

DIAGNOSTIC AND THERAPEUTIC APPROACHES IN PATIENTS WITH SECONDARY HYPEROXALURIA

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1. ABSTRACT

Secondary hyperoxaluria is due either to increased intestinal oxalate absorption or to excessive dietary oxalate intake. Certain intestinal diseases like short bowel syndrome, chronic inflammatory bowel disease or cystic fibrosis and other malabsorption syndromes are known to increase the risk of secondary hyperoxaluria. Although the urinary oxalate excretion is usually lower than in primary hyperoxaluria, it may still lead to significant morbidity by recurrent urolithiasis or progressive nephrocalcinosis. A clear distinction between primary and secondary hyperoxalurias is important. As correct classification may be difficult, appropriate diagnostic tools are needed to delineate the metabolic background as a basis for optimal treatment.

We developed an individual approach for the evaluation of patients with suspected secondary hyperoxaluria. First, 24 h urines are examined repeatedly for lithogenic (e.g. calcium, oxalate, uric acid) and stone-inhibitory (e.g. citrate, magnesium) substances, and the patients are asked to fill in a dietary survey form. Urinary saturation is calculated using the computer based program EQUIL2, and the BONN-Risk-index is determined. The measurement of plasma oxalate and of urinary glycolate helps to distinguish between primary and secondary

hyperoxalurias. If secondary hyperoxaluria is suspected, the stool is examined for *Oxalobacter formigenes*, an intestinal oxalate degrading bacterium, as lack or absence may lead to increased intestinal oxalate absorption. The last diagnostic step is to study the intestinal oxalate absorption using [¹³C₂]oxalate.

Depending on the results, various therapeutic options are available: 1) a diet low in oxalate, but normal or high in calcium, 2) a high fluid intake (> 1.5 L/m²/d), 3) medications to increase the urinary solubility, 4) specific therapeutic measures in patients with malabsorption syndromes, depending on the underlying pathology, and 5) intestinal recolonization of *Oxalobacter formigenes* or the treatment with other oxalate degrading bacteria.

2. INTRODUCTION

As the prevalence of both urolithiasis and nephrocalcinosis steadily increased over the last years, e.g. from 4 % in 1979 (incidence 0.54 %) to 4.7 % in 2000 (incidence 1.47 %) according to recent data from Germany (own unpublished survey), it is of utmost importance to search for the pathophysiological background of stone disease in every patient. This is the only adequate

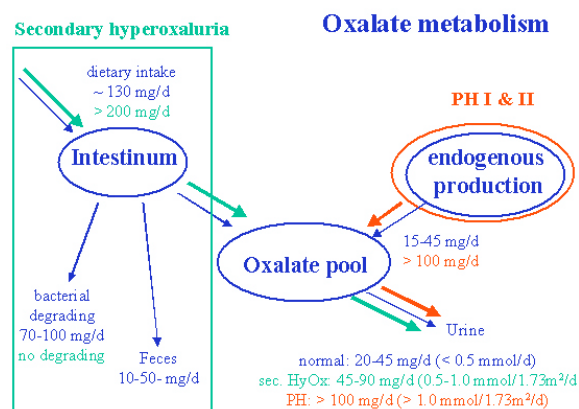


Figure 1. Normal oxalate metabolism (in blue) and reasons for increased urinary oxalate excretion. Red: primary hyperoxalurias with endogenous overproduction of oxalate. Green: secondary hyperoxalurias due to increased dietary intake or to elevated intestinal absorption of oxalate.

procedure to prevent stone recurrence or growth and progressive nephrocalcinosis.

2.1. Hyperoxaluria as a risk factor for urolithiasis and nephrocalcinosis

In more than 70 % of patients with urolithiasis calcium oxalate is the main component, with hyperoxaluria being one of the main risk factors. Oxalic acid is an end product of human metabolism. It does not appear to be needed for any process in the human body and is normally completely excreted via the kidneys. Hyperoxaluria is the main risk factor for calcium oxalate (CaOx) urolithiasis and nephrocalcinosis. Supersaturation of CaOx is mainly determined by the relative concentrations of calcium and oxalate in the urine. As the molar oxalate concentration is usually ten times lower than that of calcium, even a slight increase of the former has a much stronger effect than calcium on the urinary saturation level and hence on the crystallization process (1, 2). Hence, it is important 1) to determine the urinary oxalate excretion in patients with stones or nephrocalcinosis, 2) to look for the metabolic background of hyperoxaluria, if present, and 3) to start specific therapeutic measures with the aim to prevent recurrent stone formation or progression of nephrocalcinosis.

