Kidney International, Vol. 62 (2002), pp. 971–979

Pathophysiologic basis for normouricosuric uric acid nephrolithiasis

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Pathophysiologic basis for normouricosuric uric acid nephrolithiasis.

Background: Low urinary pH is the commonest and by far the most important factor in uric acid nephrolithiasis but the reason(s) for this defect is (are) unknown. Patients with uric acid nephrolithaisis have normal acid-base parameters according conventional clinical tests.

Methods: We studied steady-state plasma and urinary parameters of acid-base balance in subjects with normouricosuric pure uric acid stones. We also tested the ability of these subjects to excrete ammonium in response to an acute acid load. We compared these parameters in patients with pure uric acid stones to patients with mixed uric acid/calcium oxalate stones, pure calcium stones, and normal volunteers.

Results: Pure uric acid stone formers have a much higher incidence of either diabetes or glucose intolerance. After equilibration to a control diet, patients with uric acid stones have lower urinary pH and they excrete less of their acid as ammonium. This is compensated by higher titratable acidity and hypocitraturia. Despite their low baseline urinary pH, uric acid stone formers further acidify their urine after an acid load because of a severely impaired ammonia excretory response. Their characteristics are significantly different from normal volunteers and pure calcium stone formers. Patients with mixed uric acid/calcium stones exhibit intermediate characteristics.

Conclusion: We propose that certain patients with normouricosuric uric acid nephrolithiasis have a renal acidification disease. The primary defect lies in renal ammonium excretion, which may be linked to the insulin-resistant state. Although net acid excretion is maintained at the expense of increased titratable acidity and to some degree hypocitraturia, the compromise is acid urine pH and may result in uric acid nephrolithiasis.

Uric acid nephrolithiasis can result from different etiologies [1, 2]. Patients can present with uric acid and/or calcium oxalate kidney stones [3]. Biochemically, patients with pure uric acid stones can have normal or high plasma uric acid levels with normal or high urinary uric acid excretion [1–6]. A low urinary pH contributes to the formation of uric acid-containing kidney stones and appears to be the most invariant feature of the syndrome [1, 2, 7]. A low urinary pH <5.5 results in a significant increase in the concentration of the sparingly soluble nondissociated uric acid that precipitates directly to form uric acid stones [8, 9]. Calcium oxalate stones can also develop from uric acid-induced crystallization of calcium salts [9–11].

Low baseline urinary pH levels in uric acid stones formers have been consistently described in uric acid stone formers [12–19], although contradicting results have also been reported [17, 20, 21]. The best explanation for the low urinary pH is low ammonium ion (NH_4^+) concentrations in the urine [12, 14, 15] leaving the free H^+ relatively unbuffered. An implicit, but yet unproven, basis for uric acid nephrolithiasis in this syndrome is that there is an intrinsic defect in urinary NH_4^+ excretion. Some studies showed that the low urinary NH_4^+ in uric acid stone formers is only detectable under special circumstances such as high protein loading [21] or limitation of titratable acid precursors such as phosphate restriction [17]. Urinary NH_4^+ excretion was shown to be blunted following chronic ammonium chloride (acid equivalent) load [12, 14, 21], but ammoniagenic responses similar to control subjects have also been reported using chronic [13, 15, 20] as well as acute acid loading protocols [17]. The reasons for the variability of these findings are not clear but may potentially be related to differences in baseline acid excretion rate and availability of non-ammonia buffers. Caution must be exercised to interpret urinary pH and NH_4^+. Variable intake of acid/alkali load can influence the renal excretion of NH_4^+ as well as urinary pH [22]. In the presence of increased buffering power by NH_3 in response to chronic acid loading, urinary pH is actually much higher in situations of chronic acid loading compared to acute acid loads [23, 24]. A study performed under strictly controlled dietary conditions has not been performed.

The purpose of the present study was to determine whether defective urinary NH_4^+ excretions exist in pa-

Key words: Nephrolithiasis, uric acid, ammonium, urine pH, citrate, insulin resistance.

Received for publication October 19, 2001
and in revised form March 14, 2002
Accepted for publication March 29, 2002

tients with uric acid stones with a given dietary acid load and whether this defect in NH_4^+ excretion and acid urinary pH can be amplified or unmasked after a single acute acid load. We studied 21 patients with "pure" uric acid stones and compared the parameters to those of normal volunteers. We also compared the uric acid stone formers to patients with calcium oxalate stones (who should have a completely different clinical phenotype) as well as patients with mixed uric acid/calcium stones (who may exhibit some features similar to patients with uric acid nephrolithiasis).

METHODS

Experimental subjects

We studied four groups of patients. The normal group was composed of 18 normal volunteers (12 males and six females; mean age, 39 years; range, 23 to 61 years). The three groups of stone-forming patients were identified by records of stone analysis. The uric acid stone-former group consisted of 21 patients with "pure" acid stones (18 males and three females; mean age, 56 years; range, 36 to 77 years). We excluded two patients with pure uric acid stones because their daily urinary uric acid excretion were consistently above 600 mg. Eight patients had "mixed" uric acid/calcium oxalate nephrolithiasis (five males and three females; mean age, 53 years; range, 39 to 62 years). Twenty-three patients with "pure" calcium oxalate nephrolithiasis (19 males and four females; mean age, 48 years; range, 17 to 69 years). None of the patients had daily urinary uric acid exceeding 600 mg.

