

**Original article:**

## **Studies on circadian periodicity of serum ascorbic acid and uric acid in healthy Indians**

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### **ABSTRACT**

**Background:** Mapping the circadian rhythm of serum ascorbic acid and uric acid is needed to establish reference values and their status and rhythm in healthy population.

**Materials and Methods:** The circadian periodicity of serum ascorbic acid and uric acid concentration was studied in healthy volunteers. 150 healthy volunteers (90 males and 60 females) mostly medical students, staff members and members of their families were included in this study. They were divided into 4 age groups i. e., Group A (08-20 years), Group B (21-40 years), Group C (41-60 years) and Group D (61-75 years) each comprising of 35, 45, 40, and 30 participants respectively. They followed a diurnal activity from about 06:00 to about 22:00 hours and nocturnal rest. Blood samples were collected from each subject every 6 h for complete 24-hour period. Serum ascorbic acid and uric acid were measured spectrophotometrically. Data from each subject were analysed by population-mean cosinor procedures.

**Results:** A marked circadian variation was demonstrated for ascorbic acid and uric acid concentration in each group by population-mean cosinor analysis (almost invariably  $p < 0.001$ ). Furthermore, both the MESOR and circadian amplitude underwent significant changes with advancing age for both studied variables. The circadian acrophase also underwent marked changes as a function of age.

**Conclusion:** Mapping the circadian rhythm of serum ascorbic acid and uric acid is needed to explore their role in different physio-pathological conditions.

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### **INTRODUCTION**

Ascorbic acid is perhaps one of the most important single nutritional factors in terms of its influence on world history. Naval battles have been won or lost, based on the number of crewmembers sick with scurvy as well as on military prowess. The race to explore faraway places like the Antarctic was made much harder for explorers like Captains Cook and Scott by the consequences of scurvy. Vitamin C,

also known as L-ascorbic acid, is a water-soluble vitamin that is naturally present in some foods, added to others, and available as a dietary supplement. Humans, unlike most animals, are unable to synthesize vitamin C endogenously, so it is an essential dietary component<sup>1</sup>.

Uric acid is a product of the metabolic breakdown of purine nucleotides, and it is a normal constituent of urine. It is synthesized mainly in the liver,

intestines and other tissues such as muscles, kidneys and the vascular endothelium as the end product of an exogenous pool of purines, derived largely from animal proteins. In addition, live and dying cells degrade their nucleic acids, adenine and guanine into uric acid. Deamination and dephosphorylation convert adenine and guanine to inosine and guanosine, respectively. The enzyme purine nucleoside phosphorylase converts inosine and guanosine to the purine bases, respectively hypoxanthine and guanine, which are both converted to xanthine via xanthine oxidase-oxidation of hypoxanthine and deamination of guanine by guanine deaminase. Xanthine is further oxidized by xanthine oxidase to uric acid<sup>2</sup>. High blood concentrations of uric acid can lead to gout and are associated with other medical conditions including diabetes and the formation of ammonium acid urate kidney stones. Humans cannot oxidize uric acid to the more soluble compound allantoin due to the lack of uricase enzyme. The enzyme uricase (urate oxidase) can metabolize uric acid to highly soluble 5-hydroxyisourate that is further degraded to allantoic acid and ammonia, easily excreted by the kidneys. However, several primates, including man have lost the functional activity of the enzyme uricase, as uricase mRNA may be detected in human livers but it displays two premature stop codons, and the encoding gene is, thus, a pseudogene.

Vitamin C or ascorbic acid exists in reduced (ascorbate) and oxidized forms as dehydroascorbic acid, which are easily inter-convertible and biologically active. It thus acts as an important antioxidant. Due to its function as an antioxidant and its role in immune function, vitamin C has been promoted as a means to help prevent and/or treat numerous health conditions. Diseases and disorders in which vitamin C might play a role include cancer (including prevention and treatment),

cardiovascular disease, age-related macular degeneration (AMD) and cataracts, and the common cold. Under certain conditions, vitamin C can act as a pro-oxidant, potentially contributing to oxidative damage<sup>3</sup>. A few studies in vitro have suggested that by acting as a pro-oxidant, supplemental oral vitamin C could cause chromosomal and/or DNA damage, and possibly contribute to the development of cancer<sup>3,4</sup>. However, other studies have not shown increased oxidative damage or increased cancer risk with high intakes of vitamin C<sup>3,5</sup>. Blood is a commonly used biofluid for biomarker discovery because it is a data-rich source containing several thousands of hydrophilic and hydrophilic metabolites that likely reflect many complex biological processes in the body. However, these metabolites' concentrations and time structure in different age groups in healthy populations has not been extensively studied. There are only few reports regarding the circadian nature of serum ascorbic acid in health and disease<sup>6,7</sup>.

