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A new approach to calculate the risk of calcium oxalate crystallization from unprepared native urine

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Abstract This work focuses on the in vitro calcium-oxalate (CaOx) crystallization behaviour of native and synthetic urine samples in order to establish a CaOx crystallization risk index for unprepared native urine. Native 24-h urine samples from healthy persons and from stone-formers were examined. Within a $[Ca^{2+}]$ versus added oxalate (Ox^{2-}) diagram, we observed fields which allow the discrimination of each urine sample in terms of more or less risk. The $[Ca^{2+}]/(Ox^{2-})$ ratio is calculated and termed the ''Bonn-Risk Index'' (BRI; per litre). We propose that BRIs >1/l denote samples ''at risk'', whereas BRIs ≤ 1/l denote those ''without risk''. Second, the effects of different concentrations of citrate and Mg^{2+} on BRI were investigated in artificial urine. The transferability of BRI between native and synthetic urine samples is proved. To evaluate the impact of the proposed BRI, it is compared with the more familiar relative urine saturation index calculated for CaOx and brushite. Urine sampled from stone-formers shows risk indexes between 0.278 and 23.0/l (mean 2.87/l), while urine from healthy persons varied between 0.060 and 4.890/l (mean 1.05/l). Comparing the results of healthy volunteers and patients, the significance of BRI and relative urine supersaturation (RS) with respect to CaOx is $P < 0.0005$ and $P = 0.013$, respectively. Fast and easy to perform, determination of the risk index is a suitable tool for estimating the actual CaOx formation ''status'' – ''at risk'' or ''without risk'' – from the native urine of any person.

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Introduction

For a number of decades, urolithiasis research focused on the development of a simple criterion to discriminate healthy persons from calcium oxalate (CaOx)-forming patients, or – in the sense of an improved prophylaxis – to assign a factor to any person indicating the actual degree to which he or she is at risk of producing CaOx stones in the future.

A variety of approaches were developed to estimate the risk of urinary stone formation. A comprehensive and excellent review of this topic by Tiselius [25] was recently published. The author provides a review of the common risk formulae published in recent years, emphasizes their characteristic differences and classifies them into specific groups.

One disadvantage of most of the existing approaches dealing with native urine is the fact that determination is based on urine that has to be prepared before the experiment is performed (e.g. dilution, pH adjustment, centrifugation). Therefore, they do not reflect the complex interplay of macromolecular (chemical dynamical factors of stone formation) and ionic (thermodynamic driving forces of stone formation) constituents. The macromolecular constituents are, however, of special interest as, for example, urinary glycoproteins are considered to be responsible for 70–90% of the total inhibitory effect in human urine (e.g. [16, 19]).

The scope of this work is to investigate the CaOx crystallization behaviour of unprepared native 24-h urine in vitro to establish a new crystallization risk index that may represent a diagnostic marker for everybody.

Only unprepared urine exhibits the individual ratio of inhibitory/lithogenic urinary components of a single person; this is specifically reflected at the starting point of CaOx crystallization. In our study, CaOx crystallization is triggered by the addition of ammonium oxalate.

Materials and methods

Twenty-four hour urine samples from 48 persons (32 CaOx and/or calcium-hydrogene-phosphate stone-formers, 16 controls; Table 1) were analysed. The following parameters were determined: volume, pH, specific weight, and the concentrations of Na, K, Ca, Mg, NH_4^+, Cl^-, PO_4^{3-}, SO_4^2, creatinine, uric acid, citric acid and oxalic acid. These data were included in the mainly inorganic ion equilibrium program EQUIL [9] to calculate the relative urine supersaturation (RS) with respect to both CaOx and brushite. In addition, the concentration of free (synonym: ionized) calcium, $[Ca^{2+}]$, was measured by a calcium-selective electrode according to the instructions given in the manual (METROHM AG, Herisau, Switzerland; relative accuracy $\pm 3\%$).

The effects of different inhibitors (citrate and Mg^{2+}) on the proposed risk index were examined at various concentrations in synthetic urine to test the sensibility of BRI on a standardized system.