Patients and normal volunteers with chronic diarrheal illness, renal disease, urinary tract infection, liver disease, cardiovascular disease, abnormal thyroid function tests, or proteinuria were excluded. Subjects who were receiving converting enzyme inhibitors, angiotensin receptor blockers, β blockers, non-steroidal anti-inflammatory agents, lipid-altering drugs, as well as participating in strenuous physical exercise program, were also excluded from the study. The patients with nephrolithiasis were instructed to discontinue all medications for renal stones (thiazide, allopurinol, alkali). No one received drugs that could affect urate metabolism (such as high-dose acetylsalicylate). Institutional Review Board committee approved the study and informed consent was obtained from each of the participating subjects.

Study protocol

The study included an outpatient evaluation and a stabilization phase during which subjects were maintained on a constant frozen metabolic diet with a daily composition of 400 mg calcium, 800 mg phosphorus, 100 mEq sodium, 40 mEq potassium, sufficient fluid (distilled water) to ensure about 2 L of urine, with a fixed acid ash content for 4 days. After 4 days of stabilization, subjects were maintained on same constant metabolic diet at the General Clinical Research Center (GCRC) for 3 days (study days 5 to 7). During the last two days of metabolic diet (study days 6 and 7), 24-hour urine samples were collected under mineral oil and refrigerated for measurements of total volume, pH, creatinine, sodium, potassium, calcium, phosphorus, chloride, uric acid, oxalate, citrate, sulfate, ammonium, titratable acidity, and bicarbonate/carbon dioxide). Fasting venous blood samples were obtained before breakfast on days 7 and 8 for electrolytes, creatinine, urea nitrogen, calcium, phosphorus, uric acid, glucose, cholesterol, and triglycerides. On the evening of day 7, subjects fasted except for 300 mL distilled water at bedtime.

On the morning of day 8, an ammonium chloride (NH_4Cl) loading test was performed. Breakfast was withheld. Urine was collected hourly from 7:00 a.m. to 12:00 noon under mineral oil. At 8:00 a.m., 50 mEq NH_4Cl in gelatin capsules was administered orally with 250 mL water. To ensure adequate urine output, 250 mL water was drunk at 7:00, 8:00, 9:00, 10:00, and 11:00 a.m. Hourly urine samples were analyzed for the following components: total volume, pH, creatinine, ammonium, titratable acidity and bicarbonate. Arterialized venous blood was obtained (venous blood drawn without stasis from an antecubital vein after a 30-minute application of electric warm pack to the forearm) for chemistry and pH and blood gases were obtained at 7:00, 10:00 and 12:00 a.m.

Analytical procedures and calculations

Serum sodium, potassium, chloride, total carbon dioxide, calcium, phosphorus, glucose, triglyceride, cholesterol, blood urea nitrogen, uric acid, and creatinine concentrations were obtained as a part of 24-hour chemistry (GCRC Core Laboratory using Beckman CX9ALX, Fullerton, CA, USA). Urinary calcium (performed in an acidified aliquot to prevent precipitation) was determined by atomic absorption spectrophotometry. Sodium and potassium were measured by flame photometry. Urinary chloride was measured coulometrically by silver precipitation. Uric acid (done in an alkalinized aliquot to prevent precipitation) was analyzed by the uricase method and creatinine by the picric acid method. Urinary phosphorus was measured by a colorimetric technique. Urinary pH was measured with a pH electrode. Urinary citrate was determined enzymatically using reagents from Boehringer-Mannheim Biochemicals (Indianapolis, IN, USA). Urinary ammonium was determined by the glutamate dehydrogenase method. Urinary sulfate was determined by ion chromatography. Urinary titratable acidity was measured directly using automated burette end-point titration system (Radiometer, Copenhagen, Denmark) and was also calculated from concentrations of different ionic species and complexes obtained from urinary stone risk factors using the computer

Table 1. Plasma chemistries

		Stone formers		
	Normal	Uric acid	Mixed	Calcium oxylate
Number	18	21	8	23
Gender *male/female*	12/6	18/3	5/3	19/4
Age *years*	39 ± 11[c]	56 ± 11	53 ± 9	48 ± 13
BMI kg/m^2	29 ± 6[a]	35 ± 7	33 ± 8	34 ± 7
Diabetic %	0	33.3	37.5	0
Glucose Intolerant %	0	23.8	0	8.7
Triglyceride *mg/dL*	100 ± 53[c]	299 ± 223	187 ± 105	229 ± 160
Median	76	215	164	193
Cholesterol *mg/dL*	189 ± 35	210 ± 46	210 ± 49	212 ± 40
Blood urea nitrogen *mg/dL*	11 ± 3[b]	16 ± 7	12 ± 4	13 ± 3
Creatinine *mg/dL*	1.0 ± 0.2	1.1 ± 0.4	1.0 ± 0.2	1.0 ± 0.2
Sodium *mEq/L*	137 ± 2	136 ± 3	139 ± 3	138 ± 3
Potassium *mEq/L*	4.0 ± 0.3	4.1 ± 0.4	3.9 ± 0.3	4.2 ± 0.7
Chloride *mEq/L*	105 ± 3	104 ± 3	105 ± 4	104 ± 3
$H_{CO_3^-}$ *mEq/L*	27 ± 2	26 ± 2	28 ± 2	27 ± 2
P_{CO_2} *mm Hg*	46 ± 4	45 ± 5	47 ± 3	46 ± 4
pH	7.38 ± 0.02	7.37 ± 0.03	7.37 ± 0.05	7.39 ± 0.03
Calcium *mg/dL*	9.2 ± 0.4	9.3 ± 0.5	9.2 ± 0.3	9.3 ± 0.7
Phosphorus *mg/dL*	3.4 ± 0.5	3.3 ± 0.4	3.6 ± 0.8	3.1 ± 0.5
Uric acid *mg/dL*	5.6 ± 2[c]	8.1 ± 1.8	7.7 ± 2.1	6.6 ± 1.5[b]

