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EFFECTS OF ACUTE ACID LOADING ON THE RISK OF CALCIUM PHOSPHATE AND CALCIUM OXALATE CRYSTALLIZATION IN URINE

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Abstract

The aim of this study was to examine the risk of calcium phosphate and calcium oxalate crystallization during acute acid loading under controlled conditions.

The effects of acute acid loading on rates of renal excretion of calcium, magnesium, phosphate, citrate, oxalate and urine pH were studied in healthy subjects. The risk of calcium phosphate and calcium oxalate crystallization were evaluated by estimates of the ion activity products of calcium phosphate [AP(CaP)-index] and calcium oxalate [AP(CaOx)-index] according to Tiselius. In addition, the risk of brushite [AP(Bru)-index] crystallization was estimated.

An acute acid load administered as ammonium chloride (NH_4Cl) produced increased urinary excretion of calcium, phosphate and oxalate, decreased urinary excretion of citrate, and a decrease in urine pH. Consequently, calcium-citrate-ratio in urine increased markedly in response to acid loading. AP(CaP)-index decreased markedly due to a fall in urine pH. AP(Bru)-index decreased slightly and remained low throughout the study. AP(CaOx)-index increased significantly, and acid loading is suggested as a risk factor for calcium oxalate stone formation.

Key Words: Acid-base, urine, calcium phosphate, calcium oxalate, kidney calculi, citrate, oxalate.

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Introduction

Considerable evidence suggests that dietary protein is an essential factor in the pathophysiology of calcium stone formation. The exact mechanism whereby dietary protein leads to renal stone formation remains, however, to be elucidated.

It has been shown by several investigators that ingestion of a protein load results in an increase in urinary calcium excretion (Adams *et al.*, 1979; Robertson *et al.*, 1979a, b; Breslau *et al.*, 1988) and a decrease in urinary citrate excretion (Breslau *et al.*, 1988, Goldfarb, 1988), which theoretically would be expected to enhance the crystallization of calcium phosphate and calcium oxalate (Nicar *et al.*, 1987; Berg and Tiselius, 1989; Tiselius *et al.*, 1993). Concerning the role of dietary protein on urinary oxalate excretion the data in the literature are conflicting. Some studies have shown that a rise in the consumption of animal protein markedly increases renal oxalate excretion (Robertson *et al.*, 1979a, b; Brockis *et al.*, 1982; Fellström *et al.*, 1983; Urivetsky *et al.*, 1987; Holmes *et al.*, 1993). Other investigators have been unable to confirm these findings (Breslau *et al.*, 1988, Marangella *et al.*, 1989, Trinchieri *et al.*, 1991).

A protein load results in an increased endogenous production of non-metabolizable acid (NA) to be excreted by the kidneys (Lemann *et al.*, 1961; Lennon *et al.*, 1962; Kildeberg, 1981). It has been shown that there is a significant positive correlation between increased renal acid excretion and hypercalciuria (Lemann *et al.*, 1966). Furthermore, it is well-known that renal citrate excretion falls during non-carbonic acidosis due to increased proximal tubular citrate reabsorption (Simpson, 1964; Adler *et al.*, 1971). The increased stone risk of protein consumption, therefore, may be a consequence of increased endogenous acid production. On the other hand, a lower urinary pH during acid loading reduces the ionization of phosphoric acid and may thereby counteract calcium phosphate stone formation (Breslau *et al.*, 1988). Furthermore, neither the effect of an acid load on urinary oxalate excretion has been examined, nor has the effect of acid loading on the risk of calcium phosphate and calcium oxalate crystallization been evaluated.

The aim of this study was to examine the risk of calcium phosphate and calcium oxalate crystallization during acute acid loading under controlled conditions.

Material and Methods

Symbols

Quantities are presented as complete symbols, for example: cMg(U) = concentration (c) of magnesium (Mg) in urine (U) (mmol/l); nMg(U) = amount of (n) magnesium in urine (mmol), $\dot{n}$Mg(U) = rate of ($\dot{n}$) magnesium excretion in urine (mmol/hour), $\dot{V}$ = flow (volume rate, l/hour) and pH(aB) = pH in arterialized blood (aB), according to recommended chemical and physiological usage (McGlashan and Paul, 1975).