Uric acid, or more correctly (at physiological pH values), its monoanion urate, is traditionally considered to be a metabolically inert end-product of purine metabolism in man, without any physiology value. However, this ubiquitous compound has proven to be selective antioxidant, capable especially of reaction with hydroxyl radicals and hypochlorous acid, itself being converted to innocuous products (allantoin, allantoate, glyoxylate, urea, oxalate). There is now evidence for such processes not only in vitro and in isolated organs, but also in the human lung in vivo. Urate may also serve as an oxidase co substrate for the enzyme cyclooxygenase. As shown for the coronary system, a major site of production of urates is the microvascular endothelium, and there is generally a net release of urate from the human myocardium in vivo. In isolated organ preparations, urate protects against reperfusion damage induced

by activated granulocytes, cells known to produce a variety of radicals and oxidants. Intriguingly, urate prevents inactivation of endothelial enzymes (cyclooxygenase, angiotensin converting enzyme) and preserves the ability of the endothelium to mediate vascular dilatation in the face of oxidative stress, suggesting a particular relationship between the site of urate formation and the need for a biologically potent radical scavenger and antioxidant<sup>2</sup>.

To our knowledge, circadian rhythm of vitamin C and uric acid concentration in healthy volunteers of different age groups and their correlation, if any, with the aging process has not been reported. The present study aims to fill this gap by providing reference values for circadian changes of circulating ascorbate and urate in different age groups in healthy Indians.

#### SUBJECTS AND METHODS

One hundred fifty healthy Indians (90 males and 60 females, 8–75 years of age) volunteered for this study. They followed a 24-hour synchronized social schedule with diurnal activity from about 06:00 to 22:00 and nocturnal rest. They were of equal socio-economic status (middle income), residing in the northern part of the country, around Lucknow. Most were medical students, staff members and members of their families who had been residing in the region for at least 2 years. Informed consent was obtained from all individual participants included in this study, which was approved by the Institutional Review Board of King George's Medical University in Lucknow, India.

Subjects were asked to refrain from taking any Vitamin C supplements, including any multivitamins with antioxidant activity before the study. All study participants were asked to avoid junk/fast food. All participants followed their usual daily routine, but abstain from strenuous activity,

such as sports or other physical exercises on the dates of investigation. All took their usual (although not identical) meals 3 times daily: breakfast around 08:00, lunch around 13:30, and dinner around 21:00, without any change in their usual fluid intake. The burden of environmental temperature and pollution, if any, was common to all participants.

The volunteers were subdivided into 4 age groups: A (8–20 y), B (21–40 y), C (41–60 y), and D (61–75 y), composed of 35, 45, 40 and 30 subjects, respectively. The 8 to 20 years age group was included to span as wide an age range as possible without necessarily focusing on changes that may occur as a function of development and maturation. The dietary pattern of subjects in this age group was about the same as that of the other age groups. Blood samples were collected in plain vials every 6 hours for 24 hours (4 samples) around 06:00, 12:00, 18:00 and 24:00. Serum was separated and ascorbic acid (vitamin C) and uric acid were measured spectrophotometrically<sup>8,9</sup>.

Data from each subject were evaluated by conventional statistical analyses and by single and population-mean-cosinor procedures to obtain estimates of the MESOR (Midline Estimating Statistic Of Rhythm, a rhythm-adjusted mean), 24-hour amplitude (a measure of half the predictable extent of daily change) and 24-hour acrophase (a measure of the timing of overall high values recurring each day)<sup>10-13</sup>.

#### RESULTS

Results are summarized in Tables 1 and 2. A marked circadian variation was recorded for ascorbic acid and uric acid concentrations by population-mean cosinor analysis for all age groups, with changed circadian characteristics in terms of MESOR, circadian amplitude and circadian acrophase. On an individualized basis, a statistically significant circadian rhythm in Vitamin

C could be documented by single cosinor in only 10 of the 150 subjects, while borderline statistical significance was reached in another 16 subjects, likely related to the poor statistical power from the 4 samples, leaving but one degree of freedom for the zero-amplitude test. On a population basis, however, a circadian rhythm is invariably documented in each age group ( $P < 0.001$ ). Parameter tests show large changes in all circadian rhythm characteristics among the 4 age groups, Table 1. Both the MESOR and 24-hour amplitude increased greatly between age groups A and B and between age groups B and C, while they underwent a drastic decrease between age groups C and D ( $P < 0.001$ ), Table 1. The 24-hour acrophase advances between age groups A and B, and again between age groups C and D ( $P = 0.001$ ), Table 1.

