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Urinary excretion substances in patients with cystic fibrosis: risk of urolithiasis?

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Abstract. Patients with cystic fibrosis (CF) have an increased risk of urolithiasis/nephrocalcinosis. To determine potential mechanisms responsible, we studied the urinary excretion of lithogenic and stone-inhibitory substances and calculated the urinary saturation for calcium-oxalate (CaOx), brushite ($CaHPO_4$), and uric acid (UA). We examined 24-h urines in 63 patients with CF (34 female, 29 male) aged 5 months to 36 years. Renal ultrasonography was performed at the time of urine collection. Hyperoxaluria was found in 25 patients (range 0.51–1.71 mmol/1.73 m^2 per 24 h). Urinary Ca was increased in 13 patients (4.1–8.22 mg/kg per 24 h). Hyperuricosuria was found in 16 patients (5.2–18.0 mmol/1.73 m^2 per 24 h) and hypocitraturia in 14 patients (0.07–1.14 mmol/1.73 m^2 per 24 h). CaOx saturation was elevated in 26 patients, related to hyperoxaluria in 19 patients. $CaHPO_4$ saturation was increased in 19 patients and UA saturation in 11 patients. Urolithiasis in situ was diagnosed in 1 patient; 3 patients previously had renal stones; 4 patients had present nephrocalcinosis. Elevated excretion of lithogenic substances and urinary supersaturation might lead to the higher risk of urolithiasis/nephrocalcinosis in patients with CF.

Key words: Cystic fibrosis – Urolithiasis – Calcium – Oxalate – Uric acid – Citrate

Introduction

Patients with cystic fibrosis (CF) are predisposed to develop urolithiasis and/or nephrocalcinosis [1–6]. The incidence of stone disease is increased in older CF patients, as a longer life expectancy increases the unfavorable results of pancreatic insufficiency and pulmonary failure [3, 4]. The prevalence of urolithiasis was found to be higher in patients with CF compared with the normal population [5]. Medullary nephrocalcinosis was shown at post-mortem autopsy in up to 92% of CF patients [6].

The mechanisms responsible for the stone disease in CF are incompletely understood. The few data published about urinary lithogenic and stone-inhibitory substances in patients with CF disclosed secondary hyperoxaluria as a risk factor [2–4]. Although a primary renal leak for calcium (Ca) was suggested [6], hypercalciuria was found only in a minority of patients with CF [2, 7, 8].

Secondary (enteric) hyperoxaluria due to pancreatic insufficiency and malabsorption is present in patients with one of several malabsorption syndromes [9, 10]. Hyperoxaluria considerably increases the urinary calcium-oxalate (CaOx) saturation and therefore the risk of CaOx agglomeration or crystal formation [11].

In an effort to determine potential mechanisms for the increased risk of urolithiasis in CF, we studied the excretion of urinary lithogenic and stone-inhibitory substances, and calculated the urinary saturation of CaOx, brushite ($CaHPO_4$), and uric acid (UA).

Patients and methods

We examined 24-h urines in 63 patients with CF (34 female, 29 male) aged 5 months to 36 years (mean 15.5 ± 7.7 years) with different grades of disease severity. Follow-up 24-h urines were collected after 8–12 months in 20 patients (10 female, 10 male) aged 15 months to 37 years (mean 15.9 ± 9.1 years), with an initial elevated urinary excretion of lithogenic factors or a decreased excretion of stone-inhibitory substances.

All patients received pancreatic enzymes, vitamins, and mucolytics (Table 1). Patients receiving diuretics or dexamethasone, patients with an additional intestinal disturbance, those with a cardiac disorder or a renal malformation, and immobilized patients were excluded from the study. Patients ingested a high-energy, high-protein diet, but food high in oxalate was avoided (e.g., spinach, rhubarb, chocolate).

