Effect of Gender, Age, Diet and Smoking Status on the Circadian Rhythm of Serum Uric Acid of Healthy Indians of Different Age Groups

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Abstract The circadian rhythm of uric acid concentration was studied under near-normal tropical conditions in 162 healthy volunteers (103 males and 59 females; 7 to 75 year). They were mostly medical students, staff members and members of their families. They were classified into 4 age groups: A (7–20 y; N = 42), B (21–40 y; N = 60), C (41–60 y; N = 35) and D (61–75 y; N = 25). They followed a diurnal activity from about 06:00 to about 22:00 and nocturnal rest. Blood samples were collected from each subject every 6 for 24 h (4 samples). Serum uric acid was measured spectrophotometrically. Data from each subject were analyzed by cosinor rhythmometry. 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. A marked circadian variation was found in uric acid concentration in healthy Indians of all age groups. Furthermore, both the MESOR and circadian amplitude underwent changes with advancing age. In addition to effects of gender and age, diet and smoking were also found to affect the MESOR of circulating uric acid concentration in healthy Indians residing in northern India. The

present observations confirmed a definite rhythm in uric acid concentrations with significant effect of gender, age, diet, and smoking status on uric acid concentration in clinical health. Mapping the circadian rhythm of serum uric acid is needed to explore their role in different pathophysiological conditions.

Keywords Circadian rhythm · Age · Diet · Gender · Smoking · Uric acid · Epidemiology · Healthy population

Introduction

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, hypoxanthine and guanine, respectively, 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 [1, 2].

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 humans, without any physiological value. However, this ubiquitous compound has proven to be a selective antioxidant, capable especially of reaction with hydroxyl radicals

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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; 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 the 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 [3]. Both uric acid and ascorbic acid are strong reducing agents (electron donors) and potent antioxidants. In humans, over half the antioxidant capacity of blood plasma comes from the hydrogen urate ion [4].

Most serum uric acid is freely filtered in kidney glomeruli, and approximately 90% of filtered uric acid is reabsorbed, implying that it has a considerable physiological role [5]. In humans, over half the antioxidant capacity of blood plasma comes from uric acid [5, 6]. Uric acid is a strong reactive oxygen species (ROS) and peroxynitrite scavenger and antioxidant [7, 8]. High concentrations of uric acid are readily detected in the cytosol of normal human and mammalian cells, especially in the liver [9], vascular endothelial cells, and in human nasal secretions, where it serves as an antioxidant [10, 11]. Urinary uric acid exhibits a circadian rhythm in healthy subjects and in stone formers, reaching a maximum between 12:00 and 18:00 and a minimum between 00:00 and 06:00 [12]. A similar rhythm has also been reported by Lang et al. [13] and by Schneeberger et al. [14], although with a different peak. Physiologic variability can be made into a powerful source of new information with clinical applications [15]. Marked circadian variations in serum uric acid and ascorbic acid concentration were recently reported in healthy volunteers and in renal stone formers [16].

To our knowledge, chronomics of uric acid concentration in healthy volunteers of different age groups and their correlation, if any, with the aging process has not been reported. Moreover, the effect of gender, age, diet, and smoking status on the MESOR (rhythm-adjusted mean) and circadian amplitude of uric acid has not been studied in healthy Indians. The present study aims to fill this gap by quantifying serum uric 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 uric acid in healthy Indians.

Materials and Methods

One hundred sixty-two healthy Indians (103 males and 59 females, 7–75 years of age) volunteered for this study. They 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° 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. 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- to 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 uric was measured spectrophotometrically [17]. Other biochemical variables of these volunteers have recently been published separately [18, 19].

Data from each subject were evaluated by conventional statistical analyses, and by the single and population-mean-cosinor procedures to obtain 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) [20–23]. Circadian rhythm characteristics were compared among the 4 age groups by parameter tests [24]. 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. Multiple regression analyses were carried out in Excel (Office 2007). Multiple-factor ANOVAs and Tukey's multiple comparisons of means were performed in R. A *P* value of 0.05 or less was considered to indicate statistical significance.

Result

A statistically significant circadian rhythm in uric acid concentration was documented by population-mean cosinor analysis in healthy Indians of all age groups. On the average, the MESOR varied between 4.26 mg/dl (in the older age group) and 5.23 mg/dl (in young adults). During the 24-h day, uric acid varied by 6.8 to 12.0% around the MESOR, reaching overall highest values in early afternoon (younger age groups) or early morning (older age groups), Table 1.

