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ORIGINAL PAPER

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Magnesium, citrate, magnesium citrate and magnesium-alkali citrate as modulators of calcium oxalate crystallization in urine: observations in patients with recurrent idiopathic calcium urolithiasis

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Abstract The effects of magnesium (Mg) and citrate on the metastable limit of calcium oxalate (CaOx) solubility (synonym: tolerable oxalate TO) were examined in artificial urine and in postprandial urine of male patients with idiopathic calcium urolithiasis (ICU). In artificial urine increasing pH, Mg and citrate elevate TO, decrease CaOx supersaturation only marginally, but elevate considerably free citrate; the effect of Mg alone was small in comparison with citrate alone, and the effects of both substances appeared additive. In ICU patients, matched for sex, age and CaOx supersaturation to non-stone-forming controls, TO was decreased (mean values 0.33 vs. 0.52 mM/l in controls, $P < 0.05$). Additional significant ($P < 0.05$) differences were found between ICU and controls: the former exhibited increased CaOx crystal growth, decreased crystal agglomeration time, a more acidic urinary pH, increased concentrations of free calcium and free Mg, and decreased free oxalate and free citrate. After ingestion of a urine-acidifying test meal, or this meal supplemented with either neutral Mg citrate or Mg-alkali citrate, by three groups of male ICU patients, matched for age and CaOx supersaturation, only the last-named preparation evoked an increase in TO and a decrease in crystal diameter, while the normally occurring pH decline from fasting urine was virtually abolished, and the ratios urinary Mg/citrate and calcium/citrate tended towards low values. In contrast, Mg citrate increased crystal agglomeration time, while changes in the other parameters were only insignificant. The crystals formed in urine were CaOx di- and monohydrate (by electron microscopy), and energy dispersive X-ray analysis showed calcium peaks exclusively. However, chemical analysis of crystals verified the presence not only of oxalate and calcium, but also of Mg, phosphate, citrate, and urate; moreover, these crystal constituents seemed to be influenced by Mg citrate and Mg-alkali citrate in different ways. It was concluded that (1) Mg and citrate are effectors of TO in artificial and natural urine; (2) in ICU, low TO and other disturbed CaOx crystallization parameters appear related to the prevailing low urinary pH and low free citrate; (3) Mg-alkali citrate inhibits CaOx crystallization, probably via actions of the citrate, but not the Mg. Because of the eminent role of Mg in human health and ICU, further studies on crystallization after oral intake of Mg in the form of citrate are warranted.

Key words Magnesium · Citrate · Alkali · Calcium oxalate crystallization · Artificial and postprandial urine · Idiopathic calcium urolithiasis

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Introduction

In Western populations the status of magnesium (Mg) and its relationship to diseases as characterized by pathological calcifications have not yet been clarified; the view that Mg nutrition is deficient is widely held [15, 34]. For example, Mg deficiency may be found to be associated with hypertension [28], increased myocardial tissue calcium [13], and deposition of calcium phosphate in the myocardium [26], arterial walls [1] and in the renal cortico-medullary region (synonymous nephrocalcinosis [14]). In idiopathic calcium urolithiasis (ICU) the status of Mg is less clear: low urinary Mg was described early on [47], but a more direct demonstration of moderate Mg deficit in red blood cells has only recently been achieved [44]. A further argument for a causal role of Mg in ICU may be seen in the reduced recurrence rate of renal stones during long-term oral medication of patients with Mg-containing drugs, such as Mg oxide [50] and Mg hydroxide [5]. At the level of urine the rationale

underlying Mg medication is seen in the facts that Mg can compete with calcium for the oxalate ligand, that Mg oxalate is more soluble than calcium oxalate (CaOx [8, 17]), and that Mg inhibits CaOx and calcium phosphate crystal growth [10, 40]; finally, it is felt that Mg decreases oxaluria via inhibition of hepatic oxalate biosynthesis [31, 41]. Thus most publications support the view that Mg per se, i.e. irrespective of the anion, is useful in the metaphylaxis of renal stones containing calcium oxalate. Only a few in vitro studies have reported that increasing urinary Mg is of little effect on CaOx solubility [18], that the formation of larger crystals may be due to Mg, and that physiological urinary Mg concentrations fail to inhibit CaOx crystallization [9, 53].

In contrast to the Mg cation, the potential action of the anion of a given Mg salt has received much less attention in stone research, in particular the role in crystallization processes. Compared with oral Mg oxide and Mg hydroxide, oral Mg citrate has been shown to exhibit superior bioavailability, as judged by the amounts of Mg absorbed intestinally and excreted in urine [33, 34]. High bioavailability was found especially when the salts were taken together with a meal [33]. Theoretically, intake of a citrate-supplemented meal should lead to increased urinary citrate, another documented inhibitor of stone substance crystallization; hence, the combined actions of Mg and citrate should be able to decrease the risk of CaOx crystallization. This has, however, not been unequivocally demonstrated. Citrate is degraded metabolically to base equivalents, but preliminary work we carried out failed to show a Mg citrate-related increase in urinary pH [27]. In the presence of less acidic renal tubular fluid, citrate reabsorption decreases; this, together with the accompanying progressive citrate deprotonation, results in a higher intraluminal concentration of charged citrate, which exhibits superior crystallization inhibition properties [16, 21, 20]. Thus, from nephrological and physico-chemical points of view a rise in urinary pH is desirable in stone metaphylaxis. Alkali-containing Mg citrate, delivering Mg, citrate and alkali in amounts up to 50 milliequivalent per dosage, is available in the USA [40], but not in continental Europe. Moreover, a study of the effects of Mg citrate and Mg-alkali citrate on urine composition, CaOx supersaturation and crystallization in humans has not been reported.

The present work pursued several aims. We wished to establish (1) the individual and combined effects of Mg and citrate on the metastable limit of CaOx solubility [synonym: tolerable oxalate concentration (TO)] in artificial urine; (2) whether in natural urine there are differences between ICU and controls with respect to TO, free (synonym: ionized) moieties of Mg, citrate, other ions, pH and CaOx crystallization; (3) whether in ICU there is a change in TO and CaOx crystallization together with an increase of urinary pH in response to intake of Mg citrate or Mg-alkali citrate. Finally, we examined the relative effect of these Mg preparations on the morphology of crystals and the substances they contained.

