# Biochemical Risk Evaluation in Patients with Urolithiasis

84

Hans-Göran Tiselius

**Abstract**

This chapter is an overview of the biochemical evaluation of patients with urinary tract stone disease. The aim is to give practical aspects on how the search for risk factors can be carried out when stone composition is known or unknown. The physical chemistry of stone formation is superficially touched.

Aspects on the importance of the medical history, the radiographic evaluation, and the stone, blood, and urine analysis are discussed. The goal of every metabolic or biochemical evaluation is to provide a basis for a reasonable and effective recurrence prevention. The intention with this chapter is to give information in this regard.

**Keywords**

Stone analysis • Blood analysis • Urine analysis • Calcium stones • Uric acid stones • Cystine stones • Infection stones • Medical history • Radiography • Preservative of urine • AP(CaOx) index • AP(CaP) index • Protein intake

## Introduction

For a rational and effective prevention (metaphylaxis) of recurrent stone formation in the urinary tract, it is necessary to identify relevant risk factors that explain or contribute to the pathology [1]. In view of the fact that almost all patients with uric acid stones, infection stones, and cystine stones and approximately at least 50 % of patients with calcium stones will continue to form new stones, measures aiming at a reduced risk are highly desirable.

It is of note that the introduction of noninvasive or low-invasive methods for active stone removal, undoubtedly, resulted in a rather nihilistic attitude among several urologists who subsequently considered both risk evaluation and recurrence prevention as unnecessary overdoing for their stone patients.

Although a definite explanation for calcium stone formation is lacking, there are several obvious risk factors, the correction of which will result in an arrest or at least a significant reduction in the rate of stone formation. For patients with uric acid, cystine, and infection stone formation, the causes are well recognized and so are the therapeutic tools.

Moreover, it needs to be emphasized that although the procedures for stone removal have become dramatically improved and relatively easy, none of such procedures are entirely without complications and definitely not without cost. Active stone removal—with a slightly increased indication during recent years—is applicable to roughly 30–40 % of the patients [2, 3]. For the remaining patients, stones are expected to pass spontaneously. In most situations, a nonsurgical treatment is superior to all kinds of surgical stone removal in its widest sense. Also for the latter group of patients, it will usually be necessary with medical support and very often repeated visits to an emergency unit. According to several economic analyses, selective recurrence prevention is cost effective [4–10].

H-G. Tiselius, M.D., Ph.D.
Division of Urology, Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, SE-141 86, Sweden
e-mail: hans-goran.tiselius@telia.com, hans-goran.tiselius@ki.se

J.J. Talati et al. (eds.), *Urolithiasis*,
DOI 10.1007/978-1-4471-4387-1_84, © Springer-Verlag London 2012

## Stone Composition

Search for factors responsible for or contributing to the stone formation requires knowledge of the stone composition. The fundamental step in the evaluation of the disease, therefore, is an appropriate stone analysis. How this analysis technically should be carried out is extensively discussed elsewhere in this book (see Chap. 85). Suffice it here to state that patients always should get instructions to collect passed stones or stone fragments. Analysis of the stone composition is recommended at least once for every patient. Repeated analysis is indicated if it can be assumed that the prerequisites for stone formation for any reason have been changed [4].

With an appropriate stone analysis, we will know whether the patient has produced a calcium stone with calcium oxalate (calcium oxalate monohydrate [COM] and/or calcium oxalate dihydrate [COD]), calcium phosphate (hydroxyapatite [HAP], octacalcium phosphate [OCP], carbonate apatite, whitlockite, or brushite), mixtures of calcium oxalate and calcium phosphate, or a non-calcium stone composed of infection stone material (magnesium ammonium phosphate + carbonate apatite, ammonium urate), uric acid, or cystine. The stone analysis also makes it possible to identify stones composed of less commonly encountered crystal phases such as 2,8-dihydroxyadenine, xanthine, and silicates.

Not unexpectedly a large number of patients never bring a stone to analysis, because the stone material has been lost, passed without any obvious symptoms, or remains in the renal collecting system inaccessible to appropriate analysis. Also in these cases it is desirable to draw reasonable conclusions on the stone composition.

In the absence of a stone analysis, qualified indirect assumptions are necessary [11]. An algorithm for such a procedure is shown in Fig. 84.1.

Today, the stone diagnosis is established by urography; plain radiograph of kidneys, ureters, and bladder (KUB); or non-contrast helical computerized tomography (NCCT). Ideally, when both KUB and NCCT have been carried out, it can be concluded that a stone visible on the NCCT image and not visible on the KUB most likely is composed of uric acid [4, 12]. It should be noted, however, that very large stones composed of uric acid give a weak density also on the KUB.

When only a KUB is available, some features are useful. Infection stones (staghorn or non-staghorn stones) usually, but not always, have a layered morphology. Cystine stones have a radiodensity that is low relative to the size of the stone. Stones with a very compact structure with a high density are usually composed of COM or brushite, whereas stones with burdock (spiky) morphology suggest COD. It is of note that complete staghorn stones can develop with any crystal phase, and the finding of a staghorn stone does not necessarily mean an infection etiology.

When an NCCT examination is available, measurement of Hounsfield units (HU) can be very helpful for the decisions on the stone composition [13]. Unfortunately, there is an overlapping of HU recordings for different stone constituents. The latter problem has recently been addressed by applying dual photon energy technique [14, 15], but such

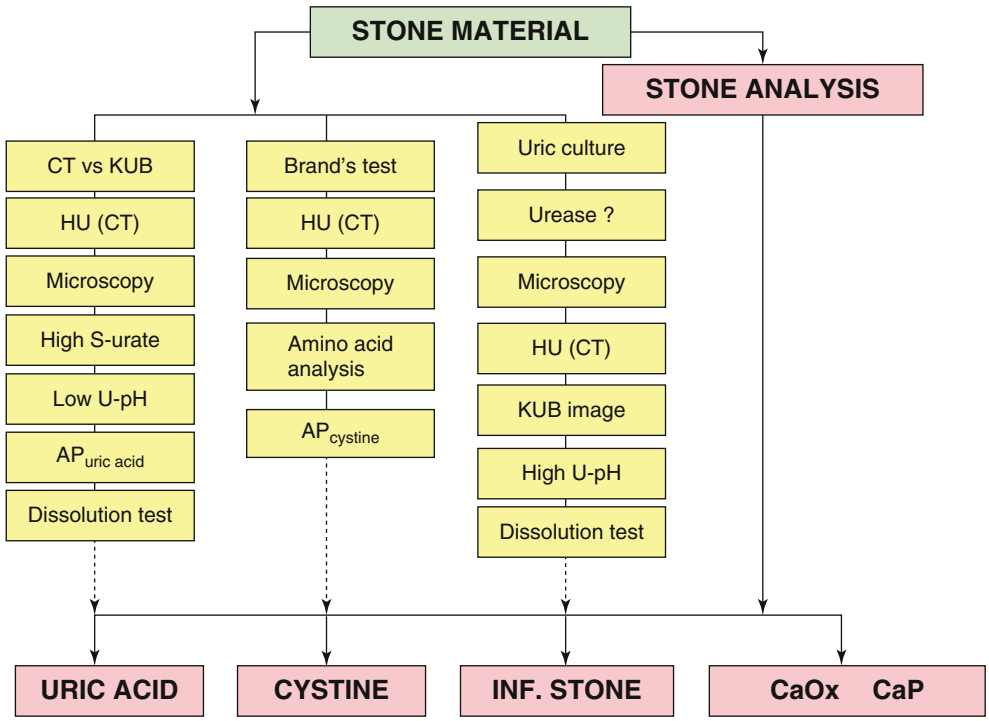


**Fig. 84.1** An algorithm for deciding what kind of stone the patient has formed

advanced facilities presently have limited availability. Three HU intervals can roughly be used for practical conclusions on stone composition: high (HU > 1,000), medium (HU = 500–1,000), and low (HU < 500). The low values correspond to stones composed of uric acid, cystine, and struvite (magnesium ammonium phosphate) and the highest for COM and brushite. Stones with intermediate HU values might be composed of COD, HAP, and carbonate apatite. Further support for an appropriate conclusion on stone composition can be made with other tools.