Circadian rhythm characteristics (MESOR, 24-h amplitude and acrophase) were found to change as a function of age, as evidenced from parameter tests, Table 1. Using age as a continuous variable, these changes can also be assessed by linear regression, as summarized in Table 2. Results

are also visualized in Figs. 1 and 2. Based on a second-order polynomial model, the MESOR is estimated to reach a maximum around 35 and 39 years of age in women and men, respectively, Fig. 1. The circadian amplitude, however, decreases linearly with age, Fig. 2. The circadian acrophase also undergoes marked changes as a function of age. In the youngest age group, the acrophase occurs around 14:15. It progressively advances to peak around 12:00, 07:15, and even 03:00 in progressively older age groups. The phase of the circadian rhythm is almost reversed between the oldest and the youngest age groups.

Tables 3 and 4 summarize results from the multiple-factor ANOVA applied to the MESOR and 24-h amplitude of uric acid. From Table 3, it can be seen that all four factors (gender, age, diet and smoking status) affect the MESOR of uric acid with statistical significance, with only a small interaction between gender and age (*P* = 0.024). Effects on the circadian amplitude are less pronounced: except for changes as a function of age reported above, only diet has an effect of borderline statistical significance (*P* = 0.068). Some slight interactions among the four factors that do not reach statistical significance are also present.

Results from Tukey's multiple comparisons of means are also illustrated in Figs. 2 and 3 to visualize interactions between two of the four factors considered separately. It

Table 1 Age effects on circadian rhythm characteristics of uric acid in healthy Indians

Age group	k	MESOR	SE	24 h Amplitude	SE	24 h Acrophase	(95% CI)	<i>P</i> value ($H_0: A = 0$)
<i>Population-mean cosinor results</i>								
A	42	4.46	0.104	0.305	0.111	− 213° (14:15)	(− 176°, − 261°)	0.029
B	60	5.23	0.081	0.498	0.063	− 182° (12:00)	(− 166°, − 198°)	< 0.001
C	35	4.92	0.084	0.591	0.060	− 108° (07:15)	(− 89°, − 124°)	< 0.001
D	25	4.26	0.101	0.424	0.093	− 42° (03:00)	(− 14°, − 69°)	0.001
Comparison	MESOR		24 h Amplitude (A)		24 h Acrophase (φ)		(A, φ)	
	F	(P)	F	(P)	F (P)		F	(P)
<i>Parameter tests</i>								
A, B, C, D	22.40	(< 0.001)	8.08	(< 0.001)			13.55	(< 0.001)
A, B	34.99	(< 0.001)	2.55	(0.114)	2.75 (0.101)		2.82	(0.062)
B, C	6.07	(0.016)	4.83	(0.031)	43.20 (< 0.001)		19.31	(< 0.001)
C, D	26.68	(< 0.001)	4.78	(0.033)	13.41 (< 0.001)		9.38	(< 0.001)
A, D	1.70	(0.197)	15.47	(< 0.001)			10.04	(< 0.001)

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

Parameter tests (equality of MESOR, A, φ, considered separately, and of (A, φ) considered jointly among populations being considered); test of equality of acrophases between two populations cannot be performed when they differ by more than 90°

Table 2 Regression with age of circadian characteristics of uric acid

Gender	R ²	P	A ₀ ± SE	A ₁ ± SE (P)	A ₂ ± SE (P)
<i>MESOR</i>					
Males	0.480	< 0.001	3.23 ± 0.16	0.089 ± 0.010 (< 0.001)	− 0.0011 ± 0.0001 (< 0.001)
Females	0.450	< 0.001	4.20 ± 0.27	0.089 ± 0.016 (< 0.001)	− 0.0013 ± 0.0002 (< 0.001)
<i>24-h Amplitude</i>					
Males	0.036	0.053	0.920 ± 0.053	− 0.0026 ± 0.0013 (0.053)	–
Females	0.091	0.021	1.023 ± 0.066	− 0.0037 ± 0.0016 (0.021)	–

R²: coefficient of determination (proportion of overall variance accounted for by fitted model)

P: P value (model)

a₀, a₁, a₂: regression coefficients, listed with their standard error (SE) and P values testing the null hypothesis H₀: a₁ = 0 (where a₁ is the slope) or a₂ = 0 (where a₂ is the curvature)

Fig. 1 Changes with age in the MESOR of uric acid are nonlinear: a maximum is reached around 35 (women) or 39 (men) years of age, based on a second-order polynomial fitted to the individual MESOR estimates. Note that women have higher concentrations of uric acid as compared to men

Fig. 2 Steady decline in the circadian amplitude of uric acid as a function of age in both males and females

can be seen that females have a higher MESOR than males ($P < 0.001$), Figs. 1 and 3. A vegetarian diet is also found to be associated with a lower MESOR ($P < 0.001$), Fig. 3. Smoking is found to be associated with a slight increase in MESOR ($P = 0.033$), Fig. 4. Whereas the effects of gender and diet remain consistent in all age groups (Figs. 5

