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Urinary oxalate excretion in female calcium oxalate stone formers with and without a history of recurrent urinary tract infections

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Abstract Therapy with antibiotics in recurrent urinary tract infections may destroy colonies of *Oxalobacter formigenes* in the intestinal tract. A lack of oxalate degradation caused by the absence of this bacterium is suggested to contribute to the hyperabsorption of dietary oxalate and to the increase in urinary oxalate excretion. The present study was performed to evaluate the effect of recurrent urinary tract infections and subsequent changes induced in the urinary excretion profile in female calcium oxalate stone formers. Serum biochemical profiles, 24-h urinary parameters, and the personal characteristics of 57 female calcium oxalate stone patients with recurrent urinary tract infections (RUTI) were compared with 78 female calcium oxalate stone patients without a history of urinary tract infection. All subjects were recruited during the same period. In female patients with RUTI, urinary oxalate excretion was significantly higher (0.374 mmol/day) than in females without urinary tract infection (0.308 mmol/day) ($P < 0.05$). Moreover, the mean 24-h pH value and urinary sodium excretion were significantly higher in women with RUTI than in women without a history of urinary tract infection. The significantly higher urinary oxalate excretion in female calcium oxalate stone formers with recurrent urinary tract infections may be associated with the application of antibiotics and a subsequent temporary or permanent decolonization of *Oxalobacter formigenes*.

Keywords Urinary oxalate excretion · Calcium oxalate stone formation · Recurrent urinary tract infections

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Introduction

An increase in urinary oxalate concentration and excretion is a much more important determinant for the formation of calcium oxalate stone than a similar increase in urinary calcium (12). Under normal circumstances, about 90% of urinary oxalate is derived from the endogenous metabolism of its major precursors, glyoxylate and ascorbic acid, and 10% from the intestinal absorption of exogenous oxalate in the diet (18). The gastrointestinal transport of oxalate is suggested to be mainly a non-energy-dependent, non-saturable, passive process occurring in the whole intestine (2), but there is also evidence for a carrier-mediated active transport process of oxalate (5). The rate of oxalate absorption from food is determined by the concentration and availability of soluble oxalate forms in the gut. Dietary components, especially calcium and magnesium, are known to reduce intestinal absorption of oxalate by forming insoluble complexes and therefore lowering the concentration of free oxalate (10).

More than half of the dietary oxalate in humans is metabolized by *Oxalobacter formigenes*, obligatory anaerobic Gram-negative rods , that degrade oxalic acid in the gastrointestinal tract to formate and CO_2 (1). Treatment with antibiotics may lead to a reduced rate or deficiency of oxalate degradation by the colonic flora and therefore may contribute to the hyperabsorption of dietary oxalate and increased urinary oxalate excretion (15). The aim of the present study was to assess the relationship between repeated antibiotic therapy in recurrent urinary tract infections (RUTI) and calcium oxalate stone formation by the determination of urinary oxalate excretion and other risk factors that promote stone formation. The study was conducted in female patients since recurrent urinary tract infections occur at a higher prevalence in female compared to male calcium oxalate stone formers.

Materials and methods

The study population comprised 135 female calcium oxalate stone patients participating in the 'Bonn Urolithiasis Follow-up Study' between July 1988 and June 1994. Patient data were obtained from the attending urologists by standardized questionnaires, which inquired after the medical history, age, body size and weight of each subject. Among the participants, 57 females had recurrent urinary tract infections requiring antibiotic treatment, whereas 78 females had no history of urinary tract infection. At the time of 24-h urine collection, 7% of patients with RUTI experienced an active urinary tract infection.

Potential cases were excluded from the study if they had intestinal disorders that might influence the intestinal absorption of oxalate. These disorders included intestinal disturbances, e.g. inflammatory bowel disease, chronic diarrhoea, duodenal ulcer, and short bowel syndrome.

Samples from 24-h urine collections from the patients on their self-selected diets were analysed in our laboratory. Urine volume, pH (potentiometry) and the concentrations of creatinine (Jaffé reaction), oxalate (ion chromatography), calcium and magnesium (atomic absorption spectrophotometry), chloride (coulomb metric titration), sodium and potassium (flame emission spectrophotometry), sulphate (nephelometry), phosphate (phosphate molybdate reaction), ammonium (ion selective electrode), citrate (enzymatically, citrate lyase) and uric acid (enzymatically, uricase) were measured. Serum parameters were available for calcium, creatinine and uric acid.

