Levels of Serum Ascorbic Acid in a Population of North Jordan

Fatima-Azzahra Delmani[1*]

[1]*Department of Biology, Faculty of Science, Jerash University, 26150 Jerash, Jordan.*

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Original Research Article

ABSTRACT

Aim: Total serum vitamin C (L-ascorbic acid) concentration was measured in a group of 499 individuals (males and females age range 18-60 years) with different health conditions (diabetes, high blood pressure, smoking, pregnancy) and a group of 432 healthy individuals (males and females age range 5-60 years) to assess whether serum vitamin C concentrations are affected (or not) by these medical conditions.
Methodology: Total serum vitamin C concentration was measured by HPLC. Sera were extracted from blood samples collected from a population from a northern part of Jordan.
Results: The total vitamin C concentration in male healthy individuals was found to be 43.6±1.75 µmol/l compared to 23.0±1.7 µmol/l in male smokers, 23.6±1.4 µmol/l in male diabetes and 24.8±2.3 µmol/l in males with high blood pressure. The total vitamin C concentration in female healthy individuals was found to be 47.2±2.1 µmol/l, whereas these concentrations were found to be lower in female smokers with 29±1.5 µmol/l, 28.4±0.8 µmol/l in females with diabetes, 29±1.5 µmol/l in females with high blood pressure, and 30.2±0.9 µmol/l in pregnant women. These values are significantly lower ($p < 0.05$).
Conclusion: Vitamin C absorption by the body is influenced by the health status of the individual. This was clearly shown by the differences between healthy individuals from the test group and those with different health conditions.

_____

Corresponding author: E-mail: delmanisa@yahoo.com;

1. INTRODUCTION

Vitamins are trace-amount organic compounds that regulate the physiological functions of an organism. Vitamins are classified into two main groups, water-soluble (vitamins B and C) and fat-soluble (vitamins A, D, E and K). Water soluble vitamins are not stored by the body and therefore should be supplied daily in our diet. On the other hand, fat soluble vitamins are dissolved in fat before they are absorbed in the bloodstream to carry out their functions. Excess of these vitamins is stored in the liver, and is not needed every day in the diet. Vitamin C, also known as ascorbic acid (AA) or ascorbate, is an essential nutrient obtained from the food we eat (fruits and vegetables such as oranges, peppers, dark green leafy vegetables…) and needed by the body to hold cells together through collagen synthesis [1] and numerous other catalytic functions. vitamin C is required for the synthesis of different structures within the body: collagen, a protein representing the main component of connective tissue, is the most abundant protein in mammals, making up about 25% to 35% of the whole-body protein content [2,3]; AA also acts as an antioxidant by lowering oxidative damage and thereby lowering the risk of certain chronic diseases [4-6]. It has been shown that vitamin C helps in decreasing blood glucose, serum lipids & serum insulin in type 2 diabetic patients [7]. Serum ascorbic acid was also inversely associated with several markers of chronic diseases including glucose homeostasis [8], C-reactive protein [9] and obesity [10]. Vitamin C was also related with the synthesis of some hormones (norepinephrine and epinephrine) [11], and carnitine, a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine, required for the transport of fatty acids from the inter-membranous space in the mitochondria, into the mitochondrial matrix during the breakdown of lipids (fats) for the generation of metabolic energy [12]. Insufficient vitamin C intake causes scurvy, which is characterized by fatigue or lassitude, widespread connective tissue weakness, and capillary fragility [13,14]. The recommended daily dietary allowance (RDA) for vitamin C is 90 mg/ day for men and 75 mg/day for women, for smokers the RDA is 125 mg/day for men and 110 mg/day for women [15]. Despite the fact that the dietary intake of vitamin C is the primary determinant of serum ascorbic acid concentrations [16], these concentrations can be influenced by other factors such as age [17], sex [6,18], smoking [16,19], body weight [20,21], physical activity [22], season [19], dietary iron [23], serum lipids [19], and prior vitamin C depletion [21]. Vitamin C was also found to be toxic to tumor cells [2,24,25].

The aim of this study is to investigate the concentrations of serum ascorbic acid in a population of individuals with different age and health conditions (diabetes, high blood pressure, pregnancy, and smoking) and compare them with healthy individuals (people with no medical condition) from the northern parts of Jordan.