2.2. Primary and secondary hyperoxalurias

Primary hyperoxaluria (PH) results from endogenous (primary) overproduction of oxalic acid (3). In contrast, secondary hyperoxaluria is due to increased intestinal absorption (enteric) or to excessive (dietary) intake of oxalate (4, 5).

The autosomal-recessive inherited **primary hyperoxalurias** are liver specific defects of the glyoxylate metabolism (6-8). Early diagnosis is mandatory but is often delayed or overlooked and therefore it appears, as if PH is easily underestimated. Data from Europe (UK, Switzerland and France) suggest that 1 in 60,000 to 120,000 children

suffer from the primary form of PH. The disease is, however, far more common in other countries like Tunisia, where PH I is the cause of ESRF in 13% of pediatric patients as compared to only 0.3% in Europe (3).

Two forms are currently distinguished on a genetic level, but other types of primary hyperoxaluria likely exist (9, 10). Primary hyperoxaluria type I (PH I, MIM 259900), characterized by elevated urinary excretion of both oxalate and glycolate, is due to low or absent activity of the liver-specific peroxisomal alanine: glyoxylate aminotransferase (AGT, AGXT-gene on chromosome 2q37.3) (6). Primary hyperoxaluria type II (PH II, MIM 260000) is caused by diminished activity of glyoxylate reductase, an enzyme also possessing D-glycerate dehydrogenase and hydroxypyruvate reductase activity, leading to elevated urinary excretion of both oxalate and L-glyceric acid (gene on chromosome 9p11, (7, 8)). The urinary excretion of oxalate is strongly elevated (> 1 mmol/1.73m²BSA/day, normal < 0.5 (3)) in both forms of primary hyperoxaluria. Hence, the urine is supersaturated with respect to calcium-oxalate ($\beta_{\text{UCaOx}} > 10$ rel. units), which results in recurrent stone formation and/or nephrocalcinosis, both leading to progressive kidney damage (3). The concentration of plasma oxalate and the plasma calcium-oxalate saturation (β_{PCaOx}) correlate inversely with the glomerular filtration rate (11). The more the plasma is supersaturated ($\beta_{\text{PCaOx}} > 1$) the more crystals are deposited in the parenchyma of most solid organs, the bones, joints, and in the retina (= systemic oxalosis) (3, 11). Although PH I is a monogenic disease, there is a wide clinical, biochemical and genetic heterogeneity, with some patients presenting in the first years of life with kidney failure and others who only have sporadic passage of stones with preserved kidney function (3).

The metabolic background and the prevalence of the **secondary hyperoxalurias** have been less well studied than that of the primary form (3). In our population of stone patients 18 % of adults and 8.6 % of children are found to have secondary hyperoxaluria as their single risk factor, with up to 43 % of patients incorporating hyperoxaluria next to other parameters to their personal stone risk profile (20). Secondary hyperoxaluria is either due to increased intestinal oxalate absorption (enteric) or results from excessive dietary oxalate intake (dietary hyperoxaluria, Figure 1 (1, 5)). Patients with intestinal disease have an increased risk of hyperoxaluria, particularly after bowel resection (short bowel syndrome), after bypass operation, in chronic inflammatory bowel disease or cystic fibrosis, and in other malabsorption syndromes (12-18). In addition, intoxication with ethylene glycol leads to hyperoxaluria (3). Although the urinary oxalate excretion is usually lower in patients with secondary (< 1 mmol/1.73m²BSA/24 h) as compared to primary hyperoxaluria, the former may nevertheless lead to significant morbidity, i.e. to recurrent urolithiasis or progressive nephrocalcinosis (12-18). Distinction between primary and secondary forms of hyperoxaluria is essential, but may be difficult. Appropriate diagnostic tools are therefore required for the correct classification and management of such patients.

