The diurnal variation in urine acidification differs between normal individuals and uric acid stone formers

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Many biological functions follow circadian rhythms driven by internal and external cues that synchronize and coordinate organ physiology to diurnal changes in the environment and behavior. Urinary acid–base parameters follow diurnal patterns and it is thought these changes are due to periodic surges in gastric acid secretion. Abnormal urine pH is a risk factor for specific types of nephrolithiasis and uric acid stones result from excessively low urine pH. Here we placed 9 healthy volunteers and 10 uric acid stone formers on fixed metabolic diets to study the diurnal pattern of urinary acidification. All showed clear diurnal trends in urinary acidification, but none of the patterns were affected by inhibitors of the gastric proton pump. Uric acid stone formers had similar patterns of change throughout the day but their urine pH was always lower compared to healthy volunteers. Uric acid stone formers excreted more acid (normalized to acid ingestion), with the excess excreted primarily as titratable acid rather than ammonium. Urine base excretion was also lower in uric acid stone formers (normalized to base ingestion), along with lower plasma bicarbonate concentrations during part of the day. Thus, increased net acid presentation to the kidney and the preferential use of buffers, other than ammonium, result in much higher concentrations of undissociated uric acid throughout the day and consequently an increased risk of uric acid stones.

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A property intrinsic to living organisms is the biological circadian clock, which is organized and oscillates at multiple hierarchies at both cellular[1,2] and multicellular levels[3,4] in both the animal and plant kingdoms.[5,6] In mammalian biology, many aspects of behavior and physiology follow cyclic rhythms, which are generally presumed to confer adaptive advantage by coordinating behavior and organ physiology to ambient day-and-night cycles.[4] In the kidney, significant circadian rhythms exist for multiple renal hemodynamic, glomerular, and tubular parameters.[7,8] These renal circadian rhythms are influenced by external cues such as feeding, ambient light, and activity, as well as inherently by intrinsic clocks.[9,10]

In 1845, Henry Bence Jones, who is considered to be the pioneer of urinary chemistry for his studies of urinary light chains, glucose, and cystine in disease states,[11] noted diurnal variation in urine pH (UpH) in normal individuals.[12] Subsequent studies also demonstrated morning alkaline and evening acidic trends of urine although this finding was not always uniformly observed.[13–18] However, the precise circadian profile of urine acidification remains incompletely defined, and the factors responsible for hour-to-hour fluctuations in pH are not known. Gastric acid secretion with the concomitant alkalization of plasma has been proposed to be the origin of postprandial changes in plasma pH and UpH.[19,20] In addition, it is unclear whether renal disorders affect circadian patterns of urinary chemistry and whether such derangements in rhythmic changes in urinary acidification contribute to pathophysiology.

Although the kidney is capable of elaborating urine at an enormously wide range of hydrogen ion concentrations when stressed (pH from <5 to >8; $[H^+]$ from $<10\,nM$ to $>10\,\mu M$), normal day-to-day UpH is poised within a much narrower span in humans somewhere between pH 5.5 and 6.5. Acidification of urine is of critical importance for prevention of calcium phosphate crystallization in the urinary space.[21–23] However, UpH cannot be lowered too

much because of another constraint in higher primates who maintain relatively high plasma and urinary uric acid.[22,24] Urinary acidification to below pH 5.5, although protective against calcium phosphate precipitation, poses a substantial risk of uric acid precipitation.[22] Precipitation of calcium phosphate and uric acid thus set the upper and lower limits of UpH, respectively, and calcium phosphate and uric acid nephrolithiasis in fact represent quintessential clinical disorders of UpH.

In humans with uric acid nephrolithiasis, excessively acidic urine causes titration of urate to the highly insoluble uric acid despite normal or even low total uric acid content in urine. The pathogenesis of low UpH has been ascribed to both increased acid load to the kidney and defective utilization of ammonia in urinary buffering.[25–31] It is not clear whether the unduly acidic urine in uric acid stone formers (UASFs) occurs at specific intervals or persists throughout the day. Current standard clinical practice, clinical investigations, and clinical trials, all use 24-h urine collections to assess risk for uric acid stones and adequacy of response to therapy. We reported that during treatment of uric acid nephrolithiasis with alkali, excessive nocturnal and early-morning urinary acidity can linger despite apparent alkalinization of pooled 24-h urine.[32] This can potentially result in a false sense of security for the clinician that uric acid stone risk is eliminated, but an elevated propensity for uric acid precipitation still persists in the patient during specific periods of the day. Although most patients likely respond to daytime alkali therapy, the possibility remains in some individuals where persistent early-morning aciduria can sustain the elevated stone risk despite alkali therapy.