2. MATERIALS AND METHODS

2.1 Experiment Design and Population

Blood samples from 931 participants were collected from the northern part of Jordan; participants were male (n= 389) and female (n= 542) between 5 and 60 years old, 133 of which 35 female and 98 male were smokers, 125 were diabetic, and 165 were suffering from high blood pressure. The study protocol was done according to the Jordanian legislation regarding research ethics.

2.2 Blood Samples Collection

Blood samples were collected by venipuncture and stored in glass tubes at the medical laboratories in Al Mafraq hospital (Al Mafraq, Jordan), samples were then centrifuged to separate the serum form other blood constituents. Sera were stored in vials at -20℃ until use.

2.3 Sample Preparation

Ascorbic acid in serum was extracted by mixing 100 µl sera with 400 µl of 60% methanol/EDTA then incubated for 10 min at 4℃ before centrifuging at 12.000 rpm for 8 min. the clear phase was transferred to another polypropylene tube. All extracts were dissolved in 100 µl methanol and stored at -20℃ until analysis. Total ascorbic acid in sera was measured by High Performance Liquid Chromatography (HPLC).

2.4 Chromatographic System

A water mode 600 solvent delivery system was used together with a Thermo Hyper Keystone, C18 (4.6 x 250 mm) column packed with (5 µm

particle size). Samples of vitamin C were injected using a Rheodyne injector with a 20 µL sample loop. Detection was done with a UV/Vis diode array detector (Water PD486), the absorbance detector was operating at 254 nm. Peak evaluation and quantitization were made using a water millennium software. The mobile phase consisted of 75% (v/v) methanol: water and flow rate of 1.0 ml/min.

2.5 Chemicals and Reagents

Ascorbic acid (AA), $C_6H_8O_6$, was obtained from Sigma-Aldrich and used without further purification. All solvents were Merck grade and used as received.

2.6 Statistical Analysis

The Two-Way ANOVA test was used for the data analysis; data was reported as mean ± Standard Deviation (SD). P-value < 0.05 was considered to be significant. The software used was Origin Pro 8.

3. RESULTS AND DISCUSSION

3.1 Validation of the Analytical Method

The validation strategy for the optimization of the chromatography methods includes the study of the repeatability of injection with at least six replicates, the determination of the linear range by dilution of standard solutions, and the estimation of the limit of quantification using standards. The above strategy was achieved by the following steps: (1) Spiking of each matrix that is subject to extraction to determine the recovery of each extraction method and compare with blanks, (2) Determine within-day reproducibility, (3) Determine day-to-day recovery. The chromatography method was used to analyze and quantize the concentration of vitamin C in serum samples.

The total area under the curve is directly related to the concentration of the Vitamin C. A set of calibration standard solutions containing Vitamin C was made (5 to 600 µM) and analyzed using HPLC instrument with 254 nm UV detector. The chromatographic data produced are graphically presented in Fig. 1. The calibration follows a linear down to 5 µM. The calibration curve of this compound following a linear relationship and it is represented by equation 1

$$y = 3.28 + (11542)x \quad \text{with RD is } 0.992 \quad (1)$$

From all the calibration experiments, the smallest concentration of Vitamin C that can be detected is estimated to be 0.1 µM. from all above, we can conclude that used method for the determination of Vitamin C is sensitive and rapid and can be used for the purpose of this study.
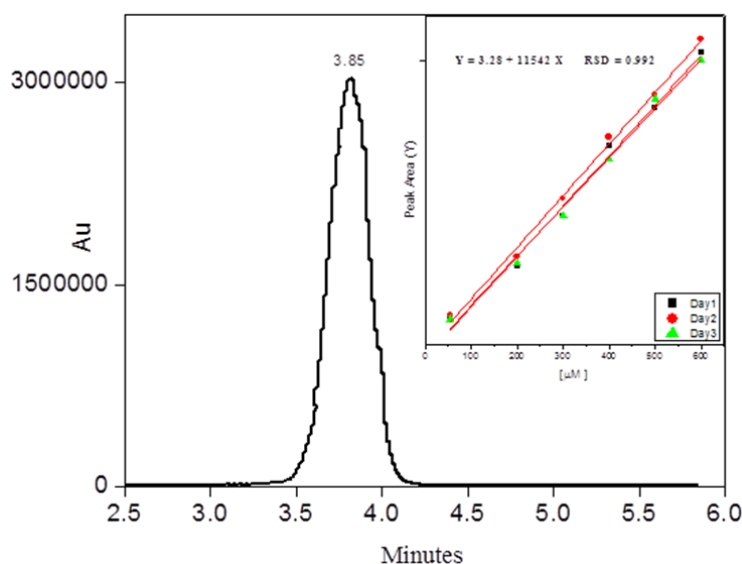