MESOR and circadian amplitude underwent significant changes with advancing age for serum uric acid. The circadian amplitude further underwent marked changes as a function of age, Table 2. The acrophase occurred around 13:45 hours in youngest age group which advanced to peak around 12:52, 06:35 and even 03:19 progressively in older age groups. Parameter tests further showed significant changes in all circadian rhythm characteristics among the 4 studied age groups, Table 2. MESOR increased greatly between age groups A and B and between age groups C and D, while they underwent a drastic decrease between age groups C and D ( $P < 0.001$ ). The 24-hour acrophase advanced between age groups A and B, and again between age groups C and D ( $P = 0.001$ ), Table 2.

**Table 1: Circadian periodicity of serum ascorbic acid in Healthy Indians**

<i>Population-Mean Cosinor Results</i>								
Age Group	n	MESOR	SE	24h Amplitude	SE	24h Acrophase	(95% CI)	P-value
A	35	0.59	0.02	0.13	0.01	-278°	(-270, -289)	<0.001
B	45	0.71	0.01	0.17	0.02	-232°	(-238, -259)	<0.001
C	40	0.89	0.02	0.32	0.02	-238°	(-221, -239)	<0.001
D	30	0.62	0.06	0.08	0.01	-149°	(-128, -186)	<0.001

Age groups: A (<20 years of age); B (21-40 years of age); C (41-60 years of age); D (> 60 years of age).

n: Number of subjects; MESOR: Midline Estimating Statistic Of Rhythm (a rhythm-adjusted mean) (mg/dl);

SE: Standard Error; 24h Amplitude (mg/dl); 24h Acrophase (measure of timing of overall high values recurring each day) (negative degrees, with  $360^\circ \equiv 24$  hours.  $0^\circ = 00:00$ ; CI: Confidence Interval.

**Table 2: Circadian periodicity of serum uric acid in Healthy Indians**

<i>Population-Mean Cosinor Results</i>								
Age Group	n	MESOR	SE	24h Amplitude	SE	24h Acrophase	(95% CI)	P-value
A	35	4.41	0.02	0.30	0.11	-213°	(-171, -268)	=0.02
B	45	5.27	0.08	0.52	0.07	-189°	(-156, -185)	<0.01
C	40	4.99	0.11	0.59	0.05	-118°	(-86, -184)	<0.01
D	30	4.02	0.10	0.48	0.08	-52°	(-14, -72)	<0.02

Populations: A (<20 years of age); B (21-40 years of age); C (41-60 years of age); D (> 60 years of age).

k: Number of subjects; MESOR: Midline Estimating Statistic Of Rhythm (a rhythm-adjusted mean) (mg/dl);

SE: Standard Error; 24h Amplitude (mg/dl); 24h Acrophase (measure of timing of overall high values recurring each day) (negative degrees, with 360° ≡ 24 hours. 0° = 00:00; CI: Confidence Interval.

#### DISCUSSION

A marked circadian variation was recorded in serum ascorbic acid and uric acid concentration in healthy Indians of different ages. The MESOR increased significantly until about 45 years in both men and women. The circadian amplitude also started to decrease around 40 years of age, whereas the circadian acrophase advanced steadily throughout the lifespan, occurring in the oldest age group almost 9 hours earlier than in the youngest age group. Such changes with age in the circadian amplitude and acrophase of ascorbic acid are also found in many other variables and are thought to reflect the aging process. Vitamin C plays an important role as antioxidant in human health. It takes part in multiple oxidation-reduction reactions as a powerful reducing agent. Most of these reactions help coordinate both circulating and hepatic lipid physiology. Vitamin C can decrease mitochondrial ROS formation and improve the activity of manganese superoxide dismutase (SOD) and glutathione peroxidase (GPx) in isolated rat liver mitochondria<sup>14</sup>. It has also been suggested to