1. Investigation of CaOx crystallization

To investigate the crystallization behaviours of synthetic and native urine, a CaOx crystallization process was triggered under standardized conditions in 200 ml aliquots of urine with the step-by-step addition (0.5 ml, 1.5 ml/min) of ammonium oxalate, $(NH_4)_2C_2O_4$, 40 mmol/l. During the experiment, the urine samples were kept at 37 °C and were continuously agitated (180 rpm) by a stirrer with low shear forces.

The onset of crystallization and its extent were determined in situ by a laser-probe crystal system analyser (Messtechnik Schwartz, Düsseldorf, Germany). The (absolute) accuracy of the estimation of the onset of crystallization is estimated to be ± 0.5 ml or ± 0.02 mmol, respectively. The laser-probe device determines the number of suspended particles online (in vitro) and simultaneously estimates particle size in the detection range of 0.5–250 μm. From these data, a statistical evaluation program calculates the actual particle size distribution (PSD). The onset of crystallization is accompanied by a dramatic change in PSD and, therefore, can be easily detected. Bongartz et al. [2, 3] showed the advantages of such a laser-probe particle analysis in urinary stone crystallization models.

2. Native urine

Urine samples from 16 healthy persons and 32 stone-forming patients were collected and examined. A number of patients were under medical treatment to avoid recurrent stone formation. Most were tested after 3 days of standardized diet (based on directions supplied by the German Society of Nutrition, DGE [8]). To record representative situations in daily life, some persons were sampled under individual diet up to three times.

Foreign particles were roughly filtered from the urine using a disposable "uro-filter" designed for clinical use (mesh size 0.3–0.4 mm; Medic-Eschmann, Hamburg, Germany). To avoid the effects of chemical contamination and to ensure a valid $[Ca^{2+}]$ determination during crystallization experiments, thymol was not used for preservation. Two 200 ml aliquots of each sample were taken and individually tested for their tendency to crystallize CaOx. The remnant urine was preserved with thymol and was later analysed chemically.

Table 1 Patients and healthy volunteers included in the study

	Females	Males	Sum	Age (years)
Patients	7	25	32	29–70
Healthy persons	14	2	16	26–54
Total	21	27	48	26–70

3. Synthetic urine

In addition to the discrimination approach for native urine, we also investigated the crystallization behaviour of synthetic urine (200 ml) containing various additional concentrations of well-known inhibitory substances with clear mechanisms of inhibition. The chemical composition of the synthetic urine was based on the paper of Griffith et al. [11] (Table 4). The concentration ratio of ionized calcium to total calcium, $[Ca^{2+}]/[Ca]$, was 0.55 at the initial stage. This corresponds fairly well to Finlayson's [9] estimation that a fraction of about 50% of the Ca is present as free ion in normal urine.

Two inhibitory ions, Mg^{2+} (as $MgCl_2 \times 6H_2O$) and citrate (as Na_3-citrate $\times 2H_2O$), were added to the urine. These ions are of great interest as their inhibitory effect on CaOx formation is based on different mechanisms: the magnesium ion forms a soluble chelate complex with oxalate, whereas the citrate anion complexes free calcium ions to form calcium citrate.

Two experimental series for Mg^{2+} and citrate were performed. At the beginning of each step of the experiment, we measured both the free calcium concentration and the amount of ammonium oxalate that would induce CaOx precipitation. Starting with an inhibitor concentration of zero, we increased the inhibitor concentrations within the reactor vessel up to 12 mmol/l by increments of 2 mmol/l.

Results

1. Native urine

Ninety-three percent of all 24-h urine samples from CaOx and calcium-phosphate stone-formers, and those of the healthy persons, fall in the range between the hyperbolae $[Ca^{2+}] = 0.1/(Ox^{2-})$ and $[Ca^{2+}] = 0.3/(Ox^{2-})$, respectively (Fig. 1). These hyperbolae represent lines of constant products $[Ca^{2+}](Ox^{2-})$ between 0.1 and 0.3 $mmol^2/l$. The product of $[Ca^{2+}](Ox^{2-})$ is similar to the calculation of the formation product (FP), which takes into account the total urinary concentration of calcium and oxalate at the onset of crystallization ($FP = [Ca^{2+}][Ox^{2-}]$). However, we are interested in the amount of oxalate that has to be added to the urine to trigger CaOx crystallization. This amount is dependent on the unknown ratio of all inhibitory and lithogenic substances within the urine and, thus, reflects the individual CaOx risk situation of a person.

