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Crystalluria in idiopathic recurrent calcium urolithiasis

Dependence on stone composition

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Summary. A retrospective study was done on the nature and degree of crystalluria in fasting and postprandial urine in patients with recurrent idiopathic calcium urolithiasis (RCU) for whom stone analysis was available. RCU was stratified into subgroups in accordance with stone analysis. The crystals were obtained and identified using a filter technique and polarization microscopy, respectively. Crystalluria score, relative saturation products (RSPs), and low-molecular-weight inhibitors were assessed. Calcium oxalate crystals were never observed in either male or female patients with stones composed exclusively of calcium oxalate, and only sporadically in patients with mixed stones (the additional component was calcium phosphate in most cases). Other crystalluria phases, such as amorphous calcium phosphate, a urate-containing phase, and a phase presenting as spherolytic particles, were slightly more frequent in patients with mixed stones. In contrast to crystalluria, RSPs and inhibitors differed in male and female patients, suggesting that crystalluria may not be under the exclusive control of these factors. The following conclusions were reached. (1) Calcium oxalate crystalluria is absent from RCU with pure calcium oxalate stones; hence, calcium oxalate crystalluria does not qualify as a diagnostic aid. (2) The co-existence of the isotropic phase and mixed stones may indicate that the formation of these concretions is characteristic for a major RCU subgroup. (3) On the basis of clinical chemistry and physicochemical data in urine and of crystalluria, it appears that the pathogenesis of RCU differs in male and female subjects.

It is generally held that crystalluria (synonym: microlithiasis) results from a supersaturation of urine with stone-forming substances, or, alternatively, may reflect an imbalance between inhibitors and promoters of crystallization [7]. Crystals of stone-forming minerals should permit certain inferences about the accompanying concrement within the urinary tract, and vice versa. According to this view, crystalluria might be expected to be of diagnostic value, but our present knowledge relevant to this is not conclusive.

Crystalluria is a frequent finding in healthy controls and in stone patients, and in stone patients qualitative and quantitative deviations from normal were reported [27]. Crystals of calcium oxalate, the most frequent component of urinary stones [12, 24], were shown to be more frequent and also larger in stone patients [19, 20, 27]. In a study of our own on healthy subjects and patients with idiopathic recurrent calcium urolithiasis (RCU) broken down not by stone analysis but by the degree of calciuria, we observed calcium oxalate crystals only exceptionally – in contrast to other particulate phases [11]. This unexpected finding of virtual absence of calcium oxalate crystals led us to search for the underlying cause(s), especially in regard to a possible relationship between the type and degree of crystalluria and the material predominating in the urinary concrement.

Therefore, the present study was undertaken to elucidate the question as to whether there is a relationship between the stone composition and crystalluria in RCU. In addition, crystalluria was determined both in fasting and postprandial urine. Also, a relationship of physicochemical supersaturation and low-molecular-weight inhibitors in urine was sought, because crystals and stone formation may depend on some of the latter substances as well. On the basis of the results obtained, the concept "crystalluria synonymous with microlithiasis" appears to be in need of revision, since there are seldom calcium oxalate crystals in the urine of RCU patients, and the phenomenon is seemingly characteristic for patients with stones composed purely of calcium oxalate.

Materials and methods

1. Patients

A retrospective study was done on RCU patients of both sexes. In all, 78 male and 38 female patients (for median age and range see

Table 1. Characteristics of study patients (means ± SEM or median/range)

	Men			Women		
	Ox	Ox + P	Ox + S	Ox	Ox + P	Ox + S
Individual data						
Number of patients	38	26	14	19	12	7
Age, years	44/20–64	39/22–64	45/21–56	47/26–57	48/25–63	40/25–62
Normocalciuria: hypercalciuria	27:11	14:12	8:6	10:9	3:9	6:1
Stones; present:absent	19:19	16:10	8:6	11:8	6:6	5:2
Metabolic activity of stone disease, score	19 ± 1	24 ± 2	18 ± 2	18 ± 2	15 ± 2	24 ± 4
Data in fasting blood						
Creatinine (mg/dl)[a]	1.13 ± 0.02	1.16 ± 0.02	1.09 ± 0.04	0.86 ± 0.03	0.88 ± 0.07	1.01 ± 0.07
Total calcium (mg/dl)[a]	9.53 ± 0.06	9.58 ± 0.07	9.70 ± 0.08	9.40 ± 0.06	9.47 ± 0.08	9.18 ± 0.17
Total magnesium (mg/dl)[a]	2.13 ± 0.02	2.04 ± 0.03*	2.10 ± 0.05	2.05 ± 0.03	2.05 ± 0.05	1.90 ± 0.07**