Table 3 Multiple-way ANOVA testing effects of gender, age, diet and smoking status

Factor	ndf	MESOR		24-h Amplitude	
		F	P	F	P
Gender (G)	1	130.081	< 0.001	2.161	0.144
Diet (D)	1	100.265	< 0.001	3.376	0.068
Smoking (S)	1	4.645	0.033	1.552	0.215
Age group (A)	3	51.838	< 0.001	5.387	0.002
G × D	1	< 0.001	0.992	0.380	0.539
G × S	1	0.018	0.893	1.057	0.306
D × S	1	0.035	0.852	0.129	0.720
G × A	3	3.245	0.024	0.899	0.444
D × A	3	1.772	0.156	0.035	0.991
S × A	3	1.552	0.204	2.290	0.081
G × D × S	1	0.501	0.480	3.752	0.055
G × D × A	3	2.360	0.075	1.365	0.256
G × S × A	3	0.848	0.470	2.452	0.066
D × S × A	3	0.676	0.568	1.614	0.189
G × D × S × A	3	0.358	0.784	0.172	0.915
Residuals	130				

ndf: number of degrees of freedom; F: F value from Fischer's test; P: corresponding P value testing equality of group means

and 6), this is not the case for smoking status: a slightly elevated MESOR of uric acid in smokers versus non-smokers overall is not observed in all four age groups (Fig. 7). The circadian amplitude of uric acid is also slightly influenced by diet ($P = 0.068$).

Discussion

A marked circadian variation was recorded in serum uric acid concentration in healthy Indians of different age groups. The MESOR increased significantly until about

Table 4 Effects of gender, age, diet and smoking status on circadian characteristics of uric acid

Model	MESOR		24-h Amplitude	
	Coefficient	<i>P</i> value	Coefficient	<i>P</i> value
Intercept	5.355	–	0.930	–
Gender	– 0.853	< 0.001	– 0.022	0.886
Diet	– 0.726	0.002	0.188	0.194
Smoking	0.130	0.692	0.090	0.658
Age group B	0.835	0.005	– 0.233	0.195
Age group C	0.145	0.659	0.025	0.902
Age group D	– 0.655	0.048	– 0.095	0.641
Gender × Diet	0.213	0.473	– 0.281	0.128
Gender × Smoking	0.278	0.538	0.292	0.298
Diet × Smoking	– 0.789	0.130	– 0.558	0.085
Gender × Age(B)	– 0.100	0.781	0.168	0.450
Gender × Age(C)	0.496	0.227	– 0.036	0.888
Gender × Age(D)	0.737	0.084	– 0.180	0.494
Diet × Age(B)	0.433	0.264	– 0.378	0.117
Diet × Age(C)	0.509	0.207	– 0.263	0.293
Diet × Age(D)	0.721	0.107	– 0.358	0.196
Smoking × Age(B)	– 0.272	0.510	0.218	0.394
Smoking × Age(C)	0.238	0.468	– 0.153	0.596
Smoking × Age(D)	– 0.230	0.647	0.105	0.736
G × D × S	0.707	0.271	0.308	0.439
G × D × Age(B)	– 0.364	0.433	0.383	0.184
G × D × Age(C)	– 0.450	0.368	0.279	0.367
G × D × Age(D)	– 1.029	0.065	0.371	0.281
G × S × Age(B)	– 0.362	0.514	– 0.630	0.066
G × S × Age(C)	– 0.567	0.351	– 0.282	0.453
G × S × Age(D)	– 0.242	0.742	– 0.440	0.334
G × S × Age(B)	1.019	0.131	0.693	0.098
G × S × Age(C)	0.291	0.662	0.392	0.342
G × S × Age(D)	0.672	0.350	0.454	0.308
G × D × S × Age(B)	– 0.785	0.333	– 0.279	0.578
G × D × S × Age(C)	– 0.236	0.792	– 0.051	0.927
G × D × S × Age(D)	– 0.487	0.609	0.033	0.955

G gender, *D* diet, *S* smoking, Age groups: A (7–20 y), B (21–40 y), C (41–60 y), D (61–75 y)

Gender: F = 0, M = 1; Diet: Omnivores = 0, Vegetarians = 1; Smoking status: Non-smokers = 0, Smokers = 1; Age groups: A = 0, B = 1, C = 2, D = 3

37.5 years of age in both men and women. The circadian amplitude also started to increase around 42 years of age, whereas the circadian acrophase advanced steadily throughout the lifespan, occurring in the oldest age group almost 11 h earlier than in the youngest age group. Such changes with age in the circadian amplitude and acrophase of uric acid are also found in many other variables and are thought to reflect the aging process [25].