Data presented as mean ± SD. Significant differences from uric acid stone formers depicted by [a]for $P < 0.02$; [b]for $P < 0.01$; and [c]for $P < 0.001$.

program of Finlayson [25]. The milliequivalents of OH^- required bring the original pH to pH 7.4 yielded titratable acidity (TA). Net acid excretion was calculated as $(NH_4^+ + TA) - (H_{CO_3^-} +$ ionized citrate); all expressed in milliequivalents. Urinary HCO_3^- was calculated from urinary pH and P_{CO_2} and milliequivalents of ionized citrate were calculated from urinary pH and a pKa of $citrate^{2-}/citrate^{3-}$ of 5.6. Arterialized venous blood and urinary pH and blood and urinary carbon dioxide were determined aerobically at 37°C [Radiometer BMS-3 pH electrode (Copenhagen, Denmark) and Servinghaus P_{CO_2} electrode]. The arterialized venous plasma bicarbonate concentration was calculated from blood pH and P_{CO_2} (Henderson-Hasselbach equation).

Statistical analysis

Two sample *t* tests were used to compare pure uric acid stone formers to normal volunteers, mixed uric acid/calcium oxalate stone formers, and pure calcium oxalate stone formers. For skewed data, log transformations were employed prior to analysis. The level of significance was alpha = 0.02 level, using the Bonferroni inequality to adjust for multiple testing. Statistical analysis was performed with SAS 8.0 (SAS Institute, Cary, NC, USA). Results are expressed as mean ± standard deviation. Analysis of covariance models were used to adjust for the potential confounding effects of covariates age and body mass index on group comparisons.

RESULTS

Patient characteristics

Comparisons were made between the uric acid stone formers and normal volunteers, patients with mixed stones, and patients with pure calcium oxalate stones. Characteristics of the study patients are shown in Table 1. Uric acid stone formers were older than normal controls. Ethnic origin (overall distribution was 80% Caucasians, 5.7% African Americans, 8.5% Hispanic, and 5.7% Asian) and gender distribution was not significantly different among the four groups. The mean body mass index (in kg/m^2) was higher in uric acid stone formers than in normal volunteers. The mean body mass index was not different among the stone-forming groups. Age and body mass index were not significant covariates. Analysis of covariance results adjusting for age and body mass index were similar to the unadjusted analysis. Since age and body mass index did not affect the results, only unadjusted analyses are shown. Since the 24-hour urinary creatinine excretion was similar in all groups reflecting similar lean body mass, the difference in body mass index was likely due to differences in body fat. Uric acid stone formers had a much higher incidence of diabetes (on treatment with insulin or oral hypoglycemic agents) or glucose intolerance (fasting blood glucose >110 mg/dL) compared to all other groups.

Plasma chemistry

Serum triglyceride concentration was significantly higher in uric acid stone formers than normal controls (Table 1). No significant difference was found in serum total cholesterol concentration among all four groups. Serum uric acid concentration exceeded the upper limit (7.5 mg/dL) and was significantly higher in uric acid stone group than normal volunteers and pure calcium oxalate stone formers but similar to the patients with mixed stones. This degree of hyperuricemia is similar to our previous