Fig. 1. Chromotogram of Vitamin C in serum samples. Inserted curve: The Calibration curve for the analysis of ascorbic acid standard samples

3

3.2 Levels of Vitamin C Detected in Participants

Serum ascorbic acid concentrations were associated with different health conditions, diabetes [7], high blood pressure [26], smoking [27], and cardiovascular diseases [28,29].

We represented the results of the study depending on sex (male versus female), medical status (healthy versus non healthy), age (5-12, 13-18, 19-29, and 30-60 years old subjects), and number of smoked cigarettes per day among tested smokers (less than 10 cigarettes/day, 10-20 cigarettes/day, and more than 20 cigarettes/day). Results were distributed into tables representing the above categories.

The total number of the participants in this study was 931 aged 5 to 60 years old of which 389 were males and 542 were females. Of the males, 161 were healthy nonsmoking individuals, 98 were smokers aged between 18 and 60 years, 40 had diabetes between 30 and 60 years old and 90 individuals had high blood pressure problems and aged 30 to 60 years. Of the female

investigated population, 271 were healthy nonsmokers, 35 were smokers between 18 and 60 years old, 85 were diabetes between 30 and 60 years old, 75 had blood pressure problems and aged between 30 and 60 years old, and 76 were pregnant (18 to 40 years old).

Table 1 summarizes the numbers of men and women investigated in this study as well as their distribution depending on their medical status. It was reported that the concentration of ascorbic acid in serum was considered to be adequate if >28 µmol/l, suboptimal if between 11 and 28 µmol/l and deficient if <11 µmol/l [30,31]. Table 2 summarizes the serum ascorbic acid concentrations in the tested population with different health conditions and age variation from 18 to 60 years; the mean values of serum ascorbic acid among healthy individuals was found to be 43.6 µmol/l in males and 47.2 µmol/l in females, both values were within the adequate range (> 28 µmol/l) although, females seem to have a slightly higher concentrations than men (Table 2). The statistical data were performed using the two way ANOVA test and the results were significant if $P < 0.05$.

Table 1. Distribution of the tested population depending on their sex, age and medical status

Participants description	Sex	Sample number	Age range / years
Total number		931	5-60
Sex	Male	389	5-60
	Female	542	5-60
Healthy individuals (non-smokers, neither high blood pressure nor diabetic problems)	Male	161	5 - 60
	Female	271	5 - 60
Smokers	Male	98	18 - 60
	Female	35	18 - 60
Diabetics	Male	40	30-60
	Female	85	30-60
High Blood pressure	Male	90	30-60
	Female	75	30-60
Pregnant women		76	18-40

Table 2. Mean concentrations of serum ascorbic acid in the collected samples from 931 individual, males and females, healthy and with different health conditions

Sample description	Sex	Sample number	Mean concentration of vitamin C (µmol/l)
Healthy individuals	Male	161	43.6±1.75
	female	271	47.2±2.1
Smokers	Male	98	23*±1.7
Age 18 - 60	Female	35	29*±1.5
Diabetes	Male	40	23.6*±1.4
Age 18 - 60	Female	85	28.4*±0.8
High Blood pressure	Male	90	24.8*±2.3
Age 18 - 60	Female	75	29*±1.5
Pregnant females Age 18 - 40		76	30.2*±0.9

$P < 0.05$, significantly different from the control (healthy individuals)

Among smokers the mean concentration of serum ascorbic acid was found to be suboptimal with 23 μmol/l in males and 29 μmol/L in females. In the diabetes group, the mean concentration of serum ascorbic acid was 23.6 μmol/l in males and 28.4 μmol/l in females considered to be suboptimal too. For the high blood pressure in the tested subjects, the mean concentration for serum ascorbic acid was found to be 24.8 μmol/l in males and 29 μmol/l in females and was considered to be in the suboptimal group. Pregnant women tested for ascorbic acid levels in their serum had a mean concentration of 30 μmol/l which was considered to be in the adequate group. It was found that low dietary intake of vitamin C during pregnancy was associated with a trend towards an increased incidence of severe pre-eclampsia, eclampsia [32] (Table 2).

