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The relation of urinary Tamm-Horsfall-Protein on CaOx-crystallization under the scope of the Bonn-Risk-Index

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Abstract In previous papers we introduced the Bonn-Risk Index (BRI) as a new method of evaluating an individual's actual risk of forming calcium oxalate (CaOx). A comparison of our results with the calculated urinary relative supersaturations (RS) with respect to CaOx, showed that samples with similar values of RS can have different values of BRI. We suggested that this may reflect the individual influence of the urinary macromolecular constituents which are not taken into account at the calculation of RS. To estimate the role of macromolecules on the value of BRI, we examined 45 unprepared 24-h urine samples from 24 persons (16 healthy subjects, eight CaOx stone-formers) with respect to BRI, RS, and the concentration of urinary Tamm-Horsfall-protein ([THP]). The crystallization experiments were carried out by the use of a laser-probe. Based on the data of BRI and RS, the effect of THP may shift from a minor promoter in stone-formers and persons ''at risk'', to an inert urinary constituent in healthy subjects and persons ''without risk''. However, the observed effects are small and at least in the latter group close to zero.

Key words THP · Native urine · Calcium oxalate · Crystallization risk

Introduction

The Bonn-Risk-Index approach (BRI) [14, 15] is a suitable tool for calculating the risk of calcium oxalate (CaOx) crystallization from the 24h-urine of any person. In comparison to the needs of relative supersaturation calculations (EQUIL) [5], the BRI-approach can easily be performed through the determination of only two parameters: the initial urinary concentration of ionized calcium, $[Ca^{2+}]$, and the amount of oxalate (Ox^{2-}), which has to be added to the sample to induce the crystallization of CaOx. The experimental procedure has been evaluated for unprepared 24-h urine; thus, the determination of BRI takes the whole urine composition into account.

A scatterplot of BRI vs. RS showed that samples with similar values of RS can have different degrees of risk according to BRI. We suggested that this could reflect the individual influence of the urinary macromolecular constituents which are not taken into account by the calculation of RS [15], although they may act as important promotive and/or inhibitive agents with respect to pathological crystal formation. To prove this hypothesis, we tested in this study the relation between urinary Tamm-Horsfall protein (THP) concentration and BRI on unprepared 24 h urines from known stone-formers and healthy persons.

THP, also known as uromodulin, was chosen because it represents the most prominent protein in human urine. THP is secreted only by the kidneys [11]. The glycoprotein tends to form aggregated polymeres. The extent of such a THP polymerization is directly influenced by the concentration of free calcium ($[Ca^{2+}]$), hypocitraturia, ionic strength, and osmolarity, and it varies inversely with pH and THP concentration [23].

The role of THP as an influencing factor with regard to the main progressive stages of urinary stone formation (i.e., nucleation, crystal growth, and crystal aggregation) is still discussed extensively [18] and the results of investigations are controversial [3, 9–12, 17, 21, 22]. It seems that THP functions differently in different urine environments [7, 20].

In its monomeric form, THP inhibits, depending on the concentration process of crystal aggregation [9], whereas in the polymeric form, this property is reduced or changed into a promotive role [8]. In the urines of stone-formers higher concentrations of THP are

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observed, additionally increasing the degree of polymerization [8].

The data about the THP 24 h excretions of healthy persons vary considerably between 23.8 ± 2.2 mg [4], 38.9 ± 16.5 mg [19], and 40.28 ± 14.8 mg [6]. Considering an average urine volume without dietary restrictions of 1.5 l per 24 h, the range of THP concentrations is between 15.9 mg/l and 26.9 mg/l. Khan [12] and Kunar and Muchmore [13] report wider THP excretion ranges of 20–100 mg per 24 h and 20–200 mg per 24 h. These excretions correspond to urinary THP concentrations between 13.3 mg/l and 66.7 mg/l, and between 13.3 mg/l and 133.3 mg/l, respectively.