Twenty-four-hour urines were collected during an admission for routine parenteral antibiotic therapy if the patients had neither a pulmonary exacerbation of disease, nor an acute infection. Urines were collected in attached plastic bags in small infants and emptied at

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Table 1. Patients with cystic fibrosis – clinical data [mean (SD)]

Number	Age (years)	Pancreatic enzymes (IU/kg body weight)	Vitamin E (mg/kg per 24 h)	Vitamin C (mg/kg per 24 h)	Serum creatinine[a] (μmol/l)	GFR[a] (ml/min per 1.73 m²)
6	5 months to 4.9	18,959 (21,385)*	10.6 (1.4)*	7.95 (5.6)	31.4 (12.9)	123.8 (34.7)
10	5–10.9	6,846 (3,340)	7.7 (2.6)	10.6 (7.4)	43.2 (6.6)	127.7 (37.5)
28	11–20	5,823 (3,846)	6.3 (2.6)	8.4 (2.8)	54.1 (13)	117.3 (23.8)
19	>20	3,660 (3,185)	5.4 (2.3)	7.2 (3.1)	58 (14)	120.4 (27.9)
Mean	15.5 (7.7)	6,420 (8,175)	6.6 (2.8)	8.4 (4.3)	51.6 (14.7)	120.2 (32)
Follow-up	15.9 (9.1)	5,129 (3,806)	6.5 (3.4)	9.7 (5.8)	44.4 (15.3)	141.2 (40.5)

GFR, glomerular filtration rate
*$P < 0.05$ versus all other groups
[a] Normal values according to references [18, 20, 21]

2-h intervals into bottles containing Thymol preservative [12], or urine was directly voided into prepared sampling bottles. The urines were immediately stored at –20° C and analyzed within 1 months. The adequacy of urine collection was assured by comparing the measured creatinine excretion with normal values, recognizing that creatinine excretion might be diminished in malnourished children and that the individual excretion varies from day to day [13, 14]. Renal ultrasonography was performed at the time of urine collection using a real-time sector scanner with a 5- or 7.5-MHz transducer (Acuson Computed Sonography, 128XP/10, Mountain View, Calif., USA).

Separation and quantification of urinary oxalate was performed using a Dionex Series 4000i gradient ion chromatography system (Dionex, Sunnyvale, Calif., USA, [15]). Urinary concentrations of Ca and magnesium were determined by atomic absorption spectroscopy, phosphorus and sulfate by ion chromatography [16]. Citric acid and UA were analyzed enzymatically using the citrate lyase and uricase method, respectively [17]. Creatinine was measured by a standard kinetic Jaffé procedure; sodium, chloride, and potassium were measured by flame photometry. Glomerular filtration rate (GFR) was calculated using the formula of Schwartz et al. [18].

All measurements of lithogenic and stone-inhibitory substances, and urinary creatinine, sodium, chloride, potassium, the 24-h urine volume, pH, and specific gravity were entered in the EQUIL 3.0 program to calculate the urinary CaOx, $CaHPO_4$, and UA saturation [19].

Statistical analyses were performed using the Wilcoxon rank or sum-rank test, and P values less than 0.05 were judged as significant. Clinical data were expressed as mean (SD). Results with regard to urinary lithogenic and inhibitory substances and the urinary saturation are shown in Fig. 1 and 2. (Urinary excretion parameters are expressed in mmol/1.73 m² per 24 h, calcium excretion is shown in mg/kg body weight per 24 h, and urinary saturations are expressed in relative units.) Follow-up data refer to the 20 patients with initial abnormalities who were restudied after a period of 8–12 months.

The study was approved by the ethical committees of the participating hospitals and consent was obtained from the patients or their parents before urines were collected.

Results

The youngest patients received a higher ($P < 0.05$) substitution of pancreatic enzymes and vitamin E than all other age groups (Table 1). However, pancreatic enzyme substitution in each group resulted in a normal fecal fat excretion in all patients. Renal function was normal in all patients, according to serum creatinine and calculated GFR (Table 1, [20, 21]). The 24-h urine volume was normal for age in all but 1 patient [22, 23]. Neither the most common mutation in CF, the ΔF508 allele, nor differences in clinical severity influenced urinary excretion parameters.

