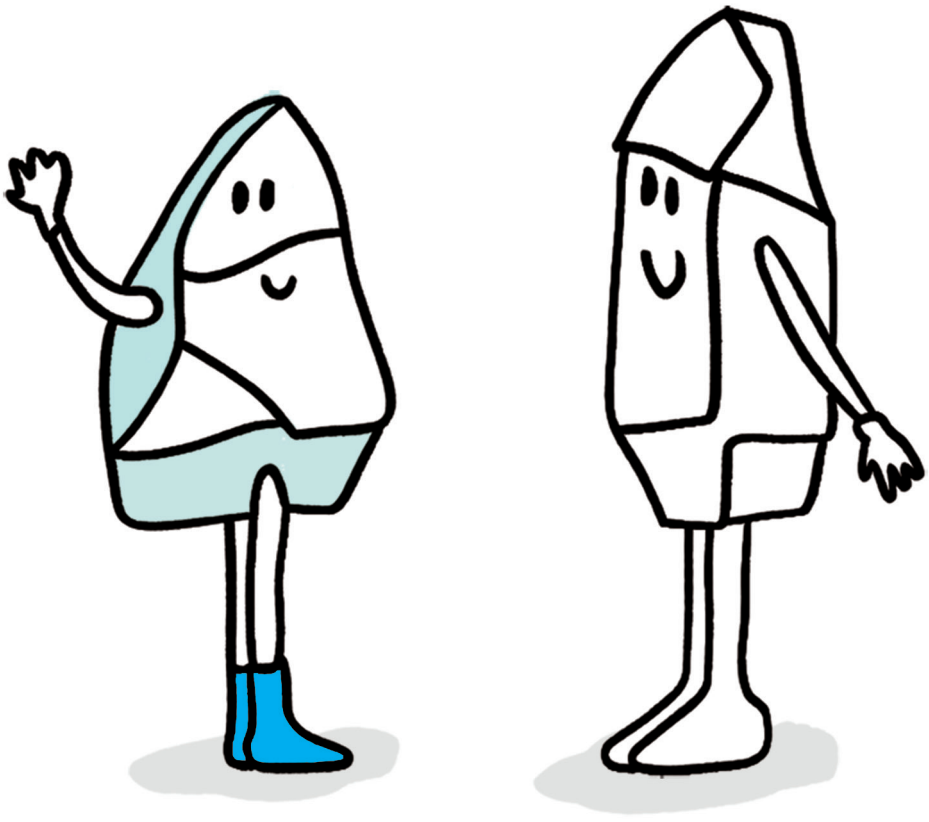
Enteric hyperoxaluria



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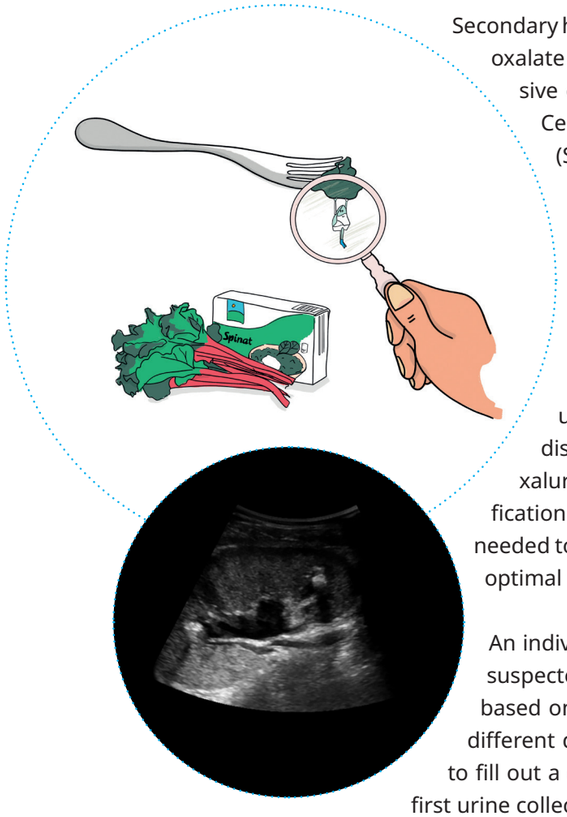
1. Abstract



Secondary hyperoxaluria is due either to an increased intestinal oxalate absorption (enteric hyperoxaluria, EH) or to excessive dietary oxalate intake (dietary hyperoxaluria, DH). Certain intestinal diseases like short bowel syndrome (SBS), chronic inflammatory bowel disease (IBD), cystic fibrosis (CF), or other malabsorption syndromes are known to increase the risk of secondary hyperoxaluria. The urinary oxalate excretion can be massively elevated (above 1 mmol/1.73m^2/d, normal < 0.5) and then be comparable to that of patients with primary hyperoxaluria. Therefore it may lead to significant morbidity due to recurrent urolithiasis or progressive nephrocalcinosis. A clear distinction between primary and secondary hyperoxalurias is important and is possible. As correct classification may be difficult, appropriate diagnostic tools are needed to delineate the metabolic background as a basis for optimal treatment.

An individual approach for the evaluation of patients with suspected secondary hyperoxaluria should primarily be based on repeated analysis of 24-h urines collected under different dietary regimens for which the patients are asked to fill out a dietary survey form. If hyperoxaluria is found in a first urine collection and under the patients' normal diet, two consecutive urine collections should follow under a low and then a high oxalate diet. With this, and with the evaluation of other markers of primary hyperoxaluria (glycolate, glyceric acid and hydroxy-oxoglutarate), a clear distinction of secondary from primary hyperoxaluria is possible. If a primary hyperoxaluria is suspected according to urinary metabolites, genetic testing should proof diagnosis. If a patient is in chronic kidney disease and urine analysis is no longer applicable, the measurement of plasma oxalate and glycolate helps to distinguish between primary and secondary hyperoxalurias.

Currently the following treatment options are available for secondary hyperoxaluria:
1) a diet low in oxalate, but normal or enriched in calcium,
2) a high fluid intake (> 1.5 L/m^2/d),
3) medications to increase the urinary solubility,
4) specific therapeutic measures in patients with malabsorption syndromes, depending on the underlying pathology. Hopefully in the near future treatments with oxalate-degrading bacteria or enzyme medications should be available.

2 Introduction

2. Introduction

2.1. Hyperoxaluria as a risk factor for urolithiasis and nephrocalcinosis

Oxalic acid is an end product of human metabolism. It is not needed for any process in the human body and is normally excreted via the kidneys and to some extend also via the intestinal tract. Hyperoxaluria is one of the main risk factors for calcium oxalate (CaOx) urolithiasis and nephrocalcinosis (Figure 1). Supersaturation for CaOx is mainly determined by the relative concentrations of calcium and oxalate in the urine (or in blood). As the molar oxalate concentration is usually ten times lower than that of calcium in urine, even a slight increase of the former has a much stronger effect than the latter on the crystallization process. Hence, it is important 1) to determine the urinary oxalate excretion in all patients with CaOx-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.