If we compare healthy individuals (males and females) with those with different health conditions that were tested in this study, we can see a significant difference in their mean vitamin C concentrations which was 1.9 fold lower in male smokers compared with healthy ones, 1.84 fold lower in male diabetics, and 1.75 times lower in males with high blood pressure compared with healthy ones from the tested population. In females, the same pattern was observed compared with healthy ones, the mean concentrations of ascorbic acid were found to be 1.62 times lower in female smokers, 1.66 times lower in female diabetics, and 1.62 fold lower in females with high blood pressure. Pregnant women were found to have their ascorbic acid levels 1.56 times lower compared with healthy females (Table 2). These results show clearly that the maintenance of AA in the body is related to its health status showing that people with different health conditions do not have adequate levels of serum AA compared with healthy people. These findings are in accordance with studies on ascorbic acid concentrations done by other research groups around the world [33,34].

Table 3 summarizes the differences in the concentrations of ascorbic acid among the healthy individuals from the tested population related to age and sex; we found that younger children from the healthy tested population had higher levels of ascorbic acid than adults regardless of their sex, we found that the levels of serum vitamin C were decreasing within age with a mean serum concentration of 50-52 μmol/l in children aged 5-12 years compared with 33.4-40.3 μmol/l in adults aged 30-60 years (Table 3).

This could be explained by the fact that as we age our body does not assimilate vitamins the same way as when we were young, this is due to the fact that metabolism slows down with age, also, older adults are more likely to take medications that prevent the absorption of certain vitamins.

Table 3. Mean serum ascorbic acid concentrations of males and females aged 5-60 years stratified by sex and age

Age	Males Mean concentration of vitamin C (μmol/l)	Females Mean concentration of vitamin C (μmol/l)
5-12	50±1.32	52±2.25
13-18	48±2.4	49.6±1.34
19-29	43±1.4	47±2
30-60	33.4±1.9	40.3±2.9

Regarding sex, females have higher concentrations of ascorbic acid than males as we stated before. A study conducted by Itoh et al showed that women has hight levels of serum aa than men [35]; another study conducted by Loria et al. [30] showed that men with low serum ascorbate concentrations may have an increased risk of mortality. In contrast, serum ascorbate concentrations were not related to mortality among women.

Among the population tested for serum vitamin C concentrations, 98 males and 35 females were smokers, Table 4 summarize the levels of ascorbic acid among smokers depending on the number of cigarettes smoked daily.

Males from the test group who smoke less than 10 cigarettes/day had their mean serum vitamin C concentrations of 26.2 μmol/l, which is 1.66 fold less than the healthy people among those who took the survey with a mean serum vitamin C concentrations of 43.6 μmol/l; with increasing numbers of smoked cigarettes/day, the concentrations of serum vitamin C were found to be decreasing to reach 20.4 μmol/l which is about 2 fold less than those found in healthy individuals with more than 20 cigarettes/day (Table 4). Females that smoked less than 10 cigarettes/day had a mean vitamin C concentrations around 29 μmol/l, which was 1.6 fold less than the healthy people among those who took the survey with a mean concentrations of serum vitamin C of 47.2 μmol/l. Unfortunately, there were no heavy female smokers among the tested group to study the impact of high number

Table 4. Mean serum ascorbic acid concentrations of smokers (males and females) aged 18-60 years and stratified by number of cigarettes smoked

		Males (n = 98)		Females (n = 35)	
		Sample Number	Mean concentration of vitamin C (μmol/l)	Sample number	Mean concentration of vitamin C μmol/l)
Smokers	< 10	13	26.2*±0.32	35	29*±1.5
(No of	10- 20	40	22.5*±2.3	0	-
cigarettes)	> 20	45	20.4*±1.4	0	-
Healthy individuals		161	43.6±1.75	271	47.2±2.1