Experimental subjects

Ten healthy men with a median age of 33 years (range 23-47 years) were included in the study. Body weight ranged from 65-115 kg (median 84 kg), and body mass index (BMI) from 22-32 (median 26) kg/m^2. Each subject was examined clinically, including physical examination and laboratory screening. All had sterile urine, and none of the subjects was taking any medication. All had normal endogenous creatinine clearance (median 131 ml/min, range 71-153 ml/min).

Study protocol

An acute acid loading study using oral ammonium chloride (1.9 mmol/kg body mass) as crushed tablets in order to ensure rapid and uniform absorption (Backman *et al.*, 1976) were performed from 6 a.m. to 1 p.m. The participants voided at 6 a.m., and urine and samples of blood were collected at 8 a.m. (*control period*). The ammonium chloride tablets were ingested between 8.00 and 8.45 a.m. To ensure adequate diuresis 150 ml of demineralized water was given every hour during the study. Urine, and venous and arterialized capillary blood samples were collected hourly. Measurements included creatinine (*serum*); standard bicarbonate and pH (*arterialized capillary blood samples*); calcium, phosphate, citrate, magnesium, oxalate, creatinine, pH and volume (*urine*).

The acid loading study was preceded by an eight-hour fasting period, and for 24 hours prior to the fasting period the participants ate a fixed diet (calcium 7.5 mmol, phosphorous 25 mmol, sodium 81 mmol, potassium 86 mmol, and total protein 50 g, oxalate 200 mg[1]). Protein constituted 11%, fat 40% and carbohydrate 48% of the total energy intake.

Analytical procedures

Concentrations of calcium and magnesium in urine were measured by atomic absorption spectrophotometry. Concentrations of citrate in urine was measured by the citrate lyase method (Boehringer). The intra-assay variation was 1.8%. All measurements were performed using only one analytical kit. Concentrations of oxalate in urine was measured by an enzymatic colorimetric method after removal of ascorbate with sodium nitrite (Kasidas and Rose, 1987). The intra-assay variation was 4.4 %. All measurements were performed with only one analytical kit. Plasma standard bicarbonate and pH were determined immediately after sampling using ABL 520 (Radiometer; Siggaard-Andersen, 1974). Urine pH was measured anaerobically at 37°C immediately after sampling using a pH meter (Radiometer PHM 93). Concentrations of phosphate and creatinine were determined by autoanalyzer methodology (Technicon RA 1000). All measurements were performed in duplicate, and estimates of analytical accuracy were obtained by analysis of an aqueous standard solution with each batch of samples showing a coefficient of variation of less than 2.4% unless stated otherwise.

Estimates of the risk of crystallization in urine

The risk of calcium phosphate and calcium oxalate crystallization in urine were estimated by means of the AP(CaP)-index and the AP(CaOx)-index (Tiselius, 1984, 1985, 1986, 1991):

$$\text{AP(CaP)-index} = 6.1 \times 10^{-3} \times \dot{n}\text{Ca}^{1.07} \times \dot{n}\text{tP}^{0.7} \times (\text{pH} - 4.5)^{6.8} \times \dot{n}\text{Ci}^{-0.2} \times \dot{V}^{-1.31}$$

$$\text{AP(CaOx)-index} = 8.8 \times \dot{n}\text{Ca}^{0.84} \times \dot{n}\text{Ox} \times \dot{n}\text{Mg}^{-0.12} \times \dot{n}\text{Ci}^{-0.22} \times \dot{V}^{1.03}$$

where rates of renal excretion of calcium ($\dot{n}$Ca), total phosphate ($\dot{n}$P), citrate ($\dot{n}$Ci), magnesium ($\dot{n}$Mg) and oxalate ($\dot{n}$Ox) are expressed in mmol per hour, and urine flow ($\dot{V}$) in liters per hour.