be inversely associated with C-reactive protein (CRP) and myeloperoxidase, which are inflammatory markers<sup>15,16</sup>. Moreover, Vitamin C may impact the coordination of adiponectin, which may be able to decrease hepatic lipid accumulation, systemic insulin resistance and inflammation, and produce protective effects against NAFLD<sup>17,18</sup>. Vitamin C also acts as a co-factor in the conversion of cholesterol to 7 $\alpha$ -hydroxycholesterol, which is the rate-limiting step in bile acid formation. Hence, the deficiency of vitamin C can result in decreased excretion of cholesterol in animals<sup>19</sup>. Several animal studies suggested that vitamin C treatment could effectively relieve hepatic oxidative stress. Nambisan et al.<sup>20</sup> reported that vitamin C deficiency accelerated the dyslipidemia and hepatic consequences, while increased vitamin C intake could reduce the severity of both dyslipidemia and hepatic lipid accumulation in guinea pigs. Rezazadeh et al.<sup>21</sup> showed that vitamin C supplementation significantly decreased hepatic markers of oxidative stress, hepatocellular

ballooning and inflammation, while SOD and catalase were increased in rat's model.

MESOR reached a maximum around 35 and 39 years of age in healthy men and women for serum uric acid. The circadian amplitude, however, decreased linearly with age. In support, plasma low uric acid levels, leading to decrease in antioxidant molecules, were evident in patients with multiple sclerosis. Peroxynitrites and ROS are believed to be responsible for myelin degradation in multiple sclerosis (MS) and can be blocked by high uric acid levels, while gout patients almost never present with MS disease<sup>22</sup>. Several reports documented association of low uric acid serum levels with MS disease<sup>23-25</sup>.

Urate crystals are deposited principally in connective tissues of the joints, tendons, kidney, and rarely in heart valves and pericardium, and readily interact with serum proteins<sup>26</sup>. A group of mouse antibodies of the IgM class were recently shown to facilitate in vitro uric acid crystallization and to bind to the MSU crystals<sup>27,28</sup>. Deposited MSU crystals in the joints cavities interact with resident macrophages and mast cells, recruited neutrophils and monocytes, and non- haemopoietic synovial and endothelial cells. All these cells may phago- or endocytose MSU crystals leading to their activation and injury and release of hydrolytic enzymes, reactive oxygen species, and a plethora of danger-associated molecular patterns (DAMP) that might be sensed by the cells surface membrane and cytoplasmic receptors of the innate immune system<sup>29,30</sup>.

Increased uric acid production, impaired renal uric acid excretion, or a combination of the two lead to hyperuricemia<sup>2,31</sup>.

Additionally, uric acid may accumulate in the kidney, leading to formation and deposition of stones. Kidney stones and urinary tract infections are the most common urinary tract problems. Uric

acid stones occur in 10% of all kidney stones and are the second most-common cause of urinary stones after calcium oxalate and calcium phosphate calculi. The most important risk factor for uric acid crystallization and stone formation is a low urine pH (below 5.5) due to impaired urinary uric acid excretion. Main causes of low urine pH beside high uric acid excretion are chronic diarrhoea, severe dehydration, and diabetic ketoacidosis. The contribution of uric acid to development and progress of gout and metabolic syndrome appears to be well-established. The pivotal role of uric acid in preservation of the human species and the individual may be anticipated by the loss of the enzyme uricase in humans and the eagerness of the kidney to retrieve filtered uric acid. Yet, studies are needed to document the paramount importance of uric acid in resistance to infectious, neurological and autoimmune diseases.

The present study highlighted the Indian scenario. Whereas more evidence is needed for combined effects of gender, age, diet and smoking status on serum ascorbic acid concentrations in other populations to allow results found in this study to be generalized, studies investigating individual effects of gender, diet, and smoking status on vitamin C support our findings<sup>32,33</sup>. The report that serum vitamin C was higher in female volunteers, and was not age related may be accounted for by the fact that in this study, subjects ranged in age between 35 and 60 years of age<sup>34</sup>. This age range corresponds to maxima in both MESOR and circadian amplitude of Vitamin C in our study, when age-related changes are minimal. The discrepancy in gender differences may also be accounted for by the fact that in Faure et al.'s study<sup>33</sup>, men (50-60 years) were older than women (35-60 years). Herein, a vegetarian diet was noticed to be associated with a higher MESOR of Vitamin C ( $P < 0.001$ ), confirming the dietary significance in

vitamin C. Smoking was found to be associated with a slightly lower MESOR of Vitamin C ( $P=0.033$ ), illustrating the negative role of smoking on vitamin C concentration and thus the necessity of intake of this vitamin as supplement for smokers. Our results are consistent with those of other authors<sup>34,35</sup>. The relation between vitamin C and serum triglycerides was previously reported by the Ness team that also found an increase of HDL cholesterol in volunteers with high blood vitamin C concentrations<sup>36</sup>. In our study as well, the MESOR of Vitamin C was found to correlate positively with HDL cholesterol ( $r=0.342$ ,  $P<0.001$ ).