Table 1. Oxalic acid content of specific food (4, 19-22)

Food	Oxalate content mg/100 g		Food	Oxalate content mg/100 g	
	Soluble	Total		Soluble	Total
Fruits:			Bread:		
Banana	0.1-2.2	0.5-23.9	Rye bread	0.9	
Apple	0.3-1.8	0.4-5.8	White bread	4.9-8.6	
Orange	0.2	1.8	Cornflakes	1.9-2.3	3.0-3.1
Strawberry	0.6-1.9	1.5-4.3	Sweets:		
Gooseberry	3.2	27	1 bar with chocolate	7.1	37.9
Vegetables:			Cocoa powder		154-980
Asparagus (boiled)	0.5-1.1	1.8-3.1	Drinks:		
Potatoes (cooked)	8.8-18.9	8.8-35.3	Coffee	0.5-0.7	
Beans (fresh)	1.5	13.9	Black Tea	2.5-6.2	
Spinach (cooked)	33.3-168	100-627	Ice Tea	0.46-1.72	0.35-2.05
Rhubarb	380	570-1900	Beer	1.7-1.8	
Tomato	2.5-4.5	3.7-13.7	Coke	0.05	

3. METABOLIC BACKGROUND OF SECONDARY HYPEROXALURIA

3.1. Dietary hyperoxaluria

Oxalate is found in many foods in varying amounts, but from the daily oxalate intake of approximately 80 – 130 mg (normal Western diet 50-100 mg/d, vegetarian diet ~150 mg/d) only a small fraction is absorbed at the intestinal tract (4, 19, 20). The oxalate content of some food is shown on Table 1 and may also be found at webpages like <http://www.ixion-biotech.com>. Certain foods have a very high oxalate content, particularly dark-green leafy vegetables (spinach, rhubarb), tea, beet roots, nuts and cocoa (20, 21). However, our ability to obtain reliable and complete data is hampered by differences in analytical methods and because the oxalate content of vegetables depends greatly on the age and maturity of the plant as well as the plant species (22). In addition, the amount of dietary oxalate that is finally absorbed is strongly influenced by the presence of other nutrients (19-22). For example, if food with a high oxalate content is ingested together with a calcium-rich drink, e.g. milk, less free and therefore soluble oxalate is available for absorption, as it is bound to calcium and excreted via the feces. Nevertheless, in general any additional dietary oxalate ingested does lead to an increase in urinary oxalate excretion (19, 23-25). Even a normal portion of spinach (150–200 g) will raise the urinary oxalate excretion (26).

Considerable controversy exists whether pharmacological doses of ascorbic acid, a precursor of oxalic acid, may cause dietary hyperoxaluria (27-29). Megadoses of ascorbic acid have been reported to lead to increased urinary oxalate excretion (27). This has, however, been contested by other authors, who claim that these findings are erroneous and are caused by non-enzymatic conversion of urinary ascorbate to oxalate before analysis in urines collected inadequately (3, 28). In addition, vitamin B6 deficiency was found to downregulate the alanine: glyoxylate aminotransferase activity, which led to hyperoxaluria and hyperglycollic aciduria. This seems to be due to the impaired metabolism of glyoxylate, comparable to the situation seen in patients with primary hyperoxaluria (3, 30). Finally, long-term parenteral nutrition may lead to

hyperoxaluria, particularly in preterm infants receiving amino-acid infusion (31).

3.2. Enteric hyperoxaluria

3.2.1. Malabsorption syndromes

It is well known that patients with malabsorption syndromes may develop hyperoxaluria (12-18). Normally, oxalate binds intestinally to calcium, and these nonabsorbable complexes are later excreted via the feces (Figure 2A, (5)). Hence, the intestinal calcium concentration has a strong effect on the amount of oxalate absorbed. Low calcium diets, which are still recommended for whatever reasons, result in hyperabsorption of oxalate, even when the dietary oxalate is normal, as more soluble oxalate is available for intestinal absorption. In malabsorption syndromes, calcium binds intestinally to malabsorbed fatty acids instead to oxalate and again, more soluble oxalate is available for absorption (Figure 2B, (5, 12)). Oxalate is normally absorbed within the first 2-4 hours after ingestion, because absorption takes place in the upper part of the intestinal tract (5). Malabsorption of bile acids, however, tends to enhance oxalate absorption at the distal colon.

