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CAT

Critically Appraised Topic

Biochemical and metabolic investigation in uro- and nephrolithiasis

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Clinical bottom line

Kidney stones are prevalent (8-15%) and have a high recurrence rate, rising to 50% after 5 years (1–3). Symptoms include pain, vomiting and hematuria and complications (e.g. sepsis, renal scarring) can occur, often leading to a loss of renal function. Consequently, kidney stone care has been gaining ground in the medical community in recent years with a focus on personalized and preventive care. The importance of identifying lithogenic risk factors through anamnesis, clinical examination, stone analysis, imaging, and initial biochemical assessment is emphasized, as this allows for targeted treatment, leading to a reduction of the recurrence rate. In addition, extensive metabolic screening may help to detect systemic disease manifesting as uro- or nephrolithiasis (4–6). Obviously, extensive screening is only advantageous for a selective group of high-risk patients and this evaluation must always be performed in accordance with the most recent guidelines on collection, storage, and analysis. This article discusses the current guidelines for 24-hour urine collection in the context of kidney stones and takes a closer look at the use of additives including hydrochloric acid (HCl).

Clinical/Diagnostic scenario

When the saturation point of certain urinary ions is exceeded (= supersaturation), crystals will form and attach to the wall of the tubules or the papillary epithelium, resulting in stone formation (1,7).

Apart from the ionic concentration, the formation of kidney stones is influenced by the urinary pH, by substances that promote or inhibit aggregation (e.g. citrate which binds urinary calcium and is thus a protective factor), or by bacterial infection. There are different types of kidney stones, and a stone can consist of several components. The calcium component is isolated in 70-80% of cases, often in combination with oxalate (8). The monohydrate calcium oxalate stone is more prevalent than the dihydrate stone. The former usually arises at an acidic pH as opposed to the dihydrate stone which is predominantly formed in an alkaline urinary environment (9). A limited amount of calcium phosphate is also regularly found at an alkaline urinary pH. Pure calcium phosphate stones are less frequent. Supersaturation of uric acid occurs at lower acidity (figure 1). A struvite stone consists of magnesium, ammonia, and phosphate and is formed in the presence of urease-producing bacteria such as Proteus mirabilis or Klebsiella pneumoniae. The urinary pH will be higher due to the production of ammonia. Struvite is more common in women as the prevalence of urinary tract infections is higher in this group. Cystine and drugs can also crystallize and lead to lithiasis (3). Cystine dissolves at an alkaline pH. Consequently, alkalinisation of the urine is used as a treatment strategy for patients with cystinuria (figure 2) (10,11).

Diet plays a crucial role in the process of stone formation. A diet high in animal protein may lead to higher urinary excretion of calcium and uric acid and lower excretion of

citrate. Furthermore, an increased calcium excretion can be caused by a diet rich in sodium, in a metabolic pathway not yet fully understood. One hypothesis states that chronic overload of sodium intake can lead to the downregulation of the protein claudin-2, which is responsible for sodium and water reabsorption but also for calcium reabsorption in the proximal tubule (12). It is important to note that a lower calcium intake paradoxically leads to an increased risk of kidney stones because fewer calcium oxalate complexes are formed in the intestine, allowing more oxalate to be absorbed. The (excessive) consumption of foods rich in oxalate (e.g. spinach, nuts, chocolate...) or vitamin C also increases the urinary concentration of oxalate (13). Low fluid intake or high fluid loss, e.g. through diarrhea or sweating, may contribute to stone formation (4,14). Hypercalciuria is diagnosed in 40% of patients and is thus the most common metabolic disorder (4). In addition to dietary risk factors, granulomatous diseases or malignancies are a frequent cause of hypercalcemia and various medications can lead to hypocitraturia or hypercalcemia (1).

Based on anamnesis, clinical examination, stone analysis if possible, imaging (low dose CT), and initial blood and urine tests, high-risk patients can be identified. Only this group of high-risk patients need additional extensive metabolic examination (figure 3) (15,16). Stone analysis is performed at the university hospitals of Leuven using Fourier transform infrared spectroscopy. Patients are advised to filter the urine to retrieve stones.