Materials and methods

1. Native urines

Altogether 45 × 24-h urines without any chemical pre-treatment from 16 clinically healthy volunteers (12 females, four males) and eight known CaOx stone-formers (one female, seven males) were collected with no dietary restrictions issued. The ages of the patients were between 36 and 66 years (mean = 52 years); the healthy subjects were aged between 29 and 37 years (mean = 33 - years). From most healthy subjects 24 h urines were repeatedly collected (3 × 1, 5 × 2, and 8 × 3) on different days.

After measurement of the total volume, the 24-h urine samples were divided into two portions. From the first portion, foreign particles were roughly filtered by a disposable "uro-filter" for clinical use (mesh-size 0.3–0.4 mm, Medic-Eschmann GmbH, Hamburg). The initial concentration of ionized calcium ($[Ca^{2+}]$) was measured by a calcium-selective electrode (Metrohm, Herisau, Switzerland, relative accuracy ±3%) and crystallization experiments to test for the risk of CaOx-formation according to the BRI [15] were immediately carried out as described below.

The second urine fraction became preserved with thymol and routine chemical analyses were performed. The following parameters were determined: pH, specific weight, Na, K, Ca, Mg, NH_4^+, Cl^-, PO_4^{3-}, SO_4^{2-}, creatinine, uric acid, citric acid, and oxalic acid. From the volume and these data we calculated the RS with respect to CaOx, using the EQUIL [5].

The urinary THP concentrations (μg/ml) were measured using the Syn^{elisa} THP enzyme immunoassay according to the principle of ELISA (Pharmacia & Upjohn Diagnostics *AB*, Cat. No. 8430/8460/8496). The detection limit of the Syn^{elisa} THP assay is 0.2 μg/ml; the precision of the assay is given to be around 5% and 7% for the intra-assay and interassay variability, respectively.

From the results of each individual person, the mean values were calculated and used for further evaluations.

2. Crystallization experiments

A CaOx-crystallization process was induced under standardized conditions (37 °C, stirring at 180 rpm) within 200 ml aliquots of urine with the step-by-step addition (0.5 ml, 1.5 ml per minute) of 0.04 N ammonium oxalate. The amount of oxalate ([mmol]) which has to be added to induce the crystallization process (Ox^{2-}) was measured.

The onset of the crystallization process was determined in-line by a laser-probe crystal system analyzer (Meßtechnik Schwartz, Düsseldorf, Germany), which continuously recorded the particle size distribution (PSD) of those particles sized between 0.5 μm and 250 μm. The initial count rates within the urine samples were around 90 particles per sec and treated as background signal. The formation (and growth) of crystals can be clearly detected by a rapid increase in the count rate and a dramatic change of PSD [1, 2]. Random tests were performed to scrutinize the nature of the formed crystals using infrared-spectroscopy; in all cases the crystals were composed of 100% calcium oxalate.

3. Calculation of the BRI

From the results of $[Ca^{2+}]$ and (Ox^{2-}) determination, the BRI per liter was calculated according to the following equation [14, 15]:

$$BRI = [Ca^{2+}]/(Ox^{2-})$$

Persons indicated by a BRI > 1/l are termed "at risk"; persons showing BRI ≤ 1/l are assumed to be "without risk" of forming CaOx stones [15]. To increase the accuracy of BRI determination, the crystallization behaviour of each urine sample was measured twice.

Results

The yielded average THP concentrations in the native urines of stone-formers and healthy persons were 16.34 μg/ml ($n = 8$) and 21.37 μg/ml ($n = 37$), respectively (Table 1). These concentrations are within the range given by Khan [12]. The determination of lower mean [THP] in urines from patients corresponds to the results evaluated by Ganter et al. and Romero et al. [6, 16].