Patients with malabsorption syndromes have a higher prevalence of urolithiasis and nephrocalcinosis. Recurrent urolithiasis and/or progressive nephrocalcinosis have been detected in up to 20 % of patients with Crohn's disease and in 11 % of patients with cystic fibrosis (own surveys). A study of the latter group of patients showed that they have indeed secondary, enteric hyperoxaluria (12).

3.2.2. Oxalate degrading bacteria

Another recently reported problem in patients with malabsorption syndromes, as well as in patients with recurrent urolithiasis due to hyperoxaluria, is the absence of oxalate degrading bacteria in the gut (32, 33). Especially the obligate anaerobe bacterium *Oxalobacter formigenes* is able to degrade oxalate via two enzymes (oxalyl-coenzyme A decarboxylase and formyl coenzyme A transferase (34)) to formate that is further metabolized. Other species with probable oxalate degrading capacity are *Enterococcus faecalis* and lactic acid bacteria (35, 36). However, the metabolic background of how they degrade oxalate is still a matter of debate. Nevertheless, it appears that the larger

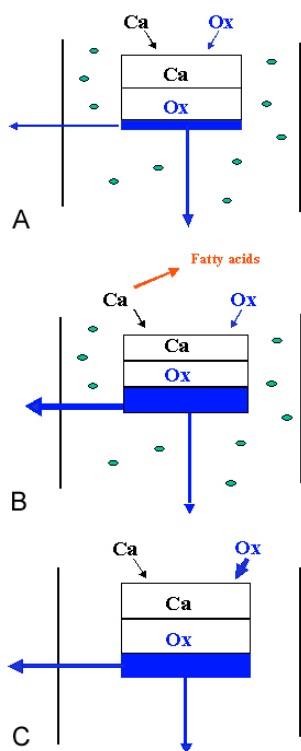


Figure 2. Normal intestinal oxalate handling (A), intestinal oxalate handling in patients with malabsorption syndromes (B) and in patients with a lack of oxalate degrading bacteria (C) (4, 5, 27). ● = Oxalobacter or other intestinal oxalate degrading bacteria.

part of the adult population (70-80 %) has such bacteria within the intestinal tract and is to some extent protected against oxalate hyperabsorption and elevated urinary oxalate excretion (32, 33). In fact, recurrent stone formers and patients with malabsorption syndromes or with hyperoxaluria of unknown origin mostly lack any kind of oxalate degrading bacteria (Figure 2C, (33)). This might be due to repeated administration of antibiotics (e.g. in CF patients), or to intestinal medication (e.g. in Crohn's disease) which prevents persistent intestinal colonization (37).

4. DIAGNOSTIC APPROACHES

We developed an individual approach for the evaluation of patients with suspected secondary hyperoxaluria. The first step is the repeated analysis of 24-h urine samples to determine the urinary risk profile.

4.1. Urine analysis

4.1.1. Lithogenic and stone inhibitory substances

Repeated analysis of both lithogenic and stone inhibitory substances is indicated in patients with stone disease, particularly in those with hyperoxaluria (38). During urine collection the intake of oxalate-rich food needs to be avoided, but patients should otherwise remain on their usual diet so that a personal risk profile can be established. We have repeatedly observed extreme intra-

individual variations when further urine specimens were examined. In such a situation we prefer to perform the evaluation on an inpatient basis with a standardized diet according to the recommendations of the German Society for Nutrition. This protocol yields reproducible results and will show the patient's risk profile. It is beyond the scope of this article to focus on all excretion parameters that influence the risk profile, but besides the urinary oxalate other lithogenic factors like calcium and uric acid and stone inhibitory substances like citrate and magnesium need to be analyzed.

