin C in south Asia. *Pak J Med Sci*. (2006) 22: 347-355.
46. Saha N, Tan PY. Serum vitamin C and total cholesterol levels in healthy adults. *Singapore Med J*. (1983) 24: 150-1.
47. Jain A, Agrawal BK, Varma M, Jadhav AA. Antioxidant status and smoking habits: relationship with diet. *Singapore Med J*. (2009) 50: 624-7.

Corresponding Author:
Mannan Hajimahmoodi*,
Email: *hajimah@sina.tums.ac.ir*