CIRCADIAN RHYTHMS OF URINARY SATURATION LEVELS OF CALCIUM OXALATE AND CALCIUM PHOSPHATE IN NORMAL MALE INDIVIDUALS

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The circadian rhythms of the calcium oxalate, calcium phosphate, and brushite saturation levels as estimated by the AP(CaOx), AP(CaP), and AP(Bru) indices (Tiselius), respectively, were studied in 5 healthy males on three different occasions. These indices were calculated from the data of urinary specimens collected in 2.5-hour fractions except during sleep. The calcium oxalate and brushite saturation levels peaked between 5:30 and 8:00 am, and these were significantly higher than in the other periods. The calcium phosphate saturation level had two peaks that occurred between 8:00 and 10:30 am and between 1:00 and 6:00 pm. The ion-activity products of octacalcium phosphate and hydroxyapatite, calculated from the AP(CaP) index, exhibited a pattern similar to the AP(CaP) values, indicating a high risk of crystallization for both substances at around the same peak periods.

In conclusion, early morning was found to be the high risk period for calcium oxalate and brushite crystallization, while two high risk periods for octacalcium phosphate and hydroxyapatite were detected between 8:00 and 10:30 am and between 1:00 and 6:00 pm.

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Key words: Circadian rhythm, Calcium oxalate, Calcium phosphate, Supersaturation, Urinary calculi

INTRODUCTION

The urinary concentrations of lithogenic and inhibitory substances have a marked role in the development of urolithiasis. Most researchers agree that 24-hour urine collection data as well as the urinary concentrations of various lithogenic and inhibitory substances only provide a vague indication of the risk of lithogenesis. These urinary substances have also been shown to express different circadian rhythms[1,2], these different patterns have not been routinely integrated into a single saturation level.

Previously, I used a nomogram developed by Marshall and Robertson[3] to measure the diurnal variation in the calcium oxalate saturation level[4]. This nomogram involved only two parameters (the calcium and oxalate concentrations) and was at that time the most reliable predictive index of the urinary saturation level. As reported previously, late night and early morning are the periods associated with the greatest risk of calcium oxalate stone formation[4]

Since that time, more parameters have been incorporated into assessment of the risk of calcium oxalate crystallization in the urine, such as 5 parameters by Tiselius and 14 by Werness et al. in EQUIL2[5,6]. However, these methods are of limited use because of the inconvenient urine collection period in the former case and because of the incorporation of too many parameters for clinical use in the latter case.

I have modified the former method to make it usable for any collection period, and the circadian rhythms of the calcium oxalate (CaOx), calcium phosphate (CaP), and brushite (Bru) saturation levels were determined using fractional urine collections in normal male volunteers.

MATERIALS AND METHODS

Five healthy male volunteers between 21 and 27 years old and weighing 60.8±4.6 kg

(mean±SD) were studied for at least a 24-hour period after an overnight stay during three different weeks. They had ordinary meals at 7:00 am, noon, and 5:00 pm and a snack at 9:30 pm. They were also allowed to drink a glass of water at 8:00 am, 1:00 pm, 6:00 pm, and 8:30 pm. Urine was collected for 2.5-hour periods except during sleep (from 11:00 pm to 5:30 pm). For each sample, the pH and volume (V: expressed in liters) were measured immediately after voiding. The urinary concentrations of calcium (Ca), magnesium (Mg), and phosphate (P) (mmol) were routinely analyzed as described previously using a Hitachi 705 autoanalyzer[7]. Aliquots of the specimens were frozen and preserved until analysis for the urinary concentrations of oxalate (Ox) and citrate (Cit), which were determined by respective enzymatic methods[8,9]. The AP(CaOx), AP(CaP), and AP(Bru) indices are estimates of the ion activity products of CaOx CaP, and Bru, respectively; they were calculated as follows:[5,10,11]

AP(CaOx) index: $K \times Ca^{0.71} \times Ox/Mg^{0.14}/Cit^{0.10}/V^{1.2}$

AP(CaP) index: $A \times Ca^{1.07} \times P^{0.70} \times (pH-4.5)^{7.0}/Cit^{0.20}/V^{1.31}$

AP(Bru) index: $B \times Ca^{1.07} \times P^{0.82} \times pH^{6.8}/Cit^{0.46}/V^{1.53}$

In these equations, the K, A, and B factors were interpolated from the literature[10,11] as follows (linear regression analysis: $r=0.9959 \sim 0.9899$, $p<0.01$). The K value was 7.171 for the 2.5-hour urine collections and 5.632 for the 6.5-hour collection. The A values were 4.869×10^{-3} for the 2.5-hour urine collection and 3.860×10^{-3} for the 6.5-hour collection, and the B values were 5.125×10^{-7} and 5.643×10^{-7}, respectively. The ion-activity products of octacalcium phosphate (OCP) $\{-\log AP_{OCP}=1/0.021/(AP(CaP)\ index)^{0.025}+0.5\}$ and hydroxyapatite (HAP) $\{-\log AP_{HAP}=1/0.0185/(AP(CaP)\ index)^{0.035}\}$ were also calculated using the formulae of Tiselius[10].

