Oxalate and calcium excretion in cystic fibrosis

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Abstract

Background—A patient with cystic fibrosis (CF) and repeated calcium oxalate renal stones prompted us to investigate other children for risk factors for this recognised complication of CF.

Methods—Twenty four hour urinary excretion of calcium, oxalate, and glycolate was measured in children with CF and no symptoms of renal tract stones. Normal diet and treatments were continued.

Results—In 26 children (aged 5–15.9 years) oxalate excretion was correlated with age; 14 of 26 children had oxalate excretion above an age appropriate normal range. There was a positive correlation between oxalate excretion and glycolate excretion. Mean calcium excretion was 0.06 mmol/kg/24 h with 21 of 24 children having calcium excretion below the normal range.

Conclusions—Hyperoxaluria may reflect malabsorption although correlation between excretion of oxalate and glycolate suggests a portion of the excess oxalate is derived from metabolic processes. The hypocalciuria observed here may protect children with CF from renal stones.

(Arch Dis Child 2000;83:244–247)

Keywords: cystic fibrosis; oxalate; calcium; renal stones

Symptomatic calcium oxalate nephrolithiasis in patients with cystic fibrosis (CF) has been reported on several occasions.^{1–4} In a study of untimed postprandial urine collections in 43 patients with CF and no symptoms of urinary tract stone disease, mean urinary oxalate concentration in the whole patient sample was increased and mean urinary calcium excretion reduced.⁵ This report did not specify how many individual CF patients had altered urinary excretion of calcium or oxalate.

Microscopic nephrocalcinosis was observed using stains for calcium at postmortem light microscopy in kidneys obtained from 35 of 38 patients who had died from CF; no controls were examined in this study.⁶ Another group found evidence of sparse microscopic nephrocalcinosis in five of 14 CF kidneys, with six of 12 control kidneys showing nephrocalcinosis.⁷

Calcium oxalate stones have been found in other malabsorptive states^{8,9} in which there is thought to be enhanced uptake of oxalate leading to excessive urinary excretion of oxalate. Excess urinary oxalate has been reported in CF patients with stones,² although the incidence of this finding in the CF population as a whole is not clear. A change in the pattern of intestinal bacterial colonisation that could lead to alterations in oxalate handling in patients with CF has recently been reported.¹⁰ One of our

patients developed two calcium oxalate renal stones and this prompted us to examine the incidence in our patient population of two factors that could influence stone formation: urinary excretion of calcium and oxalate.

In this case CF had been diagnosed in infancy and was subsequently complicated by diabetes mellitus. The patient presented on two occasions with unilateral loin pain and frank haematuria. On each occasion (at 12 and 14 years) a calcium oxalate stone was passed. On the first occasion a 24 hour urine collection revealed an oxalate excretion of 0.99 mmol per 24 hours (upper limit of normal at this age is 0.35 mmol per 24 hours). On the second occasion an oxalate excretion of 1.11 mmol per 24 hours was found. In addition, the glycolate excretion was found to be 0.46 mmol per 24 hours (normal range 0.10–0.33 mmol per 24 hours).

The aims of this study were: (1) to investigate the prevalence of hyperoxaluria in children with CF; (2) to ascertain whether hyperoxaluria could be attributed to metabolic causes by measuring glycolate excretion as a marker for precursors of oxalate; (3) to determine the level of calcium excretion; and (4) to relate the excretion of calcium and oxalate in each child in order to assess the likelihood of stone formation in this group.

Patients and methods

Twenty nine consecutive patients attending routine follow up appointments at the regional paediatric cystic fibrosis clinic at Booth Hall Children's Hospital, Manchester were approached about the study (total clinic population 100). Two of these patients declined to take part in the study. The remainder gave verbal consent to the 24 hour urine collection. All 27 children were pancreatic insufficient. Collections were carried at while the children were at home, on routine medication, but not on intravenous antibiotics. Children under the age of 5 years were not included; otherwise patients were unselected. Approval from the local ethics committee was not sought as the investigation involved was not invasive.