Materials and methods

Chemicals and equipment

The chemicals used for the analyses were all of analytical grade, and were purchased from Fluka, Buchs, Switzerland. Mg citrate, and the Mg citrate and alkali-containing preparation used in the clinical trials were of the highest degree of purity available (for details see Study protocols). Conventional laboratory facilitities were used throughout, with the exception of specialized software (see Analysis and calculations) and scanning electron microscopy (SEM) (Camscan CS 24-91 Compact) equipped with energy-dispersive X-ray analysis (EDX) (PV 9800 EDAX with Econ IV detector), for probing elements in crystals.

CaOx crystallization

Details of this single-step, small-scale test procedure requiring 490 µl undiluted urine have been described elsewhere [20, 21, 43]. In brief: when postprandial urine is incubated (37°C; 30 min) and agitated (120 rpm), the addition of sodium oxalate (98% final urine concentration) evokes CaOx nucleation and the formation of visible (by light microscopy) CaOx crystals; the TO level is reached when oxalate concentration surpasses the upper limit of CaOx solubility; after further addition of oxalate the crystal growth achieved within 30 min post-nucleation and crystal agglomeration time are determined under the microscope. TO is not confounded by simultaneous oxalate depletion [43]; the diameter of the first visible crystals formed in urine of ICU patients or non-stone-forming individuals is similar (1.8–4.0 µm), and the post-nucleation crystal growth is linear, with the slope of regression lines (up to 60 min post-nucleation) being somewhat steeper in urine of ICU patients (unpublished data). For studies of crystal morphology and crystal element content a urine volume four times that of the routine sample volume, together with the corresponding amount of oxalate, was incubated over 120 min, the urine vacuum-suctioned through a 0.22 µm pore size cellulose nitrate filter (Millipore, Eschborn, Germany), and the filter kept in a dry place until SEM. Further samples (four times the routine volume) were incubated (see above) and centrifuged (12 000 *g*), the supernatant carefully suctioned off, and the crystallized pellet dissolved in 1 M HCl, for analysis of substances.

Participants and procedures

A total of 58 adult males gave their informed consent and were enrolled in trials (see below). All remained on their usual free home diet, with no vitamin supplementation of food in the 2 weeks preceding their attendance at the laboratory. They were studied after an overnight 12- to 15-h fasting period.

Healthy volunteers ($n = 11$) were stone free and not suffering from any clinical disorder known to predispose to renal stones or osteoporosis (for age and other general features see Table 1).

Renal stone patients ($n = 47$) were diagnosed as ICU, on the basis of medical history, stone analysis, and a plain X-ray film of the kidney–ureter–bladder region documenting the formation of radio-opaque stones. About two thirds were stone free at the time of the investigation. Stone analysis revealed either pure calcium oxalate (approx. 60%), or admixtures of calcium phosphate. Systemic metabolic disorders such as overt diabetes, primary hyperparathyroidism, or other disturbances frequently associated with stones, were absent. Overall renal function was normal (serum creatinine < 1.4 mg/dl). Metabolic activity of stone-forming processes was scored in accordance with a previously described procedure [46].

Laboratory examination: while in the laboratory, participants underwent a standardized program (for details see ref. 43). After voiding the bladder at about 7:00 and 9:00 a.m. a standardized calcium-rich (1000 mg) meal (for details see ref. 43, 46) was taken together with a 200- to 300-ml drink, the composition of which differed depending on the study protocol (see below); 3 h after the meal the bladder was voided into a pre-warmed (37°C) beaker and the urine immediately filtered through a 7 μm pore size filter paper (Whatman no. 2) at room temperature to remove gross particles such as mucus. Thereafter, aliquots of urine were further processed for analyses and CaOx crystallization.

Study protocols

Several trials were carried out in an attempt to elucidate whether there is a role for among others, urinary Mg, citrate and pH in CaOx crystallization. In none of the trials was the possible specific role addressed for so-called macromolecules that can play a part in CaOx crystallization, because at present there is incomplete documentation as to whether these substances inhibit [11] or enhance [4] TO via binding sites for cations and anions. Within this limitation, results obtained in trials 2 and 3 are considered as preliminary.

Trial 1: TO in artificial urine

An artificial urine (for basic composition see ref. 42; for other details see legend to Fig. 1) was used to show up whether in this environment Mg, citrate and pH principally can interact at the TO level, in the present work considered as CaOx solid formation. Since the association of citrate with either Mg or calcium shares similar constants [22], any change in TO observed with varying Mg, citrate, pH and combinations thereof, can theoretically be ascribed to changes in different CaOx supersaturation, citrate complexation of calcium and Mg ions, or direct inhibition by citrate of calcium and oxalate ion pair formation. Available techniques do not permit these events to be studied separately in complex solutions such as artificial or natural urine.

Trial 2: CaOx supersaturation, TO, free ions in natural urine

Previously, we found low TO in ICU vs. non-stone-forming healthy controls, but CaOx supersaturation was not sufficiently matched and, theoretically, could therefore have confounded TO [20, 43]. Therefore, we attempted to identify – indirectly via regression analyses – factors associated with a given level of TO at a similar degree of CaOx supersaturation; so doing rules out the likelihood that TO, should it differ between ICU patients and non-stone-forming healthy controls, merely reflects differences in pre-existing CaOx supersaturation. Also assessed were pH, the total concentration and the free ionic moiety of several speciated urine constituents, and possible interrelationships. A knowledge of the ions remaining in solution after formation of soluble complexes is indispensable, if the factors controlling TO are to be interpreted.

Male controls ($n = 11$), and male ICU patients ($n = 11$) were matched for age, but not body weight and height (see Table 2). When examined in the laboratory they were loaded with a urine-acidifying calcium-rich test meal of fixed composition, delivering 67 mg elemental Mg, and approx. 120 milliequivalent protons (by titration of acid-dissolved meal), largely from non-phosphate sources [46]. It was assumed that the low postprandial urinary pH, as achieved with this meal, was accompanied by effective calcium suppression of parathyroid gland function, hence a minimized risk of calcium phosphate co-precipitation. The degree of calciuria [normocalciuria, combination of absorptive and fasting hypercalciuria (synonym: idiopathic hypercalciuria)] was assessed from the calcium/creatinine ratio in fasting (< or > 0.12 mg/mg) and post-calcium load (>0.27 mg/mg) urine.

