IDIOPATHIC RECURRENT CALCIUM UROLITHIASIS (IRCU): VARIATION OF FASTING URINARY PROTEIN IS A WINDOW TO PATHOPHYSIOLOGY OR SIMPLE CONSEQUENCE OF RENAL STONES IN SITU?

A TRIPARTITE STUDY IN MALE PATIENTS PROVIDING INSIGHT INTO OXIDATIVE METABOLISM AS POSSIBLE DRIVING FORCE TOWARDS ALTERATION OF URINE COMPOSITION, CALCIUM SALT CRYSTALLIZATION AND STONE FORMATION*

P. O. Schwille, A. Schmiedl, J. Wipplinger
Mineral Metabolism and Endocrine Research Laboratory, Departments of Surgery and Urology, University of Erlangen-Nurnberg, Germany

Abstract

Background: In IRCU it is uncertain whether variation of urinary protein, especially non-albumin protein (N-Alb-P), is due to the presence of stones or reflects alteration of oxidative metabolism.

Aims: To validate in a tripartite cross-sectional study of 187 ambulatory male patients, undergoing a standardized laboratory programme, whether stones impact on N-Alb-P or the state of oxidative metabolism interferes with IRCU pathophysiology.

Methods: In part 1 the strata low and high of fasting urinary excretion rate per 2 h of N-Alb-P, malondialdehyde, hypoxanthine, xanthine, pH and other urine components were compared, and association with renal stones in situ evaluated; in part 2 the co-variation of oxidatively modulated environment, fasting urinary pH, calcium (Ca) salt crystallization risk and the number of patients with stones in situ was examined; in part 3, the nucleation of Ca oxalate and Ca phosphate was tested in undiluted postprandial urine of patients and related to the state of oxidative metabolism.

Results: In part 1, N-Alb-P excretion >4.3 mg was associated with increase of blood pressure, excretion of total protein, hypoxanthine (a marker of tissue hypoxia), malondialdehyde (a marker of lipid peroxidation), sodium, magnesium, citrate, uric acid, volume, pH, and increase of renal fractional excretion of both N-Alb-P and uric acid; when stones were present, urinary pH was elevated but other parameters were unaffected. Significant predictors of N-Alb-P excretion were malondialdehyde, fractional N-Alb-P and hypoxanthine. In part 2, urine pH >6.14 was associated with unchanged blood pressure and plasma vasopressin, increase of blood pH, urinary volume, malondialdehyde, fractional excretion of N-Alb-P, uric acid, Ca phosphate, but not Ca oxalate, supersaturation; this spectrum was accompanied by decrease of concentration of urinary total and free magnesium, total and complexed citrate, plasma uric acid (in humans the major circulating antioxidant) and insulin; the number of stone-bearing patients was increased. Significant predictors of urine pH were body mass index, plasma insulin and uric acid (negative), and urinary xanthine (positive). In part 3 low plasma uric acid, not high urinary malondialdehyde or high ratio malondialdehyde/uric acid was significantly associated with diminished Ca but not oxalate tolerance, with the first nucleating crystal type being mostly Ca phosphate (hydroxyapatite), in the rest Ca oxalate dihydrate; uricemia correlated marginally positively (p=0.055) with Ca tolerance of urine, stronger with blood pressure and insulin, and negatively with urinary xanthine, fractional N-Alb-P, volume, sodium.

Conclusions: In IRCU 1) not renal stones in situ, but disturbed oxidative metabolism apparently modulates nephron functionality, ending up in higher renal N-Alb-P release, urinary volume, sodium and pH of fasting urine; 2) etiologically unknown decline of uricemia may represent antioxidant deficiency and cause a risk of hydroxyapatite crystallization and stone formation in a weakly acidic or alkaline inhibitor-deficient and N-Alb-P-rich milieu; 3) several observations, linking oxidative and systemic metabolism, are compatible with Ca stone initiation beyond tubules.

Key words: Calcium urolithiasis – Stones in situ – Uinary protein – Oxidative and systemic metabolism –Uriney pH – Calcium salt crystallization