Analysis of oxalate concentration in urine was performed by suppressed ion chromatography. The chromatographic system (Dionex, Sunnyvale, Calif.) applies a membrane suppressor for chemical reduction of the background conductivity of the eluant. It consists of an eluant pump APM-1, conductivity detector CDM-1, micromembrane suppressor AMMS-1, autosampler ASM-2, 100 µl sample loop, computer interface ACI and software AutoIon 450. For separation, a Dionex AS4A analytical column (4×250 mm) and a Dionex NG1 guard column (4×50 mm) were used. The mobile phase consists of 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$, the flow rate was 1.8 ml/min (7). The preparation of the samples was carried out immediately after urine collection along the lines suggested by Robertson and Scurr (13): 0.5 ml 1 mol/l HCl was added to 1 ml thoroughly mixed urine. Acidified urine (100 µl) was then added to 4.9 ml 0.3 M boric acid in a 5-ml autosampler vial. Oxalate was quantified via peak area and external calibration curves (7).

Differences in urinary parameters, serum values, age, body size and weight between groups were compared by Student's non-paired t test. Categorical comparisons were performed using the X^2-test. Data are presented as means ± SEM unless otherwise stated. The α level for significance was set at $P < 0.05$.

Results

Among the female calcium oxalate stone patients no significant difference was observed in patient characteristics (age, weight, height, and body mass index) between groups (Table 1). A family history of stones was found with a similar frequency both in female patients with and without RUTI (43.6% vs 42.3%). In 29.1% of female patients with RUTI and in 25.6% of females without a history of RUTI parents were also affected. Stones were less frequent in siblings and grandparents. The differences in frequency between groups were not statistically significant.

In female patients with RUTI, 22.8% had one to three anomalies that might be of importance with respect to the stone formation. In female stone formers without a history of urinary tract infection this value was 12.8%. The most frequent anomalies diagnosed were ureteral and calyx stenosis (12.3% vs 5.1%), duplication (5.3% vs 2.6%) followed by nephroptosis, fusion kidney and renal cyst. The frequency of anatomic anomalies of the urinary tract did not significantly differ between groups.

Table 1 Characteristics of female calcium oxalate stone patients with and without recurrent urinary tract infections (RUTI). *n.s.* not significant

	Without RUTI ($n = 78$)	With RUTI ($n = 57$)	P
Age (years)			
Mean	47.4	48.5	n.s.
Range	21.0–69.0	25.0–71.0	
Weight (kg)			
Mean	65.8	67.4	n.s.
Range	42.0–100.0	50.0–140.0	
Height (m)			
Mean	1.64	1.64	n.s.
Range	1.48–1.85	1.45–1.87	
Body mass index (kg/m^2)			
Mean	24.5	24.8	n.s.
Range	16.9–36.3	17.9–45.7	

Pure calcium oxalate stones were found among 74% of female calcium oxalate stone patients with RUTI and among 73% of patients without RUTI. Other minor constituents of the stones in patients with RUTI were calcium phosphate, struvite, uric acid and ammonium urate. In patients without RUTI calcium phosphate and uric acid were determined as other components of the calcium oxalate stones.

In female calcium oxalate stone formers with RUTI urinary oxalate excretion was significantly higher (0.374 mmol/day) than in females without a history of RUTI (0.308 mmol/day) ($P < 0.05$). Of the other urine parameters measured in female calcium oxalate stone formers only the urinary pH value and sodium excretion differed significantly between the two groups. Moreover, urinary ammonium and chloride excretion tended to be higher in female patients with RUTI. The biochemical data obtained from the female patients are listed in Tables 2 and 3.

Discussion

A family history of stones is common in stone formers (3, 9, 11). In our study 43.6% of patients with RUTI and 42.3% of patients without a history of urinary tract infection reported a family history of stones. In the literature percentages vary between 39% (3) and 55% (11). The lack of a difference in the frequency of family history of stone disease in both groups suggests that genetic factors are of minor importance in determining the risk of stones among female patients with RUTI.

Although no significant association was established, anomalies of the urinary tract were more frequently

Table 2 Daily urine composition of female calcium oxalate stone patients with and without recurrent urinary tract infections (RUTI) (mean ± SEM). *n.s.* not significant