It has been shown by Tiselius (1984, 1991) that the AP(CaP)-index and the AP(CaOx)-index correspond to the ion-activity products of calcium phosphate (AP_{CaP}) and calcium oxalate (AP_{CaOx}) (Werness *et al.*, 1985), respectively, as follows:

$$AP_{CaP} \approx \text{AP(CaP)-index} \times 10^{-13}$$

$$AP_{CaOx} \approx \text{AP(CaOx)-index} \times 10^{-8}$$

AP(CaP)-index and AP(CaOx)-index, therefore, represent estimates of the state of supersaturation with respect to calcium phosphate and calcium oxalate, respectively. From the AP(CaP)-index the ion-activity products of octacalcium phosphate (AP_{OCP}) and hydroxyapatite (AP_{HAP}) can be estimated (Tiselius, 1984):

$$-\log AP_{OCP} = (1/0.021 \times (\text{AP(CaP)-index})^{0.025}) + 0.5$$

$$-\log AP_{HAP} = 1/\ 0.0185 \times (\text{AP(CaP)-index})^{0.035}$$

The risk of brushite crystallization in one-hour urine samples was also estimated using the AP(Bru)-index (Tiselius, 1984):

$$\text{AP(Bru)-index} = 4.7 \times 10^{-7} \times \dot{n}\text{Ca}^{1.07} \times \dot{n}\text{tP}^{0.82} \times \text{pH}^{6.8} \times \dot{n}\text{Ci}^{-0.46} \times \dot{V}^{-1.53},$$

[1]The dietary content of oxalate was estimated from Kasidas (1980).

Table 1. Effects of acute NH_4Cl loading on blood and urine acid-base status. Values are means (standard error of mean, SEM).

	Hours after NH_4Cl loading					
	0	1	2	3	4	5
pH(aB)	7.40 (0.004)[a]	7.35 (0.007)	7.33 (0.006)	7.34 (0.007)	7.35 (0.008)	7.37 (0.007)
$cHCO_3(aB_s)$ mmol/l	25.0 (0.5)[a]	21.1 (0.6)	20.1 (0.4)	20.8 (0.4)	21.0 (0.4)	21.3 (0.4)
pH(U)	5.7 (0.1)[b]	5.6 (0.1)	5.2 (0.1)	5.0 (0.1)	4.9 (0.05)	4.8 (0.04)

[a]Values at the end of control period.
[b]pH(U) was measured in one freshly voided urine sample during the control period.

Table 2. Effects of acute NH_4Cl loading on urine composition. Values are means (SEM).

	Hours after NH_4Cl loading					
	0[a]	1	2	3	4	5
$\dot{n}$Ca(U) mmol/hour	0.2 (0.06)	0.2 (0.03)	0.5 (0.05)	0.5 (0.04)	0.4 (0.04)	0.3 (0.04)
$\dot{n}$Mg(U) mmol/hour	0.2 (0.0.07)	0.2 (0.04)	0.5 (0.05)	0.4 (0.05)	0.3 (0.05)	0.3 (0.04)
$\dot{n}$tP(U) mmol/hour	0.4 (0.1)	0.7 (0.1)	0.8 (0.1)	0.7 (0.1)	0.9 (0.2)	1.1 (0.2)
$\dot{n}$Ci(U) mmol/hour	0.1 (0.03)	0.06 (0.01)	0.04 (0.01)	0.04 (0.01)	0.05 (0.01)	0.05 (0.01)
$\dot{n}$Ox(U) μmol/hour	2.5 (1.0)	1.6 (0.3)	7.7 (1.6)	11.4 (2.1)	15.8 (2.3)	12.0 (2.7)

[a]values for control period.

Table 3. Crystallization risk: Effects of acute NH_4Cl loading on risk of calcium phosphate [AP(CaP)-index], octacalcium phosphate (AP_{OCP}), hydroxyapatite (AP_{HAP}), brushite [AP(Bru)-index] and calcium oxalate [AP(CaOx)-index] crystallization. The risk indices have been calculated according to Tiselius (1984, 1991). Values are means (SEM).

	Hours after NH_4Cl loading					
	0[a]	1	2	3	4	5
AP(CaP)-index	0.8 (0.4)	0.08 (0.04)	0.06 (0.003)	0.004 (0.002)	0.0002 (0.0001)	0.0001 (0)
AP(Bru)-index	1.9 (0.5)	1.48 (0.4)	2.0 (0.4)	1.6 (0.3)	1.0 (0.2)	1.0 (0.2)
-log AP_{OCP}	52 (2)	53 (1)	55 (1)	58 (1)	60 (2)	61 (2)
-log AP_{HAP}	61 (2)	63 (2)	66 (2)	72 (3)	76 (3)	79 (3)
AP(CaOx)-index	0.2 (0.1)	0.1 (0.02)	0.9 (0.2)	1.4 (0.3)	1.7 (0.3)	1.0 (0.2)

[a]values for control period

where rates of renal excretion of $\dot{n}$Ca, $\dot{n}$tP and $\dot{n}$Ci were expressed in mmol/hour, and $\dot{V}$ in l/hour.