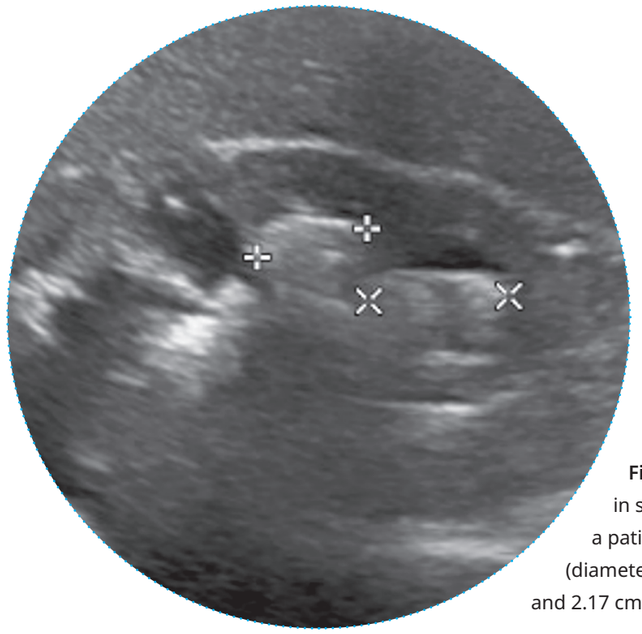


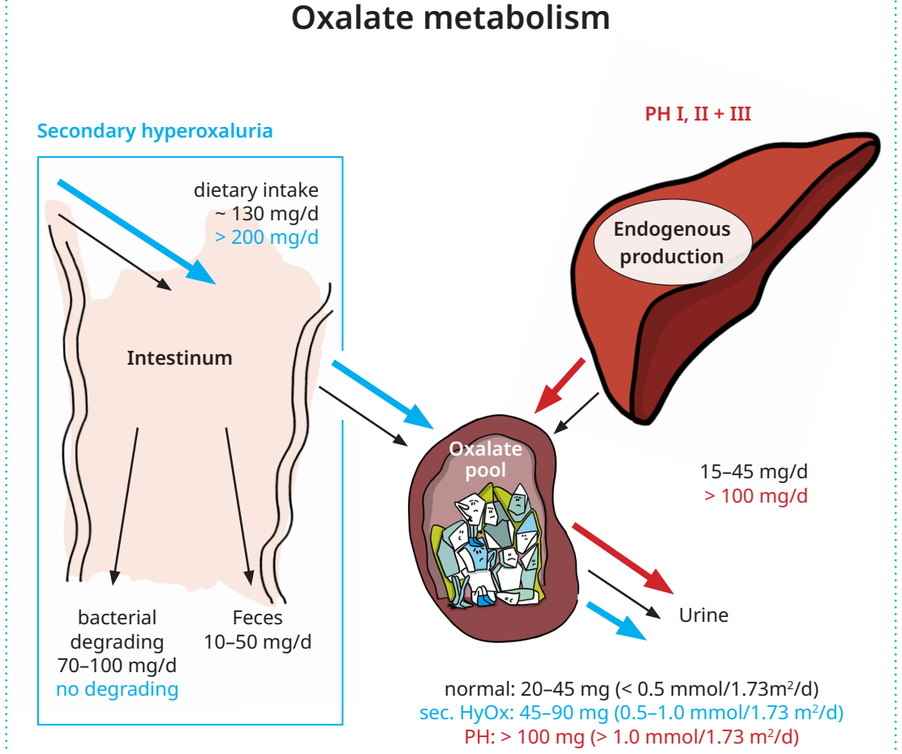
Figure 1: Two kidney stones in situ in the right kidney of a patient with Colitis ulcerosa (diameter of stones were 1.76 and 2.17 cm, respectively).

Hyperoxaluria = elevated risk of the formation of kidney stones

2.2. Primary hyperoxaluria

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

The autosomal-recessive inherited **primary hyperoxalurias** are liver-specific defects of the glyoxylate metabolism. Three forms are currently distinguished on a genetic level, but other types of primary hyperoxaluria are likely to exist. Primary hyperoxaluria type I (PH I, MIM 259900), characterized by elevated urinary excretion of both oxalate and glycolate, is due to low or absent, or mislocalized activity of the liver-specific peroxisomal enzyme alanine:glyoxylate aminotransferase (AGT, *AGXT*-gene on chromosome 2q37.3). Hallmarks of the disease are recurrent urolithiasis, progressive nephrocalcinosis and all too often early end stage renal failure (ESRF).



Primary hyperoxaluria type II (PH II, MIM 260000) is caused by diminished activity of glyoxylate reductase (GR), an enzyme also possessing D-glycerate dehydrogenase and hydroxypyruvate reductase activity, leading to elevated urinary excretion of both oxalate and L-glyceric acid (*GRHPR* gene on chromosome 9p11). We now know, that up to 50 % of PH II patients also develop chronic kidney disease (CKD) and about 25% finally experience kidney failure.

Primary hyperoxaluria type III is known since 2010 when a third causative gene (HOGA1), coding for hydroxy-oxo-glutarate aldolase 1, was described. Patients with PH III present with frequently recurring kidney stones even in early childhood, remain stone formers also in adulthood and, in the contrary to previous reports, can eventually develop CKD, but also end stage renal failure.

The urinary excretion of oxalate is strongly elevated (> 1 mmol/1.73m^{2}BSA/day, normal < 0.5) in all forms of primary hyperoxaluria. Hence, the urine is supersaturated with respect to calcium-oxalate, which results in recurrent stone formation and/or nephrocalcinosis, both leading to progressive kidney damage. The concentrations of plasma oxalate and the plasma calcium-oxalate saturation ($ß_{PCaOx}$) dramatically increase in severe CKD (stage 5) and especially in hemodialysis. The more the plasma is supersaturated ($ß_{PCaOx}$ > 1), the more crystals are deposited in the parenchyma of most solid organs, the bones, joints, and in the retina (= systemic oxalosis). Although the PH's are monogenic diseases, there is a wide clinical, biochemical and genetic heterogeneity, with some patients presenting in the first years of life with kidney failure function and onset only in adulthood.

Enteric hyperoxaluria

2.3. Secondary hyperoxaluria

The metabolic background of the secondary hyperoxalurias has been less studied than that of the primary forms. It is either due to increased intestinal oxalate absorption (enteric) or to excessive dietary oxalate intake (dietary hyperoxaluria, Figure 2).

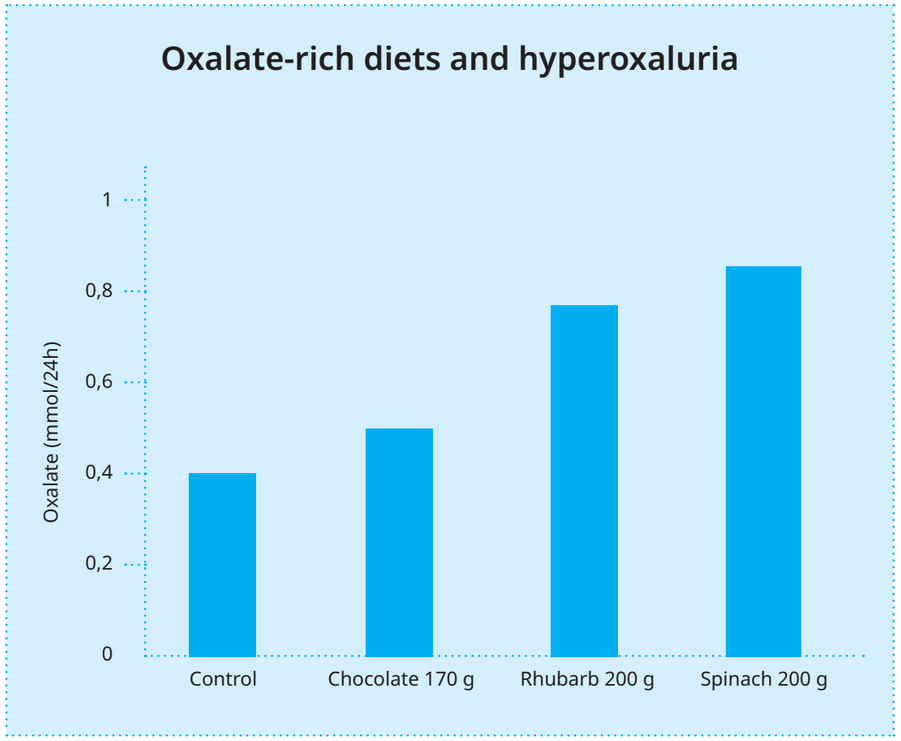


Figure 2: Increase in urinary oxalate excretion after ingestion of food with high oxalate content as compared to a normal diet with less than 100 g of oxalate (=control).

Patients with intestinal diseases 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. In addition, intoxication with ethylene glycol leads to hyperoxaluria. Although the urinary oxalate excretion is usually lower in patients with secondary (< 1 mmol/1.73m^{2}BSA/24-h) as compared to primary hyperoxaluria (> 1), the former may nevertheless lead to significant morbidity, i.e. to recurrent urolithiasis or progressive nephrocalcinosis.