Microscopic identification of typical cystine (hexagonal) or struvite (coffin-shaped) crystals is diagnostic for cystinuria and infection stone disease, respectively [11, 16, 17]. Demonstration of COD crystals might indicate calcium oxalate stone formation, but such crystals are commonly encountered also in urine from non-stone-forming subjects and thus of limited diagnostic value. Urine sediment with brown/red color (from a sample without hematuria) is typically found in association with uric acid stones.

When cystine is a possibility, the sodium nitroprusside test (Brand's test) is a useful qualitative analysis to confirm cystinuria [18].

A low urine pH is associated with uric acid stone formation [19, 20] and a high pH with infection stones and calcium phosphate stones [21]. In the absence of standardized principles for pH measurements, it is usually difficult to use urinary pH for conclusions, unless the pH recordings are extreme. Fasting morning urine samples might be most useful in this regard and also of value for decisions on whether the patient has an acidification defect or not [4, 22–24], but it is not always possible to get such samples.

A high serum or plasma level of urate (in patients with normal renal function) might give further support to uric acid stone formation, provided other observations do not exclude that type of stone.

When still in doubt of the kind of stone disease, measurements of supersaturation levels with uric acid and cystine can give valuable information. This approach is further discussed as follows.

## Medical History

Like in most other fields of medicine, a careful medical history can give valuable clues to or a full explanation of the stone disease. There are several medical diseases as well as various forms of pharmacological treatment that are more or less closely associated with an increased risk of abnormalities in urine composition, crystallization, and stone formation. The most important of these factors will be summarized below.

### Diseases Associated with Stone Formation

One of the best recognized explanations for calcium stone formation is *hyperparathyroidism* [22, 25–27]. Adenomas or hyperplasia of the parathyroid glands is responsible for an excessive production of parathyroid hormone (PTH). The biochemical consequence that leads to stone formation is hypercalciuria caused by hypercalcemia. The importance of a correct diagnosis is best understood by the fact that a correction of this abnormality usually results in arrest of stone formation.

There are also other conditions with hypercalcemia that result in an increased urinary excretion of calcium and accordingly an increased risk of calcium stone formation. In this regard, *sarcoidosis* and *immobilization* need attention. High urinary calcium levels also are encountered in patients with *hyperthyroidism* [22].

Abnormalities in intestinal function with fat malabsorption, loss of water, and alkali are seen in patients with Crohn's disease, intestinal resection, different forms of bypass procedures used for weight-reducing purposes, pancreatic insufficiency, and other conditions with intestinal malfunction [22, 25–27]. The risk of stone formation is based mainly on high urinary concentrations of oxalate (*enteric hyperoxaluria*), but the small urine volumes together with low pH levels also contribute to a pronounced crystallization propensity. These patients also have low excretion of calcium, but since oxalate is a relatively more powerful determinant of calcium oxalate supersaturation, very high crystallization driving forces are obtained.

Although the majority of patients with intestinal malfunction and diarrhea form calcium oxalate stones, it is of note that uric acid stones commonly are seen in patients with *ileostomy*, because of the very low pH levels encountered as a result of extreme losses of alkali. Similarly in patients with *ulcerative colitis*, both uric acid and calcium oxalate stones might form.

Whereas 24-h oxalate excretion levels in the range of 0.6–1.2 mmol are typical for patients with enteric hyperoxaluria, higher oxalate values might suggest *primary hyperoxaluria* [22]. This rare inborn error of metabolism can present with different degrees of severity. In the most advanced form, it is a life-threatening condition with both excessive calcium oxalate stone formation and calcium oxalate tissue deposits. Primary hyperoxaluria must be excluded when stone formation starts very early in life.

There are some less common disturbances in purine metabolism leading to increased excretion of urate and uric acid stone formation. In *Lesch-Nyhan syndrome*, the treatment with high doses of xanthine oxidase inhibitors might result in precipitation of *xanthine* [22]. Xanthine oxidase is responsible both for the conversion of hypoxanthine to xanthine and of xanthine to urate. Another abnormality in

purine metabolism (defect function of adenine phosphoribosyltransferase) is the origin of 2,8-dihydroxyadenine stones [22].

Distal renal tubular acidosis (dRTA) in a complete or partial form causes stone formation by a combination of hypercalciuria and alkaline urine. The acidification defect also leads to hypocitraturia. The condition that is most common in women should be suspected in case of calcium phosphate stone formation [24]. The inability to acidify urine below pH 5.8 is of diagnostic importance [4, 23]. A fasting morning urine pH, analysis of urine pH in repeated collections during the day (pH profile), or analysis of urine after an acid load can be used for diagnostic purposes (discussed later) [24]. Proximal renal tubular acidosis (pRTA) is not associated with stone formation.

Cystinuria is an inborn error of metabolism that explains stone formation in 1–2 % of stone formers. The homozygous form is necessary for cystine concentrations leading to stone formation. The increased excretion of the amino acids lysine, ornithine, and arginine that also are excreted in large quantities is not important for stone formation, and the loss of these amino acids is generally considered to be without important physiological or metabolic consequences.

The increased risk of stone formation in patients with *metabolic syndrome* is well recognized [28, 29], and so is the risk of stone formation in patients with *hypertension* and *diabetes mellitus* [29].

## Pharmacological Agents Associated with Stone Formation

There are some forms of pharmacological treatment to which attention should be paid as a possible explanation of stone formation.

Supplements of *calcium* and *vitamin D* commonly used in the treatment of patients with osteoporosis can give rise to hypercalciuria [30]. The intake of these agents together with meals—and not between—should be advised.

Vitamin C in large (orthomolecular) quantities can result in an increased excretion of oxalate. Individual variations most certainly exist, and the allowed amount of vitamin C has remained a matter of debate. It is commonly considered safe if the daily amount of ascorbate does not exceed 2–4 g [31–33].

Thyroid hormone causes hypercalciuria. Acetazolamide increases urine pH while simultaneously reducing urinary citrate in a way similar to that seen with dRTA [34]. These alterations lead to an increased risk of calcium phosphate precipitation and stone formation.

The low solubility of sulfonamides, triamterene, and indinavir might result in precipitation and stone formation with that composition.

Treatment with corticosteroids increases the risk of stone formation by an increased calcium excretion.

## Identification of Anatomical and Morphological Abnormalities

Factors causing stagnation of urine or a turbulent flow are probably of great importance in the stone-forming process, and their presence needs to be identified.