The calculated mean BRI and RS with respect to CaOx are 2.402 l^{-1} and 6.33 for stone-formers, and 0.986 l^{-1} and 2.11 for the healthy persons, respectively. The urinary pH-values of the samples when entering the crystallization test, range between 5.79 and 6.89 (mean = 6.23); no linear relation (y = ax + b) between [THP] and pH was observed (a = +0.0008; $r = 0.03$). RS and BRI are related to a high degree ($r = 0.86$). Remarkable differences in the correlation between [THP] and BRI were observed once the data were sorted into different categories. Calculating first the linear relationship between [THP] and BRI by taking into account all samples, no relation between [THP] and BRI ($a = +0.007$, $r = 0.05$) was determined (Fig. 1). However, considering the results of the stone-formers and healthy subjects individually, the calculated relations

Table 1 Overview statistics of the parameters investigated. *H* and P indicate results from healthy subjects and patients only, respectively. *BRI* Bonn-Risk-Index according to [15], *RS* relative supersaturation with respect to CaOx calculated using EQUIL [5], [*THP*] concentration of Tamm-Horsfall protein in 24-h urine sample

Parameter	Samples	Unit	Min.	Max.	Mean
BRI	84	1/l	0.083	6.188	2.217
BRI-H	70	1/l	0.083	4.890	0.986
BRI-P	14	1/l	1.583	6.188	2.402
RS	45	–	0.073	11.410	2.832
RS-H	37	–	0.073	5.346	2.112
RS-P	8	–	1.036	11.410	6.330
[THP]	45	μg/ml	7.5	76.7	20.53
[THP]-H	37	μg/ml	7.5	76.7	21.37
[THP]-P	8	μg/ml	7.8	26.2	16.34

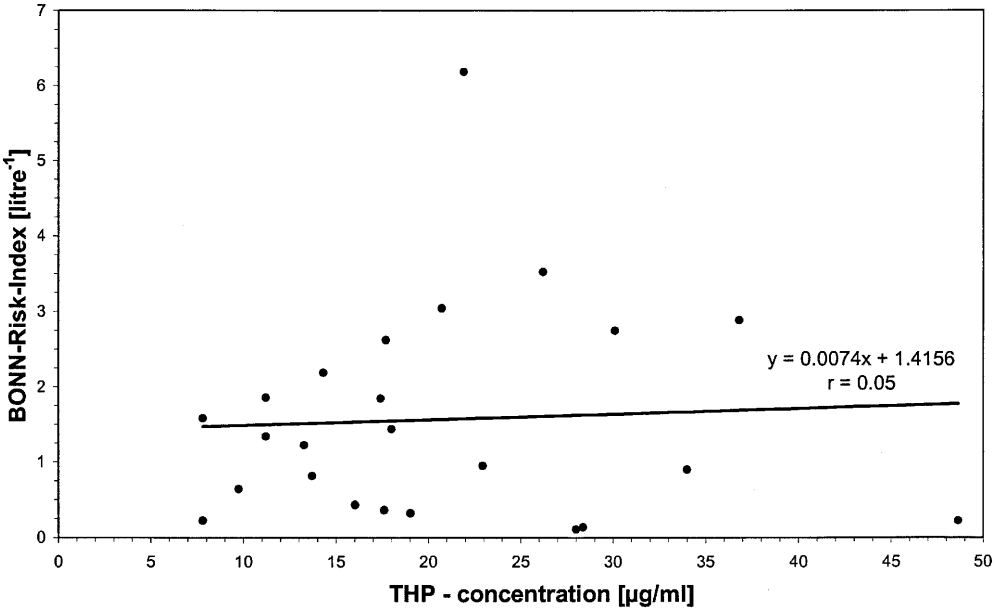

Fig. 1 Plot of mean urinary THP-concentration vs. mean Bonn-Risk-Index. The regression line is calculated without grouping of the persons

contrast remarkably. The data from healthy persons are fairly scattered and show only a negligible inverse linear correlation between [THP] and BRI ($a = -0.016$; $r = 0.19$); in contrast to that, the patient's data are directly related and show clearly higher values for a and r ($a = +0.095$; $r = 0.55$) (Fig. 2). The same correlations, yet with different values of r, are obtained when all samples are divided into the categories "without risk" (i.e., BRI $\leq$ 1/l) and "at risk" (BRI $>$ 1/l) (Fig. 3); the linear regression analysis reveals r coefficients for [THP] vs. BRI of 0.29 at the first group and 0.43 at the latter.