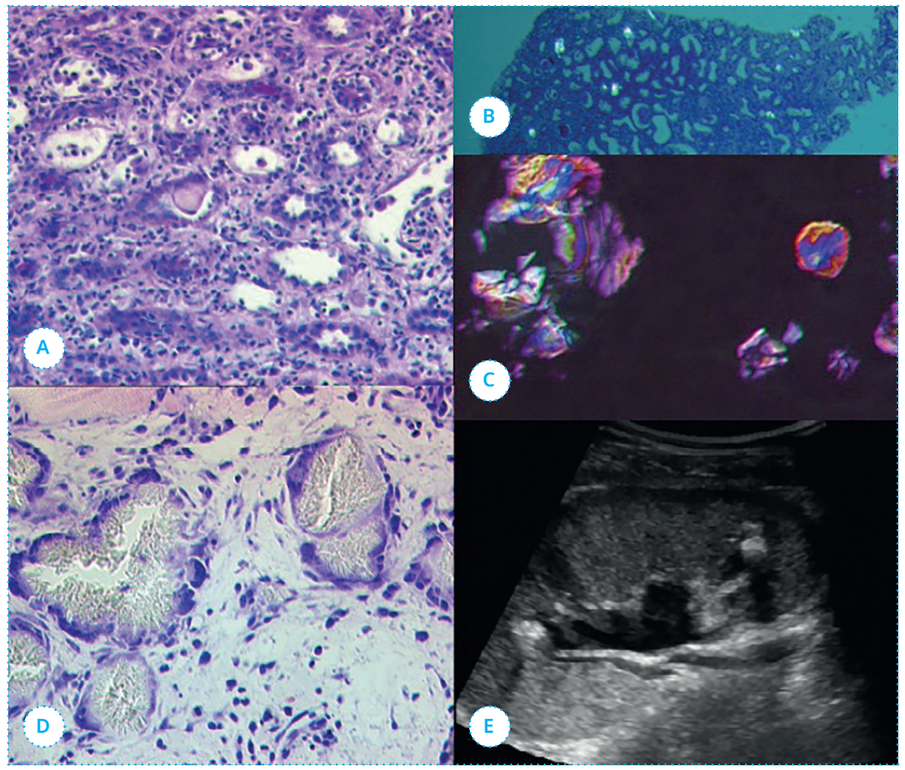


Figure 3: A) Oxalate deposition mimicking interstitial nephritis in a patient with Crohn's disease post kidney transplantation. B) oversight of transplant biopsy, C) calcium-oxalate crystal deposition made visible in polarized light, D) systemic oxalate deposition with bone marrow deposits and treatment resistant anemia and E) kidney stone post second transplantation.

Also, patients with secondary hyperoxaluria, especially patients with Crohn's disease and post ileocecal resections, do have an increased risk of chronic kidney failure and end-stage kidney disease. In the contrary to patients with PH, there is no curative treatment or transplant option in patients with EH. Hence, they have an increased risk of recurrent oxalate deposition again in the kidney transplant (Figure 3), which makes transplantation procedures difficult. It clearly shows the necessity of new treatment options in patients with EH.

3

Metabolic background of secondary hyperoxaluria

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 from the intestinal tract. The oxalate content of some food is shown in Table 1 and may also be found on a variety of webpages. Certain foods have a very high oxalate content, particularly dark-green leafy vegetables (spinach, rhubarb), tea, beet roots, nuts and cocoa. However, obtaining 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. In addition, the amount of dietary oxalate that is finally absorbed is strongly influenced by the presence of other nutrients. 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. Figure 4 shows the correlation of calcium intake and the intestinal oxalate absorption – the higher the calcium intake, the less the absorption. Hence, adding calcium to an oxalate-rich meal will help to reduce absorption and urinary excretion. Nevertheless, in general, any additional dietary oxalate ingested does lead to an increase in intestinal oxalate absorption and hence urinary oxalate excretion. Even a normal portion of spinach (150–200 g) will raise the urinary oxalate excretion in all humans.

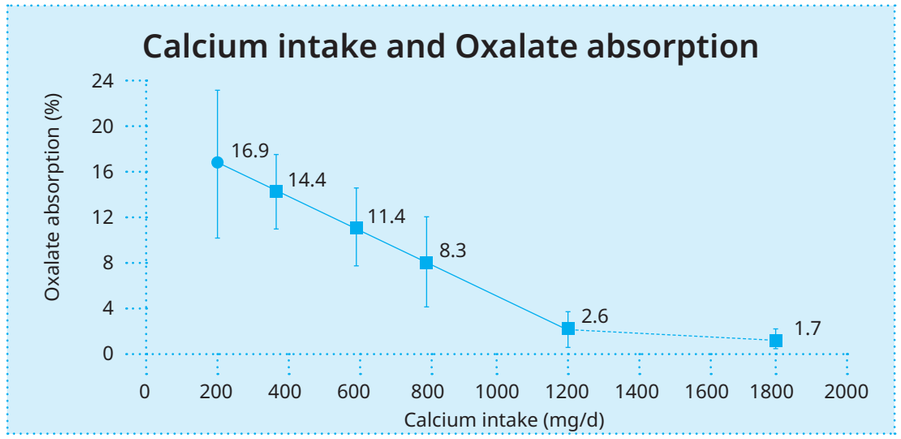


Figure 4: Correlation of calcium intake to oxalate absorption. The latter is elevated when the calcium intake is reduced below the recommended daily allowances. (Modified according to von Unruh et al, 2004, J Am Soc Nephrol. 15:1567)

Considerable controversy exists whether pharmacological doses of ascorbic acid, a precursor of oxalic acid, may cause dietary hyperoxaluria. Mega-doses of ascorbic acid have been reported to lead to increased urinary oxalate excretion. Finally, long-term parenteral nutrition may lead to hyperoxaluria, particularly in preterm infants receiving amino-acid infusions.

So, what should be observed next to a diet low in oxalate?

- Calcium intake should remain according to current recommendations – oral calcium binds intestinal oxalate. If dietary calcium is restricted higher intestinal oxalate absorption takes place.
- Excessive intake of vitamin C and D has to be avoided. Vitamin C is metabolized to oxalate. High and/or unnecessary supplementation of vitamin D may lead way to increase in calcium absorption and then excretion, thus increasing the urinary calcium-oxalate supersaturation.

Table 1: Oxalic acid content of specific food

Food	**Oxalate content mg/100 g**		**Oxalate content mg/100 g**
Fruits		**Breads**	
Bananas	0.7	Rye bread	0.9
Apples	1.5	White bread	4.9–8.6
Oranges	6.2	**Sweets**	
Strawberries	15.8	Marmalade (jam)	4.5–10.8
Gooseberries	19.3	Cocoa powder	623
Vegetables		**Beverage**	
Asparagus (boiled)	1.7	Coffee	1.0
Sweet potatoes	280–570	Coffee powder	57–230
Beans (fresh)	43.7	Beer	1.7
Beetroot (boiled)	96.8–121	Wine	3.1
Spinach (boiled)	356–780	Tea (2 min.)	7.0–10.8
Rhubarb	537	Tealeaves	375–1450