Conditions with obstruction of the ureteropelvic junction, ureteral strictures, horseshoe kidneys, and malrotated kidneys are usually associated with hydronephrosis. Crystalline material that develops in retained urine of a dilated collecting system cannot easily be eliminated [35, 36]. In sufficiently supersaturated urine, the crystals grow and aggregate to clinically important stones. Intrarenal obstructions to the urine flow, such as narrow calyx necks and calyx diverticula, are other risk factors of stone formation.

All of the mentioned anatomical abnormalities can usually be detected by NCCT.

Another common clinical entity is tubular ectasia (medullary sponge kidney disease [MSK]). This abnormality might occur in the whole kidney or only in part of the kidney. The best procedure for discovering MSK is probably by a standard urography [37, 38].

With NCCT, the diagnosis can be indirectly suspected from the distribution of calcifications. For correct diagnosis of MSK with NCCT, special image manipulation is necessary, and with the less common use of contrast medium, there is a risk that the diagnosis of MSK often will be overlooked.

It also needs to be emphasized that those patients who have been subjected to invasive surgical procedures might have scar tissue and various iatrogenic intrarenal abnormalities of great importance for stone formation.

## Basic Blood Analyses

For all patients with urolithiasis, it is essential to get information on the renal function. Thereby, analysis of *serum(S-) creatinine* is a sufficiently accurate guide.

As mentioned previously, detection of hyperuricemia can give support to an otherwise suspected risk of uric acid stone formation. It is of note, however, that S-*urate* is increased when the renal function is reduced, and a simultaneous *S-creatinine* analysis is necessary for conclusions. Moreover, a normal urate level in no way excludes the possibility of uric acid stone formation. A relationship between hyperuricemia and calcium oxalate stone formation also has been suggested [39]. There are, unfortunately, no recent studies of such a mechanism, and possibly, a high urate level only reflects one

of several abnormalities associated with the metabolic syndrome [21, 28, 29].

Inasmuch as most patients form calcium stones, it is important to find those in whom hypercalcemia is an underlying reason. Of conditions with hypercalcemia, it is most essential to identify patients with hyperparathyroidism, because that is in most cases a surgically curable condition. When the serum or plasma calcium exceeds 2.50–2.60 mmol/L, there is good reason to repeat the measurement and to add analysis of *ionized calcium* and *PTH*. Moreover, analysis of *S-phosphate* might be of diagnostic value in these patients.

Another important serum variable is *S-potassium*, since hypokalemia causes hypocitraturia [40, 41] and thereby an increased risk of calcium oxalate and calcium phosphate precipitation, growth, and aggregation [40, 42].

The blood analyses mentioned here are the only ones that I personally find unavoidable in the work-up of patients with stone disease. It is of course important to note that specific circumstances might require other blood analyses, but the variables listed are those that should be considered as a basic set for every stone former.

## Solution Chemistry of Uric Acid

Precipitates in which urate is an important constituent are most often composed of uric acid. Although sodium urate theoretically can form stones, that crystal phase is rarely encountered clinically, and the same is true for potassium urate. The most common crystal phase beside uric acid is ammonium urate, but the formation of an ammonium urate precipitate is a result of infection with urease-producing bacteria at sufficiently high concentrations of urinary urate. The latter precipitate therefore should be considered to reflect an infection stone problem (see later) [4].

There are two prerequisites for formation of uric acid stones. Firstly and most important, the pH should be low. Secondly, there must be a reasonably high concentration of urate, either caused by an excessive excretion of urate or by a small urine volume. But it needs to be emphasized that uric acid precipitation can occur also with normal urinary urate concentrations, provided the urine is sufficiently acid. With this basic understanding, the ion-activity product of uric acid $AP_{uric\text{-}acid}$ can be calculated from the following formula [21, 22]:

$$AP_{uric\text{-}acid} = \frac{C_{urate} \cdot 10^{-pH} \cdot 0.53}{(1 + 1.63 \cdot 10^{5} \cdot 10^{-pH})}$$

In this formula, the concentration of urate ($C_{urate}$) is expressed in mol/L. The formation (FP) and solubility (SP) products of uric acid are approximately $5.0 \cdot 10^{-9}$ $(mol/L)^2$ and $2.0 \cdot 10^{-9}$ $(mol/L)^2$, respectively [21].

From a clinical point of view, $AP_{uric\ acid}$ can be derived from analyses of a 24-h urine sample only by measuring urate and pH. It is essential, however, to get a representative measurement of urine pH, and it has been the author's own routine to measure the urine composition in one 16-h and one 8-h urine sample [4, 11]. Even such an approach is not ideal, but in most clinical situations, it gives a rough idea of the supersaturation with uric acid. This analytical step also can be very helpful to confirm or exclude uric acid stone disease. The therapeutic goal in uric acid stone-forming patients should be to decrease $AP_{uric\ acid}$ to a level below $SP_{uric\ acid}$.

Sodium azide (0.3 mmol/L) is an appropriate preservative to add to the collection bottles: 30 mL for a 24-h urine sample, 20 mL for a 16-h sample, and 10 mL for an 8-h sample [11]. Moreover, it needs to be emphasized that the pH should be measured with a glass electrode as soon as possible after completion of the urine collection. That means that the sample should be taken care of within the first few hours after delivery of the sample to the laboratory. It goes without saying that urate cannot be measured in acidified samples!

Corresponding formulas for $AP_{ammonium\ urate}$ and $AP_{sodium\ urate}$ have been derived and can be found elsewhere [21].

The estimate of $AP_{uric\ acid}$ shown previously can be used as part of a risk evaluation, but it is also an excellent tool for follow-up of patients during recurrence prevention or stone dissolution.

## Solution Chemistry of Cystine

An estimate of the ion-activity product of cystine ($AP_{cystine}$) is obtained from information on the concentration of cystine in urine and the pH. Although the expression for calculating $AP_{cystine}$ looks complicated, the formula can easily be stored in a computer and only requires information on the concentration of cystine ($C_{cystine}$) and pH in any urine sample [22]:

$$AP_{cystine} = \frac{(10^{-pH})^2 \cdot C_{cystine} \cdot 0.155}{\left[1 + (0.39 \cdot 10^{9} \cdot 10^{-pH}) + ((10^{-pH})^2 \cdot 3.51 \cdot 10^{16})\right]}$$

Roughly and at normal urine pH levels, the risk of forming cystine crystals occurs when the cystine concentration exceeds 1 mmol/L [43]. The solubility of cystine is increased when the pH is increased, and a rule of thumb tells us that approximately 2 mmol/L can be held in solution at pH 7 and 3 mmol/L at pH 8. It is, however, difficult to maintain a urine pH of 8 in a consistent way, and such pH levels can only be expected with powerful pharmacological alkalinizing therapy [43]. Nevertheless, it is important always to include a pH measurement in the biochemical work-up and follow-up of patients with cystinuria.

Similar to what was stated previously for $AP_{uric\ acid}$, $AP_{cystine}$ can be derived from analytical data in 24-h urine, from any other short-term urine collection, or even from a spot urine sample. Sodium azide (3 mmol/L) is an excellent preservative, and 30 mL is recommended for a 24-h sample.

The concentration of cystine should be analyzed with amino acid chromatography, whereby also the concentrations of lysine, ornithine, and arginine are obtained [43]. The latter three amino acids are important for diagnostic purposes, but they are otherwise thought to be without clinical importance. The possible long-term effects of constant loss of all four amino acids, however, have been poorly studied.