Using $[Ca^{2+}]$ instead of [Ca] improves the approximation that the concentration is equal to the ion activity for that component, and that this is the thermodynamically correct parameter to use in such a calculation.

The hyperbola approach (in general: $y = a/x + b$) shows a distinct functional relationship between $[Ca^{2+}]$ and the amount of "added oxalate". The value of the linear correlation coefficient r of the linear regression is 0.90 if $1/(Ox^{2-})$ is plotted against $[Ca^{2+}]$, which means that only 10% of the variance is not related to $1/(Ox^{2-})$ and $[Ca^{2+}]$. The best-fit hyperbola calculated from all samples is described by

$$[Ca^{2+}] = 0.2079 \times 1/(Ox^{2-}) - 0.1322$$

The $[Ca^{2+}]/[Ca]$ ratio varies between 0.072 and 0.876 without any significant correlation to either pH ($r = 0.08$) or added oxalate ($r = 0.16$). Table 2 presents

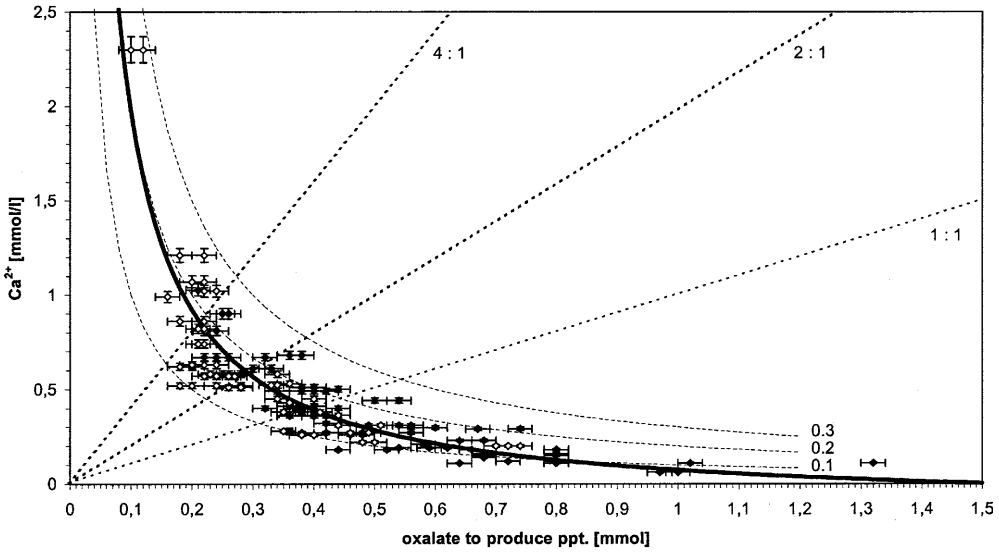

Fig. 1 Crystallization behaviour of 134 24-h urine samples of 32 CaOx stone patients and 16 healthy persons. The *X-axis* shows the amount of ammonium oxalate [mmol] added to induce CaOx precipitation within the urine. Error bars are ±0.5 mmol. The *Y-axis* indicates the free calcium concentration [mmol/l] (±3%) at the start of the experiment. The numbers 0.1, 0.2 and 0.3 denote lines of constant $[Ca^{2+}](Ox^{2-})$ products in $[mmol^2/l]$. *Open circles*, patients; *filled circles*, normal subjects. *Straight lines* show constant ratios of $[Ca^{2+}]/(Ox^{2-})$ of 1:1, 2:1 and 4:1, respectively. Samples with plots *left of the line* $[Ca^{2+}]/(Ox^{2-}) = 1$ are denoted "at risk", whereas those to the *right* of it are assumed to be "without risk"

the means and further statistics of $[Ca^{2+}]$, (Ox^{2-}), pH, and volume for patients and normals.