Ox, stones composed exclusively of calcium oxalate; Ox + P, stones containing at least 75% calcium oxalate and otherwise calcium phosphate; Ox + S, stones with less than 75% calcium oxalate and one other component or several (calcium phosphate, uric acid, urate)
[a] Upper limits for healthy normal volunteers in this laboratory (mg/dl): creatinine 1.4; total calcium 10.2; total magnesium 2.12
Comparison with Ox in same sex, respecting the Bonferroni principle [17] * $P<0.01$; ** $P<0.001$

Table 1) passed through our standardized ambulatory laboratory program [23]. On the basis of the stone analyses (see below) this sex distribution was incidental, but it does confirm that men suffer more frequently from urinary tract stones than women [12, 26]. In all cases a stone analysis stemming from an event dating back not more than 2 years was available. Among the analytical methods only infrared spectroscopy, polarization microscopy and X-ray diffractometry were accepted. In 72 male and 35 female patients filters that could be used for determining the nature and degree of crystalluria were available (see below). Patients with anatomical abnormalities of the kidney and the urinary tract, or with additional diseases such as urinary tract infection, renal tubular acidosis, primary hyperparathyroidism, overt gout or enteric hyperoxaluria, and patients with impaired kidney function (serum creatinine >1.4 mg/dl; creatinine clearance <60 ml/min) were excluded from the study. None of the participants had taken drugs during the week before the laboratory examination, and all patients were on a normal home diet and fluid intake while collecting the 24-h urine on the day before examination. From the laboratory program [23] the following data were chosen: 24-h urine, fasting blood (8:a.m.) after 12- to 15-h fasting period, 2-h fasting urine (8:00 a.m. to 10:a.m.) after prior stimulation of diuresis by drinking 600 ml distilled water, 3-h postprandial urine (10:00 a.m. – 1:00 p.m.), after consumption of the test meal contained in 300 ml distilled water. The 1264 kJ synthetic balanced meal (Vivasorb, Pfrimmer, Erlangen, FRG) was rich in carbohydrates (25.5 g glucose, 45.5 g oligomeric amylose), 6.5 g amino acids, 0.222 g essential fatty acids, with elemental calcium supplemented to a total delivery of 1000 mg (further details see [23]).

2. *Measurement of crystalluria*

Crystalluria was determined in 2-h and 3-h urine using a filter technique, as recently described [27]. In brief: freshly voided urine was vacuum-suctioned through a 0.2-μm Nuclepore filter, the temperature being kept constant; the surface of the filter was then rinsed with distilled water, air-dried and carefully stored until identification of the various phases by polarization microscopy. Both identification of the crystal nature and calculation of the crystalluria score (see below) were done blind by two independent observers (1 physician, 1 mineralogist). The phases were: amorphous calcium phosphate (tentatively named "isotropic"), a urate-containing phase (tentatively named "uric"), an organic spherolytic phase (tentatively named "spheroid phase"), whewellite and weddellite (for details see [11]). A semi-quantitative score, based on the number of observed particles on one half of the filter, was used to express the degree of crystalluria: 0 particles (score 0), 1–3 (0.25), 4–10 (0.5), 11–20 (1), 21–50 (1.5), 51–100 (2), 101–200 (2.5), >200 (3). Diameter and shape of the crystals were not taken into account.