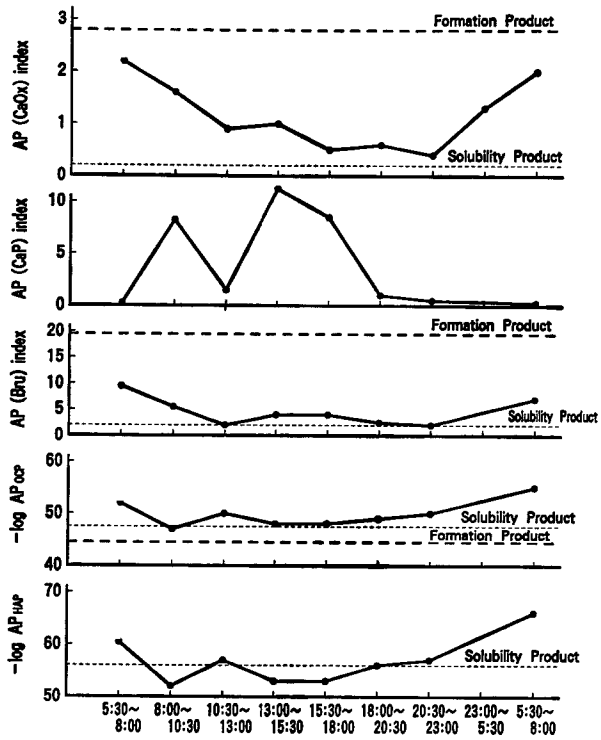
Statistical analysis was performed by the paired t-test for paired samples from each sequential urine fraction.

RESULTS

The peak AP(CaOx) index was observed between 5:30 and 8:00 am, and was significantly higher than the values at other periods. Most of the index values of AP(CaOx) were in the metastable zone (between the solubility and formation products of 0.2 and 2.8, respectively), and no values exceeded the formation product during the observation periods. AP(CaP) had two peaks, which were between 8:00 and 10:30 am and between 1:00 and 6:00 pm. Both peaks were significantly higher than the baseline values obtained between 5:30 and 8:00 am. A similar pattern was obtained for the ion-activity products of OCP and HAP, indicating a high risk of crystallization around the period. With these two indices, a higher value means a decrease in the saturation. The peak $-\log AP_{OCP}$ values remained in the metastable zone ($-\log SP=47.6$) and did not exceed the formation product ($-\log FP=44.6$). The mean fractional $-\log AP_{HAP}$ values were around the value of the solubility product ($-\log SP=55.96$), suggesting that these values remained in the metastable zone. The AP(Bru) peak occurred between 5:30 and 8:00 am, and was higher than most other values with a significant difference in several cases (Fig. 1).

DISCUSSION

The urinary saturation level varies in relation to physical exercise, diet, fluid intake, and other factors. Therefore, it cannot be expressed exactly by the saturation level in a 24-hour urine specimen. Vahlensieck et al.[1] and our group[2,4] both consider that the 24-hour represents only an average profile of the saturation levels over a day. Both groups detected circadian rhythms of lithogenic substances in the urine and found that the excretion of these constituents usually peaked after meal times, particularly in terms of calcium and phosphate, and that the urinary concentrations of these elements were highest early in the morning. The 6 different parameters could not be integrated to produce a