Trial 3: CaOx crystallization in ICU patients after test meal supplementation with Mg citrate or Mg-alkali citrate

Our major focus was on the comparative anti-crystallization effects of the two Mg preparations. Another aspect of this trial was that the above-mentioned meal alone does not create the postprandial "alkaline tide" situation, as has been held to be the case, but rather mimics the "endogenous acid rain" frequently resulting from the composition of natural food eaten in most Western civilizations. Many investigators in the field of nutrition assume that unless the proton excess generated by meals is reduced by adequate concomitant alkali supply the body is forced to acidify urine and develop mechanisms of adaptation which, themselves, harbour risk factors for disease. Of central importance therefore was the question whether the decrease in urinary pH seen with the unsupplemented meal can be prevented by supplementation with Mg preparations, and how TO and other criteria of CaOx crystallization behave in this situation.

Mg citrate, marketed as a water-soluble granulate (Boehringer, Ingelheim; Germany), was added to the drinking liquid (see Participants and procedures above) at a frequently prescribed therapeutic dosage, delivering 296 mg elemental Mg (total supply of Mg 362 mg) and 24 milliequivalent bases (from metabolic degradation of citrate). Mg-alkali citrate, a mixture of substances not available commercially (European Patent number EP 9410-9609.1), delivering (from Mg citrate) 185 mg elemental Mg (total supply of Mg 252 mg), 22 mmol potassium and 7 millimol sodium (both as bicarbonate, from Fluka, Buchs, Switzerland), and 45 milliequivalent bases (from the sum of bicarbonate equivalents). Thirty-six males were studied (meal alone, $n = 13$; meal + Mg citrate, $n = 11$; meal + Mg-alkali citrate, $n = 12$); mean age, body mass index, and metabolic activity were all similar.

Trial 4: SEM and EDX wet chemical analysis of crystals

Because of limited resources the studies were restricted to one individual in each of the three ICU subgroups in trial 3; the TO in these individuals was close to the group mean of this parameter.

Analyses and calculations

Established methodologies, described elsewhere [21, 20, 43, 46], were used for the analyses in urine: calcium (AAS), magnesium (AAS), potassium, sodium (FES), chloride (potentiometric titration), phosphorus, ammonium and sulfate (all by colorimetry), citrate and uric acid (all enzymatically), creatinine (autoanalyser), oxalate (ion chromatography), pH (glass electrode), titratable acid (auto-titration with 0.1 M NaOH to pH 7.4). Supersaturation with stone-forming substances as well as free ions was calculated using EQUIL-2 software [51]; for comparative purposes the relative CaOx supersaturation was also calculated on the basis of published activity products [36]. Results are given as mean value and standard errors. The software package STATISTICA (Stat-Soft, Tulsa USA) was used for testing bivariate and multivariate stepwise logistic regression analysis, total variance (ANOVA or, in the case of non-Gaussian distribution, the Kruskal-Wallis procedure), and the significance of intergroup differences ($P \leq 0.05$) (test of least square differences), as appropriate.

Results and discussion

Mg, citrate and pH as effectors of TO in artificial urine

In the absence of Mg or citrate, and at a pH of between 4.5 and 7, TO is <0.2 mM/l and remains

unchanged, indicating that in this urine-like solution the influence of constituents other than Mg and citrate on this parameter is negligible (not shown). Calcium phosphate co-precipitation – in several reports dealing with natural urine shown to induce heterogeneous CaOx nucleation above pH 6.5 [7, 23] – can be ruled out. When Mg is present in the absence of citrate, it exerts an isolated effect with onset at pH 4.5 (TO increases dose-dependently), and more pronounced at pH >6 (Fig. 1B); however, the overall Mg effect appears weak. On the other hand, at pH 4.5–5.0, citrate alone does not increase TO more than Mg; at higher pH citrate evokes a dramatic rise of TO (about 0.75 mM at pH 5.5, about 1 mM at pH 7; Fig. 1A). When both Mg and citrate are present, their combined effect appears additive, the highest value of TO being about 1.1 mM at pH 7 (Fig. 1C). CaOx supersaturation, when assessed for pH 5, 6 and 7, varies between 4.1 (Fig. 1A, pH 5, 1.6 mM citrate) and 3.2 (Fig. 1A, pH 6.75, 3.6 mM citrate), with all other values, including those in parts B and C of Fig. 1, ranging within these limits. In contrast, free citrate concentration, when assessed for pH 5, 6 and 7, 3.85 mM Mg, 1.6, 3.2 and 6.4 mM citrate (Fig. 1C), was 0.2, 0.4 and 0.5 mM, respectively; these figures substantiate a 2.5-fold increase of free citrate, as compared with the 22% maximal decline of CaOx supersaturation.

In Fig. 1D an integrated view of effectors of TO is given for pH 6, around which the majority of values are scattered in fasting urine of healthy humans. When citrate is kept constant at 4 mM – a physiological concentration – increasing Mg above 4 mM is ineffective; conversely, when Mg was kept at 3.85 mM – also a physiological concentration – increasing citrate further increases TO. At lower levels of effectors TO is lowest in the absence of citrate.

These combined observations prove that to effect an increase in TO in artificial urine the presence of both Mg and citrate is superior to either substance alone, and that the less acidic the pH, the more free citrate is available. However, no inference is possible as to the mode of action of effectors, i.e. supersaturation, complexation, occupation of growth sites of CaOx crystals of sub-light microscopy size or even non-crystallized CaOx molecular clusters. Further information was obtained by studying natural human urine.