Hyperoxaluria was found in 25 patients (11 female, 14 male, range 0.51–1.71 mmol/1.73 m² per 24 h, normal < 0.05 [24], Fig. 1). Oxalate excretion tended to be higher in males than females and the prevalence of hyperoxaluria was higher in older patients.

Urinary Ca excretion was elevated in 13 patients (5 female, 8 male), with a range of 4.1–8.2 mg/kg body weight per 24 h (Fig. 1, normal < 4 mg [25]). Hypercalciuria was equally distributed throughout all age groups.

Hyperuricosuria was found in 16 patients (7 female, 5 male, range 5.2–18.0 mmol/1.73 m² per 24 h or > 0.5 mg/dl of glomerular filtrate, respectively, Fig. 1, [22, 23, 26]).

Urinary citrate excretion was diminished in 14 patients (7 female, 7 male, range 0.07–1.14 mmol/1.73 m² per 24 h) when compared with normal age- and sex-related values obtained in previous studies (Fig. 1, [22, 23]. Hypocitraturia was linked with hyperoxaluria in 2 patients and with an increase in CaOx saturation in 1.

Urinary CaOx saturation was elevated in 26 patients (13 female, 13 male, Fig. 2, [22, 27]) and attributable to secondary hyperoxaluria in 19 patients (76%). CaOx supersaturation was equally distributed by age and sex, except for the youngest, where an extremely elevated saturation was present in only 1 female patient (24.84). $CaHPO_4$ saturation was elevated in 19 patients (9 female, 10 male, Fig. 2). UA supersaturation was found in 11 patients (6 female, 5 male, Fig. 2) and was always related to low urinary pH values.

Repeat urinary examination after a period of 8–12 months in 20 patients showed persistently elevated concentrations of lithogenic substances or hypocitraturia. Urinary CaOx and $CaHPO_4$ supersaturation also remained increased, but interestingly UA supersaturation persisted in only 1 patient.

Bilateral, radiopaque kidney stones in situ were diagnosed in 1 patient with a history of hematuria and colicky abdominal pain. A previous history of urolithiasis was known in 3 different patients; stone analysis in 1 of them showed CaOx-monohydrate/-dihydrate urolithiasis. Medullary hyperechogenicity was diagnosed in 4 other patients with microhematuria; 1 of them had marked nephrocalcinosis. Renal biopsies were not performed. All family histories were negative for either urolithiasis or nephrocalcinosis.

All 8 patients with a history of urolithiasis or nephrocalcinosis were older than 15 years of age at the time