Subjects were asked to provide a 24 hour urine sample. The sample bottle contained 20 ml of 5 M hydrochloric acid as acidic conditions would prevent metabolism of citrate to oxalate. In all cases the sample pH was less than 2.0, confirming adequate acidification. The volume of the 24 hour sample was determined by weighing. Two aliquots (25 ml and 10 ml) were stored at –20°C. Samples were batch processed in order to reduce variability.

Urine oxalate assays were performed using a high pressure liquid chromatography assay of a quinoline derivative with monitoring at

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Accepted 10 March 2000

Table 1 Results of assays for oxalate, calcium, and glycolate

Solute	Minimum (mmol/24 h)	Maximum (mmol/24 h)	Mean (mmol/24 h)	SD	Number above normal
Oxalate (n = 26)	0.26	1.03	<13 y, n = 14, 0.43 >13 y (M), n = 7, 0.66 >13y (F), n = 5, 0.44	0.17 0.24 0.18	14/26
Glycolate (n = 25)	0.015	0.71	0.232	0.16	2/25
	Minimum (mmol/kg/24 h)	Maximum (mmol/kg/24 h)	Mean (mmol/kg/24 h)	SD	Number below normal
Calcium (n = 24)	0.007	0.139	0.06	0.03	15/24

310 nm, according to a modification of the method of Koolstra *et al.*¹¹ Ascorbate was removed prior to denaturation by solid phase extraction. All samples were assayed in duplicate together with aqueous standards containing 0.5 and 1.0 mM oxalate.

Urine glycolate assays were performed according to the method of Kasides and Rose¹² by the biochemistry department at University College Hospital London. Interfering substances were removed by the addition of activated charcoal. Lactate contributes to the assayed products so the level of lactate was determined and taken into account. Urine calcium excretion was measured by atomic absorption spectroscopy at 515 nm.

The following clinical data were collected for each patient from the clinical records: age, FEV₁ corrected for height (expressed as a percentage of that expected for height), height (in cm and as a standard deviation score), weight (in cm and as a standard deviation score), body mass index (weight/height² expressed as a standard deviation score), and lipase dose (units of lipase per kg per 24 hours). Standard deviation scores were calculated by the LSM method.^{13 14}

The normal range for urinary concentration of oxalate after a 24 hour collection is dependent on the age and sex of the subject. In this laboratory normal concentrations in males and females under 13 years old are less than 0.35 mmol per 24 hours. For 13 years and over the upper range of normal for males is 0.48 mmol/24 h and for females 0.52 mmol/24 h. In this laboratory, normal amounts of glycolate excretion are at most 0.34 mmol/24 h. Urinary calcium excretion was measured in 24 children. The normal range in this laboratory for

this measurement is greater than 0.1 mmol/kg/24 h for children weighing less than 25 kg or 0.25–0.75 mmol/24 h in children weighing more than 25 kg and in adults.

The data were analysed using SPSS. Parametric tests were used for the continuous variables: all of these variables had a distribution not significantly different from a normal distribution as tested with a Kolmogorov–Smirnov test ($p > 0.1$).

Twenty seven urine samples were collected over a two month period. One patient did not return the sample bottle. This gave 26 samples: 12 from boys, and 14 from girls. The patients' ages ranged from 5 years to 15.9 years (mean 11.8 years, SD 3.41). The height corrected FEV₁ was available in 25 cases and ranged from 32% to 108% (mean 76%, SD 19.9%). Lipase dose varied from 2250 units/kg/day to 31 300 units/kg/day (mean 16 900, SD 6960). Weight standard deviation score varied from -1.38 to 1.08 (mean -0.38, SD 0.84). Height standard deviation score varied from -1.7 to 2.4 (mean -0.25, SD 1.1). Body mass index (BMI) standard deviation score varied from -1.90 to 1.05 (mean -0.30, SD 0.71).