3. *Stratifications, calculations*

Despite the documented admixture of small amounts of calcium phosphate to concrements composed mainly of calcium oxalate (1), for practical reasons this fact was not taken into account in the classification of the following stone types:
(a) Calcium oxalate stones consisting of whewellite (calcium oxalate monohydrate) and/or weddellite (calcium oxalate dihydrate), i.e. no phosphate component was identifiable with either of the above-mentioned analytical methods (Ox; $n=57$, ♂:♀ = 38:19).
(b) Stones containing at least 75% calcium oxalate (Ox + P; n = 38, ♂:♀ = 26:12); the remaining portion was always calcium phosphate.
(c) Stones containing less than 75% calcium oxalate; the remaining portion could have comprised one component or several, such as calcium phosphate, uric acid, urate (Ox + S; $n = 21$,♂:♀ = 14:7).

In Table 1, the term "stones present/absent" indicates whether a stone was detectable by X-rays or ultrasound at the time of examination. A case history of the last 24 months preceding the study was taken and the metabolic activity of stone disease [5] determined. Criteria for the classification into normocalciuria (NC) and hypercalciuria (IHC) were: calcium in 24-h urine >250 mg (IHC); calcium in 2-h urine <0.12 (NC) or >0.12 (IHC) mg/mg urinary creatinine; calcium in 3-h urine <0.27 (NC) or >0.27 (IHC) mg/mg urinary creatinine. In Table 2, data recorded in 20 male and 20 female healthy controls are added for comparison, but according to the main objective of the study (see above) they were excluded from statistical testing.

The physicochemical activity products of calcium oxalate, brushite, octacalcium phosphate, uric acid and sodium urate in the urine were calculated using a computer program based on published association constants [16], and expressed as relative supersaturation products (RSP; synomym: negative decadic logarithm of the activity product relative to the solubility product in water). RSPs <0 indicate undersaturation, 0–1 metastable supersaturation, >1 spontaneous precipitation [16].

Table 2. Crystalluria, crystalluria score, urinary pH and relative saturation products (RSP) of CaOx in 2-h fasting and 3-h postprandial urine of men and women with recurrent calcium urolithiasis (RCU). Note that for both whewellite and weddellite the individual score on filters is depicted

	Ox				(Ox + P) + (Ox + S)				Controls	
	%	$\bar{x}$	$\tilde{x}$	Range	%	$\bar{x}$	$\tilde{x}$	Range	%	$\bar{x}$
Men	$n=35$				$n=37$				$n=20$[a]	
Fasting urine; 2 h										
Score										
Isotropic	46	0.579	0	0–3	41	0.561	0	0–3	26	0.211
Uric	57	0.157	0	0–0.5	54	0.216	0.25	0–3	68	0.171
Spheroids	49	0.157	0	0–0.5	68	0.243	0.25	0–1	63	0.224
Whewellite		cnd				0.25; 0.5; 1; 1.5				0.25; 0.25
Weddellite		cnd				0.25; 0.5; 0.5				cnd
χ^2-test		$p < 0.01$								
pH		6.42 ± 0.11				6.59 ± 0.11				6.29
RSP-CaOx		0.51 ± 0.07				0.52 ± 0.07				0.48
Postprandial urine; 3 h										
Score										
Isotropic	37	0.329	0	0–2	41	0.473	0	0–3	29	0.250
Uric	69	0.186	0.25	0–0.5	54	0.222	0.25	0–3	71	0.206
Spheroids	34	0.114	0	0–0.5	41	0.155	0	0–1	35	0.118
Whewellite		cnd				0.25; 0.5				0.25
Weddellite		cnd				0.25; 3				cnd
χ^2-test		$p < 0.05$								
pH		5.93 ± 0.10				5.94 ± 0.08				5.72
RSP-CaOx		0.68 ± 0.05				0.70 ± 0.05				0.70
Women	$n=17$				$n=18$				$n=20$[a]	
Fasting urine; 2 h										
Score										
Isotropic	29	0.176	0	0–1	56	0.694	0.5	0–3	55	0.950
Uric	59	0.167	0.25	0–0.5	83	0.222	0.25	0–0.5	90	0.238
Spheroids	35	0.147	0	0–0.5	78	0.208	0.25	0–0.5	30	0.088
Whewellite		cnd				0.25; 0.25				cnd
Weddellite		cnd				cnd				cnd
χ^2-test		ns								
pH		6.59 ± 0.18				6.64 ± 0.17				6.41
RSP-CaOx		0.19 ± 0.10				0.20 ± 0.68				0.07
Postprandial urine; 3 h										
Score										
Isotropic	53	0.706	0.5	0–2.5	78	1.069	1	0–3	60	0.925
Uric	47	0.118	0	0–0.25	72	0.194	0.25	0–0.5	80	0.300
Spheroids	59	0.206	0.25	0–1	61	0.167	0.25	0–0.5	25	0.063
Whewellite		cnd				0.5				cnd
Weddellite		cnd				0.5				cnd
χ^2-test		ns								
pH		5.74 ± 0.11				5.98 ± 0.14				6.15
RSP-CaOx		0.54 ± 0.08				0.61 ± 0.05				0.28