Particular attention is necessary, when urine from recurrent stone formers, who are not stone free at the time of urine collection, is analyzed. Due to further stone growth during the collection period, the urine sample might be systematically depleted for lithogenic substances (39). Hence, substantially lower concentrations e.g. of oxalate might be determined resulting in false interpretation of the urinary risk profile.

4.1.2. Calculation of urinary saturation

The analysis of additional parameters (Na^+ , K^+ , Cl^- , PO_4^{3-} , SO_4^{2-} , creatinine) is required for the calculation of the urinary saturation index using computer models like the ion-equilibrium program EQUIL 2 (40). In addition, the urine volume, the pH and the specific weight are included. Calculation of this saturation index is, however, strongly biased. For example, patients with PH always have an extremely high urinary oxalate excretion but in contrast a low urinary calcium. As the CaOx saturation derived from the EQUIL program is primarily based on the ionic strength of these two substances, the index often underestimates the saturation and does not really express the stone risk profile of the PH-patients urine (own experiences).

4.1.3. BONN-Risk-index

The BONN-Risk-index (BRI) was developed by the Division of Experimental Urology, University of Bonn (41, 42). It is an effective and reliable method to determine the risk of calcium oxalate crystallization and hence of urolithiasis or nephrocalcinosis. The BONN-Risk-index experiment is carried out with unprepared urine samples (no dilution, no preservation, and no pH adjustment). The initial concentration of free ionized calcium (Ca^{2+}) is measured by a calcium sensitive electrode immediately after urine collection. In a standardized procedure, precipitation of calcium oxalate is triggered by a step-by-step addition of an ammonium oxalate solution (43). The ratio of (Ca^{2+}) and the amount of ammonium oxalate (Ox^{2-}) added until precipitation is termed the "BONN-Risk-index"; $\text{BRI} = (\text{Ca}^{2+}) / (\text{Ox}^{2-})$, (per L).

Since freshly voided instead of preserved specimens are used, all other urinary constituents are also taken into consideration with regard to the patient's personal risk profile. Thus, BRI excellently expresses the imbalance of promoters and inhibitors at an individual level. In combination with the data of the biochemical urine analysis and the results of specific tests (e.g. the [$^{13}\text{C}_2$]oxalate absorption test, see below), a very personal risk profile can be obtained for every patient.

4.2. Plasma oxalate

The determination of plasma oxalate (P_{Ox}) and glycolate is a powerful tool to distinguish primary from secondary forms of hyperoxaluria (44). Normal levels of P_{Ox} range from 0.5 to 7.5 $\mu\text{mol/l}$ depending on the method used (3, 45), and 10 $\mu\text{mol/l}$ is the upper cutoff point.

4.3. Stool examination

Oxalate degrading bacteria are our best “friends” with regard to the intestinal oxalate metabolism (33, 35-37). Therefore, we ask all our patients with hyperoxaluria to collect stool samples, which are then analyzed for the presence of bacteria such as *Oxalobacter formigenes* by direct culture or by PCR to detect bacterial mRNA (46). This is necessary for diagnostic purposes (secondary versus primary forms, absorptive hyperoxaluria?) and – in the near future – for therapeutic options as well (treatment with oxalate degrading bacteria).

4.4. The [$^{13}\text{C}_2$]oxalate absorption test

The determination of the intestinal oxalate absorption using [$^{13}\text{C}_2$]oxalate is the next diagnostic step. Patients have to follow a fluid schedule (a minimum of 1000-1500 ml/d) and the dietary recommendations (e.g. 800-mg calcium intake per day) prescribed by a certified nutritionist before the start of the test. After a baseline 24 h urine collection and informed consent have been obtained, a capsule containing 50 mg Na-oxalate (= 33.82 mg [$^{13}\text{C}_2$]oxalate) in patients > 30 kg, or 25 mg Na-oxalate in those < 30 kg, is given one hour before breakfast, and a second 24 h urine is collected into bottles containing 30 ml 6 N HCl. Oxalate absorption is determined by gas-chromatography/mass-spectrometry (25, 47).

The application of the absorption test is one further step to differentiate better between primary and secondary forms of hyperoxaluria, and to identify hyperabsorbers who need specific therapy. The test is safe and easy to perform and without risks, as the isotope used is stable. It is even applicable in small children, as soon as it is possible to collect 24-h urines.