	5:30~8:00	~10:30	~13:00	~15:30	~18:00	~20:30	~23:00	~5:30	5:30~8:00	Mean±SE
AP (CaOx)	2.2±0.3	1.6±0.2^{+}	0.9±0.2**	1.0±0.3**	0.5±0.1**	0.5±0.1**	0.4±0.1**	1.3±0.3*	2.0±0.3	1.2±0.1
AP (CaP)	0.2±0.1	8.2±2.3*	1.5±0.6	11.2±5.6	8.6±2.5*	1.1±0.4^{+}	0.7±0.3		0.2±0.1	4.0±0.9
AP (Bru)	9.5±2.3	5.7±0.8	1.9±0.3*	4.1±1.1	3.8±0.7	2.3±0.6*	2.1±0.6*		7.2±1.5	4.6±0.5
-log AP$_{OCP}$	52.1±0.8	46.7±0.5**	49.8±0.8	47.7±0.8**	47.4±0.7**	49.3±0.5**	50.1±0.7	-	55.1±1.7	49.8±0.4
-log AP$_{HAP}$	60.5±1.4	51.8±0.8**	56.9±1.4	53.4±1.2**	52.9±1.1**	55.9±0.9**	57.2±1.2	-	65.8±3.0	56.8±0.6

$^{+}p<0.10$, $^{*}p<0.05$, $^{**}p<0.01$

Fig. 1. Diurnal variations of the Tiselius saturation index (mean±SE) in normal subjects (n=5×3). The p values (<0.1, +; <0.05, *; <0.01, **) show differences between urine fractions compared with the baseline values (5:30~8:00 am). The AP(CaOx) index values corresponding to the solubility and formation products are 0.2 and 2.8, respectively[13]. The solubility and formation products of octacalcium phosphate are 47.63 and 44.60, while those of hydroxyapatite are 55.96 and unknown, respectively[10]. The AP(Bru) index values corresponding to the solubility and formation products are 1.995 and 19.95, respectively.

single saturation profile. In addition, most urine specimens were in a state of metastable supersaturation, and both the calcium oxalate and calcium phosphate values, frequently exceeded the formation product for a few hours during the daytime[12]. By contrast, our group has suggested that the calcium oxalate saturation level increased both late at night and early in the morning[4]. Ahlstrand et al. also found two intervals of increased calcium oxalate saturation, i.e., between 6:00 and 10:00 am and between 6:00 and 10:00 pm[13].

Sophisticated methods using computer programs have now become available and usable for more accurate determination of

saturation levels, not only in 24-hour urine specimens but also in the fractional urine of shorter collection period. These methods can also determine the saturation levels for several different stone components.

This study showed that early morning (5:30~8:00 am) is the high-risk period for calcium oxalate and brushite crystallization. The risk periods for octacalcium and hydroxyapatite crystallization are between 8:00 and 10:30 am and between 1:00 and 6:00 pm. Each peak index remained in the metastable zone. However, an overshoot beyond the formation product for a transient period can not be ruled out.

The present results support the single evening dose (10:00 pm) of sodium potassium citrate for patients with resurrent CaOx stones proposed by Berg et al. They reported that the high-risk periods for CaOx were late at night and early in the morning[13,14]. Their study of calcium phosphate crystallization was based solely on the AP(CaP) index, but the present findings provide further insight in terms of 3 different calcium phosphate crystallization systems. The current data suggest that there is no benefit of citrate administered three times a day, since dosing about a half hour after each meal may simply augment the effect of food intake (i.e., reduce CaOx saturation and increase CaP saturation), without exerting any significant effect on the urinary saturation level in the critical period for lithogenesis. From the clinical point of view, it is important to lower the urinary saturation level of lithogenic components for the prevention of urinary stones. There may be some patients who need three doses of citrate a day. However, to determine its precise effectiveness, the circadian profile of the urinary saturation level must be determined. Further studies involving the use of alkalinizing agents are also needed.

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和文抄録

蓚酸カルシウムと燐酸カルシウムの尿中飽和度の日内変動

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健常男性5名を対象に3回の異なる時期に，蓚酸カルシウム，燐酸カルシウム，酸性燐酸カルシウムの飽和度をそれぞれ示すAP(CaOx)指数，AP(CaP)指数，AP(Bru)指数（Tiselius）を測定し，その飽和度の日内変動を検討した．就寝時以外2時間30分毎に採尿し，これらの指数を計算した．蓚酸カルシウムと酸性燐酸カルシウムの飽和度は5時～8時で最高値となった．燐酸カルシウム飽和度は二峰性で，8時～10時30分と13時～18時に最高値となった．燐酸オクタカルシウムと水酸化アパタイトのイオン活量積も，AP-(CaP)の値より求めたが，燐酸カルシウムの飽和度と同様なパターンを示した．

以上より，早朝が蓚酸カルシウムと酸性燐酸カルシウムに関しては，結晶化の危険性が高く，燐酸オクタカルシウムと水酸化アパタイトでは8時～10時30分と13時～18時であることが判明した．

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