Statistical analysis

Measurements are given as mean values ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance and F-test. $P < 0.05$ was considered significant.

The study was approved by the local scientific ethics committee of the counties of Vejle and Funen, and informed consent was obtained from each individual.

Results

Urine flow

Mean urine flow per hour ($\dot{V}$) was 0.10 ± 0.02 liters in the control period, and 0.13 ± 0.02 liters during NH_4Cl-loading ($p < 0.05$).

Systemic and urine acid base status

Plasma standard bicarbonate [$cHCO_3^-(aB_s)$] and blood pH [pH(aB)] decreased in all subjects in response

to acid loading compared to the control period ($p < 0.0001$; Table 1). Urine pH [pH(U)] values decreased significantly from the values observed during the control period ($p < 0.01$; Table 1).

Urine composition

Renal excretion of calcium [$\dot{n}$Ca(U)] increased during NH_4Cl-loading compared to the control period ($p < 0.01$; Table 2). Renal excretion of magnesium [$\dot{n}$Mg(U)] rose slightly but insignificantly ($p > 0.1$; Table 2). Renal excretion of total phosphate [$\dot{n}$tP(U)] rose significantly in response to acute acid loading ($p < 0.01$), as did renal excretion of oxalate [$\dot{n}$Ox(U)] ($p < 0.0001$; Table 2). Citrate excretion [$\dot{n}$Ci(U)] decreased to very low values during NH_4Cl-loading compared to the control period ($p < 0.0005$; Table 2). The molar calcium/citrate ratio (Ca/Ci-ratio), therefore, rose significantly during acid loading ($p < 0.0001$).

Risk of calcium phosphate crystallization

AP(CaP)-index decreased markedly during NH_4Cl-loading compared to the control period ($p < 0.01$; Table 3). The decrease was largely determined by the fall in pH(U). In consequence of the decrease in AP(CaP)-index, AP_{OCP} and AP_{HAP} both decreased markedly during acid loading ($p < 0.01$), whereas the AP(Bru)-index did not change significantly (Table 3). All index-values were, however, far below the corresponding formation products (Tiselius 1984, 1986).

Risk of calcium oxalate crystallization

AP(CaOx)-index increased significantly during NH_4Cl-loading compared to the control period ($p < 0.0001$; Table 3).

Discussion

Renal calcium excretion increased and renal citrate excretion decreased in response to acute acid loading in each subject. These findings are in accordance with previous observations, and the mechanisms of hypercalciuria and hypocitraturia in response to acid loading have been discussed previously (Lemann *et al.*, 1967, 1979; Lennon and Piering, 1970; Coe *et al.*, 1975; Adams *et al.*, 1979; Simpson, 1964). Consequently the Ca/Ci-ratio in urine rose significantly, which theoretically would be expected to enhance the crystallization of both calcium phosphate and calcium oxalate (Tiselius *et al.*, 1993). The increase in urinary phosphate excretion during acid loading, probably a result of mobilization of phosphorus from bone and intracellular stores (Coe *et al.*, 1975), would also contribute to an increased risk of calcium phosphate crystallization. However, due to a fall in pH(U) during acid loading, the AP(CaP)-index decreased to very low values, indicating a decreased risk of calcium phosphate crystallization. As expected, the risk-indices of octacalcium phosphate and hydroxyapatite crystallization also decreased markedly in response to acid loading, since these are the calcium phosphate crystals most frequently encountered in alkaline urine (Tiselius, 1984). The calcium phosphate crystals most frequently found in acid urine is brushite (Pak *et al.*, 1971; Tiselius, 1984). AP(Bru)-index did not increase in response to acid loading, however, and the risk of brushite crystallization remained at low levels throughout the study.