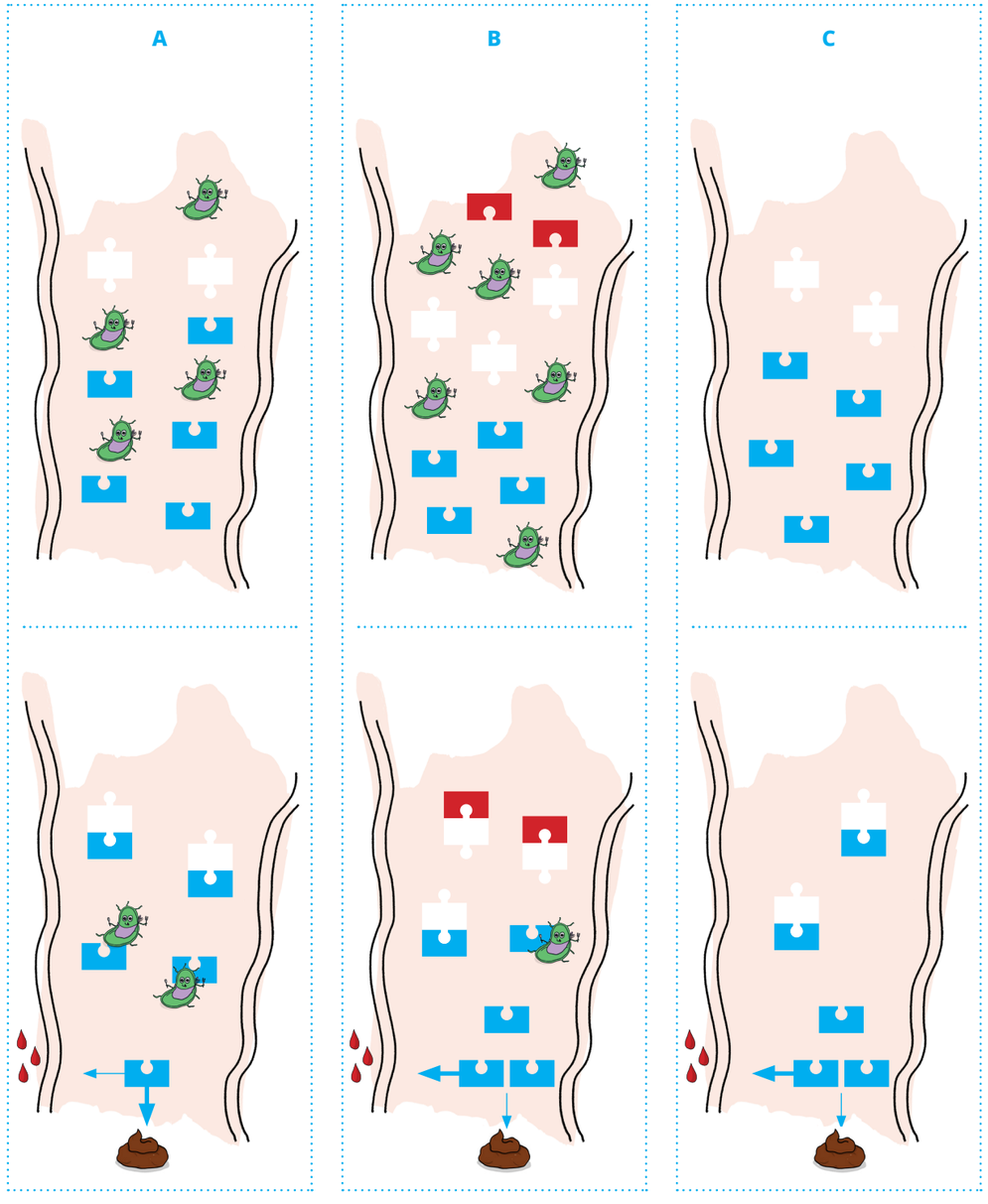


Figure 5: Normal intestinal oxalate handling (A), intestinal oxalate handling in patients with malabsorption syndromes (B) and in patients with a lack of oxalate degrading bacteria (C) [4, 5, 27].

= *Oxalobacter* or other intestinal oxalate degrading bacteria = Ca = Ox = Fatty Acids

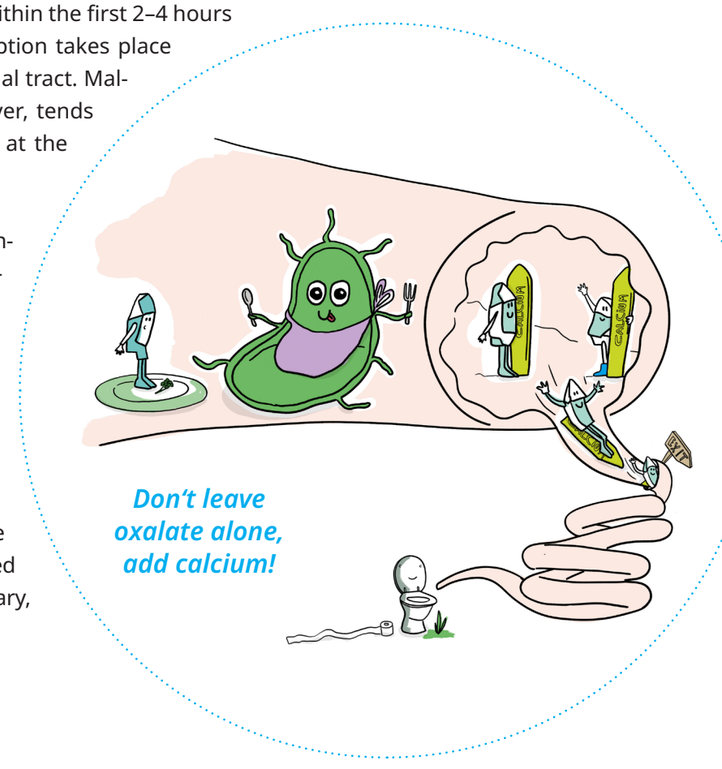
= Oxalate is absorbed into blood = Oxalate is excreted with stool

3.2. Enteric hyperoxaluria

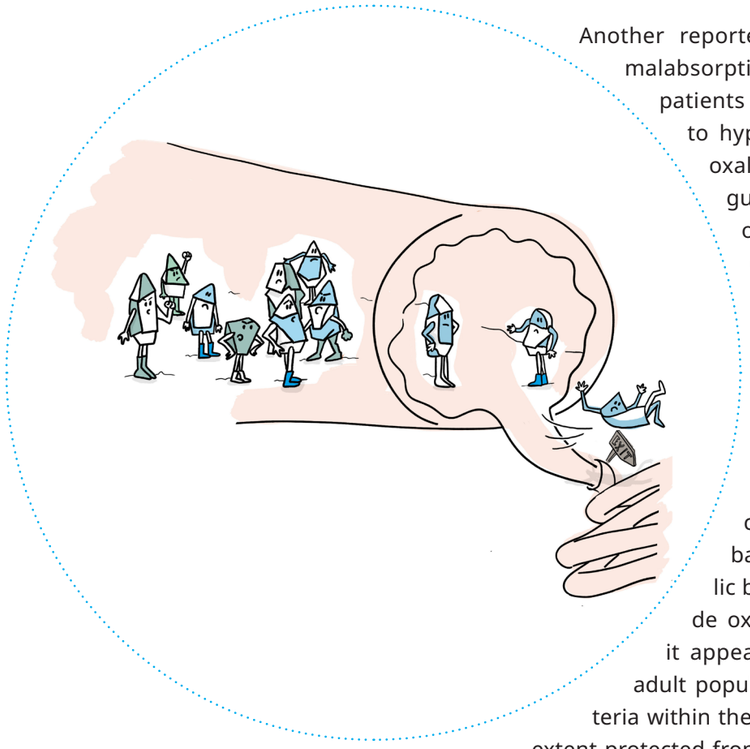
3.2.1. Malabsorption syndromes

It is well known that patients with malabsorption syndromes may develop hyperoxaluria. Normally, oxalate binds intestinally to calcium, and these non-absorbable complexes are later excreted via the feces (Figure 5A). Hence, the intestinal calcium concentration has a strong effect on the amount of oxalate absorbed. Low calcium diets, which are still recommended for obsolete 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 mal-absorbed fatty acids instead to oxalate and again, more soluble oxalate is available for absorption (Figure 5B). Oxalate is normally absorbed within the first 2–4 hours after ingestion, because absorption takes place in the upper part of the intestinal tract. 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 experiences]. A study of the latter group of patients showed that they have indeed secondary, enteric hyperoxaluria.



3.2.2. Oxalate degrading bacteria



Another 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 (Figure 5C). Especially the obligate anaerobe bacterium *Oxalobacter formigenes* is able to use two enzymes (oxalyl....) to degrade oxalate to formate A transferase) to formate that is further metabolized or excreted via the feces. Other species with probable oxalate degrading capacity are Enterococcus faecalis and lactic acid bacteria. However, the metabolic background of how they degrade oxalate is unsure. Nevertheless, it appears that the larger part of the adult population (70–80 %) has such bacteria within the intestinal tract and is to some extent protected from oxalate hyperabsorption and elevated urinary oxalate excretion. In fact, recurrent stone formers and patients with malabsorption syndromes or with hyperoxaluria of unknown origin mostly lack oxalate degrading bacteria. 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.