The excretion of cystine increases with a high sodium load [44], and if there is a clinical interest in urinary sodium, it should be noted that the collection either has to be made without sodium azide or corrected for the sodium that already is present in the bottle. In a therapeutic and follow-up perspective, it is, of course, necessary to closely look at the urine volumes produced by the patient.

## Biochemical Evaluation of Patients with Infection Stone Disease

Although it is possible to get an approximate estimate of the ion-activity product of magnesium ammonium phosphate ($AP_{MAP}$) [21], the clinical value of such calculations is usually small, partly because of the mixture of crystal phases that comprise the infection stone (struvite, carbonate apatite, and hydroxyapatite) and partly by the fact that infection stones only form and grow in urine with urease-producing microorganisms. The urease activity also brings the pH up to high levels, and it is generally considered that infection stone material does not precipitate unless the pH exceeds 7.5–8 [22]. A standard urine culture in most situations can be used for identification of the microorganism responsible for the stone formation. A specific analysis is required to show whether the microorganism produces urease or not, and the laboratory should be asked to provide that information. Occasionally, infection with *Ureaplasma urealyticum* is the responsible factor, and if no bacterial growth or history of bacterial infection can be demonstrated in patients who apparently have formed infection stones, it is worthwhile to look for that microorganism. Detection of *Ureaplasma urealyticum*, however, requires a special sampling technique with a specific medium [45].

In the work-up of patients with stones and infection, it is essential to distinguish between infection stones and stones with associated infection. The latter group of patients has stones of another composition, usually calcium oxalate, that have been secondarily infected with bacteria not producing urease. Such infection has been associated with originally sterile stones, and it is not unusual that such a development is initiated after invasive stone-removing procedures with or without residual stone material in the kidney.

When urease-producing microorganisms are the cause of secondary infection, it therefore often is necessary to search for risk factors also of calcium stone formation (see below).

Without appropriate recurrence prevention, there is a high risk of rapidly recurring and growing infection stones, and the efficacy of the treatment efforts is better followed with repeated radiographic examinations than with urine analyses. Nevertheless, urine cultures and occasionally pH measurement can be recommended for the long-term recording of these patients.

## Biochemical Risk Evaluation of Patients with Calcium Stone Disease

Although our understanding of how calcium stones form in the urinary tract is far from complete, it is undisputable that the composition of urine plays an important role. Hereby, the levels of saturation/supersaturation with calcium oxalate as well as with calcium phosphate [21, 46] together with concentrations of factors that are considered as important modifiers of crystal nucleation, crystal growth, and crystal aggregation are of interest [47]. From a clinical point of view, the available information to a large extent is limited to what we can measure in finally processed and voided urine. This shortcoming becomes particularly obvious when we consider that the initial—and possibly most important—steps in calcium stone formation appear to take place at high nephron levels, where the urine composition is much different from that recorded in caliceal, pelvic, or bladder urine. Nevertheless, it stands to reason that precipitation of calcium oxalate—the major constituent of most stones—obviously in most cases does not take place at levels above the distal part of the collecting ducts [48–51]. Recent evidence, moreover, indicates that the formation of calcium oxalate occurs either at areas of submucosal Randall's plaques exposed to urine by epithelial erosion or as trapped accumulations of calcium phosphate at the opening of the collecting ducts on the tip of the papilla [52–54].

If we disregard changes in urine composition that can be expected to occur during the passage of urine through calices, renal pelvis, ureters, and during storage in the bladder, final urine is likely—at least to some extent—to reflect the biochemical environment in which stone formation takes place.

It is thus logical that the biochemical risk evaluation should comprise analysis of the composition of one or several 24-h urine samples or any other sample of urine collected during a defined period of the day [4]. Although practically convenient, this routine is far from optimal. Urine composition varies considerably during the day as a result of food intake, drinking, and physical activities [55].

The risk of pathological or abnormal crystallization is not a continuous process, but is likely to be associated with peaks of supersaturation with either calcium oxalate or calcium phosphate or with other risk factors of stone formation. Such peaks never can be identified when urine is analyzed in long-term urine collections, in which we only can conclude whether individual urine variables, calculated risk parameters, or levels of saturation seem to be above or below an expected average or not. The ideal risk evaluation accordingly should be carried out by analysis of urine composition in a continuous series of short-term urine samples (e.g., 1, 2, or 4 h) during one or many 24-h periods. Such measurements also have been reported in the literature [55–57], but the extensive number of analyses that such approach requires is a limiting factor in the clinical routine work. Another problem is that there often is an obvious reluctance from patients to handle their own urine, and to accomplish analysis of a large number of correctly collected samples during one or several 24-h periods under normal living conditions, therefore, is less likely to be successful unless in specific cases.

The number of analyses required for useful information might be advantageously reduced by using, for instance, the Bonn Risk Index [58] or direct measurements of the risk of calcium oxalate crystallization [21]. Such procedures, however, cannot be applied without special equipment and analytical expertise that are not commonly available. There are also some test kits aiming at measurement of the crystallization propensity of urine samples, but the experience of such methods is limited [59].

The bottom line is that analysis of composition of 24-h or any urine sample collected during a defined period of the day is useful for the biochemical work-up of patients with calcium stone disease. But it is important to be aware of the limitations outlined previously because they also explain why comparison between normal subjects and stone-forming patients very often only gives discrete differences with a large overlapping of data [60, 61].

Whether one or a series of urine collections are necessary has remained a matter of debate over the years. Most certainly, the likelihood of finding one or several abnormalities increases with the number of collections [23, 59]. The reason for that is that urine composition varies not only from hour to hour but also from day to day and from week to week and is subject to a significant variation during the year. It is not easy to know how such a problem best should be handled from a clinical point of view, but the author's own preference has been that if one urine collection does not give any clues to the individual's risk of stone formation, then a repeated collection appears appropriate [62]. With such a routine, it has been possible to maintain good cooperation with the patients and still to get valuable information as a basis for recurrence preventive measures.

So what should be analyzed? In order to get sufficient information on the saturation levels, relatively accurate ion-activity products of calcium oxalate and various calcium phosphate crystal phases can be obtained by iterative approximation as published in the literature by Robertson and coworkers [63]: EQUIL2 [64], SEQUIL [65], JESS [66], or any other computerized calculation program. A major disadvantage is that all of them require a large set of urine variables.

Based on calculations carried out with the EQUIL program, it was shown that the most important determinants for the ion-activity product of calcium oxalate are the excretion of calcium, oxalate, citrate, magnesium, and the urine volume [21]. For the ion-activity product of calcium phosphate ($AP_{CaP}$), the corresponding variables are calcium, phosphate, pH, citrate, and urine volume [67]. From these urine constituents, approximate estimates (indices) of the ion-activity products were derived [21]:

$$\text{AP(CaOx) index} = \frac{A \cdot \text{Calcium}^{0.84} \cdot \text{Oxalate}}{\text{Citrate}^{0.22} \cdot \text{Magnesium}^{0.12} \cdot \text{Volume}^{1.03}}$$

$$\text{AP(CaP) index} = \frac{B \cdot \text{Calcium}^{1.07} \cdot \text{Phosphate}^{0.70} \cdot (\text{pH} - 4.5)^{6.8}}{\text{Citrate}^{0.20} \cdot \text{Volume}^{1.31}}$$

These indices, in which the excreted variables during the collection period should be expressed in mmol and the volume in liters, correspond to the ion-activity products as follows:

$$\text{AP}(\text{CaOx})\,\text{index} \sim 10^{8} \cdot \text{AP}_{\text{CaOx}}$$
$$\text{AP}(\text{CaP})\,\text{index} \sim 10^{13} \cdot \text{AP}_{\text{CaP}}$$

In the formula for calculating indices, $A$ and $B$ are numerical factors determined by the duration of the collection periods (Table 84.1). CaP does not represent a specific calcium phosphate crystal phase but reflects the ion-activity products of naturally occurring calcium phosphate crystal phases. Interpretation of $AP_{Brushite}$, $AP_{OCP}$, and $AP_{HAP}$ in terms of AP(CaP) index has been published elsewhere [67].