INTRODUCTION

In the complex pathophysiology of IRCU (for full definition see ref. [1]) the cause of variation of urinary protein, especially the non-albumin protein (N-Alb-P) fraction, and its possible contribution to calcium (Ca)
salt crystallization and Ca stone development are incompletely understood. The literature in this area, including pioneering work [2, 3], lacks information on pre-existence and nature of tissue damage that could underlie the variation of N-Alb-P and other urine components, with the development of Ca salt crystallization risk and stones being secondary events. The picture appears even more complex, as in numerous patients the renal capacity to acidify urine was found defective and accompanied by higher proteinuria [1], and as renal stones in situ can act as a foreign body leading to abrasion of tubule-lining epithelium, shedding of protein and other substances, able to modulate urinary pH [4]. On the other hand, there is now growing evidence that alteration of oxidative metabolism is widespread among various forms of nephrolithiasis, including IRCU [5-9]. More specifically, ascorbic acid and alpha-tocopherol, both documented antioxidants, were lowered in red blood cells of IRCU [6, 9], and there was decrease of plasma total antioxidative substances (TAS) that was mainly due to low plasma uric acid and albumin (Alb) concentration, in human blood the two major reactive oxygen species (ROS) scavenging antioxidants [see ref. 5]; in addition, urinary and cellular levels of malondialdehyde (MDA), a marker of ROS excess, were found increased [6, 7]. ROS excess in general manifests as peroxidation of lipoproteins [6, 10], in IRCU as damage of renal cell membranes [6] and nuclear deoxyribonucleic acid (DNA) [11, 12], and increase of proteinuria [13]. However, although the latter would help understand why in addition to incorporation of Ca oxalate (CaOx) and Ca phosphate (CaPi) the one of proteins (so-called matrix) is characteristic for Ca stones [14], numerous steps in the sequence of events leading to Ca stones under conditions of altered oxidative metabolism are far from clear. After all, it is unknown if analysing readily available biomaterials such as urine and blood for substances so far less recognized in IRCU adds to insight into pathways linking oxidative and systemic metabolism to urinary protein, pH, Ca crystal and stone forming processes and stones. To arrive at a more realistic consideration of IRCU pathophysiology, there is a need to clarify whether there are effects that can be ascribed to stones in situ, and others that can independently impact on Ca salt crystallization. Historically, for the latter to occur in urine, the pre-requisites are seen in sufficient degree of supersaturation and crystal nucleation as modulated by inhibitors and promoters (only on this basis can crystals grow and agglomerate, processes over decades considered as the first stages in stone formation [2, 3]).

From the screening of a large body of data from IRCU patients of our laboratory the impression was that in fasting urine, i.e., not compromised by prior ingestion and metabolism of nutrients and obtained under strictly controlled conditions (see below), there was enormous scatter of urinary protein, especially the N-Alb-P fraction (of which several members modulate crystallization of stone substances [15]), but also variation of urinary volume and pH, and body size (body mass index; BMI). Therefore, the present work was carried out to find a basis from which hypotheses can be formulated that are testable by future more in depth investigating controlled studies. For the first time two defined periods of a daily cycle were selected for studying the patients in the laboratory, allowing to rule out unspecific influences. Answers to the following questions were sought: 1) Is excretion of urinary N-Alb-P affected by renal stones in situ, or is there co-variation of this urine protein fraction with markers of oxidative metabolism? 2) Are there links between oxidative and systemic metabolism, urinary pH, N-Alb-P concentration, volume, Ca salt supersaturation and risk of crystallization, and the number of patients bearing stones? 3) Do excess of oxidants, deficit of antioxidants or imbalance of the two alter the propensity of urine to nucleate CaOx or CaPi, if not, are there links to systemic metabolism?

**Material and Methods**

**Study Participants**

From our ambulatory stone clinic 187 consecutively examined adult male stone patients with defined IRCU [for stone analysis and other details see ref. 1], of whom several anthropometric features, the actual presence of stones and clinical chemistry data of relevance for lithogenicity of urine were available, were included. All had normal renal function (plasma creatinine <124 µm/l) but at least one Ca stone recurrence in the past, with the last dating back more than 1 month, IRCU was diagnosed on the basis of disease history and stone analysis, showing that Ca, OX and Pi were the only constituents (see ref. 1). Patients were defined as "stone-free" (SF, n = 93) when at the time of laboratory investigation concretions were not detectable by meticulously carried out clinical techniques (X-ray, including tomography, ultrasound, etc.) in the renal pelvis, calyces, papillae or further upstream parenchyma, or as "stone-bearing" (one stone or more, but no stone nests; SB, n = 94). Exclusion criteria were females (for reasons see ref. 5), non-European ethology, residence outside North Bavaria, accompanying diseases (primary hyperparathyroidism, documented essential hypertension, diabetes mellitus, renal tubular acidosis), oxaluria >0.5 mmol in daily urine (precluding the possibility that ROS excess and subsequently elevated MDA originate from OX excess in urine [7, 16]), hematuria (dipstick-positive urine), signs of urinary tract obstruction and/or infection with urease-producing germs, and cases with potential post-renal sources of urinary protein (cystitis, prostatitis, etc.). It is emphasized that delineation of members of urinary N-Alb-P fraction in terms of structure, amount and function was not among the goals of present work. All patients had not taken specific anti-stone medication during the previous 6 weeks. A defined control group was not studied, but from a small group of adult males without a history of stones limits of normalcy for several variables are given. Upon written information all subjects gave their consent to the envisaged laboratory investigations, and the study was carried out in accordance with the principles of the Declaration of Helsinki.
LABORATORY PROGRAM