4

Diagnostic approaches

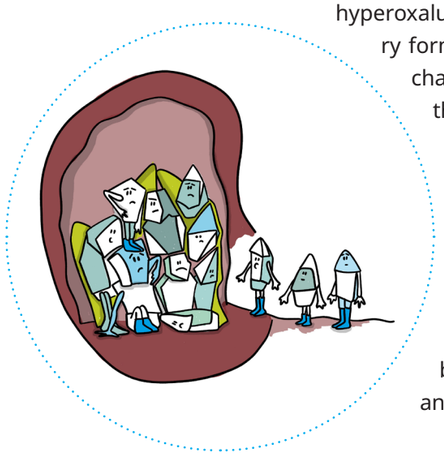
4. Diagnostic approaches

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. There should be 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

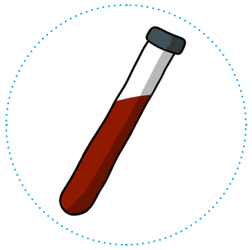
Repeated analysis of both lithogenic and stone inhibitory substances is indicated in patients with stone disease, particularly in those with hyperoxaluria. During the first urine collection the intake of oxalate-rich food needs to be avoided, but patients should otherwise remain on their usual diet and the usual fluid intake so that a personal risk profile can be established. It was repeatedly observed that extreme intra-individual variations occur when further urine specimens were examined. If hyperoxaluria is found, secondary can be distinguished from primary forms, when these collections are performed under dietary changes, e.g. one urine with food intake low in oxalate and the next collection with a diet high in oxalate. This protocol yields reproducible results and will show the patient's risk profile, with the typical urinary oxalate excretion being low under a low oxalate diet and vice versa under a high oxalate intake. It is beyond the scope of this brochure 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.



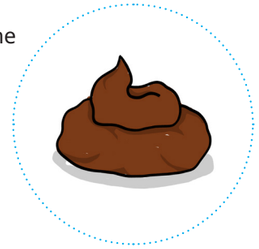
4.2. Plasma oxalate

The determination of plasma (blood) oxalate (P_{Ox}) and glycolate is a powerful tool to distinguish primary from secondary forms of hyperoxaluria with chronic kidney disease,

when urine evaluations may no longer be doable. Normal levels of P_{Ox} range from 0.5 to 7.5 µmol/l depending on the method used, and 10 µmol/l is the upper cutoff point. There are no glycolate levels yet..



4.3. Stool examination



Oxalate degrading bacteria are our best "friends" with regard to the intestinal oxalate metabolism. Therefore, stool samples can be analyzed for the presence of bacteria such as *Oxalobacter formigenes* by PCR to detect bacterial DNA. However, such analysis may only make sense, if treatment with oxalate degrading medications may be possible.

4.4. The [$^{13}C_2$]oxalate absorption test

The determination of the intestinal oxalate absorption using [$^{13}C_2$]oxalate is also possible. However, this test usually is done on an inpatient basis and may not be necessary, if the urine collections with different diets have proved secondary hyperoxaluria.

Table 2: Intestinal oxalate absorption [%] in children suffering from calcium oxalate stones (CaOx), in children suffering from primary hyperoxaluria (PH) and for healthy controls. As per Sikora et al., [$^{13}C_2$] oxalate absorption in children with idiopathic, calcium oxalate urolithiasis or primary hyperoxaluria. Kidney Int. 2008 Mar 12.

Patienten mit CaOx stones:			
	Boys (n = 33)	Girls (n = 27)	Total (n = 60)
Median (%)	17.0	14.1	15.3
Range	2.6–32.8	1.7–37.7	1.7–37.7
Patients suffering from primary hyperoxaluria:			
	Boys (n = 38)	Girls (n = 5)	Total (n = 13)
Median (%)	8.2	7.1	7.0
Range	2.0–12.4	1.8–10.0	1.8–12.4
Controls/healthy persons:			
	Boys (n = 23)	Girls (n = 12)	Total (n = 35)
Median (%)	10.6	9.6	10.4
Range	4.3–26.2	1.9–18.6	1.9–26.2

For the [$^{13}C_2$]oxalate testing patients have to follow a fluid schedule (a minimum of 1000–1500 ml/day) and the dietary recommendations (e.g. 800 mg calcium and normal oxalate intake) 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 [$^{13}C_2$]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 either by gas- or ion-chromatography/mass-spectrometry.