Table 2. Urinary data

		Stone formers		
	Normal	Uric acid	Mixed	Calcium oxylate
Number	11	187	8	19
Total volume *mL/day*	2341 ± 879	2273 ± 846	2714 ± 546	2657 ± 450
Creatinine *mg/day*	1750 ± 483	1696 ± 510	1611 ± 483	1848 ± 492
Sodium *mEq/day*	110 ± 92	80 ± 31	74 ± 26	95 ± 38
Potassium *mEq/day*	40 ± 11	35 ± 9	34 ± 16	34 ± 11
Chloride *mEq/day*	100 ± 76	75 ± 30	69 ± 25	87 ± 32
Calcium *mg/day*	151 ± 83	98 ± 49	171 ± 73[b]	198 ± 91[c]
Phosphorus *mg/day*	732 ± 298	757 ± 216	743 ± 180	728 ± 145
Oxalate *mg/day*	23 ± 7	30 ± 8	28 ± 6	35 ± 8
Uric acid *mg/day*	498 ± 104	379 ± 150	553 ± 183[a]	607 ± 134[c]
FE_{UA}	4.1 ± 1.2	3.3 ± 1.9	5.2 ± 2.3	5.0 ± 1.7[b]
Sulfate *mEq/day*	37 ± 17	40 ± 8	39 ± 13	37 ± 7
pH	5.96 ± 0.47[c]	5.40 ± 0.23	5.68 ± 0.47	6.02 ± 0.35[c]
NH_4^+ *mEq N/day*	32 ± 9	33 ± 11	36 ± 13	39 ± 13
TA *mEq/day*	23 ± 10[b]	33 ± 7	26 ± 8	23 ± 7[c]
Citrate *mg/day*	705 ± 418[a]	317 ± 219	415 ± 208	466 ± 331
Median	745	278	337	358
Citrate *mEq/day*	8.8 ± 5.3[a]	4.4 ± 3.0	5.5 ± 3.0	5.6 ± 3.9
Median	8.8	3.9	4.3	4.2
NAE *mEq/day*	46 ± 14[a]	61 ± 14	56 ± 17	55 ± 19
NH_4^+/NAE	0.74 ± 0.28[d]	0.53 ± 0.10	0.65 ± 0.10[b]	0.71 ± 0.09[c]
TA/NAE	0.50 ± 0.15	0.54 ± 0.10	0.48 ± 0.15	0.43 ± 0.09[c]
Citrate (mEq)/NAE	0.17 ± 0.10[a]	0.07 ± 0.05	0.12 ± 0.10	0.11 ± 0.08
Sulfate (mEq)/NAE	0.91 ± 0.48	0.67 ± 0.15	0.75 ± 0.34	0.72 ± 0.27
Creatinine clearance *mL/min*	135 ± 34	113 ± 41	109 ± 22	129 ± 34

Abbreviations are: FE_{UA}, fractional excretion of uric acid; TA, titratable acidity; NAE, net acid excretion. Data presented as mean ± SD. Significant differences from uric acid stone formers depicted by [a] for $P < 0.02$, [b] for $P < 0.01$ and [c] for $P < 0.001$; d = 0.01 after log transformation.

series [6]. No significant differences were found between pure uric acid stone formers and the other groups in blood urea nitrogen, serum creatinine, sodium, potassium, chloride, bicarbonate, arterialize venous pH, P_{CO_2}, calcium, and phosphorus (Table 1).

Urinary chemistry

The mean 24-hour urinary uric acid did not differ significantly between the pure uric acid stone formers and the normal controls (Table 2), even though the value was numerically lower in uric acid group. However, compared to the pure uric acid group, urinary uric acid was significantly higher in mixed uric acid/calcium oxalate stone and pure calcium oxalate stone formers (Table 2). Fractional urate/uric acid excretion was significantly higher in pure calcium oxalate stone formers compared to pure uric acid stone formers. No significant difference was found between the uric acid stone formers and other groups in urinary volume, sodium, potassium, chloride, calcium, phosphorus, oxalate, creatinine and endogenous creatinine clearance. The latter two findings indicate that all four groups of subjects had similar lean muscle mass, similar levels of renal function, and consumed equivalent diets.

The 24-hour urine data for each subject are shown in Figure 1 and the summary is in Table 2. The 24-hour mean urinary pH was significantly lower in the pure uric acid stone formers than the controls and pure calcium oxalate stone formers (Table 2). The 24-hour mean urinary pH was not different between the pure uric acid versus the mixed stone formers. Urinary ammonium did not differ significantly ($P = 0.13$) between the groups but was numerically lower in the pure uric acid group compared to the pure calcium oxalate group. We reported a similar finding of a trend toward lower total urinary ammonium in uric acid stone-formers in our previous report [6]. The mean urinary titratable acidity was significantly higher in the pure uric acid stone group than both the normal volunteers and pure calcium oxalate stone formers. Urinary citrate excretion was not different among the three groups of stone formers but was lower than the normal volunteers. Net acid excretion in the pure uric acid stone formers was slightly but significantly higher than the normal volunteers but similar to the other two groups of stone-formers.

Several patterns are appreciated in Figure 1. The primary data from each individual patient are shown in Figure 2. Note that despite prescription of fixed diet, there were considerable variations in net acid excretion. We plotted the individual urinary pH, NH_4^+, and titratable acid against net acid excretion to peruse the difference between the groups. Even in the background of overlap, one can appreciate the lower urinary pH and lower NH_4^+ and higher titratable acid content for a given net acid excretion in uric acid stone formers compared to normal controls = s or pure calcium oxalate stone for-