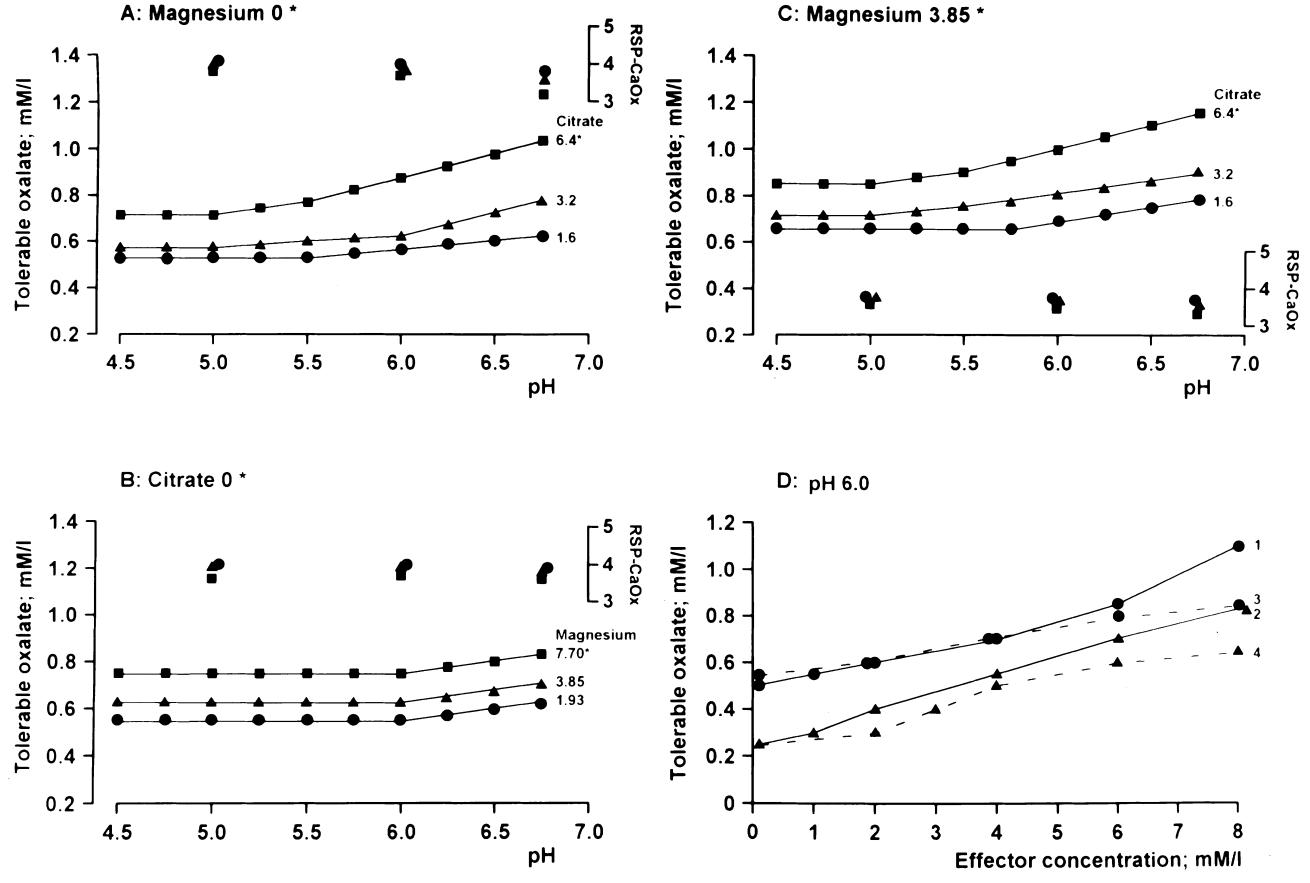

Fig. 1 Tolerable oxalate (TO) concentration and (Calcium oxalate (CaOx) supersaturation [RSP-CaOx, as free energy (ref. 51)] in artificial urine at varying pH and concentrations of Mg and citrate; note that Mg and citrate were varied by addition of Mg chloride and ammonium citrate, respectively. For other details see Materials and methods; *: mM/l. **A**, **B** in the absence (0) of Mg or citrate; **C** in the presence of Mg and citrate; **D** at pH 6.0. *Circles with solid line*: increasing citrate (Mg constant at 3.85 mM); *Circles with dashed line*: increasing Mg (citrate constant at 4 mM); *triangles with solid line* increasing citrate (no Mg); triangles with dashed line: increasing Mg (no citrate)

TO, pH, free ions, CaOx crystallization at constant supersaturation of non-stone forming and stone-forming human urine.

CaOx supersaturation is statistically indistinguishable between ICU and controls, this being true for both modes of calculation (Table 1). Urinary pH is more acidic in ICU; this indicates that if pH had influenced CaOx supersaturation, EQUIL-2, in which proton concentration is an integral part, would have led to divergent values. TO in ICU is only about 63% of that in controls; the concentrations of substances and ions are decreased (percentage of mean values ICU vs. controls: total citrate 50, free citrate 30, free oxalate 70, free urate 61), or increased (Mg 146, calcium 170). Thus, low TO was accompanied by low free citrate and urate, but not low free Mg. TO and pH correlate directly ($r = 0.58$, $P = 0.005$, $n = 22$); but TO correlates more strongly with the ratio of the free ions Mg/calcium ($r = 0.76$, $P < 0.001$, $n = 22$) – both ions that are able to compete for citrate and oxalate ligands – and with the ratio of free ions citrate/calcium ($r = 0.75$, $P < 0.001$, $n = 22$). While the slope of the regression lines is not different, there emerges a cut-off (at 0.02 mM/mM) for the ratio of the free ions citrate/calcium, separating the majority of ICU patients (9 of 11) from controls (Fig. 2). In contrast, the correlation between the ratio of total citrate and total calcium concentration and TO is weak ($n = 22$; $r = 0.57$, $P = 0.075$; data not shown). As was expected for ICU, crystal diameter and crystal growth rate are increased, while crystal agglomeration time is decreased.