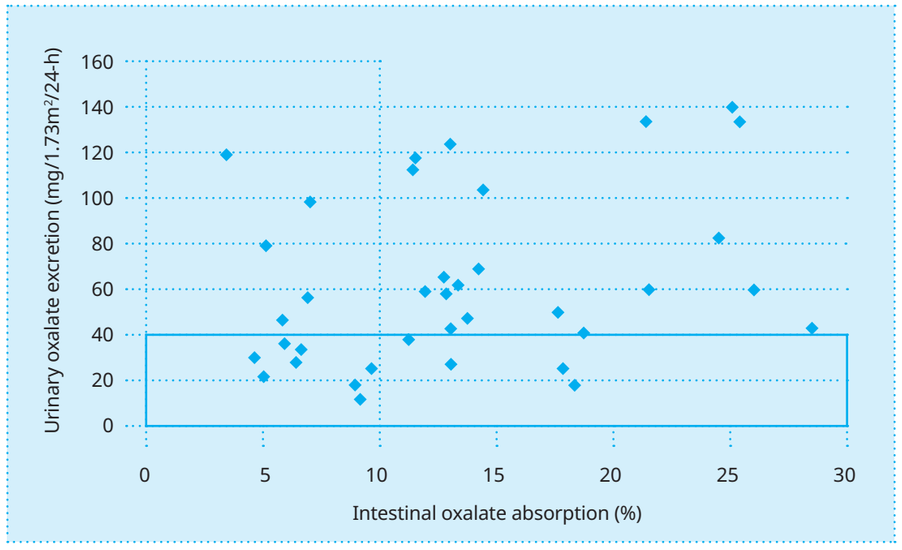


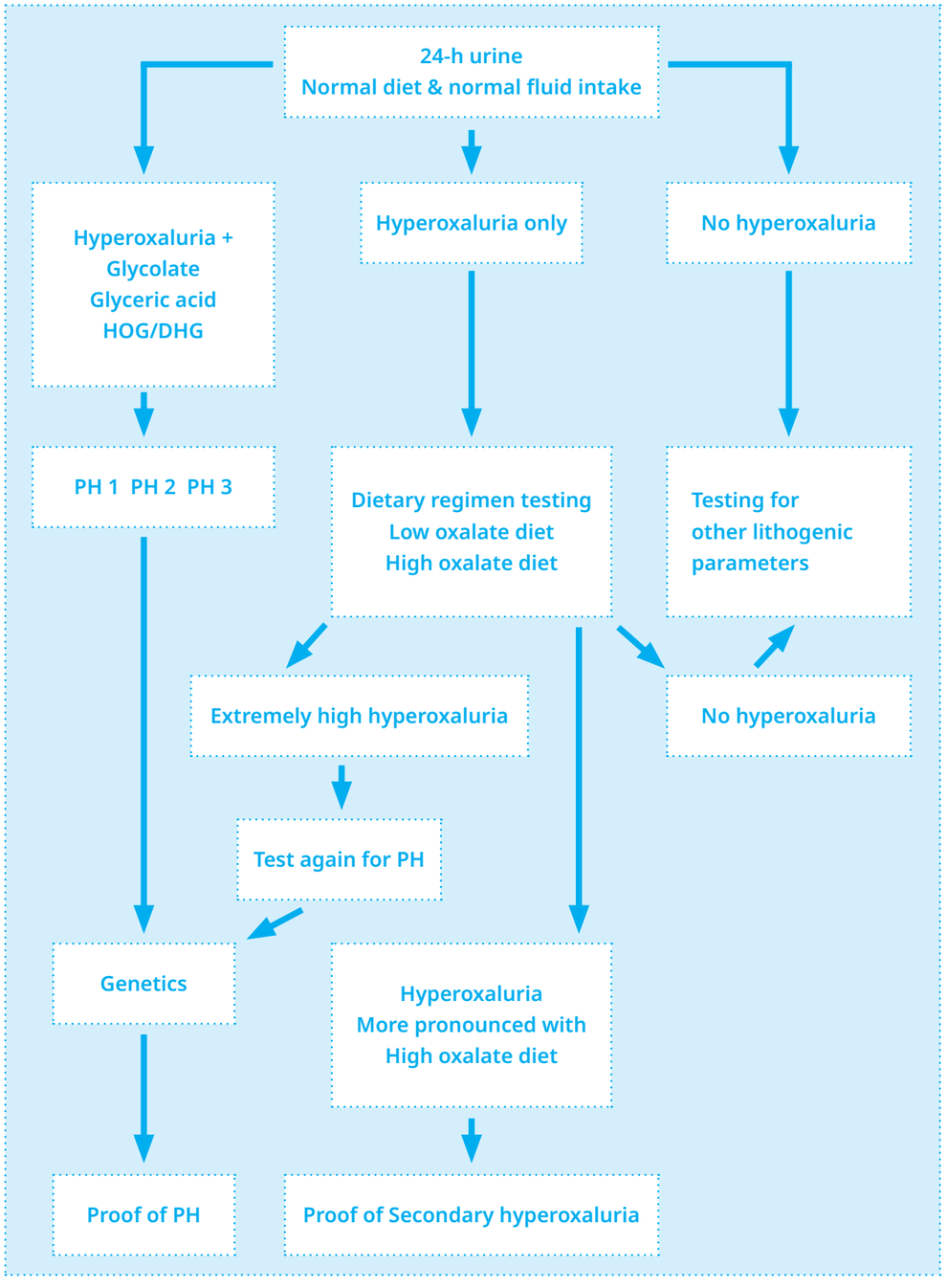
Figure 6: Although there was no correlation between intestinal oxalate absorption and urinary excretion, 19/35 patients with CF tested were hyperoxaluric and hyperabsorbers. Dashed-line, normal range of intestinal oxalate absorption (< 10 % absorption), solid line upper limit of normal urinary oxalate excretion (< 45 mg or 0.5 mmol/1.73m^2 body surface area per day). (per Hoppe et al, Absorptive hyperoxaluria leads to an increased risk for urolithiasis or nephrocalcinosis in cystic fibrosis. Am J Kidney Dis. 2005 Sep;46(3):440–5)

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, but not always easy to perform. It is without risks, as the isotope used is stable.

5

Diagnostic algorithm

5. Diagnostic algorithm



6

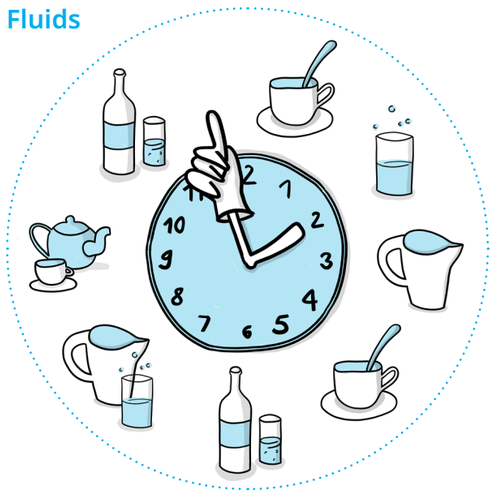
Therapeutic Implications and Perspectives

6. Therapeutic Implications and Perspectives

6.1 Current treatments

Measures to increase the urinary solubility of oxalate by a high fluid intake (> 1.5 L/m²/day) and administration of either alkali-citrate or orthophosphate are important in any form of hyperoxaluria.

6.1.1. Fluids



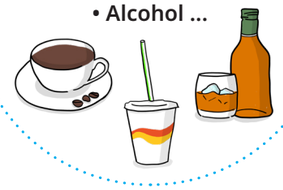
A high fluid intake has to be assured over 24 h. To drink a lot in the morning and later in the evening does not make sense. Drink every two hours (remind with a device like the alarm clock of a mobile phone)!!!

Drink, drink, drink ...

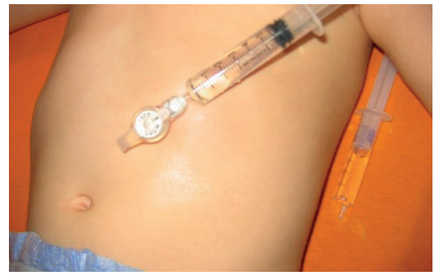
• Mineral water
(Still or sparkling)*
• Herbal Teas*
• Juice spritzers

*please ask your doctor or nutritionist for possible products and the amount you can drink.

Large amounts of...
• Black Tea
• Coffee
• Sugar-containing drinks
• Alcohol ...



In small infants and children, who are not really able to increase the fluid intake adequately, a gastrostomy tube may be placed. At times of fluid losses (vomiting, diarrhea, high fever) intravenous fluid administration may be necessary.



6.1.2. Diet

In patients with secondary (dietary and absorptive) hyperoxaluria a diet low in oxalate but normal or high in calcium is advised (Table). Specific therapeutic measures are required in patients with malabsorption syndromes, depending on their underlying pathology.

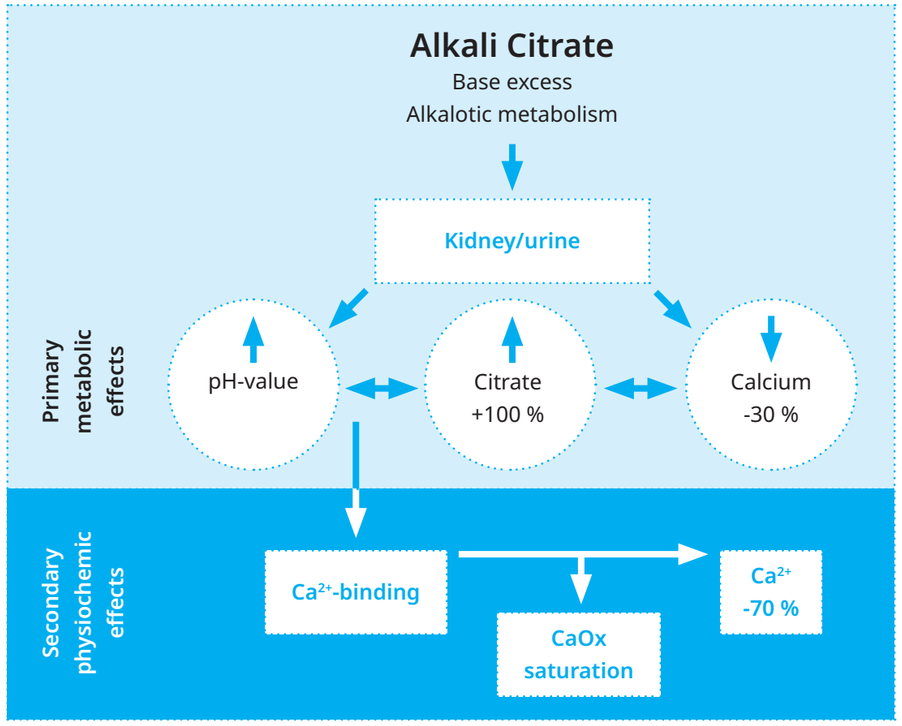
Table 3: Oxalate content of food

Food	Oxalate content ↑ mg/100 g	Food	Oxalate content ↓ mg/100 g
Spinach (cooked)	356–780	Apple	1.5
Chard (cooked)	650	Orange	6.2
Rhubarb	537	Strawberry	15.8
Cacao powder	623	Potatoes (cooked)	14.5
Sweet potatoe	280–570	Beans (fresh)	43.7
Red beet (cooked)	96.8–121	Coffee	1.0
Tea leaves (black)	375–1450	Beer	1.7
Peppermint leaves	1111	Wine	3.1
Wheat bran	457	Tea (2 min.)	7.0–10.8
Almonds	431–490		

Vegetarian diets may contain high amounts of oxalate via an increased intake of whole grain wheat, nuts, legumes (e.g. soya) and vegetables and fruits (especially as juices).