Serum vitamins are not considered to be good markers of deficiency, and this is particularly true for vitamin C<sup>37</sup>. The present study, however, aimed at assessing ascorbic acid results in clinically healthy Indians. The medical examination

conducted when recruiting study participants ascertained the health status of all participants. Persons presenting with any known or clinically documented disease were excluded. As expected, average concentrations of Vitamin C in our population averaged  $0.75 \pm 1.4$  mg/dl, and ranged from 0.54 to 1.14 mg/dl. These values are within the normal range of 0.41 to 2.00 mg/dl reported by Burtis et al.<sup>38</sup>.

In conclusion, a definite rhythm in ascorbic acid and uric acid concentrations during 24-hr period with significant effect on advancing age for both vitamin C and uric acid concentration in clinical health was noticed. Mapping the circadian rhythm of serum ascorbic acid is needed to explore their role in different pathophysiological conditions in Indian population.

#### REFERENCES

1. Li Y, Schellhorn HE. New developments and novel therapeutic perspectives for vitamin C. *J Nutr* 2007; 137: 2171-2184.
2. Becker BF. Towards the physiological function of uric acid. *Free Rad Biol Med*. 1993; 14(6): 615-631.
3. Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoid. External link disclaimer. Washington, DC: National Academy Press, 2000.
4. Podmore ID, Griffiths HR, Herbert KE, Mistry N, Mistry P, Lunec J. Vitamin C exhibits pro-oxidant properties. *Nature* 1998; 392: 559.
5. Carr A, Frei B. Does vitamin C act as a pro-oxidant under physiological conditions? *FASEB J* 1999; 13: 1007-1024.
6. Singh R, Singh RK, Mahdi AA, Kumar A, Tripathi AK, Rai R, Singh U, Cornelissen G, Schwartzkopff O, Halberg F. Circadian periodicity of plasma lipid peroxides and other antioxidants as putative markers in gynaecological malignancies. *In vivo* 2002; 17 (6): 593-600.
7. Singh R, Singh RK, Singh RK, Tripathi AK, Cornelissen G, Schwartzkopff O, Otsuka K, Halberg F. Chronomics of circulating plasma lipid peroxides, antioxidant enzymes and other related molecules in cirrhosis of liver. In memory of late Shri Chetan Singh. *Biomed & Pharmacother* 2005; 59 (Suppl 1): S229- S235.
8. Natelson S. Techniques of clinical chemistry. 3rd ed. Springfield: Charles C Thomas; 1971. p. 165-6.
9. Eichhorn F, Zelmanowski S, Lew E, Rutenberg A, Faniias B. Improvement of the uric acid determination by the carbonate method for serum and urine. *J Clin Pathol*. 1961; 14: 450-2.
10. Halberg F. Chronobiology: methodological problems, *Acta Med. Rom*. 1980; 18: 399-440.

