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Effect of gender, age, diet and smoking status on the circadian rhythm of ascorbic acid (vitamin C) of healthy Indians

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ABSTRACT

Background/Objectives: To determine effects of gender, age, diet, and smoking status on circadian rhythm characteristics of ascorbic acid (vitamin C).
Subjects/Methods: Ascorbic acid was measured spectrophotometrically in serum collected from 162 healthy volunteers (103 males and 59 females; 7–75 years) every 6 h for 24 h (4 samples). Data were analyzed by single and population mean cosinor. Effects of gender, age, diet (vegetarian vs. omnivore), and smoking status on the rhythm-adjusted mean (MESOR) and circadian amplitude were examined by multiple analysis of variance.
Results: A circadian rhythm is documented with statistical significance by population mean cosinor. In addition to effects of gender and age, the MESOR is affected by diet and smoking status. The circadian amplitude changes nonlinearly as a function of age. The circadian acrophase advances with increasing age.
Conclusion: The present observations confirm a definite circadian rhythm in ascorbic acid concentrations with significant effects of age, diet and smoking status in clinical health. Mapping the circadian rhythm of serum ascorbic acid in health can help explore its role in different pathophysiological conditions as pre-disease conditions may be characterized by alterations in the circadian amplitude and/or phase before there is a change in mean value.

Introduction

Vitamin C (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. 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 (Li and Schellhorn, 2007).

Oxidative stress generated by reactive oxygen species (ROS) is a critical component in the pathogenesis of a number of organic diseases and other neurodegenerative disorders, shown in human studies and supported by experimental models (Choudhry et al., 2012; Reddy and Beal, 2008). Reactivity of ROS can be terminated if the radical meets and reacts with an antioxidant molecule such as vitamin C or vitamin E (α-tocopherol), leading to neutralized or greatly reduced reactivity. Antioxidant molecules lose their antioxidant properties via this process and therefore must be constantly replenished or recycled. Oxidative stress is one of multiple processes that can lead to apoptosis in neurons (Mattson, 2000). Most endogenous ROS (including superoxide, hydrogen peroxide and the hydroxyl radical) are created by the electron transport chain during the production of ATP in the mitochondria (Eckert et al., 2003). Under normal circumstances, ROS are neutralized by antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase) and by antioxidants within the cell and the interstitial fluids (including vitamin C, vitamin E, and glutathione). A diet deficient in antioxidants can also lead to the impaired ability to repair neuronal DNA, which leaves the cells even more sensitive to oxidative damage (Kruman et al., 2002). Vitamin C is thought to be the most effective antioxidant in plasma,

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in part due to its water solubility and to the wide range of ROS that it can scavenge (Frei et al., 1990). If found to be protective against age-related and neurological diseases, vitamin C supplements could serve as an intervention of low cost and low toxicity. The elderly are at high risk of malnutrition, or at least sub-clinical malnutrition, for a number of reasons, including limited mobility, low income, institutionalization, reduced appetite, and poorer cognitive function. Preventing the depletion of antioxidant stores and maintaining healthy concentrations throughout the lifespan in order to improve the health of the population may be a more important strategy than trying to reverse ROS damage that has already occurred.

Vitamin C is required for the biosynthesis of collagen, L-carnitine, and certain neurotransmitters. Vitamin C is also involved in protein metabolism (Li and Schellhorn, 2007). Collagen is an essential component of connective tissue, which plays a vital role in wound healing. Vitamin C is also an important physiological antioxidant (Frei et al., 1989), which has been shown to regenerate other antioxidants within the body, including alpha-tocopherol (vitamin E) (Jacob and Sotoudeh, 2002). Ongoing research examines whether vitamin C, by limiting the damaging effects of free radicals through its antioxidant activity, might help prevent or delay the development of certain cancers, cardiovascular disease, and other diseases in which oxidative stress plays a causal role. In addition to its biosynthetic and antioxidant functions, vitamin C plays an important role in immune function (Jacob and Sotoudeh, 2002) and improves the absorption of nonheme iron (Choudhry et al., 2012), the form of iron present in plant-based foods. Insufficient vitamin C intake causes scurvy, which is characterized by fatigue or lassitude, widespread connective tissue weakness, and capillary fragility (Chambial et al., 2013; Li and Schellhorn, 2007). Although overt scurvy is now rare, there is evidence that subclinical vitamin C deficiency is still quite common (Emadi-Konjin et al., 2005).

The intestinal absorption of vitamin C is regulated by at least one specific dose-dependent, active transporter (Jacob and Sotoudeh, 2002). Cells accumulate vitamin C via a second specific transport protein. *In vitro* studies have found that oxidized vitamin C, or dehydroascorbic acid, enters cells via some facilitated glucose transporters, and is then reduced internally to ascorbic acid. The physiologic importance of dehydroascorbic acid uptake and its contribution to overall vitamin C economy is unknown.

Vitamin C 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. It has long been known that vitamin C can be used as a protection against the common cold.

Under certain conditions, vitamin C can act as a pro-oxidant, potentially contributing to oxidative damage (Institute of Medicine, 2000). 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 (Institute of Medicine, 2000). However, other studies have not shown increased oxidative damage or increased cancer risk with high intakes of vitamin C (Institute of Medicine, 2000; Podmore et al., 1998). Blood is a commonly used biofluid for biomarker discovery. However, there are only few reports regarding the circadian nature of serum ascorbic acid in health and disease (Kushwaha et al., 2017; Singh et al., 2003, 2005, 2015). Moreover, the effect of gender, age, diet and smoking status on the MESOR (rhythm-adjusted mean) and circadian amplitude of ascorbic acid has not been studied in healthy Indians. The present study aims to fill this gap by quantifying serum ascorbic acid at different time points of the 24-h cycle and by assessing any effect of gender, age, diet and smoking status on the MESOR and circadian amplitude of ascorbic acid in healthy Indians.

Materials and methods

One hundred sixty-two clinically healthy Indians (103 males and 59 females, 7–75 years of age) volunteered for this study. 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. Volunteers with a body mass index (BMI) less than 18.5 or more than 25.0 kg/m^2 were also excluded.

All subjects followed a 24-h 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. At 25.50°, Lucknow is located just north of the Tropic of Cancer. There is seasonality in this part of the country and the average temperature ranges from 10 °C to 45 °C. 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. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

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 abstained 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 (7–20 y), B (21–40 y), C (41–60 y), and D (61–75 y), consisting of 42, 60, 35, and 25 subjects, respectively. The 7–20-year 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 h for 24 h (4 samples) around 06:00, 12:00, 18:00 and 24:00. Serum was separated and ascorbic acid was measured spectrophotometrically (Natelson, 1971). Other biochemical variables of these volunteers have recently been published (Singh et al., 2016a,b).

Data from each subject were evaluated by conventional statistical analyses and by single and population mean cosinor procedures. Estimates of the MESOR (Midline Estimating Statistic Of Rhythm), a rhythm-adjusted mean, 24-h amplitude, a measure of half the predictable extent of daily change, and 24-h acrophase, a measure of the timing of overall high values recurring each day, were thus obtained (Cornelissen, 2014). Circadian rhythm characteristics were compared among the four age groups by parameter tests (Bingham et al., 1982). Multiple regression and multiple ANOVA, testing equality of group means, were used to examine any effect of gender, age, diet (vegetarian vs. omnivore), and smoking status on the MESOR and/or 24-h amplitude of ascorbic acid. The single and population mean cosinor analyses and parameter tests were performed using software developed at the

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