Questions

1. Which parameters are relevant in the initial and extensive metabolic examination of uro- and nephrolithiasis?
2. How often should an extensive metabolic examination be repeated?
3. Is it necessary to collect an acidified urine sample or can acidification be performed upon arrival at the laboratory?
4. Can a urine sample (spot urine) replace the 24-hour urine collection in uro- and nephrolithiasis?

Methods

During this literature review, the Cochrane library and Medline (Pubmed) database were systematically searched for eligible articles published between January 1st 2000 and February 1st 2022 concerning laboratory testing of patients with uro-and nephrolithiasis.

The following key words were used: "urolithiasis", "nephrolithiasis", "ureterolithiasis", "kidney calculi", "kidney stones", "ureteral calculi", "urine specimen collection", "24-hour urine", "acidification", "hypercalciuria", "oxalate".

In addition, the reference lists of the selected articles were searched.

Appraisal

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1. Which parameters are relevant in the initial and extensive metabolic examination of uro- and nephrolithiasis?

In the acute phase, biochemical evaluation includes a blood sample with determination of hemoglobin, white blood cells (total count and differentiation), electrolytes (sodium, calcium, potassium, chloride, phosphate, and magnesium), creatinine, urea, and uric acid (6). In some studies, a microscopic analysis of the urine sediment is recommended (6) but other authors consider a dipstick sufficient (15). When a urinary tract infection is suspected, CRP and urine culture are part of the work-up (15). It is not cost-effective to carry out further examinations in low-risk patients (17). Targeted dietary measures are usually started in primary care (16).

Complete metabolic screening is required in high-risk patients. This category includes patients with multiple calculi at first presentation, patients with an early-onset or family history of uro- or nephrolithiasis, active stone disease (recurrent stone formers), urological malformations, etc. Table I provides a complete overview. Due to the high recurrence rate in this group, a comprehensive diagnosis and targeted therapy are crucial to avoid chronicity (2).

If hypercalcemia or a high-normal calcium value is noticed at the initial screening, parathyroid hormone has to be determined at the next blood sampling (4). In the case of metabolic acidosis with a normal anion gap and a positive urinary anion gap, renal tubular acidosis type 1 should be suspected. Patients with a history of bypass surgery, bowel resection, Crohn's disease, or celiac disease are at risk for enteric hyperoxaluria due to fat malabsorption. Calcium will bind free fatty acids, reducing the formation of calcium

oxalate complexes in the gut and thus increasing oxalate absorption. In pediatric patients, attention should be paid to possible primary hyperoxaluria (when urinary oxalate exceeds 75 mg/day without absorption problems) or cystinuria (when cystine crystals are detected under the microscope or when the urinary cystine concentration exceeds 50 mg/day) (3,4,11).

In addition to blood sampling, the comprehensive screening includes a 24-hour urine examination. The excretion of various parameters is calculated based on the collection volume. The following tests are classically requested in this context: calcium, sodium, chloride, potassium, magnesium, citrate, phosphate, oxalate, uric acid, cystine (if stone analysis shows cystine or if there is a family history), pH value, urea, and creatinine.

The supersaturation ratio is a new index calculated by using the parameters of the 24-hour urine collection. When this ratio is equal to 1, it is called saturation, > 1 means supersaturation and < 1 undersaturation. When working with relative supersaturation, 0 means saturation, a positive ratio equals supersaturation and a negative ratio equals undersaturation. Various studies have examined the contribution of these ratios to the diagnosis and have shown a strong correlation with stone type and therapy response, as saturation decreases after treatment (18–20). In a study of pediatric patients, the relative urinary calcium oxalate supersaturation (calculated using EQUIL2) was significantly higher in the group with stone formation compared to the controls. However, the discriminatory power of the test was limited with an area under the curve (AUC) of only 0.610 (8).

Software programs including Lithorisk, EQUIL2, or JESS calculate the supersaturation ratio of various urinary parameters. Lithorisk is offered as an application whereas EQUIL2 and JESS must be written in a programming language. Although already widespread in

the United States, these programs are not yet standard practice in Flanders (table II and figure 4) (21–23).