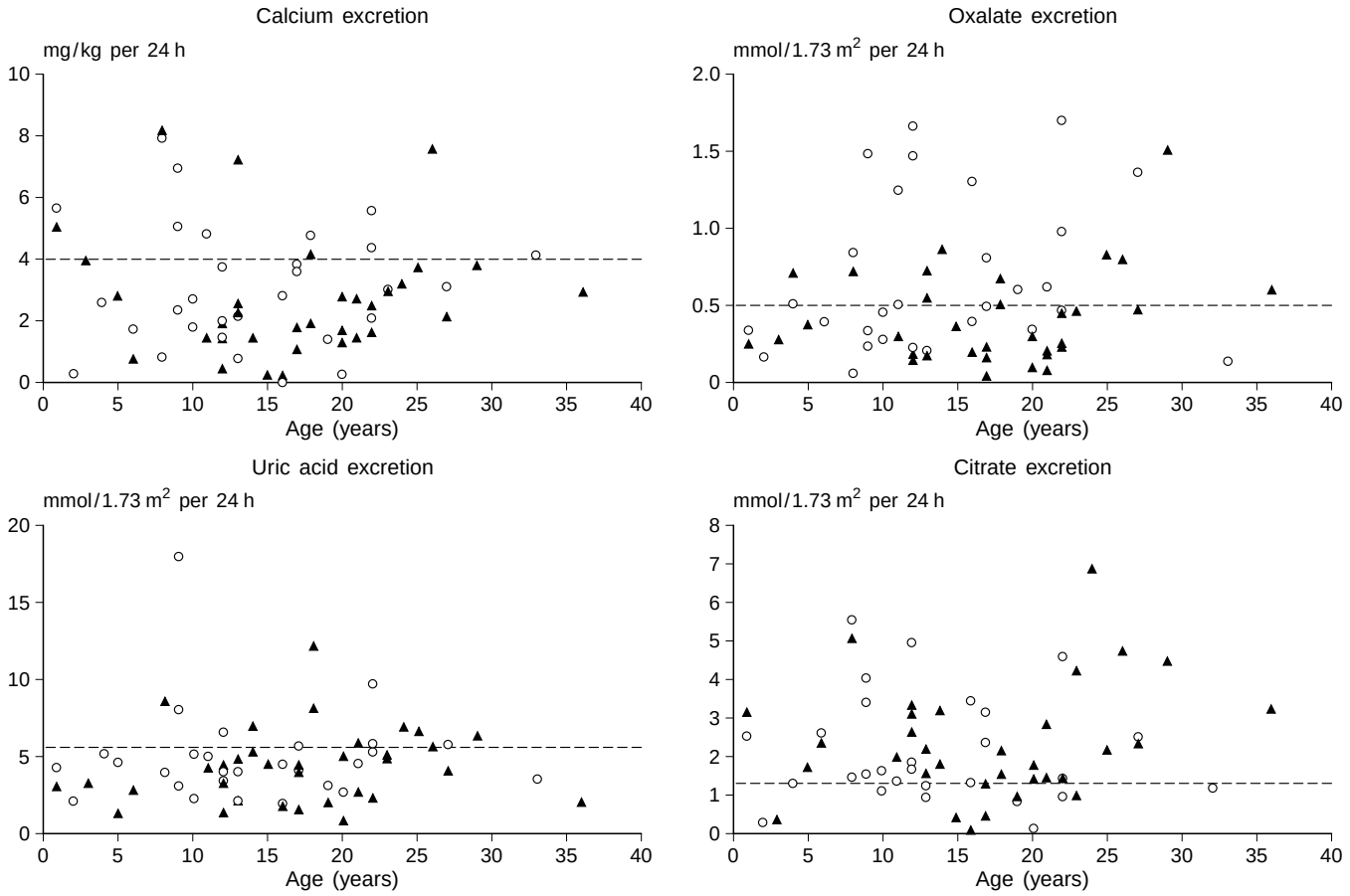

Fig. 1. Urinary oxalate, calcium, uric acid, and citrate excretion in patients with cystic fibrosis. *Lines* = upper limit of normal (citrate = *lower* limit of normal). ○, Male patients; ▲, female patients

of diagnosis. Their urinary CaOx and/or $CaHPO_4$ saturations were persistently elevated, except for 1 patient with nephrocalcinosis, who only showed hypocitraturia.

Discussion

With the aim of identifying the factors responsible for the high incidence of urolithiasis/nephrocalcinosis in patients with CF [5, 6], we examined the urinary excretion of lithogenic and stone-inhibitory substances in a contemporary population of 63 patients with CF. Secondary hyperoxaluria stood out as the main risk factor. Additionally, Ca and UA excretions were increased in up to 25% of patients. Hypocitraturia was also present in more than 20% of our patients. Consequently, we calculated a supersaturation for CaOx in 41%, for $CaHPO_4$ in 30%, and for UA in 17.5% of the CF patients.

Secondary hyperoxaluria is expected in patients with malabsorption syndromes [9, 10]. Oxalate absorption increases when Ca binds in the intestine to fatty acids rather than to oxalate and malabsorbed bile salts induce a higher permeability for oxalate in the distal colon. Oxalate is 10 to 15 times more potent than Ca in increasing the urinary CaOx saturation [11]. Therefore, a higher risk of CaOx precipitation might occur along with hyperoxaluria.

Could the pancreatic enzyme substitution be responsible for hyperoxaluria? The number of patients with hyperoxaluria increased when enzyme substitution decreased. However, hyperoxaluria was also found in the youngest patients receiving the highest enzyme substitution. Patients with or without hyperoxaluria received a comparable enzyme dosage, and all had normal fecal fat excretions, hence the amount of substituted enzyme seemed not to be of great importance as a cause of hyperoxaluria.