cnd, Crystals not detectable; χ-test, comparison of Ox (no calcium oxalate crystals) against (Ox + P) + (Ox + S) (whewellite + weddellite); ns, not significant

[a] Data recorded in normal controls [11] are given for comparison

4. *Analyses*

Clinical chemistry variables in fasting blood and urine were determined by established methods: calcium (complexometry), magnesium (AAS), sodium (FES), phosphorus (colorimetry), total protein (refractometry), creatinine, alkaline phosphatase (autoanalyzer Technicon), bone isoenzyme of alkaline phosphatase (electrophoresis), urine pH (glass electrode). Uric acid [13] and citrate [18] were determined enzymatically, pyrophosphate [4] radioenzymatically. Oxalate was measured by ion chromatography [25], cyclic AMP by protein binding assay [2].

5. *Presentation of data, statistics*

To rule out urine collection errors, citrate, pyrophosphate and magnesium were factorized for creatinine in the same sample. Differences between groups were examined for significance ($P<0.05$) with the U-test. In cases of Gaussian distribution the *t*-test for unpaired data was used, but with multiple testing the level of significance ($P = 0.05$) was corrected for the nominal significance level as described by Bonferroni ($0.05/k$, where k = number of tests; [17]). The RSPs in 2-h and 3-h urine were tested by the Wilcoxon test for paired data, the crystalluria-phase calcium oxalate in the stone groups by the χ^2-test. Several variables were regressed.

Results

1. General data

Individual data of the participants (Table 1). With respect to age, the stone analysis subgroups were comparable in males and females. In both sexes, patients with NC predominated, as did patients with detectable stone at the time of examination. The metabolic activity of urolithiasis was roughly equal in all groups (differences not significant).

Clinical chemistry data. In fasting blood (Table 1) mean creatinine was normal in both males and females, and no statistically significant differences were found within the stone analysis subgroups; the same applied to total calcium. Total magnesium was significantly decreased in the Ox group of both sexes, as compared to the groups Ox + P or Ox + S. Other variables such as total protein, phosphorus, alkaline phosphatase, uric acid (data not shown) were unchanged. The volume of 2-h and 3-h urine was comparable in all stone analysis subgroups (males, females), and the same applies to the pool groups (see below). The mean values of urine volumes were: 2-h urine, males, 202 (Ox), 253 (Ox + P), 207 (Ox + S); females, 268 (Ox), 317 (Ox + P), 259 (Ox + S); 3-h urine, males, 286 (Ox), 235 (Ox + P), 262 (Ox + S); females, 257 (Ox), 260 (Ox + P), 219 (Ox + S).

The cyclic AMP excretion in 2-h urine was unremarkable, i.e. parathyroid gland function was not affected when based on this variable (data not shown). Also not given were data of several other substances in urine the molar concentrations of which entered into the calculation of RSPs (see below), because the latter were considered the more predictive variables for the proneness of urine to form crystals or stones.

2. Crystalluria

Influence of sex and urine collection period in RCU (data not shown). In a first step the score of the various crystalluria phases was evaluated separately in male and female patients and in 2-h and 3-h urine, but no distinction was made between the stone analyses.