2. How often should an extensive metabolic examination be repeated?

Complete metabolic screening is essential in the high-risk patient group as defined in Table 1. This should be done when the patient is stone free for at least 20 days and has returned to his regular diet and activities (14,16,24). The literature recommends that in an initial comprehensive screening, a 24-hour urine collection should be performed twice on two non-consecutive days as the outcome may be influenced by diet, fluid intake, and the patient's physical activities (3,14,16,25–28). The importance of intra-individual variation was substantiated in a study of 70,192 patients where in 1 out of 4 patients a deviation of 50% or more was measured for at least one urinary parameter between two collections in one week (figure 5) (2). The study by Nayan et al. found that in 17.1% to 47.6% of patients (depending on which parameter) a value changed from normal to outside the normal reference range or from abnormal to within the normal limits between the first and second urine collection taken on two consecutive days. Although not to be underestimated, this variation must be weighed against practical feasibility and cost (27). 3 to 6 months after the start of a diet or medication, a 24-hour urine sampling should be repeated to evaluate therapy response (3,14).

The results of the metabolic urinary evaluation should be interpreted according to the clinical features, possible stone analysis, and other biochemical results. In addition to intra-individual variation, interpretation is also complicated by the unclear significance of limited abnormalities and the discussion of the reference values. Curhan et al. found biochemical anomalies in 239 healthy subjects (non-stone formers) including hypercalciuria (14% to 27%), hyperoxaluria (7% to 43%), hyperuricosuria (8% to 40%), hypocitraturia (3% to 9%) and a low urine volume (7% to 20%) (29). This bottleneck was

also registered at the university hospitals of Leuven. The determination of creatinine in 4077 acidified urine samples in our laboratory was re-evaluated. Reference values for creatinine in urine were established by Junge et al. in 2004 and are within the range of 720 to 1510 mg/24 h for women and 980 to 2200 mg/24 h for men (30). Of the 4077 patients in our evaluation, 27% of the women and 24% of the men fell outside this 5-95% range. A greater proportion of hospitalized patients compared to ambulatory patients had abnormal values for urinary creatinine. The latter could be a consequence of a higher percentage of hospitalized patients with reduced muscle mass and the higher incidence of nephrological problems in this patient group (table III and IV).

3. *Is it necessary to collect an acidified urine sample or can acidification be performed in the laboratory?*

The container for 24-hour urine collection can be acidified (= pre-acidification) or non-acidified. Acidification can also take place upon arrival in the laboratory (= post-acidification).

Hypercalciuria is the most commonly diagnosed metabolic disorder in lithiasis patients (4). The bulk of the literature on urinary additives is therefore situated around this parameter. In the study by Chenevier-Gobeaux et al., three conditions (non-acidification, pre- and post-acidification) did not appear to produce a significant change in urinary calcium concentration in ten healthy volunteers. Similarly, the effect of post-acidification vs. non-acidification was examined on 567 routine urine samples from a tertiary centre, showing a good correlation with only 4.4% of samples outside the acceptable detection limit on the Bland-Altman graph (figure 6). In addition, in none of the patients, a correction of the diagnosis of normocalciuria was needed after acidification (31). Sodi et al. confirmed the non-effect of post-acidification on urinary calcium in 133 patients. Nonetheless, this study demonstrated the value of acidified samples (with 5 mol/L HCl) when timely processing of the urine collection is not possible, as the differences in urinary

calcium after seven days compared to basal calcium are largely within two standard deviations of the mean when acidified (figure 7) (32). Various studies on spot urine samples showed no significant effects of acidification on the concentration of the parameters calcium, sodium, magnesium, and phosphate (33,34).

Oxalate is determined spectrophotometrically at the laboratory of the university hospitals of Leuven. Acidification is necessary to prevent or dissolve the formation of calcium oxalate crystals and to prevent the oxidation of ascorbic acid to oxalate (35). According to different studies, the result for urinary oxalate does not significantly differ if acidification takes place after 24 hours (figure 8) (36,37). In non-acidified samples with an excessive value of urinary oxalate, calcium may be falsely reduced because of the formation of crystals (38). However, this analytical problem does not seem to be clinically relevant as, in the case of excessively high urinary oxalate, the clinician already has sufficient information. At the university hospitals of Leuven, acidification of the aliquot intended for the measurement of oxalate happens upon arrival in the laboratory.