The study of 24-h urine profiles in UASFs will enhance our understanding of its pathophysiology and the origin of the excessive aciduria. It will also guide us in designing better clinical tests for diagnosis and monitoring of therapy in uric acid nephrolithiasis. We embarked to detail the circadian pattern of urinary acidification parameters in normal volunteers to delineate normal circadian physiology and in UASFs to identify pathophysiological defects. In addition, we tested the longstanding belief that gastric acid secretion contributes to changes in UpH by blocking gastric acid production in the two groups of subjects.

RESULTS

Demographic characteristics

The demographic characteristics of the study population are depicted in Table 1. Ten UASFs and nine healthy volunteers (HVs) participated in the study. Most of the subjects in both groups were male and non-Hispanic Caucasians, which is typical of UASFs. The mean age did not differ significantly between the two groups. The UASFs weighed more and had a higher BMI than the HVs.

Serum and urine chemistry

Table 2 shows the fasting serum chemistry for both groups in each study phase. In both phases, UASFs had a slightly higher serum creatinine level due to higher creatinine production rate, but creatinine production rate per body mass was not different between the two groups. Most importantly, creatinine clearance was not different between the two groups. Serum uric acid was persistently higher in UASFs in both phases of the study. Serum bicarbonate, chloride, and venous pH did not differ between the two groups. UASFs taking placebo demonstrated a very slight but statistically significantly higher serum potassium level than in the proton pump inhibitor (PPI) phase and higher than HVs in both phases.

Urine was collected for 24 h prior to each diurnal study and results are shown in Table 3. In both study phases, UpH in UASFs was significantly lower than in HVs (UASFs vs. HVs in placebo phase: 5.42 ± 0.36 vs. 5.86 ± 0.28; $P = 0.01$; UASFs vs. HVs in PPI phase: 5.32 ± 0.36 vs. 5.93 ± 0.23; $P < 0.001$). Urinary sodium, potassium, sulfate, and phosphate did not differ among the groups, indicating equivalence of dietary intake. In both phases, citrate excretion was numerically lower in UASFs but the difference was not statistically

Table 1 | Patient demographic data

	Healthy volunteers (HVs)	Uric acid stone formers (UASFs)
Gender: M/F	6/3	9/1
Race (white/black)	7/2	9/1
Ethnicity (non-Hispanic/Hispanic)	9/0	9/1
Age (years)	52.8 ± 13.1	57.0 ± 8.2
Weight (kg)	85 ± 19	109 ± 19*
Height (cm)	171 ± 10	172 ± 6
Body mass index (kg/m^2)	28.5 ± 4.3	36.9 ± 6.8*

Abbreviations: F, female; M, male.
*$P < 0.05$, *t*-test.

Table 2 | Fasting serum profile

	Placebo		PPI	
	HV	UASF	HV	UASF
Creatinine (mg/dl)	0.84 ± 0.13	1.06 ± 0.02*	0.88 ± 0.15	1.12 ± 0.21†
(μmol/l)	74 ± 11	94 ± 1.7	78 ± 13	99 ± 18
Creatinine clearance	139 ± 35	131 ± 39	129 ± 35	126 ± 36
(ml/min)	120 ± 20	100 ± 26	112 ± 17	95 ± 23
(ml/min per 1.73 m^2)				
Glucose (mg/dl)	97 ± 10	103 ± 25	96 ± 6	106 ± 26
(mmol/l)	5.4 ± 0.6	5.6 ± 1.4	5.3 ± 0.3	5.0 ± 1.4
Uric acid (mg/dl)	6.0 ± 1.6	8.0 ± 1.5*	6.3 ± 1.6	8.1 ± 1.5†
(μmol/l)	357 ± 95	476 ± 89	375 ± 95	482 ± 89
Sodium (mEq/l)	139 ± 3	138 ± 3	138 ± 2	138 ± 2
Potassium (mEq/l)	4.0 ± 0.3	4.5 ± 0.7*	3.9 ± 0.2	4.0 ± 0.3‡
Chloride (mEq/l)	106 ± 3	107 ± 2	107 ± 2	107 ± 3
Bicarbonate (mEq/l)	27.0 ± 1.3	26.1 ± 3.3	26.7 ± 1.5	25.9 ± 1.1
Venous pH	7.41 ± 0.02	7.40 ± 0.02	7.41 ± 0.01	7.40 ± 0.01

Abbreviations: HV, healthy volunteer; PPI, proton pump inhibitor; UASF, uric acid stone former.
*$P < 0.05$ UASF vs. HV; on placebo.
$^\dagger P < 0.05$ UASF vs. HV; on PPI.
$^\ddagger P < 0.05$ UASF placebo vs. UASF on PPI.
Comparisons made with mixed-model repeated-measures analysis.