The ratio $[Ca^{2+}]/(Ox^{2-})$ is calculated and termed the "Bonn-Risk Index" (BRI; per liter). Therefore, samples following a line through the origin have the same risk factor, despite the absolute amount of oxalate added. We propose that BRI > 1/l denote a sample "at risk", whereas BRI ≤ 1/l denote a sample "without risk". At BRI > 1/l, the $[Ca^{2+}]$ exceeds the added oxalate concentration at the onset of precipitation; here, only small changes in $[Ox^{2-}]$ relative to $[Ca^{2+}]$ result in CaOx crystallization. Hallson and Rose [12] and Robertson and Hughes [21] showed that mild hyperoxaluria is a more potent inducer of crystalluria than hypercalcuria – which is clearly reflected by a BRI > 1/l.

In contrast, a BRI < 1/l indicates a surplus of oxalate ions at the onset of CaOx crystallization. In this case, the "system urine" is relatively insensitive to an increase in oxalate. Compared with samples having a BRI > 1/l, the system must be forced to crystallize.

Urine sampled from stone-formers (CaOx and brushite, $CaHPO_4 \times 2H_2O$) shows risk indexes between 0.278 and 23.0/l (mean 2.87/l), while those from healthy persons varied between 0.060 and 4.890/l (mean 1.05/l) (Fig. 2, Table 3). Sixty-four percent of the non-stone-former samples and 74% of the patients' samples were classified into the proper group (see Discussion section).

The absolute error in BRI, Δ_{BRI}, is dependent on BRI and can be calculated according to the Gauss error-propagation method and the best fit hyperbola:

$$\Delta_{BRI} = \left\{ \left(BRI \times \Delta_{Ca^{2+}} / [Ca^{2+}] \right)^2 + \left(BRI / \left[0.0061 + \left(0.0061^2 + 0.2079/BRI \right)^{0.5} \right] \times \Delta_{(Ox^{2-})} \right)^2 \right\}^{0.5},$$

where $\Delta_{Ca^{2+}}$ indicates the error in $[Ca^{2+}]$ determination = ±3%, and $\Delta_{(Ox^{2-})}$ indicates the error in (Ox^{2-}) measurement = ±0.02 mmol. For BRI = 1/l, Δ_{BRI} amounts to ±0.053/l, while for BRI = 2/l, Δ_{BRI} = ±0.068/l.

2. Synthetic urines

The crystallization behaviour of the synthetic urine samples under various concentrations of added inhibi-

Table 2 Selected data of 24-h urine samples. N, number of samples (some persons were sampled up to three times); P, patients (n = 32); H, healthy subjects (n = 16); SD, standard deviation; $(Ox)^{2-}$, amount of added ammonium oxalate (in most cases determinations were performed twice)

Parameter		N	Minimum	Maximum	Mean	SD
Ca^{2+} (mmol/l)	H	39	0.06	1.03	0.3654	0.2197
	P	32	0.20	2.30	0.6247	0.4031
(Ox^{2-}) (mmol/l)	H	72	0.21	1.32	0.5130	0.2137
	P	63	0.10	0.74	0.3173	0.1440
PH	H	39	5.78	6.92	6.2655	0.3029
	P	32	5.04	7.27	6.2591	0.4792
Volume (ml)	H	39	790	4030	2735	589
	P	32	950	3775	2590	688

