HUMAN URINARY BIORHYTHM IN FLUORIDE METABOLISM ANALYZED BY THE COSINOR METHOD

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SUMMARY: Fluoride (F) metabolism in eight healthy men was studied by urinary excretion patterns under fasting and feeding conditions. The subjects in the fasting experiment had no food for 40 hr and drank water *ad libitum*, while those in the feeding experiment ate space food for 4 days. Urine samples were taken at 2-hr intervals during waking hours for determination of F, Na, K, Cl, Ca, Mg, P, and creatinine (Cre). Results were analyzed for biorhythm by the cosinor computation method. Under fasting conditions, no biorhythm was apparent in these components. Under feeding conditions, three rhythm groups were identified: Group 1: Na, K, and Cl; Group 2: Ca, Mg, P, and Cre; and Group 3: F. These results indicate marked differences in metabolic behavior between F and the other urinary components under study.

Keywords: Biorhythm analysis, Cosinor method, Fluoride metabolism, Urinary biorhythm, Urinary minerals.

INTRODUCTION

To study the metabolism of ingested fluoride (F) in the body, it is possible to use the radioactive ^{18}F isotope.[1-3] However, because of the short 110-min half-life of ^{18}F, it is difficult to use it to trace the fate of F in the body. Another possible way of studying F metabolism in the body is through an indirect approach involving analysis of F intake (input) and urinary excretion (output), and comparing the results with those of several substances whose metabolism is better known. This indirect approach was employed in this investigation. The use of this method, however, requires a strict control of all alimentary inputs, not just fluoride, since urine, the output, is formed continuously without appreciable interfering factors that may cause fluctuations or noises. It is possible, therefore, to introduce time into the system and to assess the rhythmic property of urinary F excretion by time-series analysis.[4,5] With regard to the 'input' employed in this study, the signals related to food intake are clearly influenced by different factors under normal feeding conditions. To minimize the effect of these factors or noises, we employed two conditions: (1) complete fasting, except for water; and (2) feeding space food of uniform composition.

MATERIALS AND METHODS

Fasting Experiment: The subjects selected for this experiment were three healthy males, aged 38-39. They were told about the nature of the experiment and took part in it voluntarily by submitting a written agreement. Five

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sessions were conducted, each lasting 40 hr. The day before each session, the subjects were fed dinner at 7 p.m. and instructed to skip the next four meals: breakfast, lunch, dinner, and breakfast. In each of the sessions, the subjects were housed together with the investigator to facilitate observations. The subjects were instructed to go to bed at 11 p.m. and rise at 7 a.m. and carry out light work from 9 a.m. to 5 p.m.

During the 40-hr session, each subject drank about 1.4 L of drinking water (F content: <0.02 ppm) daily, consumed *ad libitum.* In each of the sessions, urine samples were collected 9 times, except at night, at 2-hr intervals beginning at 7 a.m.

Feeding experiment: The subjects selected for this experiment were five healthy young adult males, aged 19. As with the fasting experiment, the subjects were briefed on the nature of the experiment and participated in it with a written consent. They were fed space food,[6] consisting of 170 g of stew and 40 g of crackers (Freeze Dry Foods Co., Oregon, USA), three times daily, at 8:30 a.m., 12:30 p.m., and 6:30 p.m., respectively, for 4 consecutive days. The amounts of stew and crackers given were normally well tolerated even by a subject consuming the space food for the first time. To facilitate ingestion the freeze-dried stew was soaked in hot water. Total water consumption per day, including the water used for soaking the stew, was approximately 1.4 L. The subjects and the investigator stayed in the same house, carrying out their routine activities. During the 4-day sessions, urine was collected every 2 hr except at night, and after measuring the volume, a portion of it was placed in a polyethylene bottle and kept cold until analysis.