Within the limitation set by failing to control for macromolecules – from reasons mentioned above (see also ref. 11, 4) – the data allow us to postulate that when ICU and controls are selected in the manner described (matching for supersaturation, age, body weight), an excess of oxalate and CaOx supersaturation at urinary pH < 6 can be ruled out. It follows that, although direct proof is still lacking, the combination of excess free calcium, low free citrate and possibly free urate and pH, but not low free Mg, appears to be crucial for the liquid-solid transition of calcium and oxalate, i.e. whether these ions remain in solution, form ion pairs, CaOx molecular clusters, or crystals at the submicroscopical level. Furthermore, total citrate concentration is low despite roughly unchanged urinary volume, adding another feature to the often reported low citrate excretion of ICU patients. One of the causes of low citrate at low urinary pH is enhanced back diffusion of non-ionized citrate [25, 45]. That low urinary citrate – primarily free citrate – is associated not only with low TO but also with high body weight relative to height (synonym: body mass index) – a nutrition-dependent variable – is a new

Table 1 Data from male healthy controls and male ICU patients, matched for age and CaOx supersaturation in postprandial urine. Data are mean values (SE). For details see Materials and methods

Variables	Controls $n = 11$		ICU $n = 11$		*P*-value
General features					
Age; years	31.6	(1.4)	33.5	(1.3)	ns
Body weight; kg	71	(4)	77	(4)	ns
Height; m	1.80	(0.02)	1.73	(0.02)	0.052
Body mass index; kg/(m)^2	21.7	(0.8)	25.8	(1.4)	0.041
Urine					
Volume; ml	207	(28)	256	(46)	ns
pH	5.88	(0.14)	5.39	(0.16)	< 0.05
Calcium; mM/l	4.59	(0.65)	5.92	(0.70)	ns
Oxalate; mM/l	0.18	(0.02)	0.16	(0.02)	ns
Citrate; mM/l	2.39	(0.40)	1.21	(0.21)	< 0.05
Magnesium; mM/l	3.6	(0.2)	3.8	(0.4)	ns
Phosphate; mM/l	15.7	(3.0)	12.0	(2.2)	ns
Urate; mM/l	2.14	(0.22)	1.83	(0.19)	ns
CaOx supersaturation					
Relative[a] (solubility = 0)	0.76	(0.07)	0.77	(0.07)	ns
Free energy[b]; ΔG	1.28	(0.02)	1.22	(0.04)	ns
Free ions[c]					
Calcium; mM/l	2.70	(0.36)	4.58	(0.54)	< 0.01
Oxalate; mM/l	0.045	(0.03)	0.032	(0.004)	< 0.05
Citrate; mM/l	0.144	(0.024)	0.043	(0.009)	< 0.001
Magnesium; mM/l	2.0	(0.2)	2.9	(0.3)	< 0.01
Urate; µM/l	0.151	(0.1)	0.092	(0.15)	< 0.05
Tolerable oxalate; mM/l	0.52	(0.07)	0.33	(0.02)	< 0.05
Crystal diameter[d]; µm	4.6	(0.2)	6.0	(0.2)	< 0.001
Crystal agglomeration time; min	50	(4)	37	(3)	< 0.01
Crystal growth rate; µm/min	0.08	(0.01)	0.09	(0.01)	< 0.001

[a] Activity products relative to solubility in water [51]
[b] According to EQUIL-II [36]
[c] Note that at urinary pH < 6.0 the fraction "free (triply negatively charged) phosphate" is negligible
[d] At 30 min post-nucleation

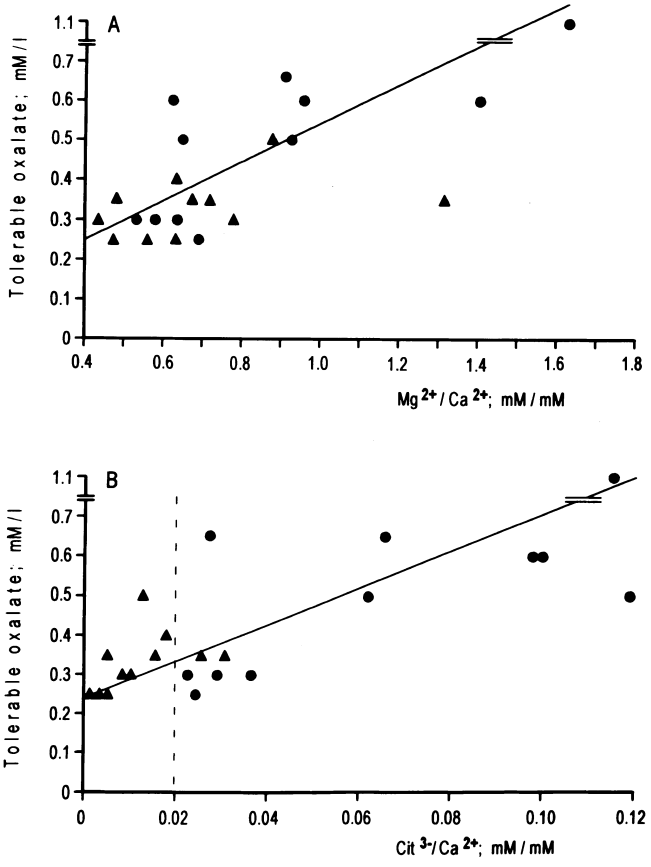

Fig. 2 TO concentration and the ratio of ionized (= free) ions, in non-stone-forming controls (*circles*) and ICU patients (*triangles*). For details see section Materials and methods. **A**: independent variable free Mg/free calcium ($r = 0.76$, $P < 0.001$, $n = 22$). **B** independent variable free citrate/free calcium ($r = 0.75$, $P < 0.001$, $n = 22$)

datum. Interrelations of obesity and lipids on the one hand, and homeostatic mechanisms for calcium and citrate on the other, have been reported [2, 38]. Therefore, measures aimed at treating ICU appear to have a sound theoretical basis when they are able to halt an acid meal-dependent decrease in urinary pH; in such a situation TO should remain unchanged via the maintenance of total and free citrate concentration at the preload level.

Based on similar considerations in ICU patients, Ashby and Györy et al. [3] ascribed the key role to free citrate, showing an average concentration of approx. 0.015 mM as compared with 0.043 mM in present work, the difference being due to different methodology. Against the widespread use of free citrate as a reliable marker before and during metaphylaxis regimens in ICU is the considerable workload imposed by the necessary clinical chemistry determinations. In the following section results are described that were obtained upon administration of Mg citrate and a urine-alkalinizing Mg citrate to ICU patients, using more traditional parameters of evaluation.