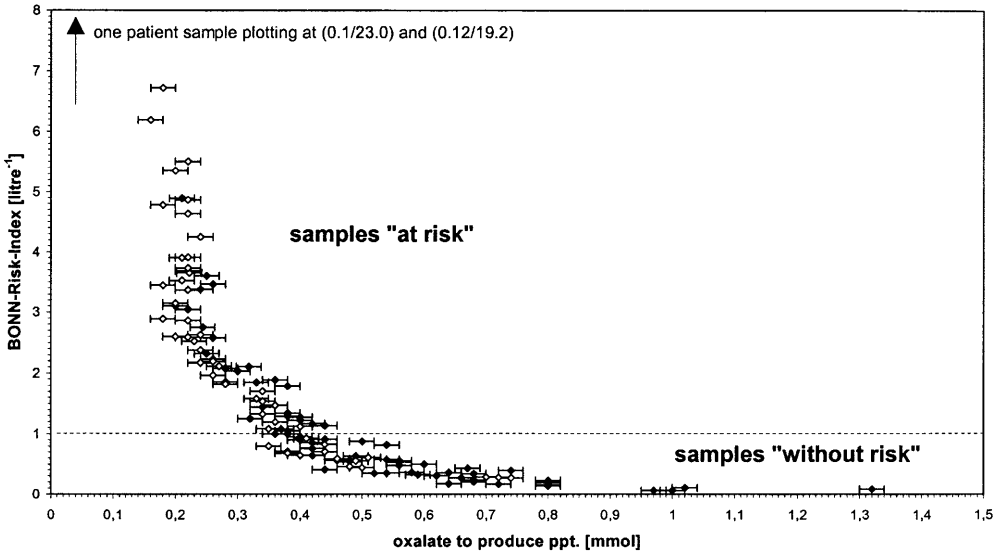

Fig. 2 CaOx crystallization risk of the samples shown in Fig. 1. The risk of each sample is calculated as the ratio of $[Ca^{2+}]/(Ox^{2-})$. The Bonn Risk Index (BRI) is the gradient of the straight line through the origin and sample point in Fig. 1. Urine samples with BRI < 1 are assumed to be "without risk". *Open circles*, patients; *filled circles*, normal subjects

Table 3 Descriptive statistics comparing the results of the BRI and RS of patients and healthy volunteers. The calculated significance (*S*) of the Mann-Whitney *U*-test (patients versus healthy persons) is given. P, patients; H, healthy subjects; SD, standard deviation. Some persons were sampled up to three times; the BRI of most of the samples was determined twice

		N	Minimum	Maximum	Mean	SD	*S* (P/H)
Bonn Risk Index [1/l]	H	72	0.060	4.890	1.05	1.038	
	P	63	0.278	23.000	2.87	3.696	< 0.0005
Relative CaOx supersaturation	H	34	0.073	6.183	1.913	1.486	
	P	32	1.036	15.700	5.370	3.361	0.013

tors are shown in Fig. 3. This clearly reveals that increased concentrations of Mg^{2+} and citrate led to reasonably different courses within a $[Ca^{2+}]$ versus "added oxalate" diagram.

As expected, $[Ca^{2+}]$ within the urine decreases with increasing citrate concentration as a result of the formation of Ca–citrate complexes; the amount of oxalate to be added to produce CaOx precipitation increases with higher citrate concentrations. An increased urinary $[Mg^{2+}]$ does not affect $[Ca^{2+}]$ but does lead to an increase in the oxalate concentration necessary for crystallization.

Furthermore, it can be seen that the initial synthetic urine (i.e. no inhibitor added) differs notably from the native urine; first, the plots are outside the product range of 0.1 and 0.3 $mmol^2/l$, and second at a considerably higher $[Ca^{2+}]$ level (Fig. 3). This is attributed to the lack of oxalate binding, complex-forming inorganic constituents, such as, for example, Mg^{2+}, or by the absence of organic Ca^{2+}-binding components, such as citrate or proteins.

Assuming that the necessary amount of added oxalate is a measure of the inhibitory effect of a specific urinary constituent, the effect of Mg^{2+} and citrate is of the same order of magnitude. The enhanced inhibitor concentrations (from 0 to 12 mmol/l, increments of 2 mmol/l) result in an increased amount of added oxalate: 0.17 mmol for Mg^{2+} and 0.205 mmol for citrate (Fig. 3A). This difference of 0.035 mmol is well within the range of experimental accuracy (± 0.02 mmol, respectively ± 0.5 ml). Citrate is known to be a powerful inhibitor; Mg^{2+}, however, is only an intermediate inhibitor [9]. Thus, the parameter (Ox^{2-}) is not a suitable measure for evaluating the different inhibitory effects of, at least, the tested substances.

Figure 3B shows a plot of BRI versus (Ox^{2-}). This clearly shows that citrate reduces the "crystallization risk" (BRI 34.5 → 1.5 1/l) to a much greater degree than Mg^{2+} (BRI 34.5 → 14 1/l). Moreover, there is clear evidence that the inhibitory effect of both citrate and Mg^{2+} does not increase proportionally with the inhibitor concentration; a step-by-step increase in concentration is accompanied by a step-by-step decline in the rise of the BRI. This indicates a supersaturation effect with respect to the inhibitory impact of the specific additive.