Other factors that obviously are of great importance for the risk of abnormal crystal formation are the influence of various small as well as large molecular inhibitors of crystallization [61, 68]. In calculations of AP indices or more accurate ion-activity products, however, no consideration is paid to the influence of urinary macromolecules [47]. Previous studies have shown that by adding an estimate of the inhibition of crystal growth and/or crystal aggregation, an improved distinction can be made between stone-forming patients and normal subjects [61, 68]. Unfortunately, there are so far no generally accepted routine methods for measuring various inhibitory properties.

**Table 84.1** Factors *A* and *B* to be used when calculating AP(CaOx) and AP(CaP) index values

| Collection period (h) | 24 | 16 | 12 | 8 | 4 | 2 | 1 |
|---|---|---|---|---|---|---|---|
| Factor *A*—AP(CaOx) index | 1.9 | 2.3 | 2.7 | 3.2 | 4.5 | 6.3 | 8.8 |
| $10^3 \times$ Factor *B*—AP(CaP) index | 2.7 | 3.0 | 3.2 | 3.6 | 4.3 | 5.1 | 6.1 |

There is, however, no consensus on whether urinary inhibiting activities exert their most important effect in diluted urine (at high nephron levels) or in whole urine (at a caliceal level). Moreover, although there is an array of large molecules that have an inhibitory or promoting activity, there are no established ways in which they can be therapeutically influenced except by changing pH and by increasing the excretion of citrate and magnesium. Small molecular inhibitors such as citrate and magnesium are already included in the list of important determinants for the ion-activity products of calcium oxalate and calcium phosphate. Therapeutically induced increments in citrate and magnesium might favorably reduce the ion-activity products of calcium oxalate and calcium phosphate and in addition to that increase the inhibition of the crystallization processes of both salts. The clinical importance of other small molecular inhibitors—such as pyrophosphate, phytate, and some metallic ions—has not been definitely established and is therefore not included in the routine risk evaluation suggested later in this chapter.

Analysis of creatinine is of great importance in order to decide whether the urine collection is complete or not. It is common that patients deliver urine samples that do not correspond to the urine produced during the intended collection period. Samples might be too small or too large, but with knowledge of the patient's body weight, the recorded creatinine excretion can be compared with predicted creatinine excretion. For 24-h urine samples, the relationship between body weight and urinary creatinine is shown in Fig. 84.2 [11]. Analysis of urea is useful because the urea level reflects the intake of protein. That value can be obtained from the following formula [11, 24]:

$$\text{Protein intake}\left(\text{g/24 h}\right) = \text{Urea}\left(\text{mmol/24 h}\right)0.18 + 13$$

By comparing the accordingly recorded protein intake with that recommended (0.8–1.0 g/kg body weight), the dietary advice can be facilitated.

It has been suggested that urinary urate concentrations are important for calcium oxalate precipitation. A salting-out effect, as demonstrated experimentally, has been put forward as the reasonable explanation for a relationship between hyperuricosuria and calcium oxalate stone disease [69]. There might be geographical variations of that risk, but it is the author's personal opinion that in most patients, a high urate excretion reflects a diet that also in other ways changes urine composition in a crystallization-promoting direction. Contradictory results also have been reported from allopurinol treatment of patients with calcium oxalate stone disease [39, 70].

Recent as well as earlier reports have indicated that urinary pH is of fundamental importance, not only for calcium phosphate precipitation but also for calcium phosphate dissolution and thereby probably also for calcium oxalate precipitation/nucleation [48]. It is, however, not easy to get a representative measurement of urine pH, which is subject to a considerable variation during the day. What is said previously about the shortcomings of analysis of different urine constituents in 24-h urine is even more relevant for pH. Moreover, pH changes during storage, and if not measured directly after completion of the urine collection, erroneous results can be obtained. Ideally, pH should be recorded as a pH profile with repeated and frequent measurement during the 24-h period [24]. Alternatively, one or several pH measurements in urine collected during well-defined periods are useful and for larger groups of patients, undoubtedly, most practical.

The following variables might be useful to include in the search of risk factors of calcium stone formation [4]:

- Calcium
- Oxalate
- Phosphate
- Citrate
- Magnesium
- pH
- Volume
- Creatinine
- Urate (optional)
- Urea

For the measurement of pH, it has been the author's own preference to collect samples between 22:00 h and 06:00 h, with sodium azide (10mL of a solution with a concentration of 3 mmol/L). Although this is not a fasting urine sample, it comes close to that and in anyway represents urine from a standardized collection period. Inasmuch as the 8-h urine sample suggested does not contain any acidifying agent as preservative, the sample also can be used for analysis of urinary urate. In the latter case, it is also recommended to measure creatinine provided that the total urate excretion, and not only the concentration, is of interest.

The other urine variables should be analyzed in a urine collection to which an acidifying agent has been added. It can be a 24-h sample or any sample collected during another defined period of the day. The author's own routine during recent years has been a 16-h urine sample

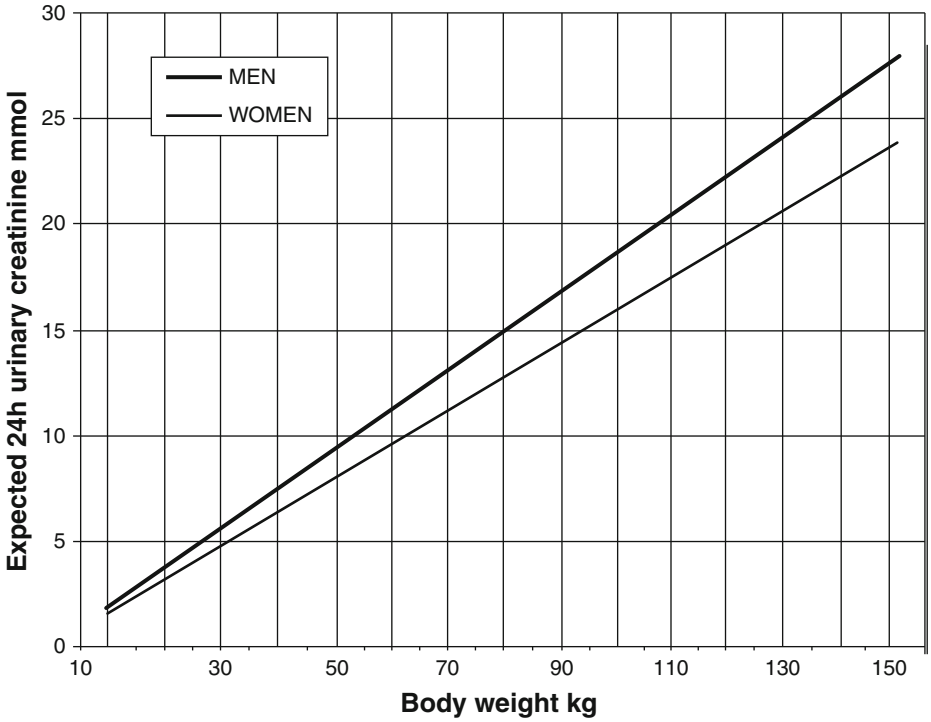


**Fig. 84.2** Relation between bodyweight and expected 24-h excretion of creatinine in men (*bold*) and women (*thin*) [11]