A limited but significant decrease in citrate was noted for acidified urine collections, probably due to hydrogenation of citrate in acidic urine (34). To mitigate this risk, the acidity of the urine sample for citrate analysis is checked and neutralised with sodium hydroxide if necessary.

Uric acid stones are formed in a low pH environment. Consequently, it might be recommended to keep the urine alkaline, but this was refuted in a study by Petit et al. (39). Acidification of urine would also not affect the uric acid concentration significantly (34,39), however, this is contradicted in other scientific studies (37). In general, acidification when determining uric acid should preferably be avoided. Furthermore, it is

unambiguously recommended not to use HCl when measuring chloride or albumin because this may affect the results (37). Adding HCl can also cause dilution of urine (40).

It remains important to keep the container at 4°C during the collection to avoid bacterial overgrowth and consequently a rise in pH (34). In the laboratory, the collection should be brought to room temperature and mixed well before analysis to prevent loss through precipitation of crystals.

4. Can a urine sample (spot urine) replace the 24-hour urine collection in uro- and nephrolithiasis?

Spot urine is an alternative to the 24-hour urine collection, mainly used in case of difficult collection (e.g. elderly or children) (16). However, several parameters determined on a spot urine sample are not reliable due to the large variation in urinary ionic concentrations throughout the day caused by meals and activity. Multiple spot urine samples or a 24-hour urine collection remain the method of choice (4,41).

Conclusion

Patients at risk for active stone disease require additional metabolic screening including blood sampling for parathyroid hormone and two 24-hour urine collections on two non-consecutive days. After 3 to 6 months, treatment response should be evaluated with an extensive metabolic screening.

The supersaturation index can be useful in case of ambiguous results due to intra-individual variation or limited abnormalities. The value of this ratio in the context of the standard urinary parameters - supplementary or nevertheless essential? - requires additional research (28). The software application Lithorisk, a tool to calculate the supersaturation ratio, will be implemented in the university hospitals of Leuven in 2022.

A universal urine collection preservative unfortunately does not exist (42). It is not necessary to acidify the aliquot intended for calcium measurement. The oxalate sample needs acidification, but this can also take place after 24 hours. Acidification in the laboratory results in better standardization (fixed quantity of acid per volume of urine), is safer for the patient and ensures that only one container has to be filled.

Attachments (tables – figures)

General	Metabolic/inflammatory disorders	Genetic disorders	Structural malformations
Early-onset (<20 yo)	Hyperparathyroidism	Cystinuria	Solitary kidney (no increased risk of kidney stones but prevention of stone formation is even more important)
Positive family anamnesis	Malabsorption (e.g. Crohn's disease, celiac disease)	Primary hyperoxaluria	Vesico-ureteral reflux
>= 3 episodes in <= 5 years	Sarcoidosis	Renal tubular acidosis	Ureterocele
Bilateral (residual) lithiasis on imaging	Cushing syndrome	Xanthinuria	Medullary sponge kidneys
Hospitalization	Metabolic syndrome, insulin resistance	Cystic fibrosis	Pyelo-ureteral junction stenosis
Chronic or acute kidney disease, nephrocalcinosis		2,8-dihydroxyadenine urolithiasis	Horseshoe kidneys

Table I Patients at high risk for recurrent kidney stones (16)

Program	Lithorisk	EQUIL2	JESS
Parameters	calcium sodium potassium chloride magnesium citrate phosphate oxalate uric acid pH creatinine urea (cystine) (ammonia) (sulphate)	calcium sodium potassium chloride magnesium citrate phosphate pyrophosphate oxalate uric acid CO_2 sulphate ammonia creatinine	Very comprehensive database of (mixed) chemical reactions
Format	Offered as an application since 2021 (lithorisk.com)	Written in programming language	Written in programming language
Outcome	Supersaturation ratio	Supersaturation ratio Delta G = Gibbs free energy	Supersaturation ratio Distribution of different elements (e.g. Ca^{2+}, CaOx, $CaCit^{1-}$, $CaCitHPO_4$)