Without grouping the data, the RS with respect to CaOx and [THP] are related by [THP] = 0.55RS + 18.6 ($r = 0.16$). In both groups, stone-formers and healthy subjects, the data of RS and [THP] are directly related; linear regression analysis showed for these groups [THP] = 3.7RS + 13.72 ($r = 0.46$), and [THP] = 1.42RS + 8.97 ($r = 0.44$), respectively.

Discussion

The present study was carried out to determine the relation between the urinary THP concentration [THP] and the CaOx crystallization risk calculated according to the BRI [15]. The urinary RS with respect to CaOx was computed [5] for comparison. Our results are in good agreement with other studies (see introduction), as they underline the ambiguous role of THP in crystal formation processes.

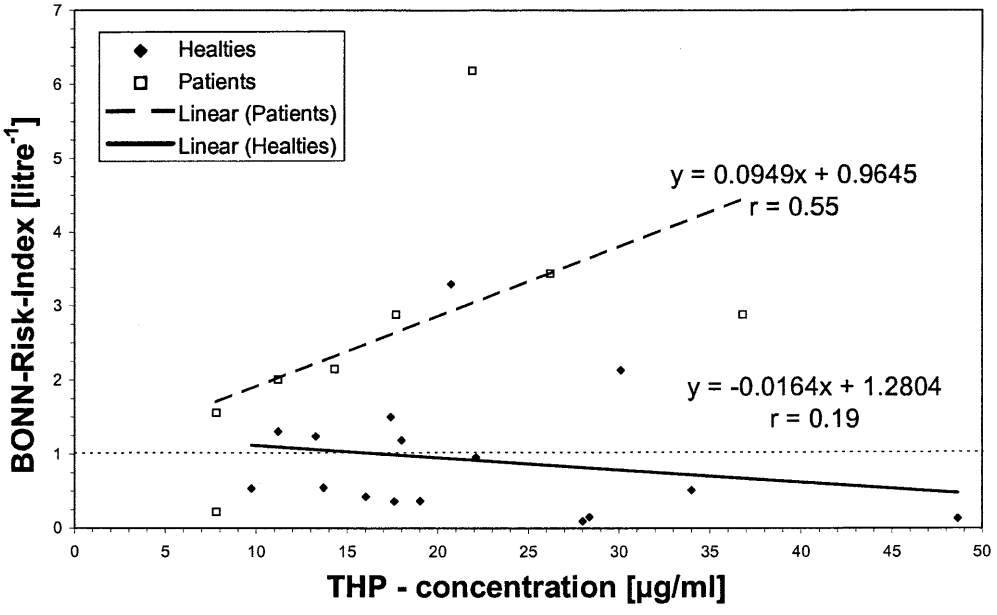

Fig. 2 Plot of mean urinary THP-concentration vs. mean Bonn-Risk-Index. Samples are grouped with respect to known stone-formers (*squares*) and healthy subjects (*diamonds*). Two regression lines are shown: (1) *dashed line*: patients only, (2) *solid line*: healthy subjects only