With respect to all the filters investigated the uric phase was the most common in both sexes, followed by the spheroid and then the isotropic phases. With respect to the mean score of a phase determined on each individual filter, that of the isotropic phase was the highest, while the uric and the spheroid scores were lower and roughly of the same order of magnitude. Calcium oxalate crystals were observed only exceptionally (see below). In 2-h urine the score was comparable in both sexes. In 3-h urine females and males differed significantly with respect to the isotropic phase ($P<0.02$), and showed a difference of borderline significance with respect to the spheroid phase ($P<0.09$).

In males, the mean score of the isotropic and spheroid phases were lower in 3-h than in 2-h urine, and that of the uric phase was higher; in females the situation was reversed (differences not tested).

On comparison with the reference group (controls), it appeared that RCU men had a predominance of the isotropic phase, RCU women of the spheroid phase.

Influence of stone analysis. In a further step the scores for the three subgroups Ox, Ox + P and Ox + S were determined separately (data not shown). The most important finding was that calcium oxalate crystals were not detectable in either 2-h or 3-h urine of men or women with pure calcium oxalate stones; only in a few cases were calcium oxalate crystals observed, but in these, stone analysis revealed additional components besides calcium oxalate. This unexpected situation could not be attributed to the presence or absence of stones within the urinary tract at the time of the examination, because the respective percentages were roughly the same in all subgroups (see Table 1). The absence of calcium oxalate crystals in Ox, and their presence in Ox + P and Ox + S, led us to pool the latter two to (Ox + P) + (Ox + S) for both men and women.

In the resulting presentation of data (Table 2) the scores were comparable in Ox and (Ox + P) + (Ox + S) with respect to the isotropic, the uric and the spheroid phases (men, women; 2-h, 3-h urine). It also becomes clear from Table 2 that the scores roughly paralleled the absolute occurrence of filters with positive crystal finding, with the latter expressed as a percentage of total filters examined. However, in both sexes the mean score of these phases was lower in Ox than in (Ox + P) + (Ox + S), with the exception of the isotropic phase in the 2-h urine of men, and the spheroid phase in the 3-h urine of females. Wherever calcium oxalate crystals were present, they were identified as whewellite or weddellite (Table 2); on the basis of the small number of observations there was a significant difference between Ox and (Ox + P) + (Ox + S) in men (χ^2-test; Table 2), but not in women (Table 2).

Urinary pH, RSP-CaOx and calcium oxalate crystalluria (Table 2). In addition to the pH, RSP-CaOx was also listed, because it is accepted that the probability of homogeneous nucleation and initiation of calcium oxalate crystal formation increases with increasing saturation of the urine with calcium oxalate [7]. However, despite the absence of calcium oxalate crystals in the Ox group the pertinent pH and RSP-CaOx were of the order shown by the pool group (Ox + P) + (Ox + S), in which calcium oxalate crystals were observed in several cases. It is worth noting that calcium oxalate crystals were no more frequent in the calcium-rich 3-h than in the fasting 2-h urine, although the mean RSP-CaOx was higher in the former. Moreover, more individual filters with this phase were found in the 2-h than in the 3-h urine (2-h urine: men + women, 9 observations; 3-h urine: men + women, 6 observations).

3. RSPs in 2-h and 3-h urine

In addition to RSP-CaOx, also RSP-Bru, RSP-OCP, RSP-NaHU and RSP-UA were evaluated in order to more broadly characterize the propensity of urine to form