Results

Measurement of intra-assay precision for the assay of oxalate gave a coefficient of variation (CV) of 9.9% at 0.17 mmol/l oxalate and CV of 8.0% at 1.12 mmol/l oxalate. Table 1 shows the results of assays for oxalate, glycolate, and calcium. Figure 1 shows the relation between oxalate excretion and calcium excretion; this illustrates that children with high amounts of oxalate excretion were likely to have low amounts of calcium excretion.

Correlations were measured between oxalate excretion and clinical variables, and between calcium excretion and clinical variables. Table 2 shows the results. Oxalate excretion was significantly correlated to age and glycolate excretion, while age and glycolate were not correlated ($r = 0.047$, $p = 0.82$). These associations persisted when only cases with high oxalate excretion were included (age: $r = 0.665$, $p = 0.009$; glycolate: $r = 0.602$, $p = 0.023$; $n = 14$). Multiple linear regression was performed using these significant variables for all cases with oxalate excretion as the dependent variable. Table 3 shows the results; age and glycolate excretion were likely to be independently related to oxalate excretion. Figure 2 shows the relation between oxalate excretion and glycolate excretion. There is one outlier with high glycolate excretion. If this data point

Figure 1 Urinary excretion of oxalate versus urinary excretion of calcium.

Table 2 Correlation between excretion of solutes and clinical variables

Variable	Correlation* with oxalate excretion	p value	Correlation* with calcium excretion	p value	Correlation* with glycolate excretion	p value
Age	0.455	0.020	-0.497	0.013	0.047	0.824
BMI SD score	-0.036	0.863	-0.025	0.909	-0.004	0.987
Calcium excretion	-0.177	0.408	—	—	-0.060	0.785
FEV ₁	0.095	0.653	0.081	0.715	-0.148	0.489
Glycolate excretion	0.485	0.014	-0.060	0.785	—	—
Lipase dose	-0.363	0.081	-0.103	0.648	-0.157	0.474
Oxalate excretion	—	—	-0.177	0.408	0.485	0.014

*Pearson's correlation coefficient; significance levels are two tailed.

Table 3 Results of multiple linear regression for oxalate

Variable	Regression coefficient	Standard error	p value
Glycolate	0.637	0.221	0.009
Age	0.0273	0.010	0.011

F = 8.371, 2 degrees of freedom, p = 0.002, R² = 0.432.

is excluded the correlation between oxalate and glycolate excretion is strengthened ($r = 0.760$, $p < 0.001$). Calcium excretion was significantly correlated with age but not with any other clinical variable.

Of the 14 patients with increased oxalate excretion, 13 had normal amounts of glycolate excretion. The exception was patient 9 who had an oxalate excretion of 1.03 mmol/24 h, which was the highest in this survey, and glycolate excretion of 0.59 mmol/24 h. Of the 12 patients with normal amounts of oxalate excretion the glycolate was above normal in one patient (patient 19; oxalate excretion 0.35 mmol/24 h, glycolate excretion 0.710 mmol/24 h). No patient had macroscopic nephrocalcinosis or any symptoms suggestive of stone disease.

Discussion

This study provides a cross sectional view of urinary oxalate excretion in children with cystic fibrosis and no clinical evidence of stone at the time of the collection. Fourteen of 26 children with CF had increased urinary excretion of oxalate during a 24 hour collection of urine. The limitations of this study include: uncontrolled diet; incomplete assay of oxalate loss (faecal loss not accounted for); non-random selection of patients (children with more problems may have had a higher frequency of "rou-

tine" clinic visits); lack of data about renal function (as oxalate excretion will depend on glomerular and tubular function); and lack of formal controls (comparisons were made to predefined laboratory ranges for normal populations).

Oxalate is the end product of vitamin C (ascorbic acid) metabolism.⁹ Glycolate is an intermediate in this process. It would be expected that if hyperoxaluria were the result of increased metabolic activity then glycolate excretion in urine would be raised.⁹ On the other hand if hyperoxaluria were secondary to increased uptake then an increase in urinary glycolate would not be expected. In the current study, the urinary glycolate excretion was used as a non-invasive measure of whether the urinary oxalate had a metabolic origin.