$P < 0.05$, significantly different from the control (Healthy individuals)

of smoked cigarettes on the concentrations of serum vitamin C. Schectman and his research group found similar results when testing a group of American volunteers for their serum ascorbic acid levels depending on the number of smoked cigarettes/day compared to non-smokers. The study showed that smokers of 20 cigarettes daily had the lowest serum vitamin C levels compared to non- smokers and smokers of 1-19 cigarettes daily had decreased levels of vitamin C compared to non- smokers [27]. In another study, Schleider et al. found that the mean concentrations of serum ascorbic acid of adult smokers were one-third lower than those of nonsmokers [34]. Our results are in accordance with those found by these scientists indicating that smoking leads to depletion of vitamin C due to higher levels of free radicals generated by inhaling smoke.

4. CONCLUSION

As a conclusion to this study, we can state that the health status of an individual affects its serum vitamin C concentrations; this was shown in the tested population of Al mafraq- Jordan where people with different health conditions had lower serum vitamin C concentrations compared to the healthy ones. People with diabetes, high blood pressure, smokers, and others who do get sufficient vitamin C in their diet should be made aware of the health risks resulting from inadequate intake of this important vitamin.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Hata RI, Senoo H. L-ascorbic acid 2-phosphate stimulates collagen accumulation, cell proliferation, and formation of a three-dimensional tissue-like substance by skin fibroblasts. J Cellular Phys. 2005;138(1):8–16.
Available:http://doi.org/:10.1002/jcp.1041380103

2. Riordan NH, Riordan HD, Casciari JP. Clinical and experimental experiences with intravenous vitamin C. J. Orthomolecular Med. 2000;15(4):201-213.

3. Sharma R, Poddar R, Sen P, Andrews JT. Effect of vitamin C on collagen biosynthesis and degree of birefringence in polarization sensitive optical coherence tomography (PS-OCT). African J. Biotech. 2008;7(12):2049-54.
Available:http://doi.org/:10.5897/ajb07.486

4. McCall MR, Frei B. Can antioxidant vitamins materially reduce oxidative damage in humans? Free Radic Biol. Med. 1999;26(7-8):1034-53.
Available:http://doi.org/:10.1016/S0891-5849(98)00302-5

5. 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(3):121-8.
Available:http://doi.org/:10.1024/0300-9831.78.3.121

6. Telang PS. Vitamin C in dermatology. Indian Dermatol. Online J. 2013;4(2):143–146.
Available:http://doi.org/:10.4103/2229-5178.110593

7. Afkhami-Ardekani M, Shojaoddiny-Ardekani A. Effect of vitamin C on blood glucose, serum lipids & serum insulin in type 2 diabetes patients. Indian J. Med. Res. 2007;126(5):471-4.

8. Paolisso G, D'Amore A, Balbi V, Volpe C, Galzerano D, Giugliano D, et al. Plasma vitamin C affects glucose homeostasis in healthy subjects and in non-insulin-

dependent diabetics. Am. J. Physiol. 1994;266(2 Pt 1):E261-8.

9. Block G, Jensen CD, Dalvi TB, Norkus EP, Hudes M, Crawford PB, et al. Vitamin C treatment reduces elevated C-reactive protein. Free Radic. Biol. Med. 2009;46(1): 70-7.
   DOI: 10.1016/j.freeradbiomed.2008.09.030

10. García OP, Ronquillo D, Elian S, de la Torre K, Caamaño MC, Rosado JL. Vitamin C deficiency is associated with obesity in rural Mexican women. FASEB J. 2009;23:917-4.

11. Sanchit P, Tiwari S, Jigar Haria J. Relationship between depression and vitamin C status: A study on rural patients from Western Uttar Pradesh in India. Int. J. Scientific Study. 2014;1(4):37-39.

12. Rebouche CJ. Ascorbic acid and carnitine biosynthesis. Am. J. Clin. Nutr. 1991;54(6): 1147S-1152S.

13. Li Y, Schellhorn HE. New developments and novel therapeutic perspectives for vitamin C. J. Nutr. 2007;137:2171-84.

14. Stephen R, Utecht T. Scurvy identified in the emergency department: A case report. J. Emerg. Med. 2001;21:235-7.

15. Available:https://ods.od.nih.gov/factsheets/VitaminC-HealthProfessional/

16. Galan P, Viteri FE, Bertrais S, Czernichow S, Faure H, Arnaud J, et al. Serum concentrations of beta-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. Eur. J. Clin. Nutr. 2005;59(10):1181-90.