Vitamin C, an important precursor of oxalate, could have influenced urinary oxalate excretion. Daily ascorbic acid intake, however, did not differ between patients with or without hyperoxaluria.

The number of hyperoxaluric patients increased from the youngest patients (33%) to adolescent and adult patients (42%). Disease severity, however, seemed not to influence oxalate excretion or any other urinary parameter. The routine antibiotic treatment necessary in CF patients might lead to a reduction or to a loss of intestinal oxalate-degrading bacteria, such as *Oxalobacter formigenes* [28]. Oxalate then would more likely be reabsorbed than excreted in the feces.

As a response to hyperoxaluria, we could not find evidence in this study to support the previously reported "protective" hypocalciuria in patients with CF [2, 7, 8]. In contrast, our findings would rather support the presence of

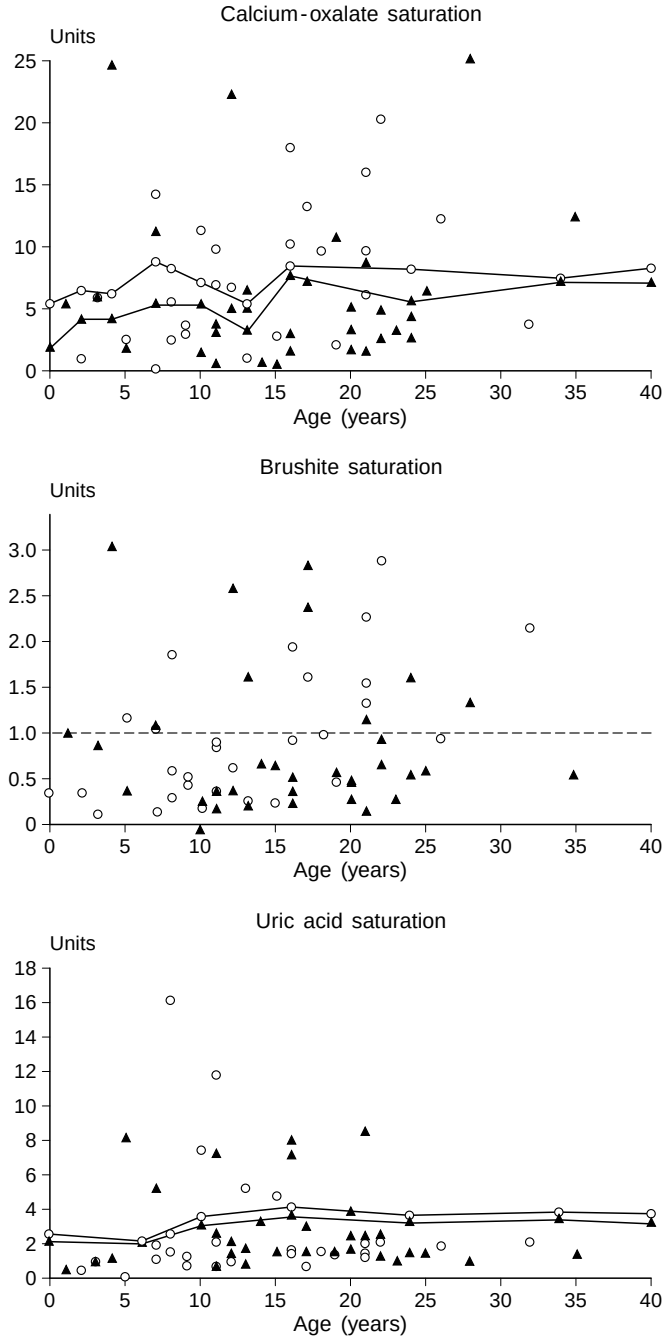

Fig. 2. Urinary calcium-oxalate, brushite, and uric acid saturation in patients with cystic fibrosis. *Lines* = upper normal limit of *normal* for male patients ❍ and female patients ▲

a renal Ca leak [6]. The high Ca excretion in our patients was neither linked to an overt acidosis nor to the use of diuretics. However, hypercalciuria might be due, in part, to the high chloride excretion in CF patients [29]. A low bone density, which was reported in patients with CF, can be correlated with hypercalciuria [30, 31].