The association of oxalate excretion with glycolate excretion (which persisted after statistical adjustment for age) suggests that the excretion of these solutes is linked. As seen in fig 2, this relation persisted in children with high amounts of oxalate excretion, suggesting that excess oxalate excretion has a metabolic component.

Alternative explanations are not supported by these data.

- The crude markers of malabsorption available from routine clinical notes (lipase dose and BMI SD score) were not correlated with urinary excretion of either oxalate or calcium. At the time of this study lipase dose was determined on the basis of symptoms relating to defaecation, which are relatively poorly linked to the extent of malabsorption.
- The pulmonary marker of disease severity used here, FEV₁, was not related to excretion of any of the solutes. However, the subjects were clustered towards relative good health.
- Medication could have altered oxalate excretion. Pyridoxine has been used to reduce urinary oxalate in mild metabolic hyperoxaluria.¹⁵ As noted, vitamin C is a source of oxalate⁹; vitamin C intake could therefore have altered oxalate excretion. Furthermore, high urinary concentrations of ascorbic acid have been shown to enhance stone formation.¹⁶ During this study all of these patients were on their normal multivitamin preparation (Ketovite) which contains both pyridoxine and ascorbic acid in the tablet form. It seems unlikely that this could explain these results, particularly as half the children have increased oxalate excretion but all the children received multivitamins.

Figure 2 Relation between urinary oxalate excretion and urinary glycolate excretion.

Our data therefore support the conclusion that at least some of the oxalate excreted by children with CF has a metabolic origin.

Some clues about the nature of this metabolic process are given by results in individual patients. The index patient was retested as part of the series and found to have normal amounts of excretion of oxalate and glycolate, suggesting that any metabolic process could be transient. Another child had increased excretion of oxalate and glycolate but did not develop renal stones, which suggests that other factors may be involved in the formation of renal stones. One child had normal excretion of oxalate but increased excretion of glycolate which could be due an atypical pattern of ingestion of sugars (for example, xylose¹⁷).

Factors not considered in this study could alter renal stone forming potential in CF. Sidhu *et al*¹⁰ recently reported that a bacterium involved in oxalate metabolism (*Oxalobacter formigenes*) was frequently present in the stool of healthy controls but infrequently present in the stool of young children with cystic fibrosis; the only child with normal amounts of *O formigenes* had not received antibiotics prior to the stool collection. We have no data on the presence of this bacterium on our sample and this issue will require further study. Some therapeutic agents used in CF could influence stone formation: gentamicin enhances oxalate crystal formation¹⁸ and damages urothelium which can enhance stone formation¹⁹ (none of these patients was on an aminoglycoside during the urine collections); high dietary intake of sodium increases calcium excretion²⁰ (none of our patients received salt supplementation during the urine collections).

In conclusion, the current report is the first study to relate urinary excretion of oxalate and the simultaneous urinary excretion calcium in 24 hour urine samples from children with CF, and to relate the excretion of each compound to that of the other in the same child. We have shown that 15 of 24 children with CF had reduced amounts of urinary calcium excretion, including several children with amounts of oxalate excretion high enough to risk stone formation. It is possible that the reduced level of urinary calcium excretion is protecting children with hyperoxaluria from forming renal stones.

Altered renal handling of calcium in cystic fibrosis could reflect several factors such as reduced uptake caused by deficiency of vitamin D or transport defects in the kidney. A direct

effect of alterations in calcium handling by the CF kidney cannot be excluded in the light of recent work that shows that other defects in ion transport affecting sodium²¹ or chloride²² also affect calcium excretion.

Dr Barbara Phillips and the late Mr Ian Ward identified the clinical issue and initiated the study. We are grateful to Manchester Grammar School for providing funding for the purchase of reagents. Glycolate estimations were carried out by the Department of Biochemistry, University College London to whom we are grateful.

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