17. Lowik MR, Hulshof KF, Schneijder P, Schrijver J, Colen AA, van Houten P. Vitamin C status in elderly women: A comparison between women living in a nursing home and women living independently. J. Am. Diet. Assoc. 1993;93(2):167–172.

18. Vioque J, Weinbrenner T, Asensio L, Castelló A, Young IS, Fletcher A. Plasma concentrations of carotenoids and vitamin C are better correlated with dietary intake in normal weight than overweight and obese elderly subjects. Br. J. Nutr. 2007;979(5):977–986.

19. Faure H, Preziosi P, Roussel AM, Bertrais S, Galan P, Hercberg S, Favier A. Factors influencing blood concentration of retinol, alpha-tocopherol, vitamin C, and beta-carotene in the French participants of the SU.VI. MAX trial. Eur. J. Clin. Nutr. 2006;60(6):706–717.

20. Drewnowski A, Rock CL, Henderson SA, Shore AB, Fischler C, Galan P, et al. Serum betacarotene and vitamin C as biomarkers of vegetable and fruit intakes in a community-based sample of French adults. Am J Clin Nutr. 1997;65(6):1796–1802.

21. Block G, Mangels AR, Patterson BH, Levander OA, Norkus EP, Taylor PR. Body weight and prior depletion affect plasma ascorbate levels attained on identical vitamin C intake: A controlled-diet study. J. Am. Coll. Nutr. 1999;18(6):628–637.

22. Johnston CS, Corte C, Swan PD. Marginal vitamin C status is associated with reduced fat oxidation during submaximal exercise in young adults. Nutr. Metab. (Lond). 2006; 3:35.

23. Lynch SR, Cook JD. Interaction of vitamin C and iron. Ann. N. Y. Acad. Sci. 1980;355:32–44.

24. Folk E, Downs TM, Ordman AR. Two grams BID ia an oral dosage of vitamin C to reduce the risk of recurrence of superficial bladder carsinoma. J. Cancer Therapy. 2015;6:169-176.

25. Kaiser J. Vitamin C could target some commun cancers. Science. 2015;350:619. DOI: 10.1126/science.350.6261.619

26. Juraschek SP, Guallar E, Appel LJ, Miller ER. Effects of vitamin C supplementation on blood pressure: A meta-analysis of randomized controlled trials. Am. J. Clin. Nutr. 2012;95(5):1079–1088.
   DOI: 10.3945/ajcn.111.027995

27. Schectman G, Byrd JC, Gruchow HW. The influence of smoking on vitamin C status in adults. Am. J. Public Health. 1989;79(2): 158-162.

28. Ness AR, Powles JW, Khaw KT. Vitamin C and cardiovascular disease: A systematic review. J Cardiovasc. Risk. 1996;3(6):513-21.

29. 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(3):516-22.

30. Loria CM, Klag MJ, Caulfield LE, Whelton PK. Vitamin C status and mortality in US

adults. Am. J. Clin. Nutr. 2000;72(1):139-45.

31. Jacob RA. Assessment of human vitamin C status. J. Nutr. 1990;120(11):1480–1485.

32. Klemmensen A, Tabor A, Osterdal ML, Knudsen VK, Halldorsson TI, Mikkelsen TB, et al. Intake of vitamin C and E in pregnancy and risk of pre-eclampsia: Prospective study among 57 346 women. B.J.O.G. 2009;116(7):964-74.
DOI: 10.1111/j.1471-0528.2009.02150.x

33. Cahill L, Corey PN, El-Sohemy A. Vitamin C deficiency in a population of young Canadian adults. Am. J. Epidemiol. 2009;170(4):464-471.

34. Schleicher RL, Carroll MD, Ford ES, Lacher DA. Serum vitamin C and the prevalence of vitamin C deficiency in the United States: 2003–2004 National Health and Nutrition Examination Survey (NHANES). Am. J. Clin. Nutr. 2009;90: 1252–63.

35. Itoh R, Yamada K, Oka J, Echizen H, Murakami K. Sex as a factor in levels of serum ascorbic acid in a healthy elderly population. Int J Vitam Nutr Res. 1989;59(4):365-72.