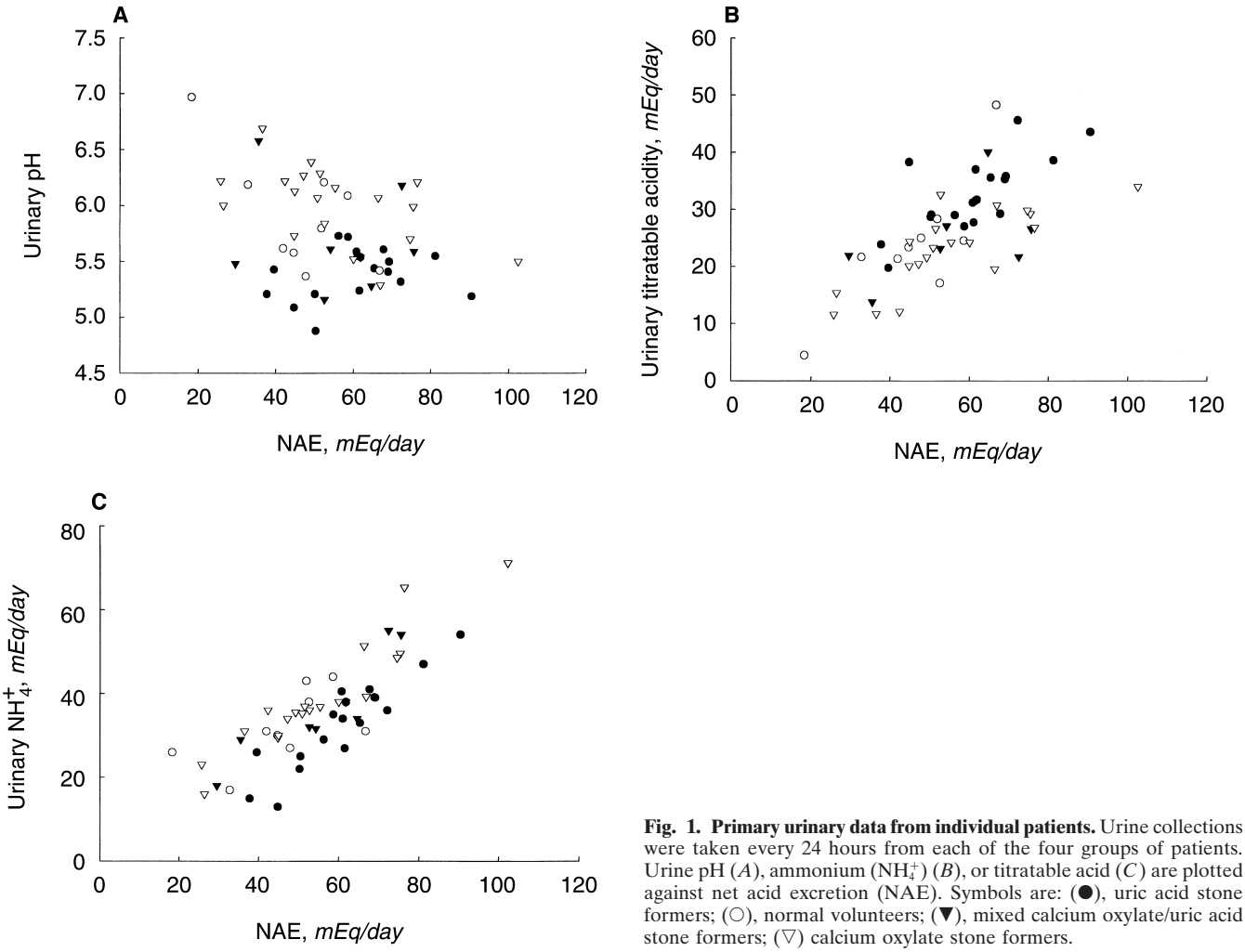

Fig. 1. Primary urinary data from individual patients. Urine collections were taken every 24 hours from each of the four groups of patients. Urine pH (*A*), ammonium (NH_4^+) (*B*), or titratable acid (*C*) are plotted against net acid excretion (NAE). Symbols are: (●), uric acid stone formers; (○), normal volunteers; (▼), mixed calcium oxylate/uric acid stone formers; (▽) calcium oxylate stone formers.

mers (Fig. 1). When urinary NH_4^+ was plotted against urinary pH (Fig. 2), one can appreciate that patients with either pure uric acid stones or mixed uric acid/calcium oxalate stones segregated from the other two groups. Although we did not find a significant difference in 24-hour urinary NH_4^+ among the groups, the fraction of net acid excreted as ammonium (NH_4^+/net acid excretion ratio) was significantly lower in patients with pure uric acid stones than in both the mixed and pure calcium oxalate stone formers (Table 2). In addition, all patients with nephrolithiasis have lower urinary citrate excretion compared to normal controls (Table 2). For all the findings described above, patients with mixed uric acid/calcium oxalate stones were either similar to patients with pure uric acid stones or somewhat intermediate between the pure uric acid stone formers and normal controls.

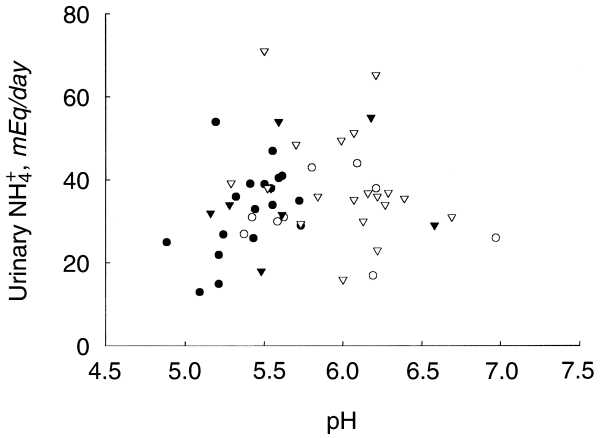

Fig. 2. Primary urinary data from individual patients. Urine collections were taken every 24 hours from each of the four groups of patients. Urinary pH is plotted against net acid excretion (NAE).