6.1.3. Citrate medication

The goal of the therapy with alkali citrates is to reduce the saturation in urine for calcium oxalate. Citrate forms soluble complexes with calcium, thus less calcium is available for binding to oxalate and urine shows a lower saturation for CaOx. In the liver, citrate is converted to bicarbonate and thus leads to an alkaline metabolic state (higher pH value in blood and urine), while more citrate is secreted with urine (not needed to keep blood pH in a good range).



In a pilot study and a long-term study in patients suffering from primary hyperoxaluria under alkali citrate therapy, this medication enabled stabilization of renal function, a reduction of the rate of kidney stone passage and/or a lesser degree of renal calcification.

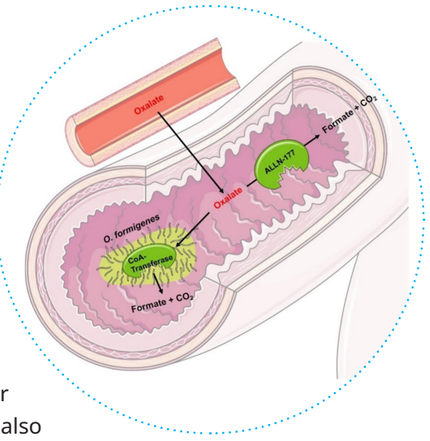
The dosage of alkali citrate is 0.1–0.15 g/kg body weight per day (0.3–0.5 mmol/kg) of a sodium and/or potassium citrate-containing preparation. In the majority of patients, who have well cooperated over an observation period of several years, above all, the renal function remained stable or even improved. The best control parameters for the less cooperative participation of patients eventually are the clinic (sharp increase of the rate of kidney stone passage), a reduced urinary citrate excretion, or an acidic pH value of the urine.

6.1.4. Phosphate binders

Theoretically medications normally given to intestinally bind phosphate in patients with chronic kidney disease should also be able to bind oxalate and hence reduce intestinal oxalate absorption. As already mentioned, adding calcium to a diet rich in oxalate will help to intestinally bind oxalate to the calcium provided, so it would be conclusive that calcium carbonate is a meaningful "medication" in enteric hyperoxaluria. This was shown also in studies, when calcium carbonate administration was compared to sevelamer, a cationic phosphate binder. Calcium carbonate was clearly better here with a mean of 41.2±17.4% reduction as compared to sevelamer with 30.4±23.8%. Conflicting results were found in another study on sevelamer, with only a 17 % reduction in urinary oxalate, but a 25 % increase in urinary calcium excretion and a 23 % decrease in urinary citrate excretion. So overall, treatment of enteric hyperoxaluria with sevelamer seems to be not reasonable, as it may even increase the risk of stone development by changes in other lithogenic factors.

Also for lanthanum carbonate, positive data on intestinal oxalate absorption, and hence urinary oxalate excretion, were published in animal studies. Recently, it was even reported that Lanthanum efficiently reduced plasma and urine oxalate levels in patients with primary hyperoxaluria. A recent paper also described the search for best cation binding to phosphate and/or oxalate by using quantum chemical calculations. For oxalate, only aluminum was a very strong candidate for complexation, while also lanthanum and Fe^{3+} showed promising results, thus trivalent cations (Fe^{3+}, Al^{3+} and La^{3+}) showed a higher affinity for oxalate. However, aluminum should not be considered a good candidate for treatment, as it is obsolete for routine/daily administration, especially in the pediatric population. Long-term aluminum exposure reduces the levels of mineral and trace elements in bone.

6.2. Future treatment

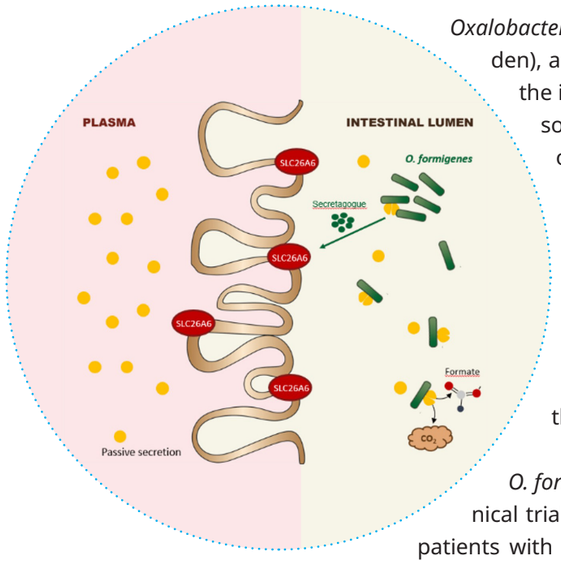


Still, the therapeutic arsenal currently available for secondary (and primary) hyperoxaluria has its limitations, hence other therapeutic approaches are welcome. For more information about the primary hyperoxalurias, please visit www.ph-europe.net.

For the secondary hyperoxalurias two treatment strategies would be possible:

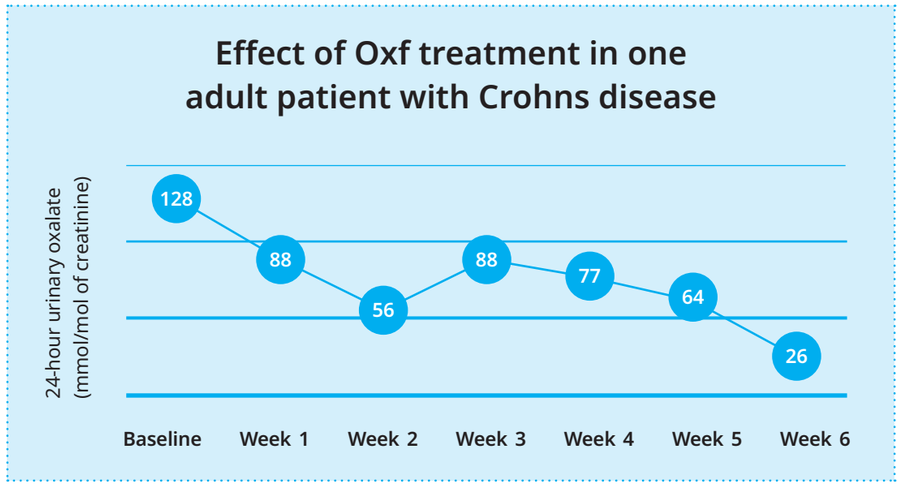
1) to provide an oxalate-degrading bacterium orally to use its action intestinally by degrading oxalate, or
2) to provide oxalate-degrading enzyme preparations also orally to the main meals to try to reduce the oxalate load ingested.

6.2.1. Oxalate degrading bacteria



Oxalobacter formigenes (Oxabact®, Oxthera AB, Sweden), an anaerobic bacterium, normally localized in the intestinal tract of humans, uses oxalate as its sole carbon source. Orally applied, it reduces oxalate concentration in the patient's intestine, leading to a relevant concentration gradient between blood and intestinal lumen, but also to activation of the intestinal oxalate transporter (SLC26A6). Both effects can lead to a significant shift of oxalate from patient's blood into the intestinal lumen, where it is being degraded by *O. formigenes* and thus excreted via the feces.

O. formigenes is already being tested in human clinical trials – however, studies were only reported for patients with primary, not with secondary hyperoxaluria. There was a pilot study done in patients with Crohn's disease, but data was inconclusive because of mal-compliance of most of the patients. However, in one patient with consistent intake of Oxf study medication urinary oxalate excretion expressed as molar creatinine ratio declined nicely over the 6 weeks under medication.



Since then, however, *O. formigenes* was tested only in PH studies and just recently in a phase III placebo-controlled study (NCT02000219) in patients with primary hyperoxaluria and still good renal function, but elevated plasma oxalate values (> 10 µmol/l).

The results from earlier studies showed that *O. formigenes* is capable to lower the plasma oxalate value. However, it resulted in an elevated oxalate excretion in urine, although the other way around was expected. The elevated urinary oxalate excretion may have been caused by the dissolution of systemic oxalate deposits. The primary endpoint of the current study therefore was the change in plasma oxalate. The company just has released a press information, that its study did not meet the final endpoint of the study, therefore, medication with *Oxalobacter* will not be available in the near future, neither in PH, nor in enteric hyperoxaluria.