Urine composition and CaOx crystallization in ICU as influenced by the meal, with and without Mg citrate or Mg-alkali citrate

Table 2 presents TO and crystallization data, general patient data (age, body mass index, metabolic activity of ICU) and a spectrum of urine parameters developed in response to the three types of load. Free energy and free ions were not determined. It should be noted that CaOx supersaturation was in a comparable order of magnitude, and that the associated brushite supersaturation (calculated for pH 6.0) is near solubility. Intake of Mg-alkali citrate, but not Mg citrate, halts the decline in pH associated with the meal alone (pH in Table 2 is defined as pH in postprandial minus pH in fasting urine), improves TO and significantly reduces crystal diameter, while the prolongation of crystal agglomeration time and the reduction in crystal growth rate differ only insignificantly. In contrast, Mg citrate leaves TO and crystal diameter unchanged, but prolongs crystal agglomeration time and reduces crystal growth rate. The crystal count tends to increase with Mg-alkali citrate, but remains unchanged by Mg citrate. Particulate calcium phosphate could not be identified. From the comparable pre-existent CaOx supersaturation in the three groups it is obvious that these changes in CaOx crystallization must have been caused by other factors.

The correlation matrix indicated that the only significant relationships were between TO and pH ($r = 0.38$, $P = 0.03$, $n = 36$), and TO and calcium concentration ($r = -0.37$, $P = 0.03$, $n = 36$). Despite the fact that free citrate was not available, multiple stepwise regression analysis, with TO as the dependent variable, revealed that about 25% of the variation of TO in the ICU patients in this study could be attributed to the variables considered [$r^2 = 0.22$ for the model (adjusted for confounding variables), $P = 0.03$]. Among the variables found to exert significant influence were Mg ($r = -0.67$, $P = 0.015$) pH ($r = 0.52$, $P = 0.018$), molar ratio calcium/citrate ($r = -0.65$, $P = 0.048$) and phosphate ($r = 0.56$, $P = 0.048$); in contrast, the influence of the molar ratio Mg/citrate was only of borderline significance ($r = 0.59$, $P = 0.085$). In view of the high correlation of TO and the ratio free citrate/free calcium (Fig. 2), the influential variables studied in the two clinical trials may explain most of the variation of TO.

TO increases when the decrease of postprandial urinary pH is prevented, when a decline in the calcium/citrate ratio results due to increasing citrate and, surprisingly, when Mg decreases. This spectrum of changes is clearly more pronounced with the Mg-alkali citrate supplemented meal (Table 2). The mean lower urinary Mg concentration that is seen with the latter load must be related to higher urine volume, smaller amount of Mg supplied, enhanced renal tubular Mg reabsorption, or some combination of these. Stimulation of Mg reabsorption is a well-documented phenomenon when intratubular pH rises or remains neutral [52]. More important is the inverse relationship of Mg and TO, since

Table 2 General features of ICU patients, variables in urine and parameters of CaOx crystallization as influenced by the meal (Meal alone), additional Mg citrate (MgC) or Mg-alkali citrate (MgAC)

	Meal alone (n = 13)		Meal + MgC (n = 11)		Meal + MgAC (n = 12)	
General features						
Age; years	39.4	(2.7)	39.7	(3.4)	44.9	(5.1)
Body mass index; kg/(m)^2	26.1	(0.8)	26.0	(0.8)	26.1	(1.4)
Metabolic activity[a]; score	32	(7)	28	(6)	30	(7)
Urine						
Volume; ml	287	(25)	290	(40)	347	(55)
pH	5.89	(0.13)	5.62	(0.19)[b]	6.18	(0.24)
Δ pH	−0.48	(0.12)	−0.46	(0.12)	−0.05	(0.17)[a]
Oxalate; mM/l	0.09	(0.02)	0.10	(0.01)	0.09	(0.01)
Calcium; mM/l	3.92	(0.46)	5.62	(1.19)[b]	3.35	(0.59)
Magnesium; mM/l	3.0	(0.37)	3.63	(0.74)	2.61	(0.65)
Citrate; mM/l	1.71	(0.28)	1.88	(0.44)	2.12	(0.35)
Calcium/citrate; mM/mM	3.19	(0.83)	3.43	(0.60)	1.87	(0.33)[b]
Magnesium/citrate; mM/mM	2.33	(0.54)	2.19	(0.39)	1.35	(0.31)[b]
Phosphate; mM/l	7.8	(1.5)	12.2	(2.6)[b]	7.4	(1.5)
RSP[b]-CaOx	0.56	(0.05)	0.56	(0.08)	0.454	(0.09)
RSP-brushite***[c]	0.02	(0.14)	−0.10	(0.15)	−0.003	(0.17)
CaOx crystallization						
Tolerable oxalate; mM/l	0.46	(0.03)	0.45	(0.04)	0.58	(0.06)[a]
Crystal count-N; number/20 nl	14	(4)	13	(3)	26	(6)[b]
Crystal diameter-N; μm	3.61	(0.30)	3.31	(0.29)	2.67	(0.24)[a]
Crystal diameter-30; μm	7.48	(0.51)	6.89	(0.48)	6.19	(0.37)[a]
Agglomeration time; min	27	(2)	34	(3)[a]	30	(4)
Growth rate; μm/min	0.16	(0.03)	0.11	(0.01)[b]	0.14	(0.02)

$P \leq 0.05$; ***$0.05 < P < 0.10$; Δ: value in 3 h minus value in 2 h urine
Mean values (SE)
[a] Statistics based on $\log_{10}$ data
[b] Relative supersaturation product (for calculation see ref. 36)
[c] Calculated for pH 6.0
N at the stage of nucleation, *30* at 30 min post-nucleation

the majority of published articles dealing with interactions of Mg and CaOx crystallization in urine or urine-like fluids support crystallization inhibition by Mg (50, 5, 17, 10). Our opposite finding may be explained by assuming that the fewer Mg ions there are to compete for citrate, the more CaOx inhibition by citrate will take place, a hypothesis which needs proof. Also, Mg actions may be indirect, e.g. via some unknown metabolic step stimulated by increasing retention of ingested Mg. This possibility appears realistic in view of Mg deficit inside cells [44].