5. DIAGNOSTIC EVALUATION IN PATIENTS WITH UNEXPLAINED HYPEROXALURIA

Taken all these diagnostic tools together, a stepwise approach to the patient with secondary hyperoxaluria is possible, even on an outpatient basis. This is best illustrated by a recent study on 29 pediatric patients with unexplained but presumably secondary hyperoxaluria (0.52-1.2 mmol/1.73m²BSA/24h), who had come to our attention because of urolithiasis, nephrocalcinosis, or persistent hematuria. We used our stepwise approach including the [$^{13}\text{C}_2$]oxalate absorption test in all 12 female and 17 male patients aged 9.8 +/- 3.3 years (range 3-16 years). The plasma oxalate was elevated (> 7.5 $\mu\text{mol/l}$) in 8/29 children. *Oxalobacter formigenes* was found only in 6 patients (21%).

Intestinal oxalate absorption ranges in normal adults from 1.7 to 20 % (mean 8 %) and in adult stone patients from 1.8 to 27 % (mean 10.2 %), thus there is quite

some overlap. As there exist no specific normal data for children, we used 10 % as the upper critical value. Using this definition, oxalate absorption was elevated in 9/29 patients (range 10.4-29.6 %), borderline in eight (8.8-9.9 %) and normal in 14 patients (1.9-8.7 %).

Hypercalciuria was found in five (range 4.4-7.2 mg/kg body weight/d) and hypocitraturia was observed in 6 other patients (range 0.07–1.2 mmol/1.73m²/24h). Renal ultrasound examination revealed hyperechoic kidneys in nine (33 %), nephrocalcinosis in 13 patients (44 %) and urolithiasis in two (7%); five were normal.

In conclusion, 17/29 patients have hyperoxaluria due to elevated or high intestinal oxalate absorption. Of the 4 patients with elevated P_{Ox} (16.1-23.0 $\mu\text{mol/l}$), three had normal intestinal oxalate absorption (3.3-7.4 %) and one had a slightly elevated value (11.5 %). However, the latter was definitively not the reason for the elevated P_{Ox} level. It remains thus to be seen whether these 4 patients have a mild form of (atypical) primary hyperoxaluria. In the remaining 4 patients with P_{Ox} levels of 7.5-9.2 $\mu\text{mol/l}$, oxalate absorption ranged from 7.7-11.6 %. Hence, the marginally elevated values of both P_{Ox} and absorption make it difficult to define the exact background of their elevated urinary oxalate excretion. Three patients with completely normal intestinal oxalate absorption and normal P_{Ox} levels presumably have a dietary form of hyperoxaluria.

6.THERAPEUTIC IMPLICATIONS AND PERSPECTIVES

Measures to increase the urinary solubility of oxalate by a high fluid intake (> 1.5 L/m²/d) and administration of either alkali-citrate or orthophosphate are important in any form of hyperoxaluria. Some patients with PH I respond to pharmacological doses of vitamin B6 (3, 38); liver transplantation is currently the only option for enzyme replacement therapy (3). In patients with secondary (dietary and absorptive) hyperoxaluria a diet low in oxalate but normal or high in calcium are advised. Specific therapeutic measures are required in patients with malabsorption syndromes, depending on their underlying pathology.

Still, the therapeutic arsenal currently available for secondary (and primary) hyperoxaluria has its limitations, hence other therapeutic approaches are welcome. The use of oxalate degrading bacteria like *Oxalobacter* to reduce the amount of oxalate available for intestinal absorption could become an option. Sidhu *et al.* reported successful and safe recolonization of *Oxalobacter formigenes* in rats, leading to a reduction of urinary oxalate excretion (46). Campiero *et al.* treated 5 hyperoxaluric patients with a preparation of lactic acid bacteria (Oxadrop) and observed a significant and persistent reduction in urinary oxalate excretion over time (36). Hence, treatment with oxalate degrading bacteria might be a safe and important future tool in the treatment of patients with hyperoxaluria. Another still hypothetical approach, suggested from experiments in rats, is the idea to enhance net colonic oxalate excretion (49).

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