Response to an acute acid (NH_4Cl) load

To further examine if there is an impediment to excrete NH_4^+, we challenged the 21 patients with uric acid stones and the 49 patients from the other three groups with a single dose of oral NH_4Cl (50 mEq) and examined the urinary acidification parameters before and every hour after the load for 4 hours. The peak urinary NH_4^+ response was observed at 2 hours in 80% of the patients with the

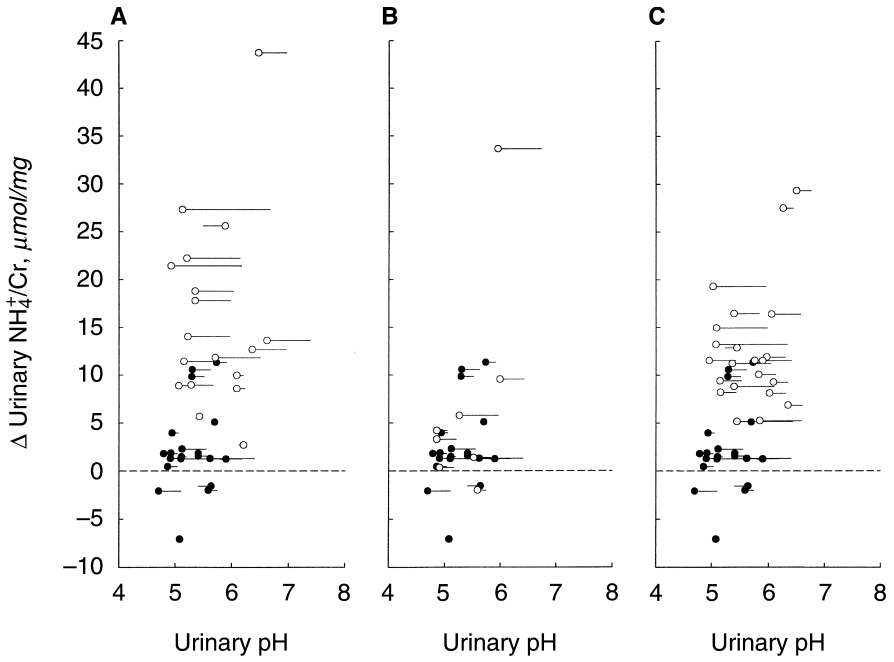

Fig. 3. Urinary response to acute acid load for each individual patient. After equilibration on a standard diet, patients were given an oral load of 50 mmol/L NH_4Cl and hourly urine samples were collected for pH, NH_4^+, and creatinine determinations. NH_4^+ were normalized to urinary creatinine (NH_4^+/Cr). Difference between the baseline and the peak ammonium excretory response (ΔNH_4^+/Cr) is on the y axis. The corresponding starting and final urinary pH is on the x axis and each pair of urinary pH's are connected by a line. The open end of the lines denote starting urinary pH and the symbol end of the lines denote the final urinary pH. Symbols are: in (*A*) (●), uric acid stone formers; (○), normal volunteers. In (*B*), (●) uric acid stone formers; (○) mixed calcium oxylate/uric acid stone formers. In (*C*), (●) uric acid stone formers; (○) calcium oxylate stone formers.

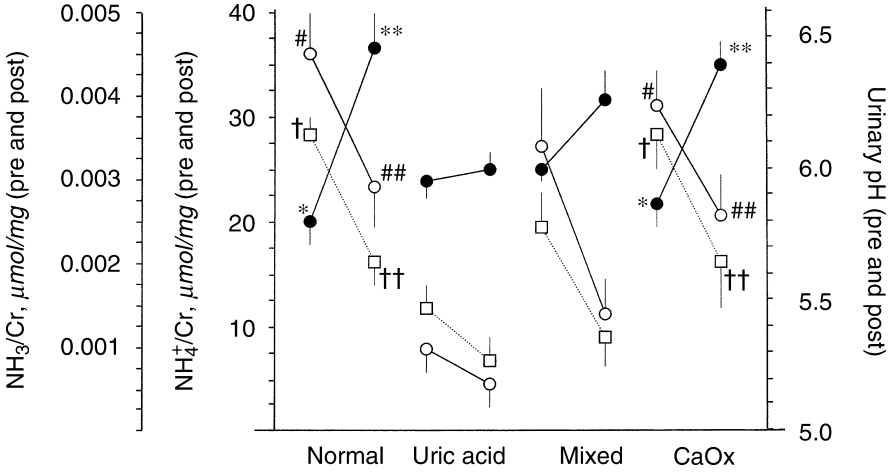

Fig. 4. Urinary response to acute acid load. After equilibration on a standard diet, patients were given an oral load of 50 mmol/L NH_4Cl. Urinary pH (□) and values for NH_4^+ (●) and NH_3 (○) (normalized for creatinine) pre- and post-acid load are plotted for the four groups of patients. Symbols and bars denote mean ± SE. Statistically significant differences ($P < 0.05$, ANOVA) are indicated as follows: *, compared to pre-load NH_4^+/Cr in uric acid stone-formers; **, compared to post-load NH_4^+/Cr in uric acid stone formers; #, compared to pre-load NH_4^+/Cr in uric acid stone formers; ##, compared to post-load NH_4^+/Cr in uric acid stone formers; †, compared to pre-load NH_4^+/Cr in uric acid stone formers; ††, compared to post-load NH_4^+/Cr in uric acid stone formers.