Discussion

We showed that the proposed BRI approach provides a good description of the crystallization behaviour of synthetic urine, which is in accordance with theoretical expectations. It was noted that the amount of added oxalate alone is not a useful measure of the inhibitory impact of a substance (Fig. 3).

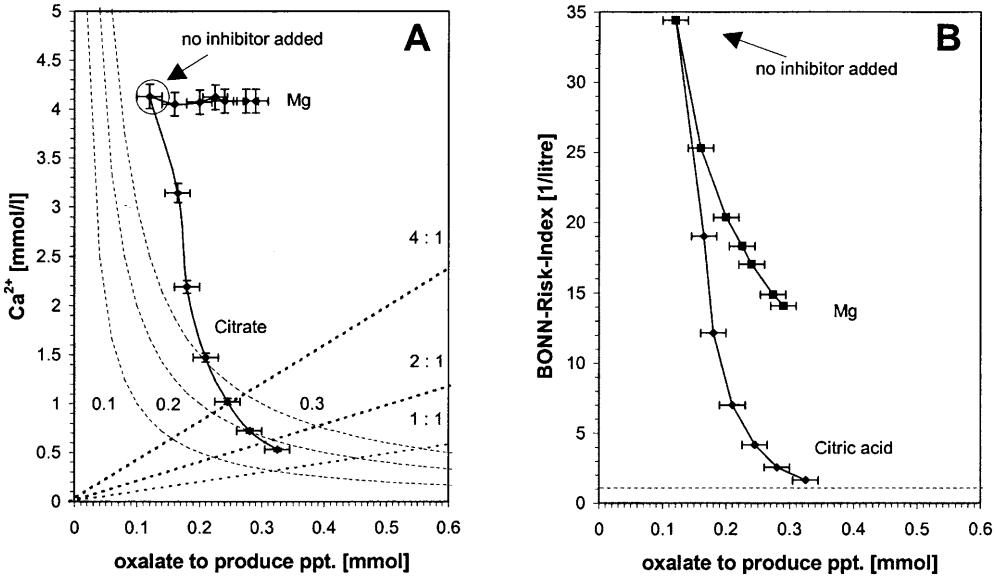

Fig. 3A Influence of citrate and magnesium on the crystallization behaviour of artificial urine. In order to vary the urinary Mg^{2+} and citrate concentration, $MgCl_2 \times 6H_2O$ and Na_3-citrate $\times 2H_2O$, respectively, were added to the urine (200 ml) in a range of 0–12 mmol/l. The *X-axis* shows the amount of ammonium oxalate [mmol] (± 0.5 mmol) titrated to induce CaOx precipitation in the urine. The *Y-axis* shows the related free calcium concentration [mmol/l] ($\pm 3\%$) after adding magnesium or citrate. **B** The CaOx crystallization risk (BRI) of samples shown in **A** (for calculation, see Fig. 2 or text). A change of the inhibitor concentration at a low total inhibitor concentration results in a more marked decrease of BRI than at a high total inhibitor concentration. This clearly indicates a supersaturation effect relative to the impact of an inhibitor in urine

Before it can be introduced as a suitable index for native urine, the results of the BRI calculation must be compared with established methods of risk evaluation.

Briellmann et al. [7] introduced an "oxalate-tolerance-value" for whole urine samples to distinguish CaOx stone-formers from healthy persons. Using synthetic urine (Table 4) with various Ca contents, they established a "standard curve" by plotting the urinary Ca concentration against the concentration of "added oxalate" ($Na_2C_2O_4$) at the starting point of crystallization. Based on this standard curve, native urine samples were differentiated as being either "at risk" or "without risk" according to the position of their plots (below or above) relative to the standard curve.

The curve and the data of Briellmann et al. [7], however, did not show a relation to our results. It is only if we assume that $[Ca]/[Ca^{2+}] = \text{constant} = 0.1$ that the curve of Briellmann et al. roughly approaches the order of magnitude of our data. The $[Ca]/[Ca^{2+}]$ ratio considered is not of the order of magnitude that we determined for either artificial (0.55, [9]:0.5) or native urine (2.2–13.9). The discriminative approach of Briellmann et al. cannot explain the distribution of our samples; according to them, all samples would be "at risk". Although CaOx solubility increases notably with the complexity of the urinary composition [18], this observed discrepancy cannot be attributed solely to the use of different synthetic urine compositions.