Fluoride analysis: Fluoride concentrations of the drinking water and urine samples were determined with the F ion specific electrode.[7] Analysis of F in the space food was carried out by the method of Itai.[8]

Analysis of urinary electrolytes and creatinine (Cre): The levels of urinary electrolytes and creatinine were determined by published conventional methods.[9-14] Na and K were analyzed by flame ionization spectrophotometry[9] (Japan Flame Spectrophotometer III), while Cl was determined by titrimetry[10] (Corning Chloride Meter). Ca and Mg were determined spectrophotometrically[11-12] by following color development with Calcium Test Wako and Magnesium B Test Wako. The absorbance of the resulting sample solution was measured at 570 and 660 nm, respectively, while P was determined by the Fiske-Subbarow method.[13] Creatinine was determined by the Jaffe method.[14]

Analysis of biorhythm by the cosinor method: To study the circadian rhythm of the concentrations of urinary components when only small amounts of data are available, the quadruple moving average method of time-series analysis is used.[15]

In this study, the cosinor method was employed to analyze the circadian rhythm[16-19] of urinary F excretion. The cosinor method is based on a mathematical computation by the least squares method of a series of data obtained from analysis of a component at certain intervals.[16-19] It gives rise to a curve exhibiting the periodicity of that component under study, such as urinary excretion of F. Data for urinary excretion of F were obtained at an interval of 2 hr, instead of hourly, and the results were analyzed by the cosinor method, yielding curves which serve to project the periodicity of F metabolism in the body.

A computer program made available to us was used in the analysis. For this purpose, the level of each of the substances obtained from the chemical analysis was substituted into the equation below:

$$y = M + A \cos(\omega t + \varnothing),$$

where, M = average daily output; A = amplitude, both in the same units as y; ω = angular frequency; t = time (hr), and Ø = computative acrophase. The values for M, A, and Ø were obtained by calculation. The cosinor method is characterized by its ability to provide a quantitative analysis of those factors affecting periodical changes and phase deviations.

RESULTS AND DISCUSSION

Circadian rhythm of urinary F excretion assessed by the cosinor method: Results of circadian rhythm analysis by the cosinor method showed no large fluctuations (amplitude = 0.014) in the levels of excreted F during fasting (Figure 1). As with the quadruple moving average method,[15] the outputs were higher around noon and they peaked at 12:22 (Table).

Table. Urinary excretion of F and various components assessed by the cosinor method. Data are shown as the peak clock time or peak phase.

Experiment	Fasting		Feeding	
Component	Concentration	Quantity	Concentration	Quantity
UrV	-	11:49	-	11:47
F	ND*	12:22	0:34	12:08
Ca	2:11	4:52	2:24	10:53
Mg	1:41	5:04	0:56	13:19
P	0:28	12:18	1:16	15:32
Cre	0:16	14:40	0:55	10:35
Na	9:47	11:05	13:40	12:27
Cl	11:41	11:51	13:10	12:20
K	12:32	11:20	9:28	11:55