remaining 20% peaking at 3 hours. The patients clearly segregated into two easily discernible groups (Fig. 3). Patients with pure uric acid stones and mixed uric acid stones were different from pure calcium oxalate stone formers and normal volunteers. Urinary pH decreased in all patients studied. The starting urinary pHs were higher in normal volunteers (mean ± SD, 6.16 ± 0.57) and patients with pure calcium oxalate stones (6.12 ± 0.43) compared to patients with pure uric acid (5.47 ± 0.42) or mixed stones (5.76 ± 0.61). There were also greater falls in urinary pH in normal volunteers (−0.52 ± 0.48) and patients with pure calcium oxalate stones (−0.48 ± 0.34) compared to patients with pure uric acid (−0.21 ± 0.30) or mixed stones (−0.39 ± 0.23). (Fig. 4). Despite the smaller decrease in urinary pH, the pure uric acid and mixed uric acid/calcium stone formers had the lowest end urinary pH after the acid load (5.26 ± 0.35) compared to normal controls, mixed stone formers, and pure calcium stone formers (5.64 ± 0.54, 5.37 ± 0.47, 5.64 ± 0.47, respectively) (Figs. 3 and 4). The rise in NH_4^+ excretion rate (normalized to creatinine) above baseline was five- to sevenfold higher in normal controls (+15.8 ± 9.7 μEq/mg) and pure calcium oxalate stone-forming patients (+12.7 ± 6.2 μEq/mg) compared to pure uric acid stone formers (+2.2 ± 4.4 μEq/mg) and twice that of mixed uric acid/calcium oxalate stone formers (+7.0 ± 11.3 μEq/mg). The poor ammonium response was due to the extremely low NH_3^+ available in uric acid and mixed uric acid/calcium stone formers compared to normal controls or pure calcium stone formers

(Fig. 4). The individual primary data points are shown in Figure 3 where the rise in NH_4^+ excretion in response to each acid load in a given individual was plotted against the beginning and end urinary pH. The dramatic difference in NH_4^+ excretion is evident in this plot where there was virtually no overlap between the pure uric acid stone formers with the pure calcium oxalate stone formers (Fig. 3C) and normal volunteers (Fig. 3A) in terms of ammonium excretory response to the acid load. The response was very similar between the uric acid and mixed stone-formers (Fig. 3B) except for one outlying point.

DISCUSSION

This is the largest study to date that examined whether defective renal ammonia excretion underlies uric acid urolithiasis under conditions of controlled dietary acid and phosphorus intake. Our data demonstrate that such a defect in ammonium excretion would account for the undue urinary acidity, the fundamental deficit leading to uric acid nephrolithiasis [18, 19]. There are three major findings in this study. First, patients with either pure uric acid or mixed uric acid/calcium oxalate stones excrete less of their net acid as ammonium due to a defect in ammonium excretion at the steady state; the end result being acidic urinary pH. Second, despite a mild defect in ammonium excretion, patients with either pure uric acid or mixed uric acid/calcium oxalate stones have normal systemic acid-base parameters and normal net acid excretion. Since a lesser portion of their acid is excreted as urinary ammonium, net acid excretion is compensated by increased titratable acidity and hypocitraturia. Finally, there is a high incidence of either glucose intolerance or frank type II diabetes in patients with pure uric acid stones compared to patients with calcium oxalate stones. The incidences of type II diabetes or glucose intolerance in patients with mixed uric acid/calcium oxalate stones are intermediate between the pure uric acid and pure calcium oxalate stone formers.

Low urinary pH is the single most invariable finding in pure uric acid nephrolithiasis. There is little doubt that the low urinary pH is pathogenic for uric acid precipitation in the urine. Low ammonium excretion as a cause of low urinary pH has been described in some studies, but not in others [6, 12–17, 20, 21]. Part of the discrepancy may arise from the small difference in ammonium excretion required to lower urinary pH at the typical urinary pH values encountered in these patients so biochemically significant differences may not be statistically significant. Another is the variation in net acid production and excretion in any given individual from day to day. Plante, Durivage, and Lemieux noted that a high protein diet (presumed high acid load) was required for the abnormal urinary parameter to manifest [21]. The variation in levels of alternative H^+ acceptors such as urinary phosphate may also account for the discrepancy [17]. In the present study, we attempted to control all variables by equilibrating the patients on the same metabolic diet but we still observed some degree of scatter in daily net acid secretion (see primary data in Figs.1 and 2). The equivalence of urinary Na^+, K^+, sulfate, and phosphate among the four groups suggests that the subjects were probably compliant with the diet. The slightly lower net acid excretion in normal volunteers may be due to lower endogenous acid production, less gastrointestinal alkali loss, or both. Patients with either pure or mixed uric acid stones have lower urinary pH and higher titratable acid. Although baseline NH_4^+/day were not statistically different, the fraction of H^+ excreted as NH_4^+ (NH_4^+/net excretion rate ratio), this number is clearly lower in patients with pure uric acid stones (53%) and mixed uric acid/calcium oxalate stones (65%) compared to normal controls (74%) and patients with pure calcium oxalate stones (71%).