The MESOR of serum uric acid reached a maximum around 35 and 39 years of age in healthy men and women, respectively. The circadian amplitude, however, decreased linearly with age. Low plasma uric acid concentrations,

leading to a decrease in antioxidant molecules have been reported 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 concentrations. By contrast, gout patients who have high concentrations of uric acid almost never present with MS disease [26]. Several reports documented an association of low uric acid serum concentrations with MS disease [27–29]. However, the rhythmic nature of serum urate has not been taken into consideration in associated pathophysiological conditions. Herein, we have demonstrated the chronomics of serum urate in healthy

Fig. 3 Interactions between gender and diet by multiple-way analyses of variance indicate that uric acid concentrations are lower in vegetarians than in omnivores and that they are lower in males than in females

Fig. 5 Interactions between gender and age by multiple-way analyses of variance indicate that uric acid concentrations are higher in females than in males, irrespective of age

Fig. 4 Interactions between gender and smoking status by multiple-way analyses of variance indicate that uric acid concentrations are higher in smokers than in non-smokers and that they are higher in females than in males

population of different age groups for better interpretation in disease conditions.

Urate crystals are deposited principally in connective tissues of the joints, tendons, kidneys, and rarely in heart valves and pericardium, and readily interact with serum

Fig. 6 Interactions between diet and age by multiple-way analyses of variance indicate that uric acid concentrations are lower in vegetarians than in omnivores, irrespective of age

proteins [30]. Increased uric acid production, impaired renal uric acid excretion, or a combination of both lead to hyperuricemia [31].

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

Fig. 7 Interactions between smoking status and age by multiple-way analyses of variance indicate that the overall slightly lower uric acid concentrations in non-smokers as compared to smokers is not observed in all age groups

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. Circadian variation in serum and urinary urate has been reported in healthy subjects and renal stone formers [12, 16]. 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 the development and progress of gout and metabolic syndrome appears to be well-established. The pivotal role of uric acid in the 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 the role uric acid plays in resistance to infectious, neurological and autoimmune diseases.

Due to limited resources and facilities, blood sampling could only be performed once every 6 h for 24 h (4 samples). To detect a circadian rhythm on an individual basis, it would have been desirable to collect data at least every 3 or 4 h for 24 h. nevertheless, even with the sparse sampling, a circadian rhythm could invariably be demonstrated on a group basis, Table 1.

Hyperuricemia and gout are commonly diagnosed in subjects with abnormal purine metabolism. Hyperuricemia

is the earliest stage of gout, which is the most common cause of inflammatory arthritis in men over 40 years of age and in women older than 60 years. The effect of vitamin C supplementation on the serum concentration of uric acid was investigated in patients suffering from either hyperuricemia or gout disease [32]. Serum creatinine concentration and glomerular filtration rate were measured before and after vitamin C supplementation to examine whether this treatment improved the functionality of the renal system of such patients. Thirty male and female patients were divided into two groups of 15 patients each, based on their disease condition (hyperuricemia or gout). Patients were given chewable tablets of 500 mg vitamin C daily for two consecutive months. At the end of treatment, the uric acid concentration decreased ($P < 0.05$) in the blood of the hyperuricemic patients, but not in patients with gout. Additionally, no statistically significant effect was found on serum creatinine or glomerular filtration rate in either group.

Uric acid is synthesized mainly in the liver, intestines, and the vascular endothelium, as the end-product of an exogenous pool of purines, and endogenously from damaged, dying and dead cells, whereby nucleic acids, adenine and guanine, are degraded into uric acid. Mentioning uric acid generates dread because it is the established etiological agent of severe, acute and chronic inflammatory arthritis, gout, and it is implicated in the initiation and progress of the metabolic syndrome. Yet, uric acid is the predominant anti-oxidant molecule in plasma and is necessary and sufficient for induction of type 2 immune responses. These properties may account for its protective potential in neurological and infectious diseases, notably schistosomiasis. The pivotal protective potential of uric acid against blood-borne pathogens and neurological and autoimmune diseases is yet to be established [33]. In summary, a definite circadian rhythm in uric acid concentrations is here demonstrated, with statistically significant effects of age, diet, and smoking status in clinically healthy males and females. Circadian rhythms in purine metabolism and their degradative pathways in the aging process may account for some of the changes observed in this investigation. Mapping the circadian rhythm of serum uric acid is needed to explore its role in different pathophysiological conditions.

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Compliance with Ethical Standards

Conflict of interest None.

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