*ND: Not determined

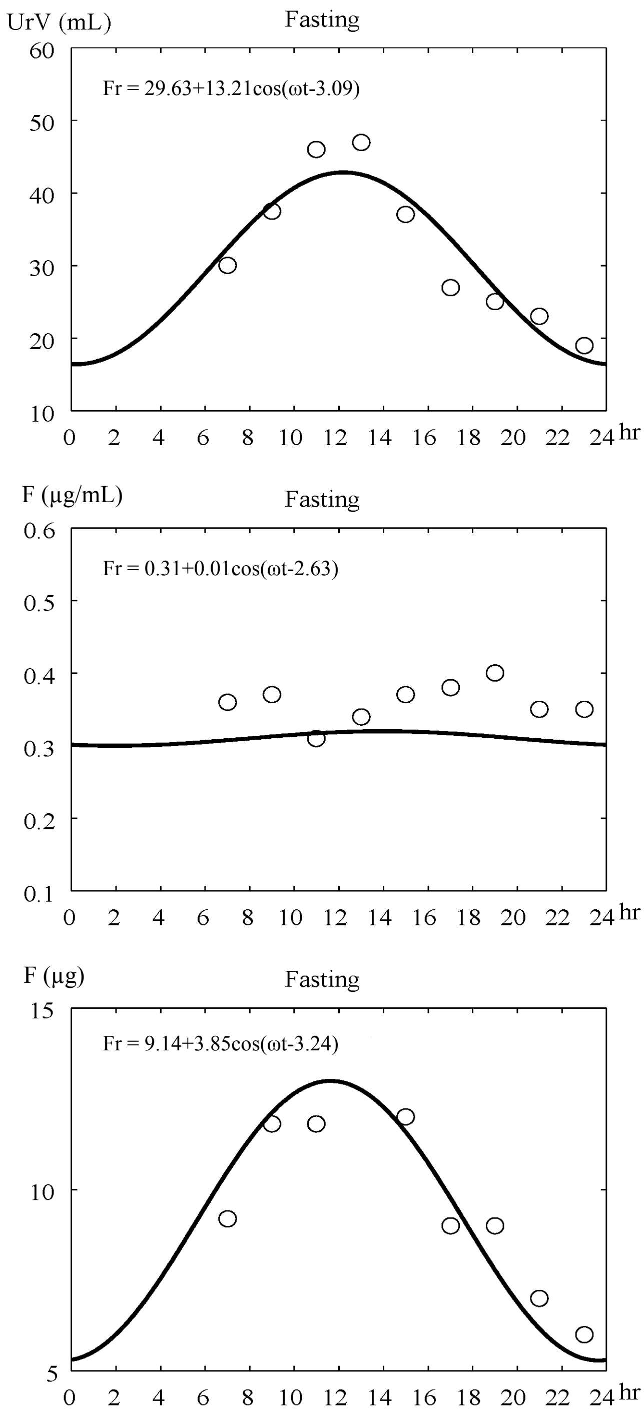

Figure 1. Circadian rhythm of urinary fluoride excretion during fasting determined by the cosinor method.