Table II Comparison of supersaturation calculation programs (21–23)

Creatinine 24-hour urine	M	F	Total
Decreased	231 (12%)	370 (17%)	601
Increased	235 (12%)	218 (10%)	453
Normal	1434	1589	3023
Total	1900	2177	4077
Aberrant	**24%**	**27%**	

Table III Percentage of urinary creatinine abnormalities in samples of adult men and women (research university hospitals of Leuven)

Creatinine 24-hour urine	Ambulatory	Hospitalized	External	Unknown	Total
Decreased	136 (13%)	220 (26%)	241 (11%)	4	601
Increased	87 (9%)	53 (6%)	311 (14%)	2	453
Normal	796	581	1639	7	3023
Total	1019	854	2191	13	4077
Aberrant	**22%**	**32%**	**25%**		

Table IV Percentage of urinary creatinine abnormalities in samples of adult ambulatory, hospitalized and external patients (research university hospitals of Leuven)

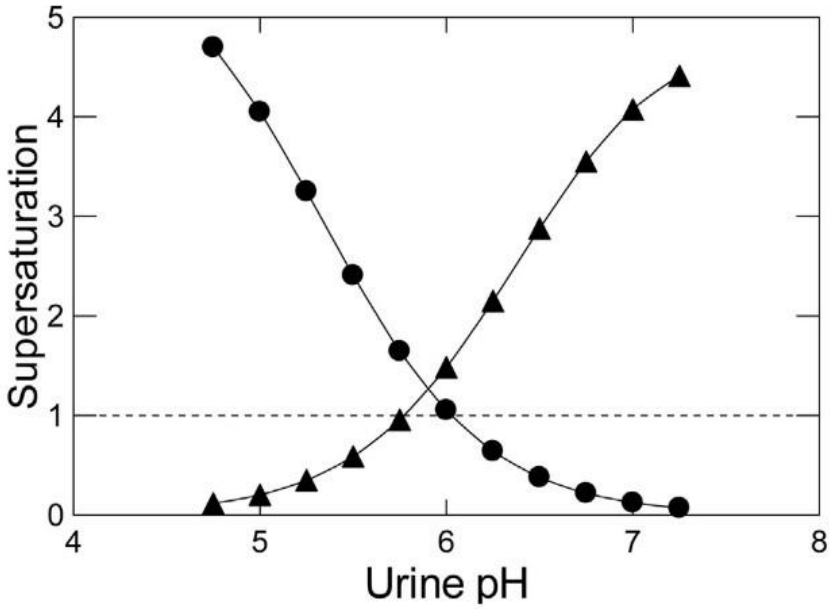

Figure 1 (Super)saturation of uric acid (dots) and calcium phosphate (triangles) in relation to the urinary pH. Values higher than 1 (100%) correspond to supersaturation (4)