It was found in the present study that an acute acid load resulted in an increase in renal excretion of oxalate. An increase in renal oxalate excretion may originate from: (**1**) increased dietary intake; (**2**) increased metabolic production; (**3**) increased intestinal absorption; and (**4**) a renal "leak" (Schwille *et al.*, 1989; Borsatti, 1991). Since the subjects in the present study fasted 8 hours prior to acid loading and were fasting throughout the observation period, it is unlikely that the hyperoxaluric response to acid loading was caused by increased intestinal oxalate absorption. It also seems unlikely that acute acidosis results in increased endogenous production of oxalate due to increased metabolization of amino acids. Plasma oxalate values were not available in this study, however, and the latter possibility cannot be excluded. Increased oxaluria in response to acid loading, however, is probably caused by a renal oxalate "leak". Oxalate is freely filtered by the glomeruli (Schwille *et al.*, 1989; Borsatti, 1991). Micropuncture studies have shown high oxalate concentrations in the proximal convoluted tubule as compared with inulin, indicating that tubular secretion plays a major role in oxalate secretion in man (Borsatti, 1991; Williams and Wandzilak, 1989). If intracellular acidosis in response to acid loading was induced in the proximal renal tubular cells as suggested by the dramatic fall in urine citrate excretion, a decreased intracellular concentration of hydroxyl (and bicarbonate) ions would have been the result, favoring oxalate secretion in exchange for chloride by the chloride/hydroxyl transporter in the brush-border membrane (Steinmetz, 1986; Borsatti, 1991). However, it has previously been shown that there exists a strong positive correlation between urine flow and oxalate excretion and a strong negative correlation between urine flow and oxalate concentration (Klän and Butz, 1987). The augmented oxaluric response in the present study, thus, may partially be explained by the increased diuresis during acid loading as compared with the control period. This cannot be the sole explanation, however, since urine oxalate concentration also increased in response to acid loading from $66 \pm 16 \mu$mol/l in the control period to a maximum at $95 \pm 11 \mu$mol/l during acid loading. Furthermore, the urine flow was included in the estimated risk-index [AP(CaOx)-index], and it seems likely that acid loading represents a true risk factor for calcium oxalate crystallization due to increased renal excretion of calcium and oxalate and decreased excretion of citrate. It is possible, therefore, that enhanced calcium oxalate stone formation due to dietary protein intake, at least in part, may be a consequence of increased endogenous acid production. It should, however, be emphasized that the risk-indices used in this study are gross estimates of the state of supersaturation, and that the use of more accurate methods may provide different results

(Tiselius 1984, 1991).

The conflicting data in the literature on the effect of dietary protein on renal oxalate excretion are difficult to explain (Robertson *et al.*, 1979a, b; Brockis *et al.*, 1982; Fellström *et al.*, 1983; Urivetsky *et al.*, 1987; Breslau *et al.*, 1988; Marangella *et al.*, 1989; Trinchieri *et al.*, 1991; Holmes *et al.*, 1993). However, if acid loading influences renal oxalate excretion as indicated by the present study, the differences could be explained by differences in acid (base) content of the different diets. It should be emphasized, however, that renal stone patients may react differently to an acid load than healthy men, and that the acid load achieved by NH_4Cl-loading probably is not identical to the acid load represented by the increased endogenous acid production resulting from protein catabolism. Also, 1.9 mmol NH_4Cl per kg body mass is a rather large acid dose compared to physiological conditions.

Other factors than supersaturation are involved in calcium stone formation, and these factors may vary differently in response to acid loading, and thus modulate the stone promoting effect. It has been shown that a high *c*Ca(U) as well as pH(U) < 7 promotes self-aggregation of Tamm-Horsefall glycoprotein (THP) (Hess *et al.*, 1991). The effects of acid loading on urine composition [increased *c*CA(U), decreased *c*Ci(U) and low pH(U)], therefore, probably would increase the risk of THP-self-aggregation, which would reduce inhibition of calcium oxalate monohydrate crystal aggregation, and increase the risk of stone formation (Hess *et al.*, 1991). This further emphasizes that acid loading probably plays a role in the pathophysiology of calcium oxalate stone formation.

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Discussion with Reviewers

B. Hess: Do calcium stone formers behave exactly as healthy men?

Authors: Possible not. We have shown that the calciuric response to acid loading was significantly greater in idiopathic calcium stone formers than in healthy controls (Osther *et al.*, 1993). Whether this applies to other stone forming salts, we do not know.