collected between 06:00 h and 22:00 h in a bottle containing 20 mL of 6 mol/L hydrochloric acid. Other acidifiers can be used, but it is essential to keep the pH in the sample sufficiently low (below pH 2) in order to avoid precipitation of calcium oxalate or calcium phosphate in the bottle and to dissolve any crystals that have been excreted with urine. The other—and equally important—role of acidification is to counteract oxidation of ascorbate to oxalate [4]. Insufficiently acidified urine otherwise might result in an overestimation of the excretion of oxalate. In case of large urine volumes, it is recommended to measure pH in the sample upon delivery and if necessary add more hydrochloric acid (or another acidifying agent) to get a pH below 2.0.

There are some points of note before proceeding to analysis of urine for risk factors:

1. After any kind of active stone removal—open surgery, shock wave lithotripsy (SWL), percutaneous nephrolithotomy (PNL), ureteroscopy (URS), retrograde intrarenal surgery (RIRS)—allow 4–8 weeks to pass before collecting urine [4].
2. Wait until there is little or no risk that fragments will be excreted with urine.
3. In case of bacteriuria, ongoing urinary tract infection, or when hematuria is present, discard the sample and wait for a better occasion.
4. Send the sample to the laboratory as soon as possible. If some delay is necessary, the sample can be stored in a refrigerator up to 24–48 h. Otherwise, keep aliquots of urine frozen until analysis.
5. It is important to note that the acidified sample cannot be used for analysis of urate. Although alkalinization is an option, it is better to measure urate in a separate sample that has been collected without acid.
6. Detailed instructions must be given to the patient in oral as well as written form, to make sure that the patient starts and finishes the urine collection in a correct way.
7. Urine collection should be undertaken during conditions that—as far as possible—reflect the normal (average everyday) situation and not that of artificial living conditions with unusual diet and excessive fluid intake.
8. It also is of utmost importance that urine samples are carefully mixed and heated to a temperature of 37 °C before aliquots are drawn for analysis. This might appear unnecessary to emphasize, but I am sure that neglecting these steps explains numerous erroneous results.

Despite careful instructions on how to collect urine, experience has shown that during this procedure, people tend to drink more than they usually do. Standardized estimates of AP(CaOx) index and AP(CaP) index therefore have been developed based on a 24-h urine volume of 1,500 mL (1.5 L in the formula) during 24 h [21]. Inasmuch as the pH cannot be measured in the 16-h sample suggested previously, the standardized AP(CaP) index is derived for a pH of 7.0. These two standardized indices are given the annotations "s": AP(CaOx) $index_s$ and AP(CaP) $index_s$. As mentioned, the factors A and B are determined by the duration of the collection period, and some relevant numbers are given in Table 84.1.

Interpretation of AP(CaOx) and AP(CaP) index values is partly hampered by our incomplete understanding of the

calcium stone-forming process. From previous calculations and experiments, the formation of calcium oxalate crystals is less likely to occur at $AP_{CaOx}$ values below 1.5–2.5 · $10^{-8}$ $(mol/L)^2$ [21]. When a 16-h AP(CaOx) index of 1.5 is recorded, it means that peak values of up to at least 2.5 can have occurred during the collection period. In case of calcium phosphate-induced precipitation caused by dissolution of calcium phosphate, very high local levels of supersaturation with CaOx are likely [48]. Such peak supersaturation levels will never be reflected in AP(CaOx) index levels in samples collected over longer periods of the day.

From a practical point of view, an AP(CaOx) index above 1.5–1.7 might indicate a need for actions to lower the supersaturation. Similarly, an AP(CaP) $index_s$ exceeding 50 indicates an increased risk of calcium phosphate precipitation. In this regard it is of note that AP(CaP) $index_s$ can be assumed to reflect the $AP_{CaP}$ in the distal part of the collecting duct.

With the result obtained from analysis of urine samples as discussed previously, a reasonable basis should be available for conclusions of factors responsible for or contributing to the stone formation. The AP indices give an impression of the concert action exerted by the various urine variables in terms of forming urine critically supersaturated with calcium oxalate and/or calcium phosphate. The individual urine variables subsequently can be used for dietary and drinking advice or for choosing the most appropriate form of pharmacological therapy. Those issues are, however, extensively discussed elsewhere in this book.

## When Should Chemical Analysis of Urine Be Carried Out?

If we first look at non-calcium stone-forming patients, it is mandatory to measure the concentration of cystine in patients with cystine stone formation. The supersaturation with cystine ($AP_{cystine}$) should be calculated both as part of the initial risk evaluation as during follow-up during recurrence preventive treatment. For uric acid stone formers, $AP_{uric\ acid}$ might be a helpful estimate in the diagnostic work-up. But recurrence prevention usually can be started and maintained without further analyses. In case of therapeutic failure, it is, however, highly recommended to measure urine urate and pH and calculate $AP_{uric\ acid}$.

Patients with infection stones—like patients with cystine and uric acid stones—always should be given recurrence preventive treatment [71]. The outcome of such a therapy usually is best followed clinically in terms of new stone formation and the presence or absence of bacteriuria. There is thus no absolute need for any further analytical efforts.

In patients with calcium stone disease, there is a great diversity in terms of the severity of stone formation. There is definitely a group of patients in whom a careful analysis of risk factors should be strongly recommended. In others it might be optional, whereas some patients have a mild disease (or what appears to be a mild disease) for whom a complete urine analysis is overdoing. It also is important that the patient is motivated to accept medical advice or treatment based on urinary findings before an extensive risk analysis is undertaken. That is, however, mostly a pedagogic problem.

Several categories of calcium stone formers can be identified [4]. A relatively small group (category Rs) has a frequently recurring stone formation, which by itself calls for effective preventive measures. It is often difficult to get a good estimate of the frequency of stone formation. There is usually insufficient data available, and the patient has only a vague idea when stones have formed. On the other hand, the total number of stones ($N$) that has formed is usually better recorded or known by the patient. With this information, a stone age index (SAI) can be calculated as follows [46]:

$$\mathrm{SAI} = \frac{N \cdot 100}{\mathrm{Age}}$$

A value above 10 indicates a severe disease (Rs). To that group—irrespective of the previous history of stone formation—should also be added those with specific risk factors such as formation of brushite stones, as well as those with medical diseases, anatomical abnormalities, and pharmacological treatment known to be associated with calcium stone formation. The patients thus referred to category Rs always should be considered for a complete metabolic risk evaluation.