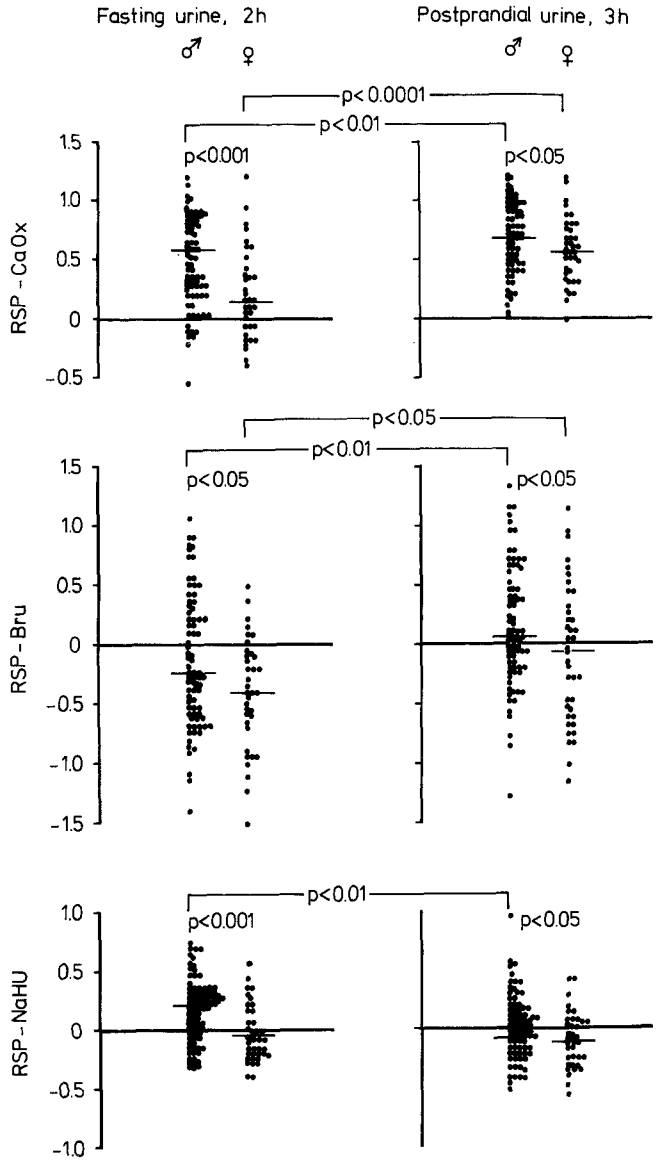

Fig. 1. Relative saturation products (RSP) of calcium oxalate (CaOx), brushite (Bru), and sodium acid urate (NaHU), in 2-h fasting and 3-h postprandial urine of men (♂) and women (♀). –, Median values

crystals. Only a few participants reached RSP values >1, thereby indicating a proneness to spontaneous precipitation. RSP-OCP and RSP-UA values were regularly <0, i.e. undersaturation predominated. With respect to all the RSPs mentioned above there were no significant differences between Ox and (Ox + P) + (Ox + S) in either male or female patients (2-h, 3-h urine); therefore, the two groups in each sex were pooled to form one group, and the individual values for RSP-CaOx, RSP-Bru and RSP-NaHU were plotted (Fig. 1). As a result, in both 2-h and 3-h urine all RSPs were significantly higher in male than in female patients. In 3-h urine, i.e. after consumption of the calcium-rich test meal, RSP-CaOx and RSP-Bru increased significantly in both male and female patients, whereas RSP-NaHU decreased significantly in men.

4. Excretion of low-molecular-weight inhibitors in 2-h and 3-h urine

On the assumption that the nature and degree of crystalluria might reflect influences of low-molecular-weight inhibitors hitherto known to be of importance alongside RSPs citrate, pyrophosphate and magnesium were evaluated in Ox and (Ox + P) + (Ox + S); a further reason for determining urinary magnesium was the elevated concentration of total magnesium in fasting serum of the Ox group (Table 1). However, irrespective of sex and urine collection period the three inhibitors showed no differences. In view of this situation, Ox and (Ox + P) + (Ox + S) were pooled for each sex. The mean excretions (per gram of urinary creatinine) of inhibitors were as follows (range of the individual values and standard error are available upon request): 2-h urine – citrate: 311 mg (♂), 572 mg (♀); pyrophosphate: 13 µmol (♂), 22 µmol (♀); magnesium: 40 mg (♂), 46 mg (♀); 3 h urine – citrate: 269 mg (♂), 405 mg (♀); pyrophosphate: 15 µmol (♂), 32 µmol (♂); magnesium: 103 mg (♂), 134 mg (♀). Accordingly, female subjects were regularly found to have more inhibitors than male ones; the differences were significant for citrate (2-h urine: $P<0.01$; 3-h urine: $P<0.01$), pyrophosphate (2-h urine: $P<0.01$; 3 h urine: borderline significance), magnesium (3-h urine: $P<0.05$).