6.2.2. Oxalate degrading enzyme treatment

6.2.2.1. Urirox/Reloxaliase

Urirox/Reloxaliase™ (Allena Pharmaceuticals, USA) is a recombinant oxalate decarboxylase, thus an oxalate-degrading enzyme in the form of tablets, which is also able to degrade oxalate in the intestinal tract. Even if Urirox does not directly activate the oxalate transport in the intestine, the difference in concentration generated by the degradation possibly suffices to reach a shift of oxalate from the blood into the intestinal tract. In healthy persons, Urirox could reduce the excretion urine with an oxalate-rich diet and it is currently analyzed in patients suffering from secondary hyperoxaluria as well as in patients suffering from PH II and PH III (NCT03391804).
Urirox/Reloxaliase (7500 units of oxalate decarboxylase) is orally administered for at least 5 times a day with the meals. It was shown in recent studies in healthy persons made hyperoxaluric, but also in enteric and idiopathic hyperoxaluria patients that urinary oxalate excretion was nicely reduced. The study in patients with enteric and idiopathic hyperoxaluria was proof of concept (Urirox 1 study). URIROX is not systemically absorbed and hence has low potential for systemic toxicity. A second pivotal Phase 3 study (Urirox 2) is ongoing now with FDA alignment on accelerated approval strategy. Interim Analysis at first quarter of 2022. Filing at Drug Agencies (FDA & EMA) is planned for early 2023.

6.2.2.2. Genetically engineered bacteria

Synlogic pharmaceuticals is targeting EH with a genetically-engineered bacterial platform. SYNB8802 is a genetically engineered strain of E.coli Nissle 1917 containing a pathway for oxalate degradation from *O. formigenes*. It is "Designed to consume oxalate in the GI tract to prevent the increased absorption of oxalate in patients with EH". In the contrary to *Oxalobacter* the genetically engineered bacteria are said to be capable to degrade oxalate in the Colon, like *Oxalobacter* is doing, but also in the stomach and the small intestine. In a phase 1 study in healthy individuals consuming a diet high in oxalate SYNB8802 administration reached the following goals: After a 4-day run-in of high-oxalate and low-calcium diet (400mg or 600mg oxalate) subjects received $3x10^{11}$ live cell dosage for 5 days and had to collect a daily 24-h urine. Results showed a 29% reduction

in urinary oxalate (Uox) from baseline vs. placebo. Safety data has not been reported in detail. Another phase 1 study is currently performed in patients post gastric bypass surgery, who are known to have severe secondary hyperoxaluria. This study evaluates N=20 patients with elevated UOx secondary to gastric bypass (Roux-en-Y) surgery. Selected dose is $3x10^{11}$ live cells and same procedures like in the healthy volunteer study. Results will be available at end of year.

6.2.2.3. Orally administered oxalate decarboxylase

OX-1 (Oxidien pharmaceuticals) is a novel enzyme to treat hyperoxaluria. It exhibits a broad pH-activity profile and a superior stability and activity in the stomach. OX-1 has demonstrated biological effect in healthy volunteers and is the first product to demonstrate meaningful effect in subjects with dietary induced hyperoxaluria at upper limit of normal (< 0.5 mmol, or 45 mg/day, respectively).

Currently only data of a study with healthy subjects made hyperoxaluric with a high oxalate diet and compared to placebo treated subjects are available. The subjects treated received ~1,000 units (µmol/min/mg) of Ox-1 or placebo with meals 3 times daily during the 4 days of treatment. A mean 29 % reduction in urinary oxalate excretion was achieved and 31/33 treated subjects showed a reduction of > 5%. The conclusion of the published was that the medication significantly decreases urinary oxalate excretion. Nevertheless, more studies are needed to show efficacy in patients with enteric hyperoxaluria.

6.2.2.4. Engineered bacterial strains

Novome is a genetically-engineered bacterial strains to perform therapeutic functions to address a wide variety of diseases including enteric hyperoxaluria. Novome's synthetic biology tools deliver precise activities, here controllable engraftment of GEMMs (Gentically engineered microbial medicines), in the gut for sustained and truly effective therapies. For enteric hyperoxaluria these microbes carry oxalate decarboxylase into the intestinal lumen, with which oxalate is later degraded. There is no clinical data currently available. However, the experimental work shows a 70 % reduction of oxalate in a culture medium by Lactobacillus plantarum which was engineered to constitutively secrete oxalate decarboxylase (OxdC) for the degradation of intestinal oxalate. This finding was also found later in rats studies treated with the engineered Lactobacillus. Currently Novome is performing a phase 1 study for proof of concept of GEMMs.

7

Conclusion

7. Conclusion

Patients with secondary hyperoxaluria and here especially with enteric hyperoxaluria have a great risk to develop recurrent kidney stones and consecutively even chronic kidney disease and end stage kidney failure. The current treatment options are scarce and more or less only include hyperhydration, a diet low in oxalate and at least normal in calcium and alkaline citrate medication. In addition to that the administration of drugs developed to target intestinal phosphate absorption, like sevelamer or lanthanum was also studied and recommended. New therapeutics target to reduce the intestinal oxalate burden by providing oxalate degrading enzymes and bacteria into the intestinal lumen so that less oxalate is available for absorption. Whether or not these medications will definitively reduce the disease burden has still to be elucidated.

Normal values

Plasma		
Oxalate in plasma	All age groups	< 6.3 ± 1.1 µmol/l (free oxalate)
Glycolate in plasma	All age groups	< 7.9 ± 2.4 µmol/l (assumed)
24-h urine collection		
Oxalate in 24-h urine	All age groups	< 0.50 mmol/1.73 m^2/24h < 45 mg/1.73 m^2/24h
Glycolate in 24-h urine	All age groups	< 0.50 mmol/1.73 m^2/24h < 45 mg/1.73 m^2/24h
L-Glyceric acid in 24-h urine	All age groups	< 5 µmol/l
Hydroxy-oxo-glutarate in 24-h urine	All age groups	< 10 µmol//1.73 m^2/24h
Controls/healthy persons:		
Oxalate/creatinine	0–6 months 7–24 months 2–5 years 5–14 years > 14 years	< 325–360 mmol/mol < 132–174 mmol/mol < 98–101 mmol/mol < 70–82 mmol/mol < 40 mmol/mol
Glycolate/creatinine	0–6 months 7–24 months 2–5 years 5–14 years > 14 years	< 363–425 mmol/mol < 245–293 mmol/mol < 191–229 mmol/mol < 166–186 mmol/mol < 99–125 mmol/mol
L-Glycerate/creatinine	0–6 months 7–24 months 2–5 years 5–14 years > 14 years	< 14–205 mmol/mol < 14–205 mmol/mol < 14–205 mmol/mol < 23–138 mmol/mol < 138 mmol/mol
HOG/creatinine	All age groups	< 2.5 µmol/mmol

Glossary

Autosomal recessive
The chromosome the patient had been transferred from both the mother and the father must possess the same change in a specific gene to be affected

CKD
Chronic kidney disease

Colonization
Population of bacteria e.g. in the gut

Dialysis
Renal replacement therapy

DNA
desoxy-ribonucleic acid

Enzyme
A protein driving a chemical reaction.

ESRF
End stage renal failure

ESWL
Abbreviation for extra-corporeal shock wave litho-trypsie crushing of stones by means of ultrasound waves from the outside

Gene
Genetic information on a chromosome

Hematuria
Blood in urine

Hemodialysis
Dialysis

Homozygous
One same mutation on both chromosomes each

Heterozygous
Only one mutation on one chromosome

Hyperoxaluria
Increased oxalate excretion in urine

Intestinal oxalate absorption
Oxalate absorption from the gut into the blood

L/m2/d
liter per square meter body surface area per day

Metaphylaxis
Treatment to prevent new development of stones or progression of nephrocalcinosis

mmol/1.73m2BSA/day
mmol/1.73m2 body surface area per day

Nephrocalcinosis
Calcification of the kidneys = deposits of calcium-oxalate crystals in the renal tissue

PCR
Polymerase chain reaction

Systemic oxalosis
oxalate deposits in all body tissue

Urolithiasis
Kidney stone disease

Imprint
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Dr. Cristina Martin-Higueras &
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PH&HP team

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