Since the relationship between urinary pH and NH_4^+ is inconsistent in a chronic steady state [26], we challenged our patients with a standard acid load in the background of equilibration to a controlled diet. All patients mounted a response with increased urinary ammonium excretion as expected. Patients with pure uric acid stones clearly have a lower ammonium excretory response compared to normal controls or patients with calcium oxalate stones, whereas the response is by-and-large indistinguishable between the pure uric acid and mixed uric acid/calcium stone formers. Despite their low baseline urinary pH, uric acid stone formers further acidified their urine in response to an acute acid load. Patients with mixed uric acid/calcium oxalate stones behaved similarly to those with pure uric acid stones in terms of ammonium excretory response. The calcium oxalate precipitation could well be secondary to uric acid-induced calcium aggregation [9–11]. We postulate that uric acid nephrolithiasis is primarily an "acidification disease" with uric acid precipitation occurring as an "innocent bystander."

It is noteworthy that all the patients have normal steady-state systemic acid-base parameters and all seem to excrete their daily acid load adequately (Tables 1 and 2). While the defect brought out by the acute acid load (which takes the system to capacity) seems severe (13% of normal, Table 3), the decrease in urinary ammonium is relatively minor at the steady state and is compensated by a higher titratable acid content, as well as a lower urinary citrate excretion. The higher titratable acid is likely accountable entirely by the lower urinary pH because there is no increase in phosphate, creatinine, or uric acid excretion in the patients with pure uric acid stones. Although hypocitraturia is well known in patients with pure calcium oxalate and mixed uric acid/calcium stones, low urinary citrate has not been previously reported in patients with pure uric acid stones, including our previous analysis using random outpatient urine col-

lection [6]. The hypocitraturia may be secondary to proximal tubule cell acidification, which increases apical membrane citrate uptake [27, 28], as well as cellular citrate metabolism [29, 30]. Although the mean urinary citrate is statistically lower in stone formers and numerically lowest in pure uric acid stone formers, there was considerable variation in the individual urinary citrate values. At present, the significance of hypocitraturia in pure uric acid nephrolithiasis is unclear since citrate does not complex or solubilize uric acid per se. One possible role of hypocitraturia is the potentiation of nucleation of calcium oxalate by uric acid. However, we did not find consistently lower urinary citrate in a subgroup analysis in patients with mixed uric acid/calcium oxalate stones.

In this study, as well as our previous study [6], we have shown that patients with pure uric acid stones share some clinical features of type II diabetes mellitus, including high body mass index (Table 1), dyslipidemia (Table 2), and hyperuricemia (Table 1 and Fig. 1). However, it is important to note that the higher age and body mass index per se cannot account for our findings because the age- and body mass index-adjusted results were completely identical. One preliminary report suggests that insulin resistance may be causing the low urinary pH (abstract; Abate N et al, *J Invest Med* 49:112A, 2001). Insulin has been shown to increase ammonia synthesis in suspended proximal tubules [31, 32], as well as the apical Na^+/H^+ exchanger [33, 34], which mediates NH_4^+ secretion in proximal tubules and cultured renal cells. It is important to note that insulin resistance cannot be the sole mechanism for the impaired ammonium excretion and aciduria because some of our patients with pure uric acid stones clearly did not have clinically detectable insulin resistance. Conversely, we do not know what fraction of patients with insulin resistance harbor defects in acidification because factors other than insulin can affect ammonium excretion. And of those with impaired acidification, how many have yet unidentified uric acid stones? Other factors such as total uric acid + urate, urinary volume, and presence of inhibitors can modify the stone forming propensity.

CONCLUSION

We have shown that in patients with either pure uric acid or mixed uric acid/calcium oxalate stones, the acidic urinary pH is due to impaired urinary ammonium excretion. The lower urinary ammonium is compensated by higher urinary tiratable acidity in all and hypocitraturia in some patients; thus, normal systemic acid-base parameters are preserved. There is a high fraction of patients with insulin-resistant states with uric acid nephrolithiasis. In these patients, the defective ammonium excretion may be in part related to the insulin resistance. We propose that uric acid nephrolithiasis is a disease of renal acidification. This mild defect does not lead to any acid-base abnormalities but uric acid precipitates as an innocent bystander in the acidic urine.

ACKNOWLEDGMENTS

The authors were supported by the National Institutes of Health [P01-DK20543 and M01-RR00633 (CYCP), R01-48482 and R01-54396 (OWM)], and the Department of Veterans Affairs Research Service (OWM). The authors wish to acknowledge the expertise of the nursing staff at the General Clinical Research Center at the University of Texas Southwestern Medical Center and, in particular, Ms. Marcia Roberts, RN.

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