5. Search for interrelation of variables

Since the urinary pH, the RSPs and the low-molecular-weight inhibitors could not explain the absence of calcium oxalate crystals in the urine of patients with pure calcium oxalate stones or their presence in patients with mixed stones, we extended our search by calculating simple correlations, regarding the isotropic and the spheroid phases as dependent variables. We did not attempt to relate the uric phase, the nature of which is still uncertain [11], to RSPs or inhibitors.

Female patients with pure calcium oxalate stones showed a positive correlation only between the isotropic phase and citrate in 3-h urine (Table 3). In male patients there were positive correlations between the isotropic phase, RSPs and inhibitors, but these were restricted to the 2-h urine (Table 3; pure stones, mixed stones). In male, but not in female patients, there were negative correlations between the spheroid phase, RSPs and citrate (pure stones; RSP-NaHU: $r = -0.34$, $P<0.05$; citrate: $r = -0.36$, $P<0.05$) in 3-h urine.

Discussion

To date the possible importance of crystalluria for the pathophysiology and diagnosis of RCU is still uncertain. With respect to calcium oxalate, a phase traditionally examined in diagnostic laboratories, our results differ greatly from those reported in the literature (see below). In a preceding study on RCU, classified in accordance to calciuria, we observed calcium oxalate crystals only exceptionally [11].

Table 3. Correlations in 2-h fasting and 3-h postprandial urine of male and female stone patients in dependence on stone analysis (pure or mixed)

	Stones Pure[a] Mixed[b]	Sex Male (♂) Female (♀)	Urine Fasting (F) Postprandial (P)	*n*	*r*	*P*
Isotropic phase (score)						
1. Relative supersaturation						
CaOx	Pure	♂	F	35	0.46	<0.01
Bru	Pure	♂	F	35	0.62	<0.0001
NaHU	Pure	♂	F	35	0.56	<0.001
CaOx	Mixed	♂	F	35	0.38	<0.05
NaHU	Mixed	♂	F	37	0.46	<0.01
2. Concentrations of citrate and magnesium (mmol/l)						
Citrate	Pure	♂	F	35	0.42	<0.02
Magnesium	Pure	♂	F	35	0.55	<0.001
Citrate	Mixed	♂	F	36	0.46	<0.01
Citrate	Pure	♀	P	16	0.64	<0.01

[a] Calcium oxalate
[b] Calcium oxalate and at least one additional compound

Therefore, in the present work the stone composition was taken into account as a possible determinant of crystalluria, and these data were compared with the degree of urinary supersaturation and excretion of low-molecular-weight inhibitors. A number of observations deserve comment.

Stone analysis and crystalluria

Several studies have revealed more and larger calcium oxalate crystals in patients with calcium urolithiasis than in normal subjects [19, 20, 27]. However, the most striking result in 2-h fasting or 3-h postprandial urine in our work was that calcium oxalate were not observed in men and women with pure calcium oxalate stones, and only in some of the patients with mixed stones. This prompted us to abandon the initial classification of patients into three stone groups (see Materials and methods) and, instead, to consider only one group with pure and one with mixed stones. Despite differences in calcium oxalate crystalluria, the two groups did not differ with respect to urinary pH, RSPs and inhibitors. Theoretically, pooling in this group might have masked existing differences, e.g. in the data of mineral metabolism. Thus, a more finely differentiated stratification, based for instance on the various components in mixed stones alone, or these in combination with associated serum chemistry data (Table 1), would be desirable. A recent study yielded similar conclusions, after separate evaluation of patients with calcium oxalate and those with calcium phosphate stones, demonstrating the predominance of the latter stone type in female subjects and other sex-related differences [9]. In the present work marked differences between men and women were revealed with respect to RSPs and urinary inhibitors, and only slight differences with crystalluria. This allows us to recommend re-evaluation of the importance of urinary supersaturation in the etiology of RCU, which may be assumed to differ between the sexes.