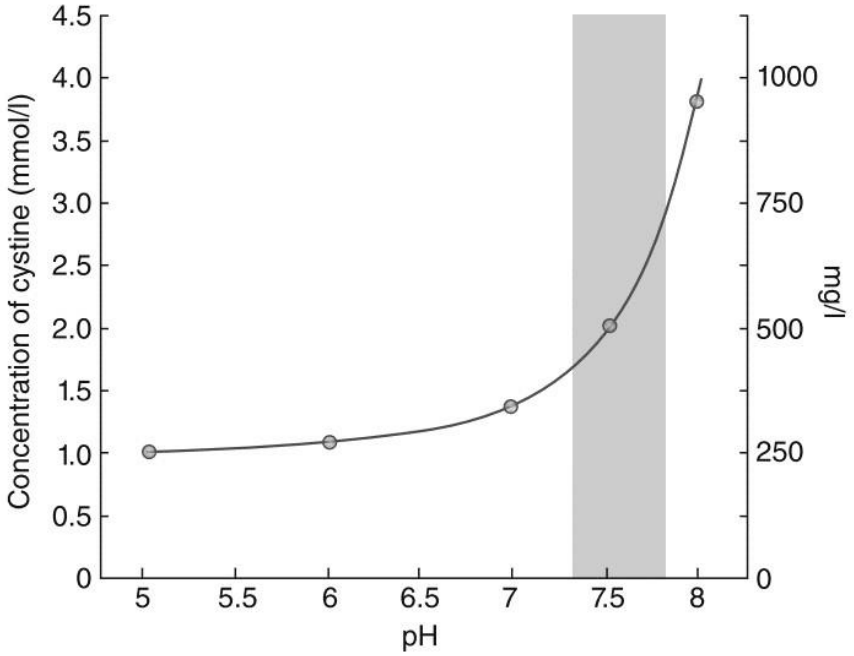

Figure 2 Solubility of cystine strongly increases at a urinary pH > 7 (10,11)

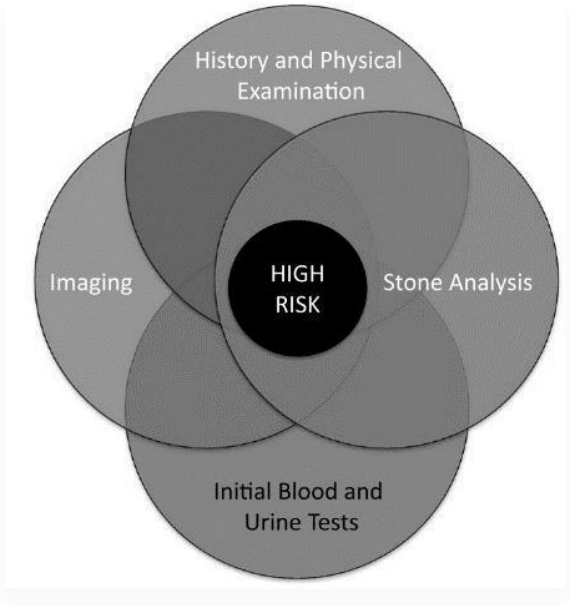

Figure 3 Diagnostic process in uro- and nephrolithiasis with identification of high-risk patients (6)

Figure 4 Supersaturation ratio of urinary parameters using Lithorisk (21)

	% Difference between Consecutive Samples			
	20% or Greater	30% or Greater	40% or Greater	50% or Greater
Primary analysis:				
Calcium	48.1	30.1	17.8	10.2
Citrate	32.5	17.2	9.4	5.7
Oxalate	30.1	14.1	6.2	2.8
Uric acid	21.3	8.3	3.6	2.0
Vol	42.4	24.9	13.9	7.4
Sensitivity analysis with 2 collections on consecutive days:				
Calcium	47.9	30.1	17.8	10.0
Citrate	32.3	17.0	9.3	5.6
Oxalate	29.9	14.0	6.2	2.8
Uric acid	21.1	8.2	3.6	2.0
Vol	42.4	24.8	13.9	7.4
Sensitivity analysis considering only clinically meaningful differences:*				
Calcium	48.3	30.3	19.0	10.1
Citrate	31.6	16.2	8.4	4.7
Oxalate	31.6	15.0	6.7	3.1
Uric acid	21.3	8.3	3.6	2.0
Vol	47.9	29.7	17.4	9.6

Figure 5 Percentage of patients with differences in urinary parameters in two consecutive samples (2)