A number of other ratios for distinguishing stone-formers from healthy persons are under discussion. For example, Ca/Mg or Mg/Ca [26], Ca/citrate [27] or more complex relationships (e.g. [25]) are used to express the risk status.

In this study, we compare the BRI with the best known parameter to describe the risk of urinary crystallization: RS calculated via the mainly inorganic ion equilibrium program EQUIL [9]. The calculations shown were performed with respect to brushite and CaOx (Fig. 4).

Only an approximate linear relation between BRI and the relative CaOx saturation, RS_{CaOx}, was detected

Table 4 Synthetic urine composition used in this study and the composition used by Briellman et al. [7]

Substance	Study (based on [11]) [g/l]	Briellmann et al. [7] [g/l]
$CaCl_2$	–	Variable
$CaCl_2 \times 2H_2O$	1.103	–
NaCl	2.925	3.51
$NaHCO_3$	–	2.27
$NaH_2PO_4 \times H_2O$	–	1.18
Na_2SO_4	2.25	–
K_2SO_4	–	4.18
KH_2PO_4	1.40	–
KCl	1.60	–
NH_4Cl	1.00	–
$(NH_4)_2$-citrate	–	0.41
$MgCl_2 \times 6H_2O$	–	0.67
Urea	25.00	13.7
Creatinine	1.10	–
D-Glucose	–	0.047
D-Fructose	–	0.009
Saccharose	–	0.014
Glycin	–	0.085
L-Ascorbic acid	–	0.045
pH	6	Variable

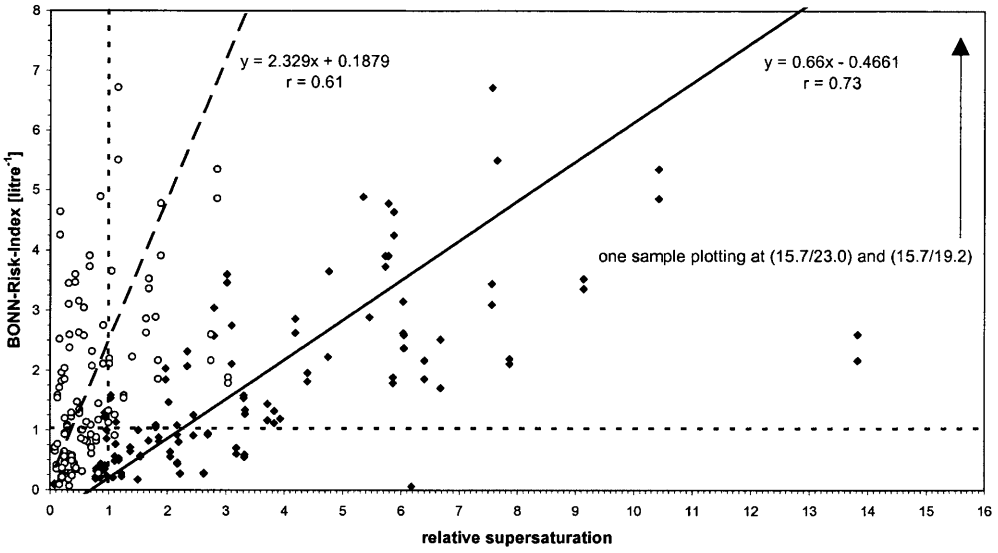

Fig. 4 Plot of relative urine supersaturations, RS, (EQUIL [9]) with respect to calcium hydrogene phosphate (brushite, *open circles*) and CaOx (RS_{CaOx}, *filled circles*) versus BRI

($r = 0.725$). The best linear relationship can be described by the equation:

$$BRI = 0.66RS_{CaOx} + 0.4661$$

Samples with low RS_{CaOx} are indicated by low BRI indexes (Fig. 4). Furthermore, the results confirm that stone-formers tend to excrete more supersaturated urine than non-stone-formers (e.g. [17]). An inverse correlation ($r = -0.56$) between RS_{CaOx} and the amount of added oxalate has been found. This confirms the results of Borghi et al. [4, 5] who showed, using a modified oxalate tolerance test [20], that a lower initial RS_{CaOx} is accompanied by a greater amount of oxalate which could be added to a prepared (pH-adjustment, filtration, etc.) urine sample without the formation of a detectable CaOx precipitation. However, comparing the correlations of BRI and (Ox^{2-}) with RS_{CaOx}, the BRI assay shows a considerably higher relationship to RS_{CaOx} than (Ox^{2-}).