The simple assumption that the concrement present in the urinary tract could be predicted from the crystalline phase prevailing in the urine, for instance that calcium oxalate crystals would be diagnostic of pure calcium oxalate stone disease, is misleading. Within limits, this interpretation in the case of calcium oxalate crystals is also valid for other stone types, such as mixed stones. Other investigators have argued along the same lines, although when considered critically their methodology might be considered inadequate [28]. Thus, the pathogenesis of lithiasis, and specifically that of microlithiasis in the upper urinary tract, probably does not rest on whether crystalluria has already developed, and the corresponding hypothesis [21] needs further specification. The absence of calcium oxalate crystalluria in RCU – as far as we know the first documentation – may give rise to the development of new concepts in stone research.

Possible early events in the pathogenesis of urolithiasis

In explaining the absence of calcium oxalate crystalluria we may assume that calcium oxalate crystals were in fact formed and were present in the urinary tract, but remained adhering to the tissue. Direct experimental proof is needed, especially in humans, but there are pointers in the literature to support this. In a clinical study on healthy subjects it was calculated that some retention of orally administered ^{14}C-oxalate must have taken place within the kidney [3]. This observation, although not necessarily valid for calcium oxalate, suggests that adherence of calcium oxalate crystals may be an elementary phenomenon in stone-forming processes, and that it would be a candidate factor at least in calcium oxalate stone formation.

The significant correlations between the isotropic phase, RSP-CaOx, RSP-Bru and RSP-NaHU in men (Table 3) suggest that calcium oxalate is the result of heterogeneous nucleation; this would imply that an initial step in the formation of these phases is the presence of another, already nucleated, phase. However, this assumption, though held by many investigators, cannot be maintained, after all since in view of the frequently negative (synonym: undersaturation) RSPs, homogeneous nucleation of brushite and sodium urate could not have taken place.

The role of the isotropic phase, identified as amorphous calcium phosphate (11), in the etiology of urinary stone disease is uncertain. On the basis of the present data, the isotropic phase was characteristic for men and women in whom stone analysis disclosed mixed stones, i.e. besides calcium oxalate other components were present, notably calcium phosphate. Furthermore, calcium oxalate crystals were sporadically observed only in the latter (Table 2). Accordingly, at least theoretically, the quantitatively predominating isotropic particles could have displaced calcium oxalate crystals from their tissue-binding sites, thereby facilitating the appearance of the former in the urine. The positive correlation between the isotropic phase and urinary citrate, a strong inhibitor of crystal and stone formation [8], was unexpected, for, assuming a causal connection between the two, a negative correlation would be expected if any. Significant correlations were also found between spheroids and RSP-NaHU and citrate. In a previous study we saw more of the lipid-like [11] spheroids in RCU females than in healthy controls, and the observed correlation of this phase and lean body mass led us to assume the existence of a still unknown metabolic disturbance. In the past, lipids have also been the subject of urolithiasis research; they are one component of the organic matrix of urinary stones [14]. Lipids extracted from stone matrix are capable of binding calcium and oxalate ions in a metastable calcium oxalate solution, and also of catalyzing calcium oxalate crystal formation [15]. When urolithiasis was induced in experimental animals, cell injury and necrosis accompanied by fatty degeneration were regularly present [22].

Choice of urine for evaluation of crystalluria

Some investigators evaluated crystalluria in so-called spot urine [6, 10], whereas we examined timed fasting (2-h) and postprandial (3-h) urine. We gained the impression that the scores for the crystalluria phases described were independent of the urinary collection period. Significant correlations between the isotropic phase and the RSPs or inhibitors were, however, found mainly in fasting urine (Table 3), while conversely, a significant relationship between the spheroid phase and the RSPs or inhibitors was limited to postprandial urine. Hence, for future investigations the choice of the urine collection period should be matched to the particular aims of the study.

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Erratum

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Environmental Factors of the Pathophysiology of Recurrent Idiopathic Calcium Urolithiasis (RCU), with Emphasis on Nutrition.

Due to an unfortunate error, Fig. 3 of this article was incorrectly lettered; instead of "Hydroxyapatite" it should have read "Hydroxypyruvate".