	Without RUTI ($n=78$)	With RUTI ($n=57$)	*P*
Urine volume (l/24 h)	2.06 ± 0.09	2.10 ± 0.10	n.s.
pH	6.22 ± 0.05	6.42 ± 0.08	< 0.05
Sodium (mmol/day)	158.8 ± 7.9	188.0 ± 11.3	< 0.05
Potassium (mmol/day)	60.3 ± 2.2	65.8 ± 3.5	n.s.
Calcium (mmol/day)	5.42 ± 0.26	5.03 ± 0.30	n.s.
Magnesium (mmol/day)	4.34 ± 0.18	4.40 ± 0.29	n.s.
Ammonium (mmol/day)	24.7 ± 1.0	38.0 ± 10.2	n.s.
Chloride (mmol/day)	153.3 ± 8.0	174.9 ± 10.1	n.s.
Phosphate (mmol/day)	24.8 ± 0.9	25.2 ± 1.0	n.s.
Sulphate (mmol/day)	19.6 ± 0.7	21.6 ± 1.3	n.s.
Creatinine (mmol/day)	10.42 ± 0.28	10.89 ± 0.48	n.s.
Uric acid (mmol/day)	3.10 ± 0.11	3.34 ± 0.17	n.s.
Oxalic acid (mmol/day)	0.308 ± 0.017	0.374 ± 0.022	< 0.05
Citric acid (mmol/day)	2.788 ± 0.158	3.075 ± 0.229	n.s.

Table 3 Serum parameters of female calcium oxalate stone patients with and without recurrent urinary tract infections (RUTI) (mean ± SEM)

	Without RUTI ($n=78$)	With RUTI ($n=57$)	*P*
Calcium (mmol/l)	2.39 ± 0.02	2.35 ± 0.02	n.s.
Uric acid (mg/dl)	4.36 ± 0.16	4.63 ± 0.20	n.s.
Creatinine (mg/dl)	0.86 ± 0.02	0.90 ± 0.03	n.s.

present in female calcium oxalate stone formers with RUTI (22.8%) than in those without RUTI (12.8%). The increased tendency to anomalies of the urinary tract among patients with RUTI suggests that this factor may contribute to recurrent urinary tract infections.

Urinary tract infections may be caused by urea-splitting microorganisms. The most important urease-producing bacterial strains are *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. The presence of these bacteria leads to a pronounced urinary alkalinization and predisposes to stone formation, mainly struvite stones (4). According to the resistance pattern, antibiotic therapy along with complete stone removal and acidification is the obligatory treatment of urinary tract infections associated with stone disease (6). Since the coincidence of recurrent urinary tract infections and urolithiasis may lead to kidney dysfunction, it is important to exclude possible risk factors.

The essential finding of this study is a significantly higher urinary oxalate excretion in the female calcium oxalate stone formers with RUTI. Urinary oxalate excretion is a major determinant of stone prevalence in the population. Any factor which leads to an increase in oxalate excretion will markedly increase the risk of calcium oxalate stone formation. A recent study in patients at high risk for calcium oxalate stone disease showed a direct correlation between the number of recurrent kidney stone episodes and the lack of *Oxalobacter formigenes* colonization (14). The lack of *O. formigenes* revealed a clear association with prophylactic antibiotic therapy. The use of antibiotics and, as a result, a low or absent intestinal colonization with *O. formigenes*, can lead to a marked reduction in oxalate degradation in the gut and an increased absorption and urinary excretion of dietary oxalate. In a further prospective study, faeces of calcium oxalate stone patients with and without a history of RUTI will be examined for detection of *O. formigenes*.

There is no evidence for the oxidation of ascorbic acid producing oxalic acid as an artefact during the collection and handling of the specimens. Our own investigations failed to demonstrate an erroneously high analytical oxalate level in the presence of thymol/isopropanol as a urine preservative, compared to HCl (8), even under conditions of high dietary ascorbate and a high urinary pH (17).

The significantly higher mean urinary pH in female patients with RUTI indicates that some of the patients suffered from urinary tract infections at the time of 24-h urine collection. As a result of urinary tract infection with urea-splitting bacteria, hydrolysis of urea by bacterial urease increases urinary pH and ammonium excretion. Urinary pH is a major determinant for the crystallization of calcium phosphate. Carbonate apatite and hydroxyapatite stones form in alkaline urine with an optimal pH of above 6.8. The most common causes of calcium phosphate stone formation are disturbances in the calcium phosphate balance, renal tubular acidosis (RTA) and urinary tract infection. Carbonate apatite and hydroxyapatite are commonly mixed with calcium oxalate. In the case of urinary tract infection with urease producing microorganisms, struvite is another component of the precipitate. Thereby urinary pH usually increases to levels above 7.0. The most likely explanation for the higher sodium excretion in women with RUTI may be a higher dietary intake of sodium chloride.

In connection with the dietary intake and intestinal absorption of oxalate, the possible role of oxalate-degrading bacteria or enzymes in the gut should be pursued with the view to developing a possible method for treating calcium oxalate stone disease. Prolonged use of antibiotic therapy is a probable cause of decolonization in many individuals, but it is not known whether

O. formigenes can re-establish itself in the gut naturally or what the ideal conditions might be that would allow recolonization. A recolonization with *O. formigenes* might lessen the risk of calcium oxalate stone formation in patients with recurrent urinary tract infections (16).

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