Samples with similar RS_{CaOx} can exhibit broad variations in their related BRI. This reflects the more complex and variable chemical situations in native urine, which are not taken into account by the EQUIL program. Macromolecules, however, are treated as important chemical dynamical factors that influence crystallization. For example, Tamm-Horsfall protein (THP) or glycosaminoglycans (GAG) are assumed to play a major role as inhibitors and/or promotors for CaOx crystallization in urine (e.g. [6, 9, 10, 13, 14, 22–24]).

We suggest that samples displaying high BRI are indicated by the relatively lower inhibitory activity of their macromolecular urinary components compared with those displaying low BRI but equal/similar RS_{CaOx}.

Brushite is assumed to be a potential promotor of (secondary) CaOx formation by initiating the nucleation of CaOx salts (e.g. [1, 15, 28]). Thus, it is apparent that samples with high BRI are correlated with high brushite supersaturations ($r = 0.62$).

Based on the non-parametric Mann-Whitney U-test for independent samples, the asymptotic two-tailed significance for the independence of the patients' data as opposed to those of healthy persons are $P < 0.0005$ and $P = 0.013$ for BRI and relative CaOx saturation, respectively (Table 3).

The BRI is a very sensitive marker for risk of stone-formation, more so than the RS index. This may be attributable to the fact that the calculation of BRI takes into account the starting point of (forced) urinary CaOx formation as well as the free calcium concentration, both in unprepared native urine. Therefore, the complex interplay of inhibitory and stone-promoting constituents within a particular urine is always considered. The BRI much more describes the subject's individual CaOx risk.

The overlap of values in BRI as well as for the RS index between both groups – healthy persons and stone-forming individuals – reflects the general situation in a population. There are patients undergoing successful medical treatment (BRIs < 1) but, on the other hand, there are individuals who, while still being regarded as "clinically healthy", are already forming stones, or who will certainly do so at some future date.

A critical BRI value, BRI_{crit}, which distinguishes with an optimal selectivity (i.e. a minimum range of overlap) between healthy persons and stone-formers in a particular data set, can be obtained by minimizing the sum S, with

$$S = \sum_{i,j}^{N} X_{i,j} \ ,$$

where i, j and N denote patients, healthy persons and the total number of persons ($N = i+j$), respectively. $X_i = |BRI_i - V|$ if $BRI_i < V$, and $X_j = |BRI_j - V|$ if $BRI_j \geq V$, with V as an iteration variable. In any other cases, $X_i = X_j = 0$.

When S reaches its minimum, V equals BRI_{crit}. For any given V, this method of calculation only includes

those samples involved in the overlap at a given V. For the data shown in Table 2, BRI_{crit} is achieved at $V \approx 1.15$ ($S = 31.05$). Calculating S for $V = 1$, i.e. the proposed general BRI_{crit}, results in $S = 31.97$; this value is very close to the optimum of the data set.

The importance of the oxalate/total calcium ratio in crystal growth and agglomeration was mentioned by Robertson and Hughes [21]. We showed that the initial concentration of urinary "free calcium", [Ca^{2+}], is a more sensitive parameter for the investigation of the CaOx crystallization behaviour than the total calcium concentration, [Ca].

Fast (only ≈30 min per sample) and easy to perform (only addition of NH_4Ox, no urine preparation, determination of only two parameters), determination of the BRI is a suitable tool for estimating the actual CaOx formation "status" – "at risk" or "without risk" – of any person using unprepared native urine.

The BRI can be used as a primary diagnostic feature at the beginning of treatment. Consecutive determination of BRI during therapy or dietary restriction enables the monitoring of the (individual) impact of these measures for any given patient.

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