11. Cornelissen G, Halberg F. Chronomedicine, in: P. Armitage, T. Colton (Eds.), *Encyclopedia of Biostatistics*, second ed. John Wiley & Sons Ltd., Chichester, UK 2005, pp. 796–812.
12. Refinetti R, Cornelissen G, Halberg F. Procedures for numerical analysis of circadian rhythms, *Biol. Rhythm. Res* 2007; 38: 275–325.
13. Bingham C, Arbogast B, Cornelissen G, Lee JK, Halberg F. Inferential statistical methods for estimating and comparing cosinor parameters. *Chronobiologia*. 1982;9:397–439.
14. Valdecantos MP, Perez-Matute P, Quintero P, Martinez JA. Vitamin C, resveratrol and lipoic acid actions on isolated rat liver mitochondria: all antioxidants but different. *Redox Rep* 2010; 15: 207–216.
15. Aasheim ET, Hofso D, Hjelmessaeth J, Birkeland KI, Bohmer T. Vitamin status in morbidly obese patients: a cross-sectional study. *Am J Clin Nutr* 2008; 87: 362–369.
16. Mah E, Matos MD, Kawiecki D, Ballard K, Guo Y, Volek JS, et al. Vitamin C status is related to proinflammatory responses and impaired vascular endothelial function in healthy, college-aged lean and obese men. *J Am Diet Assoc* 2011; 111: 737–743.
17. Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J Clin Invest* 2007; 117: 2621–2637.
18. Rose FJ, Webster J, Barry JB, Phillips LK, Richards AA, Whitehead JP. Synergistic effects of ascorbic acid and thiazolidinedione on secretion of high molecular weight adiponectin from human adipocytes. *Diabetes Obes Metab* 2010; 12: 1084–1089.
19. Ginter E. Ascorbic acid in cholesterol and bile acid metabolism. *Ann N Y Acad Sci* 1975; 258: 410–421.
20. Nambisan B, Kurup PA. Ascorbic acid and glycosaminoglycan and lipid metabolism in guinea pigs fed normal and atherogenic diets. *Atherosclerosis* 1975; 22: 447–461.
21. Rezazadeh A, Yazdanparast R, Molaei M. Amelioration of diet-induced nonalcoholic steatohepatitis in rats by Mn-salen complexes via reduction of oxidative stress. *J Biomed Sci* 2012; 19: 26.
22. Hooper DC, Spitsin S, Kean RB, Champion JM, Dickson GM, Chaudhry I, et al. Uric acid, a natural scavenger of peroxynitrite, in experimental allergic encephalomyelitis and multiple sclerosis. *Proc Natl Acad Sci USA*. 1998; 95 (2): 675–680.
23. Drulović J, Dujmović I, Stojsavljević N, Mesáros S, Andjelković S, Miljković D, et al. Uric acid levels in sera from patients with multiple sclerosis. *J Neurol*. 2001; 248 (2): 121–126.
24. Sotgiu S, Pugliatti M, Sanna A, Sotgiu A, Fois ML, Arru G, et al. Serum uric acid and multiple sclerosis. *Neurol Sci*. 2002; 23 (4): 183–188.
25. Rentzos M, Nikolaou C, Anagnostouli M, Rombos A, Tsakanikas K, Economou M, et al. Serum uric acid and multiple sclerosis. *Clin Neurol Neurosurg*. 2006; 108 (6): 527–531.
26. Martinon F. Mechanisms of uric acid crystal-mediated autoinflammation. *Immunol Rev*. 2010; 33 (1): 218–232.
27. Spilberg I. Current concepts of the mechanism of acute inflammation in gouty arthritis. *Arthritis Rheum*. 1975; 18 (2): 129–134.
28. Kanevets U, Sharma K, Dresser K, Shi Y. A role of IgM antibodies in monosodium urate crystal formation and associated adjuvanticity. *J Immunol*. 2009; 182: 1912–1918.



29. N. BussoN, So A. Mechanisms of inflammation in gout. *Arthritis Res Ther.* 2010; 12 (2): 206.
30. Busso N, So A. Microcrystals as DAMPs and their role in joint inflammation. *Rheumatology (Oxford).* 2012; 51 (7) (2012): 1154–1160.
31. Bobulescu IA, Moe OW. Renal transport of uric acid: evolving concepts and uncertainties. *Adv Chronic Kidney Dis.* 2012; 19 (6): 358–371.
32. Convento MS, Pessoa E, Dalboni MA, Borges FT, Schor N. Pro-inflammatory and oxidative effects of noncrystalline uric acid in human mesangial cells: contribution to hyperuricemic glomerular damage. *Urol Res.* 2011; 39 (1): 21–27.
33. 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 bold italic beta-carotene in the French participants of the SU.Vi.MAX trial. *European J Clin Nutrition* 2006; 60: 706-717.
34. Handelman GL, Packer L, Cross C. Destruction of tocopherols, carotenoids, and retinol in human plasma by cigarette smoke. *Am J ClinNutr* 1996; 63: 559–565.
35. Marangon K, Herbeth B, Lecomte E, Paul-Dauphin A, Grolier P, Chancerelle Y et al. Diet, antioxidant status, and smoking habits in French men. *Am J ClinNutr* 1998; 67: 231–239.
36. Ness AR, Khaw KT, Bingham S, Day NE. Vitamin C status and serum lipids. *Eur J ClinNutr* 1996; 50: 724–729.
37. Benzie IFF. Vitamin C: prospective functional markers for defining optimal nutritional status. *ProcNutrSoc* 1999; 58 (2): 469–476.
38. Burtis CA, Ashwood ER, Burns DE. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*, 4th ed., Elsevier Saunders: St. Louis, MO, USA, 2006.