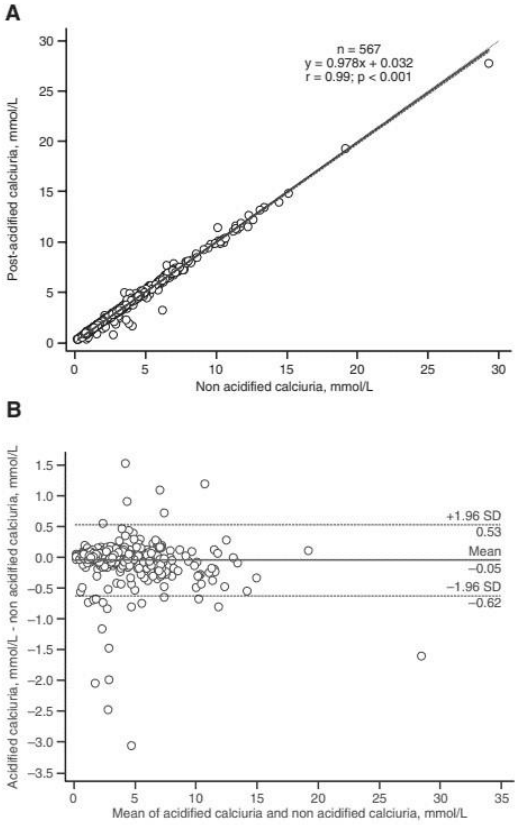

Figure 6 Calciuria before and after acidification, (A) linear regression, (B) Bland-Altman graph (31)

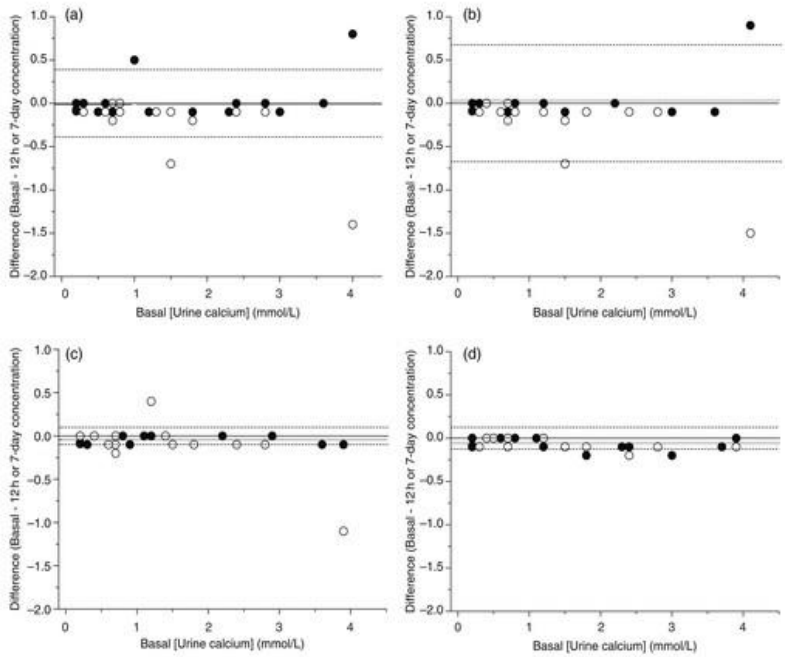

Figure 7 Bland-Altman graphs: basal concentration of urinary calcium in relation to 12-hour concentrations (filled circles) or seven-day concentrations (open circles) at basal pH (a), and with 0.1 mol/L (b), 1 mol/L (c) and 5 mol/L HCl (d) (32)

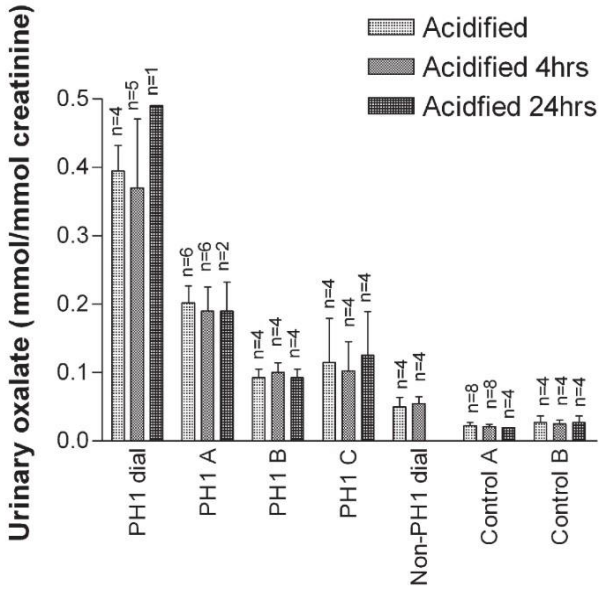

Figure 8 Effect of acidifying urine at different times on the concentration of oxalate (36)

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