Another aspect relates to the fact that the depicted physicochemical CaOx and brushite supersaturation in postprandial undiluted urine are not identical to supersaturation values prevailing in the lumen of renal tubules [49]. For example, if one assumes that the intratubular pH is higher than in urine (as a result of the higher degree of dilution), there is clearly a risk for precipitation of some calcium phosphate phase [49]; the risk should be greatest in the ascending portion of Henle's loop where the alkalinizing effect of the Mg preparation (Meal + MgAC) may become most pronounced. Such possibilities, and a subsequent risk for heterogeneous CaOx precipitation cannot be ruled out. However, along the same line of thinking would be that during increasing acidification of tubular fluid downstream of the nephron the bulk of potentially precipitated calcium phosphate may be dissolved (for phosphate inside CaOx crystals see below). Also, in both ICU patients and non-stone-forming healthy controls Mg-alkali citrate delays the precipitation of amorphous calcium phosphate via increasing the tolerated calcium concentration (P. O. Schwille; unpublished data). Finally, in work done in vitro, Mg was found able to potentiate the citrate-induced prolongation of CaOx crystal agglomeration time [30]. Therefore, in our work the prolonged agglomeration time together with reduced crystal growth (Meal + MgAC; Table 2) may indicate that treatment of ICU by the combination of Mg, citrate, and alkali attacks the three key processes thought to lead to stones: CaOx solid formation (in present work expressed as TO), crystal growth and crystal agglomeration.

Crystal morphology and crystal composition

SEM of crystals revealed that in urine of untreated ICU patients the frequency distribution of CaOx dihydrate and that of CaOx monohydrate are roughly equal. The two Mg-containing preparations appear to shift the crystal type distribution toward the dihydrate form (Fig. 3A–C). *EDX* of crystals revealed peaks for calcium and tiny amounts of potassium, but not Mg or phosphorus (data not shown). The crystal number was smaller with Mg citrate and larger with Mg-alkali citrate; however, the crystal size appeared smaller with the latter, confirming the data obtained by direct measurement of crystal diameter (Table 2).

In contrast to EDX, which failed to detect Mg and phosphorus, wet-chemical analysis of the unfiltered crystallized mass from the untreated urine (meal alone, Table 2) revealed the presence of 7.5 mM phosphate and 1.2 mM citrate; the concentration of both of these substances was low with the Mg citrate load, but phosphate concentration was high with the Mg-alkali citrate load; also oxalate concentration declined (in the order meal

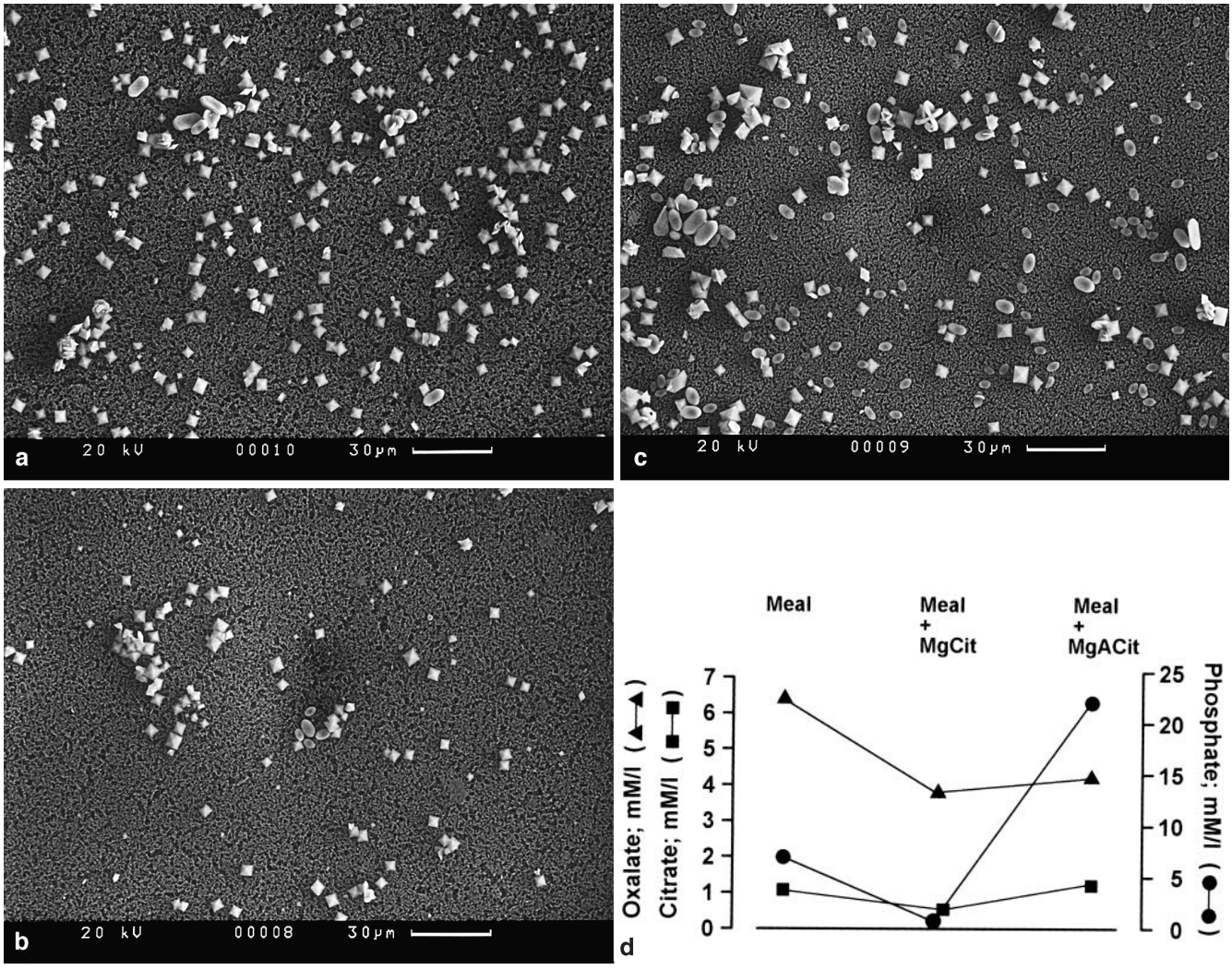

Fig. 3 Crystal morphology (SEM) and substance concentration in dissolved crystals of three idiopathic calcium urolithiasis (ICU) patients. For details see Materials and methods and Results. **a**–**c** CaOx dihydrate and CaOx monohydrate as found after ingestion of meal alone **a**, additional Mg citrate **b**, or Mg-alkali citrate **c**. **d** oxalate, citrate and phosphate. *MgCit* Mg citrate, *MgACit* Mg-alkali citrate