Mild recurrent stone formation (Rm) is defined by longer intervals between stones and SAI usually in a range between 7 and 10. Those patients who do not have any residual stones (Rmo) can probably be left with some general preventive advice. Those with residual stone material (Rmres) might definitely benefit from specific medical advice and/or pharmacological treatment and accordingly that group of patients should be offered a urine examination. The same probably is wise also for first (single) stone formers with residuals (Sres). In contrast, the first-time stone former without residuals (So) can be given general advice, but that is all needed unless the patient highly desires an evaluation in order to find a reason for the stone. Of first-time stone formers, around 75 % remain stone-free during a 10-year period [72].

For the categories (So and Rmo), the evaluation can be restricted to a set of serum (or plasma) analyses including calcium, phosphate, creatinine, potassium, and urate. Spot urine sample can be used to exclude or confirm bacteriuria or leukocyturia, and with a measurement of the pH, no further analyses are necessary.

## Acid Load for the Diagnosis of DRTA

An intake of 0.1 g of ammonium chloride per kg body weight together with 150 mL of water is followed by urine collection in five 1-h samples. At each collection, 150 mL of water is taken. The pH should be measured before acidification in each sample. If the pH is reduced to 5.4, the diagnosis of dRTA can be excluded. The distinction between complete and incomplete RTA is made from measurements of pH and bicarbonate in blood. Whereas blood pH and bicarbonate are normal in the incomplete form, low values are seen in patients with complete RTA [24].

### Conclusion

Appropriate consideration of relevant aspects of the patient's medical history and radiographic image, together with analyses of stones, blood, and urine, is extremely helpful for identifying risk factors of stone formation. This is a field that unfortunately is neglected by too many urologists, but a lot of problems and expenses can be saved by paying attention to the etiology of stone formation in the individual patient. It is recommended that the principles of risk evaluation are adapted both to the type of stone that the patient has formed (if this is known) and to the severity of the disease. These findings should provide the basis for subsequent recurrence preventive measures.

## References


1. Tiselius H-G. Epidemiology and medical management of stone disease. BJU Int. 2003;91:758–67.
2. Straub M, Strohmaier W, Berg W, Beck B, Hoppe B, Laube N, et al. Diagnosis and metaphylaxis of stone disease. Consensus concept of the national working committee on stone disease for the upcoming German urolithiasis guidelines. World J Urol. 2005;23:309–23.
3. Tiselius HG. Investigation of single and recurrent stone formers. Miner Electrolyte Metab. 1994;20:321–7.
4. Tiselius H-G, Alken P, Buck C, Gallucci M, Knoll T, Sarica K, et al. Guidelines on urolithiasis. European association of urology guidelines. EAU Guideline Office. 2009 edition; 2009.
5. Parks J, Coe F. Evidence for durable kidney stone prevention over several decades. BJU Int. 2009;103:1238–46.
6. Leslie SW. Outpatient metabolic evaluation of patients with recurrent kidney stones. Ohio Med. 1989;85:292–4.
7. Colussi G, De Ferrari ME, Brunati C, Civati G. Medical prevention and treatment of urinary stones. J Nephrol. 2000;13 Suppl 3:S65–70.
8. Robertson WG. The economics and epidemiology of urolithiasis. In: Gohel MDI, Au DWT, editors. Kidney stones: inside and out. Hong Kong: The Hong Kong Technical University; 2004. p. 368–71.
9. Tiselius HG. Routine metabolic evaluation of patients with stone disease – aspects on its cost effectiveness. In: Gohel MDI, Au DWT, editors. Kidney stones: inside and out. Hong Kong: The Hong Kong Polytechnical University; 2004. p. 372–6.
10. Parks JH, Coe FL. The financial effects of kidney stone prevention. Kidney Int. 1996;50:1706–12.
11. Tiselius HG. Medical evaluation of nephrolithiasis. Endocrinol Metab Clin North Am. 2002;31:1031–50.
12. Liebman SE, Taylor JG, Bushinsky DA. Uric acid nephrolithiasis. Curr Rheumatol Rep. 2007;9:251–7.
13. Chevreau G, Troccaz J, Conort P, Renard-Penna R, Mallet A, Daudon M, et al. Estimation of urinary stone composition by automated processing of CT images. Urol Res. 2009;37:241–5.
14. Ferrandino MN, Pierre SA, Simmons WN, Paulson EK, Albala DM, Preminger GM. Dual-energy computed tomography with advanced postimage acquisition data processing: improved determination of urinary stone composition. J Endourol. 2010;24:347–54.
15. Matlaga BR, Kawamoto S, Fishman E. Dual source computed tomography: a novel technique to determine stone composition. Urology. 2008;72:1164–8.
16. Hesse A, Tiselius H, Siener R, Hoppe R. Crystals in the urinary sediment. In: Urinary stones, diagnosis, treatment and prevention of recurrence. Basel: Karger AG; 2009. p. 213–5.
17. Shekarriz B, Stoller ML. Cystinuria and other noncalcareous calculi. Endocrinol Metab Clin North Am. 2002;31:951–77.
18. Brand E, Harris MM, Bildon S. Cystinuria: excretion of a cystine complex which decomposes in the urine with liberation of free cystine. J Biol Chem. 1930;86:315.
19. Marickar YM. Calcium oxalate stone and gout. Urol Res. 2009;37:345–7.
20. Kenny JE, Goldfarb DS. Update on the pathophysiology and management of uric acid renal stones. Curr Rheumatol Rep. 2010;12:125–9.
21. Tiselius HG. Solution chemistry of supersaturation. In: Coe FL, Favus MJ, Pak CYC, Parks JH, Preminger GM, editors. Kidney stones: medical and surgical management. Philadelphia: Lippincott-Raven Publishers; 1996. p. 33–64.
22. Tiselius HG. Aetiological factors in stone formation. In: Davison AM, Cameron JS, Grünfeld J-P, Ponticelli C, Ritz E, Winearls CG, van Ypersele C, editors. Oxford textbook of clinical nephrology. New York: Oxford University Press; 2005. p. 1199–223.
23. Hess B, Hasler-Strub U, Ackermann D, Jaeger P. Metabolic evaluation of patients with recurrent idiopathic calcium nephrolithiasis. Nephrol Dial Transplant. 1997;12:1362–8.
24. Hesse A, Tiselius H, Siener R, Hoppe R. Urinary stones: diagnosis, treatment and recurrence prevention. Basel: Karger AG; 2009.
25. Worcester EM. Stones from bowel disease. Endocrinol Metab Clin North Am. 2002;2002:997–9.
26. Asplin JR. Hyperoxaluric calcium nephrolithiasis. Endocrinol Metab Clin North Am. 2002;31:927–49.
27. McConnell N, Campbell S, Gillanders I, Rolton H, Danesh B. Risk factors for developing renal stones in inflammatory bowel disease. BJU Int. 2002;89:835–41.
28. Asplin JR. Obesity and urolithiasis. Adv Chronic Kidney Dis. 2009;16:11–20.
29. Maalouf NM. Metabolic syndrome and the genesis of uric acid stones. J Ren Nutr. 2011;21:128–31.
30. Domrongkitchaiporn S, Ongphiphadhanakul B, Stitchantrakul W, Piaseu N, Chansirikam S, Puavilai G, et al. Risk of calcium oxalate nephrolithiasis after calcium or combined calcium and calcitriol supplementation in postmenopausal women. Osteoporos Int. 2000;11:486–92.
31. Taylor EN, Curhan GC. Determinants of 24-h urinary oxalate excretion. Clin J Am Soc Nephrol. 2008;3:1453–60.
32. Auer BL, Auer D, Rodgers AL. Relative hyperoxaluria, crystalluria and haematuria after megadose ingestion of vitamin C. Eur J Clin Invest. 1998;28:695–700.
33. Massey LK, Liebman M, Kynast-Gales SA. Ascorbate increases human oxaluria and kidney stone risk. J Nutr. 2005;135:1673–7.