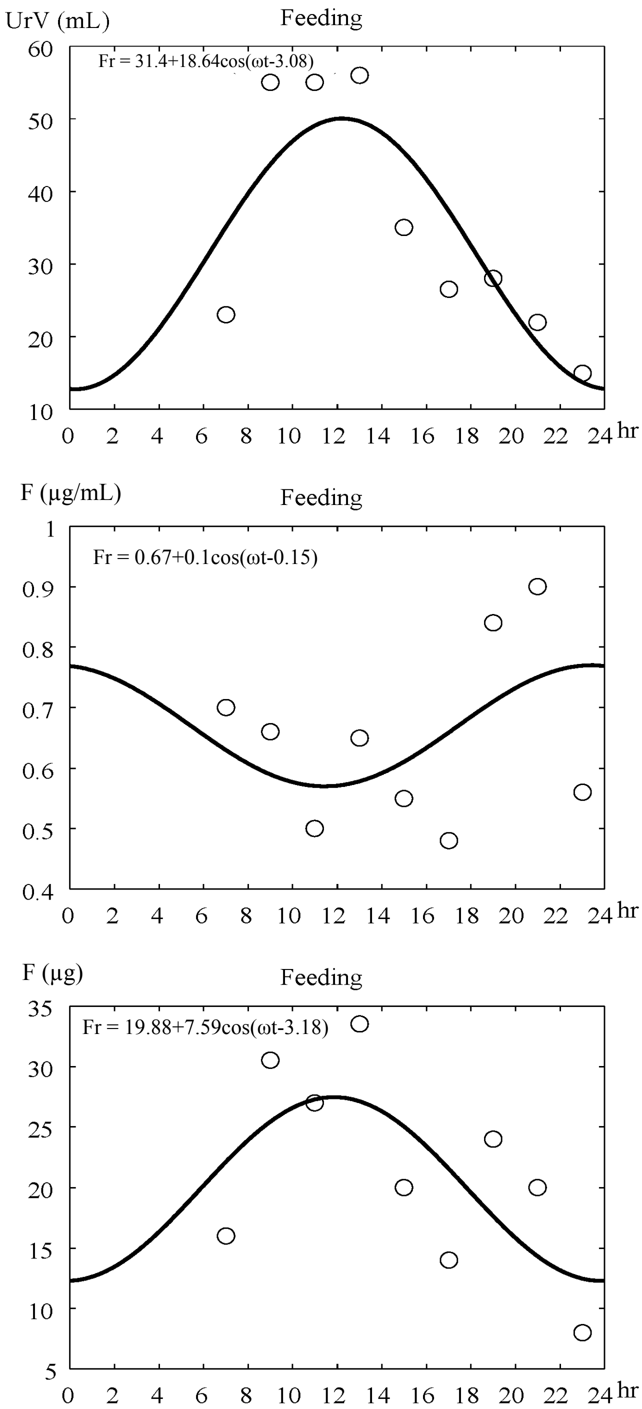

Figure 2. Circadian rhythm of urinary fluoride excretion during feeding determined by the cosinor method.

Under feeding conditions, the concentrations of urinary F yielded a gentle curve with a peak at 0:34, while the amplitude was 0.100 (Figure 2). With the quadruple moving average method, a transient peak occurred at night, while with the cosinor method it occurred after midnight. The quantity of F excretion as a whole showed a peak at about noon (12:08) (Figure 2), as with the quadruple moving average method.

Circadian rhythm of several urinary components and volume of urine: During fasting the concentrations of urinary Cre, Ca, Mg, and P were low at about noon. Their concentrations then increased, and individual peaks occurred at 0:16, 2:11, 1:41, and 0:28, respectively. In contrast, the concentrations of Na, K, and Cl were high around noon, with peaks at 9:47, 12:32, and 11:41, respectively (Table). Also during fasting, urinary volume (UrV), and the quantities of F, Na, K, Cl, and P each showed a curve, peaking at about noon. Their peaks occurred at 11:49, 12:22, 11:05, 11:20, 11:51, and 12:18, respectively. The outputs of both Ca and Mg were high in the morning, and their peaks appeared at 4:52 and 5:04, respectively, followed by declines. On the other hand, the output of Cre was high in the afternoon, peaking at 14:40.

During feeding, the urinary F concentrations determined by the quadruple moving average method[7] showed a transient peak at night. With the cosinor method, a gradually ascending curve resulted past midnight. The concentrations of Cre, Ca, Mg, and P were low late in the afternoon but then increased. They showed peaks early in the morning, at 0:55, 2:24, 0:56, and 1:16, respectively. In contrast, Na, K, and Cl peaked at 13:40, 9:28, and 13:10, respectively. Both K and Ca showed two peaks with the quadruple moving average method, but the cosinor method gave a smooth curve. The amplitude for Ca was 0.881.

Analysis by the cosinor method revealed that under feeding conditions, the time peak phases for urinary volume and the quantities of urinary excretion for Na, K, and Cl were around noon. For Na, K, and Cl, the time peak phase was at 12:27, 11:55, and 12:20, respectively. The outputs for Cre and Ca were found to be higher before noon, compared to other times, and they manifested a time peak phase at 10:35, and 10:53, respectively. The values were lower thereafter. The output for both Mg and P was higher in the afternoon, peaking at 13:19, and 15:32, respectively. The amplitudes were 0.061 and 0.044, respectively.

In conclusion, use of the cosinor method has provided accurate time peak phases for F and various urinary components (Table). Furthermore, results from the analysis suggested that, based on their output characteristics, the urinary components studied may be divided into three groups - group 1: Na, K, and Cl; group 2: Cre, Ca, Mg, and P; and group 3: F. This work has shown that the fate of ingested F in the body is similar to that of ingested Ca.

In addition, our results indicated differences in behavior between F and several other substances in the body. As already noted, the output under feeding conditions for UrV, Na, K, and Cl peaked at 11:47, 12:27, 11:55, and 12:20, respectively. Urine formation has been shown to be dependent on the osmosis of Na, K, and Cl.[20] The results obtained from this study have clearly shown that the movement of body water is also an important factor affecting such activity. Similarly, several substances besides Ca also have an influence on the fate of F in the body.

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