alone > meal + Mg citrate = meal + Mg-alkali citrate; Fig. 3D); in contrast, concentrations of calcium and Mg were 11.6, 6.7, 14 and 3.9, 0.7, 6.9 mM, respectively (in the order meal alone, meal + Mg-citrate, meal + Mg-alkali citrate), and the molar ratio calcium/oxalate was 1.8, 1.8, 3.3 (same order of loads). These figures, although not generalizable to ICU as a whole, signal a considerable excess of calcium over oxalate in the CaOx crystals formed in postprandial urine produced following ingestion of a calcium-rich and oxalate-free meal; previously, part of the excess calcium was found associated with citrate, stoichiometry ≤1:1 [21]. Also, despite the concomitant high supply of Mg by the Mg citrate preparation, the uptake of Mg by crystals lags behind that of calcium. In contrast, despite lower Mg supplied in the form of the Mg-alkali citrate preparation the Mg uptake by crystals was high. Apparently, the formation and final composition of CaOx crystals in the rather complex urinary environment depends not only on a certain degree of CaOx physico chemical supersaturation of urine, but also on such factors as the ionic composition and net charge of the crystal hydration shell [12]. The latter is tightly attached to the solid formed [12], and its components are not detectable by SEM or EDX of crystals isolated by filtration; however, according to presented data modulation of the hydration shell by Mg-containing preparations with different alkalinizing properties may occur, and may be readily detected when freshly formed crystals are dissolved and analysed.

Conclusion

The data presented herein are informative in a number of respects. The additive effects on TO concentration seen with Mg, citrate and combinations in artificial urine may serve as a basis for interpreting the effects of Mg citrate and Mg-alkali citrate seen in clinical trials.

Although direct inhibitory effects of Mg, citrate and alkali on CaOx crystallization have repeatedly been reported [5, 17, 40, 24, 32], only one report has focused on the isolated effect of Mg [24]; however, the healthy volunteers in that study had previously received sodium cellulose phosphate – a regimen known to produce hyperoxaluria [39] – which theoretically could have increased the microscopic crystal count the authors ascribed to the low urinary Mg associated with the use of that drug [24]. Similarly, in experiments using increasing amounts of Mg in the form of sulfate in artificial urine, inhibition of CaOx nucleation by Mg was deduced on the basis of preferential formation of the more soluble Mg oxalate; however the study did not control for calcium sulfate complexation [32].

On the basis of the enormous work done in the past on the pathophysiology of ICU, several lists of urinary risk factors have been established and incorporated into various medical textbooks; these variable lists have contained excess of calcium, oxalate, urate, and deficit of citrate, Mg (or Mg relative to calcium), pyrophosphate and large-molecular (>10 kDa) crystallization inhibitors, but information on the possible importance of low urinary pH for CaOx crystallization steps is sparse [48, 6]. One reason why the majority of investigators did not give sufficient consideration to pH in this context may be the fact that CaOx supersaturation varies largely independently of pH (Table 1), while at the same time low pH may exert actions of its own, which are of relevance for CaOx crystallization. Other work at our laboratory suggests that CaOx supersaturation and the underlying oxaluria seem to be overestimated as stone risk factors, while urinary pH, a factor largely determining TO, appears to be of at least equal importance (P. O. Schwille, manuscript in preparation). Intratubular citrate protonation depends on ambient pH, since it has been long known that low luminal fluid pH drives the reabsorption of protonated citrate; hence free citrate and TO are necessarily low. From the stoichiometry of substances in urine it is unlikely that the deficit of free citrate alone causes the excess of free calcium. This suggests the presence of a calcium ligand gap, which may constitute a crystallization risk factor per se. On the basis of the chemically analyzed crystals (Fig. 3), the less negatively charged phosphate species in particular may be a candidate ligand, although there are probably also others. Further more detailed studies in this area are necessary and should involve careful matching of participants for CaOx supersaturation, sex, age, obesity and metabolic activity of ICU.

Although in artificial urine Mg was able to increase TO to some degree in the presence of practically unchanged supersaturation (Fig. 1), the absence of a deficit of Mg ions in undiluted urine (Table 2) makes it difficult to assess reliably the true role of Mg in the formation of crystals presenting optically as CaOx. Previous workers reported similar conclusions [53, 9]. Since no Mg peak is observed by EDX in crystals harvested from urine of Mg-treated individuals, the Mg content of the former must be less than the 2% detection limit of the method. However, the finding cannot rule out that smaller amounts of Mg would be detectable by more sensitive methods, and that these Mg amounts exert direct inhibition of CaOx crystallization, i.e. act as crystal growth site poison [17]. Alternatively, Mg ions, apart from their presence in the hydration shell, may enter the lattice of nascent CaOx crystals thereby disturbing crystallization kinetics; a similar situation is known for Mg in calcium phosphate crystallization (for details see ref. 37). Mg-alkali citrate has been reported to reduce the recurrence rate of stones in ICU over the long term [19], but unfortunately crystallization was not studied in that work. From the present work on the response of ICU patients to acute Mg challenge it appears likely that Mg-alkali citrate evokes anti-CaOx crystallization effects via the combined direct or indirect actions of Mg, citrate, and stabilization of urinary pH. It remains to be shown whether as yet unidentified Mg-, calcium- and citrate-containing complexes contribute.

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