The high-energy, high-protein diets encouraged in patients with CF can increase UA production. Therefore, the appearance of hyperuricosuria was not unexpected and has been previously reported [32]. High-dose pancreatic extract therapy has induced hyperuricosuria in patients with CF [33]. We, however, did not find such a correlation. Importantly, UA saturation was only elevated when a low urinary pH (<6.0) led to a less-soluble form of UA.

Hypocitraturia was recently reported to be a possible risk factor for stone disease in patients with CF and, in general, in patients with malabsorption syndromes [34]. Hypocitraturia was linked with hyperoxaluria and/or CaOx supersaturation in only 2 of our CF patients. In 1 patient with nephrocalcinosis, however, hypocitraturia was the only risk factor.

Even though a supersaturated urine does not necessarily induce crystal formation, calculation of the urinary saturation might allow a better prediction of the risk of urolithiasis/nephrocalcinosis. This was substantiated, as 7 of 8 patients with a history of urolithiasis or nephrocalcinosis showed urinary supersaturation for either CaOx or $CaHPO_4$. UA supersaturation, however, did not induce urolithiasis.

The incidence of urolithiasis in our region is 0.5% in adults (4% prevalence [35]); the incidence of childhood urolithiasis is low (2%–5% of all adult stone events). Therefore, a 6.3% incidence for both a history of urolithiasis or nephrocalcinosis in CF patients was considerably high.

In conclusion, an elevation of urinary lithogenic substances could promote the development of urolithiasis and/or nephrocalcinosis in patients with CF. Whether a higher substitution of pancreatic enzymes will be useful to decrease the substantial risk of hyperoxaluria has to be evaluated further.

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Literature abstract

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Parathyroid hormone prevents 1,25(OH)$_2$D$_3$ induced down-regulation of the vitamin D receptor in growth plate chondrocytes in vitro

Günter Klaus, Tanja May, Ulrike Hügel, Barbara von Eichel, Julian Rodriguez, Porfirio Fernandez, Jörg Reichrath, Eberhard Ritz, and Otto Mehls

1,25(OH)$_2$D$_3$ has an antiproliferative effect on growth plate chondrocytes when given in high doses, whereas low doses stimulate chondrocyte proliferation. In the present in vitro study we investigated the effects of parathyroid hormone (PTH) when given concomitantly with 1,25(OH)$_2$D$_3$ on cell proliferation and vitamin D receptor (VDR) regulation. Freshly isolated rat tibial chondrocytes were grown in monolayer cultures or in agarose stabilized suspension cultures (10% charcoal-treated FCS). VDR expression was determined by RT-PCR generating a 297 bp fragment and by binding assays (Scatchard analysis) with [^{3}H]-1,25(OH)$_2$D$_3$. Cell proliferation was measured by [^{3}H]-thymidine incorporation, growth curves in monolayer cultures and by colony formation in agarose-stabilized suspension cultures. Optimal concentration of 1,25(OH)$_2$D$_3$ (10^{-12} M) and of PTH fragments [bPTH(1–34) or hPTH(28–48), 10^{-10} M] showed additive effects on DNA synthesis of and colony formation by growth plate chondrocytes. This may be explained in part by an up-regulation of VDR by PTH: PTH increased both mRNA expression of VDR and binding capacity. 1,25(OH)$_2$D$_3$ (10^{-12} M) induced an up-regulation of the VDR within 24 h followed by a down-regulation after incubation for more than 24 h. PTH fragments added concomitantly prevented the down-regulation seen with 1,25(OH)$_2$D$_3$. These findings provide evidence that PTH is a growth promoting hormone that also modulates the effects of 1,25(OH)$_2$D$_3$ by regulating the VDR status of 1,25(OH)$_2$D$_3$ target cells.