34. Ahlstrand C, Tiselius HG. Urine composition and stone formation during treatment with acetazolamide. Scand J Urol Nephrol. 1987; 21:225–8.
35. Koul HK, Koul S, Fu S, Santosham V, Seikhon A, Menon M. Oxalate: from crystal formation to crystal retention. J Am Soc Nephrol. 1999;10 suppl 14:S417–21.
36. Khan SR. Renal tubular damage/dysfunction: key to the formation of kidney stones. Urol Res. 2006;34:86–91.
37. Pritchard MJ. Medullary sponge kidney: causes and treatments. Br J Nurs. 2010;12:972–6.
38. Maw AM, Megibow AJ, Grasso M, Goldfarb DS. Diagnosis of medullary sponge kidney by computed tomographic urography. Am J Kidney Dis. 2007;50:146–50.
39. Sorensen CM, Chandhoke PS. Hyperuricosuric calcium nephrolithiasis. Endocrinol Metab Clin North Am. 2002;31:915–25.
40. Caudarella R, Vescini F. Urinary citrate and renal stone disease: the preventive role of alkali citrate treatment. Arch Ital Urol Androl. 2009;81:182–7.
41. Wuermser LA, Reilly C, Poindexter JR, Sakahee K, Pak CY. Potassium magnesium citrate versus potassium chloride in thiazide-induced hypokalemia. Kidney Int. 2000;57:607–12.
42. Tiselius HG, Berg C, Fornander AM, Nilsson MA. Effects of citrate on the different phases of calcium oxalate crystallization. Scanning Microsc. 1993;7:381–90.
43. Tiselius HG. New horizons in the management of patients with cystinuria. Curr Opin Urol. 2010;20:169–73.
44. Goldfarb DS, Coe FL, Asplin JR. Urinary cystine excretion and capacity in patients with cystinuria. Kidney Int. 2006;69:1041–7.
45. Grenabo L, Hedelin H, Pettersson S. Urinary infection stones caused by ureaplasma urealyticum: a review. Scand J Infect Dis. 1988;53(suppl):46–9.
46. Tiselius HG. Factors influencing the course of calcium oxalate stone disease. Eur Urol. 1999;36:363–70.
47. Khan SR, Kok DJ. Modulators of urinary stone formation. Front Biosci. 2004;9:1450–82.
48. Tiselius H, Lindbäck B, Fornander A-M, Nilsson M. Studies on the role of calcium phosphate in the process of calcium oxalate crystal formation. Urol Res. 2009;37:181–92.
49. Luptak J, Bek-Jensen H, Fornander AM, Hojgaard I, Nilsson MA, Tiselius HG. Crystallization of calcium oxalate and calcium phosphate at supersaturation levels corresponding to those in different parts of the nephron. Scanning Microsc. 1994;8:47–62.
50. Kok D, Schell-Feith E. Risk factors for crystallisation in the nephron: the role of renal development. J Am Soc Nephrol. 1999; 10:S364–70.
51. Kok DJ. Crystallization and stone formation inside the nephron. Scanning Microsc. 1996;10:471–86.
52. Evan AP, Lingeman JE, Worcester EM. Role of interstitial apatite plaque in the pathogenesis of common calcium oxalate stone. Semin Nephrol. 2008;28:111–9.
53. Cifuentes Delatte L, Minon Cifuentes J, Medina JA. Randall and his plaque. Urology. 1996;48:343–6.
54. Liu Y, Mo L, Goldfarb DS, Evan A-P, Liang F, Khan SR, Lieske JC. Progressive renal papillary calcification and ureteral stone formation in mice deficient for Tamm-Horsfall protein. Am J Physiol Renal Physiol. 2010;299:F469–78.
55. Vahlensieck EW, Bach D, Hesse A. Circadian rhythm of lithogenic substances in the urine. Urol Res. 1982;10:195–203.
56. Robert M, Roux JO, Bourelly F, Boularan AM, Guiter J, Monnier L. Circadian variations in the risk of urinary calcium oxalate stone formation. Br J Urol. 1994;74:294–7.
57. Ahlstrand C, Larsson L, Tiselius H-G. Variations in urine composition during the day in patients with calcium oxalate stone disease. J Urol. 1984;131(1):77–81.
58. Laube N, Hergarten S, Hoppe B, Schmidt M, Hesse A. Determination of the calcium oxalate crystallization risk from urine samples: the BONN risk index in comparison to other risk formulas. J Urol. 2004;172:355–9.
59. Conte A, Piza P, Garcia-Raja A, Grases F, Csta-Bauza A, Prieto RM. Urinary lithogen risk test: usefulness in the evaluation of renal lithiasis treatment using crystallization inhibitors (citrate and phytate). Arch Esp Urol. 1999;52:305–10.
60. Tiselius HG. Aspects on estimation of the risk of calcium oxalate crystallization in urine. Urol Int. 1991;47:255–9.
61. Tiselius HG, Bek-Jensen H, FA M, Nilsson MA. Crystallization properties in urine from calcium oxalate stone formers. J Urol. 1995;154:940–6.
62. Bek-Jensen H, Tiselius HG. Repeated urine analysis in patients with calcium stone disease. Eur Urol. 1998;33:323–32.
63. Roberston WG, Peacock M, Nordin BEC. Activity products in stone-forming and non-stone-forming urine. Clin Sci. 1968; 34:579–94.
64. Werness PG, Brown CM, Smith LH, Finlayson B. EQUIL2: a basic computer program for calculation of urinary saturation. J Urol. 1985;134:1242–4.
65. Ashby R, György AZ. Thermodynamic equilibrium model for calcium salt urolithiasis: clinical application. Exp Nephrol. 1997;5: 246–52.
66. Rodgers A, Allie-Hamdulay S, Jackson G. Therapeutic action of citrate in urolithiasis explained by chemical speciation: increase in pH is the determinant factor. Nephrol Dial Transplant. 2006;21: 361–9.
67. Tiselius HG. A simplified estimate of the ion-activity product of calcium phosphate in urine. Eur Urol. 1984;10:191–5.
68. Roberston WG, Peacock M, Marshall RW, Marshall DH, Nordin B. Saturation inhibition index as a measure of the risk of calcium oxalate stone formation in the urinary tract. N Engl J Med. 1976; 294:249–52.
69. Ryall RL, Grover PK, Marshall VR. Urate and calcium stones-picking up a drop of mercury with one's fingers? Am J Kidney Dis. 1991;17:426–30.
70. Tiselius HG, Larsson L, Hellgren E. Clinical results of allopurinol treatment in prevention of calcium oxalate stone formation. J Urol. 1986;136:5–53.
71. Leusmann DB, Niggemann H, Roth S, von Ahlen H. Recurrence rates and severity of urinary calculi. Scand J Urol Nephrol. 1995;29: 279–83.
72. Ahlstrand C, Tiselius HG. Recurrences during a 10-year follow-up after first renal stone episode. Urol Res. 1990;18:397–9.