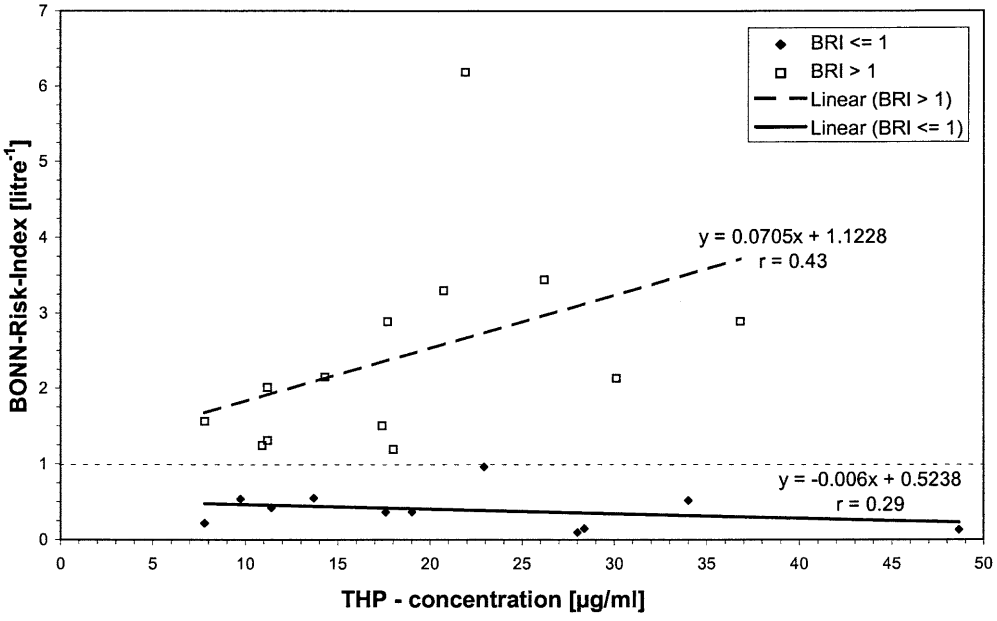

Fig. 3 Plot of mean urinary THP-concentration vs. mean Bonn-Risk-Index (BRI). In contrast to Fig. 2 the samples are divided up into "at risk" (BRI > 1/l, $n = 13$) and "without risk" ($n = 11$). The regression lines show: (1) *solid line*: only persons "without risk"; and (2) *dashed line*: only persons "at risk". *Diamonds*: "without risk"; *squares*: "at risk"

It could be shown that in urines from stone-formers the values of BRI and RS are directly correlated to [THP]; this direct correlation was also obtained for persons assigned to be "at risk" according to the BRI approach (i.e., BRI > 1/l). These results suggest a slightly promotive activity of THP for these groups of persons. In healthy subjects and also in persons indicated by BRI ≤ 1/l, an inverse and only weak relation between [THP] and BRI is given, suggesting a slightly inhibitive to neutral role of THP in CaOx crystal-formation processes in the urines of these individuals. These results are qualified as the non-stone-formers show a direct correlation between RS and [THP]. However, it must be taken into consideration that the linear regression analysis calculated for the parameter combinations RS and BRI on the one hand and [THP] on the other, yielded only very low values of a and r in each case.

This fact indicates that the crystallization risk of the healthy subjects calculated by RS and BRI is nearly independent of [THP]. The THP concentrations measured in the urines of healthy subjects and stone-formers are in the same order of magnitude, showing a broad range of overlap. This is also a clear indication that the value of [THP] itself is not an important factor that holds a decisive control over urinary CaOx-crystallization.

In conclusion, the widely discussed variable effects of THP on urinary crystallization processes are confirmed in principle by the BRI and RS method. The BRI data revealed that the effect of THP may shift from a minor promoter in stone-formers to an inert urinary constituent in healthy subjects; however, the observed effects are small, and in healthy subjects and in persons indicated by BRI ≤ 1/l, they are close to zero. Whether the variable effects of THP are caused by the urine environment or by intrinsic changes in THP itself, is still not clarified. We suggest that the contribution of THP to the process of crystal formation is much smaller than can be expected by its urinary concentration. This does not exclude a potentially important role of THP in stone formation processes, e.g., aggregation.

The "general effect" of macromolecules on the value of the BRI is still unclear and further investigations of other macromolecular constituents are necessary.

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