B. Hess: How can we be sure that acid loading by ammonium chloride loading, which is being metabolized to HCl (an inorganic acid), has the same effects as the acid loads which people usually undergo, namely excess organic acids, i.e., animal protein?

Authors: Surely, the *kind of acid load* caused by NH_4Cl loading differs from that resulting from catabolism of ingested protein: (**1**) As far as loading with *native* protein is concerned, the consequence of protein loading will include release of free amino acids, subsequent deamination with urea formation and appearance of organic acids to be converted to CO_2 and water, the transient effect on the acid-base status of extracellular fluid depending on the ratio between "acid" and "basic" proteins. (**2**) Also, any *losses of salts* of organic acids with the urine represent an equimolar loss of non-metabolizable base (NaOH) from the body. (**3**) The *state of ionization* of ingested proteins (determined by "titration" with other food constituents) will influence the ratio between amounts of metabolizable and non-metabolizable acid liberated. (**4**) Dietary loads of protein containing *neutral* (unoxidized) *sulfur* will result in endogenous production of (non-metabolizable) sulfuric acid. Surprisingly, however, methionine-loading in the young rat does not lead to "sulfuric acidosis" but rather to accumulation of metabolizable acids in blood (Wamberg *et al.*, 1987). Any difference between NH_4Cl and protein loading with respect to stone formation remains to be elucidated. We have implied a mechanism involving the extracellular pH.

W.G. Robertson: Have you measured the supersaturation of urine with respect to uric acid since that should have increased markedly because of the acidification of urine? This would also be relevant to any assessment of the risk of stones.

Authors: We have not so far measured supersaturation of urine with respect to uric acid.

W.G. Robertson: If acidification is the cause of the mild hyperoxaluria observed in this study, why did urinary oxalate not increase in all of previously reported animal protein loading studies?

Authors: One explanation to the conflicting data in the literature regarding the effect of dietary protein on renal oxalate excretion may be variations of the protein composition with respect to amino acids from which oxalate may be derived (phenylalanine, tyrosine and tryptophan). Also, it has been shown that the apparent endogenous acid production in normal subjects on self-selected diets may differ by nearly ten-fold, with a range of approximately 20 to 120 mmol/day (Kurtz *et al.*, 1983). If an increased load of non-carbonic acid influences renal oxalate excretion as suggested by our study, differences in the rate of endogenous acid production resulting from different diets might explain differences in renal oxalate excretion.

H.-G. Tiselius: The urine samples in this study are obviously collected in bottles without any preservative. There might, however, be a risk of forming crystals of calcium oxalate in the samples. Did you take any precautions to avoid analytical errors from such a mechanism?
Authors: We agree with your concern. Throughout the study urine was collected in bottles without preservative. During ammonium chloride loading, the urine was actually acidified, pH(U) falling to below 5.0, which means that more oxalate may have been available for measurement.

H-G. Tiselius: The data in Table 2 show that whereas the excretion of Ca, Mg, P and Ci extrapolated to 24 hours period is normal, that of Ox is low (2.5 x 24 = 0.06 μmol). After 4 hours, the corresponding excretion would be 0.38 μmol. How do you explain the low oxalate excretion during the 2-hour control period and what is your experience of the effects of a fasting period on urinary oxalate?

Authors: Apart from the present study, we have no experience with oxalate excretion after fasting in heathy subjects. The oxalate excretion during the 2-hour control period is rather low, possibly due to crystal formation (see above).

P.O. Schwille: The diet antecedent to acid loading was poor in calcium (300 mg/day) but somewhat rich in carbohydrates (almost 50%), why?
Authors: This diet was chosen because it was the only fixed diet available in our laboratory at that time. We do not believe that the calcium content of the diet influenced oxalate absorption significantly in this study, since the subjects fasted 8 hours prior to the acid loads.

P.O. Schwille: What is the relationship between calculated risk and observed crystallization, and was the latter evaluated by appropriate means (particle analysis, microscopy etc.)?
Authors: We did not examine the urine samples for crystalluria. The used risk-indices have been shown to give rather accurate information on supersaturation of the different calcium salts. Thus, a rise in AP(CaOx)-index would be expected to increase CaOx-crystalluria.

Additional References

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