Details of the standardized examination protocol (including analysis of daily, fasting, postprandial urine, and fasting blood, etc.) as practiced in our outpatient stone clinic have been described [17]. For the present work the composition of fasting blood and urine and postprandial urine (after intake of a purine- and oxalate-free but Ca-rich and urine acidifying meal of fixed composition [1, 18]) was studied. After an overnight fasting period of 12-14 h, diuresis was stimulated in the laboratory by drinking 2 x 300 ml distilled water (generally resulting in urine flow of 1–2 ml/min), blood pressure was measured with the patient in a recumbent position, and forearm venous blood withdrawn into heparinized prechilled tubes. After puncture of an ear-lobe for blood gas analysis, the bladder was voided to give urine from a timed period of 2 h; thereafter the meal was ingested, and urine collected from a timed 3 h period. Aliquots of plasma and paper-filtered (Whatman no. 3) urine were prepared and either analysed on the same day or stored at -80°C.

STUDY DESIGN, DATA COMPILATION

The study was tripartite, retrospective and observational, cross-sectional and correlational. The overlap of participants in the present and previous studies, using different strategies and outcomes [1, 5, 18], was 80 – 95%. As a search for the mechanism(s) underlying ROS production and ROS nature was not in the focus of present work, overproduction of ROS (by cells during hypoxia-induced ATP degradation [19]) was taken as reflected by increase of urinary hypoxanthine, and ROS excess-mediated damage of lipids and lipid-containing cell membranes, other molecules and genes [11, 12, 20] as reflected by increase of urinary MDA; low plasma uric acid, the dominant antioxidant component of human plasma [5, 21], was taken as indicating antioxidant deficiency. In part 1 variables as observed in fasting urine of the pool group (SF + SB patients, n = 187) were stratified according to the median urinary N-Alb-P excretion (strata Low and High), the protein fraction known to contain crystallization-inhibiting and -promoting species [15, 22, 23]; these data were complemented by general features, including a score roughly reflecting the activity of stone-forming processes (ASFIP) in the past 2-year period [24], urinary excretion rate of volume, minerals, MDA and the uric acid precursor oxyuripines hypoxanthine and xanthine, renal fractional excretion (FE; indicating the state of the kidney to retain or release substances) of N-Alb-P (FE-N-Alb-P), uric acid (FE-Uric acid) and oxalate (FE-oxalate), fasting plasma levels of uric acid and total antioxidants (TAS), vasopressin and insulin. Also in part 1, and using the same database as for stratification of N-Alb-P excretion (see above), SB were contrasted with SF patients. Part 2 comprised, besides general features and the state of oxidative metabolism, FE data and those on the concentration of substances that govern the propensity of urine to crystallize: the physico-chemical supersaturation with CaOx and CaPi, the latter in the form of both the molar Ca/Pi ratio (the formation of amorphous CaPi solid begins at 0.015 M [1]) and the Ca-rich hydroxyapatite (HAP; molar Ca/Pi ratio of crystals 1.60), magnesium (Mg) and citrate (Cit) in terms of total concentration and after splitting into moieties able to form soluble complexes or remaining as free ions (F-Mg, F-Cit; discussed as small-molecular CAox and CaPi crystallization inhibitors [25, 26]). Thereafter, the patients were stratified according to median pH of fasting urine (strata Low and High). In part 3 the nucleation of Ca salts, i.e., appearance of solid CaOx or CaPi visible by light microscopy, was probed in postprandial urine by means of the maximally tolerable total concentration of Ca (T-Ca) and Ox (T-Ox). This urine was chosen because in it deficient acidification in response to intake of an acid meal is frequent among IRCU patients, accompanied by unchanged Ca/Pi but dramatic rise of HAP supersaturation [1]; therefore, and in accordance with studies on CaPi solubility in urine-like solutions with varying pH [27], the formation of HAP and other CaPi solids at urine pH 6.0 upon addition of Ca in excess of Ca in native urine should be indicated by T-Ca. In contrast, at the CaOx supersaturation level prevailing at the native urine pH and Ox added in excess of Ox in native urine, CaOx solid formation should inform about T-Ox. These data were assigned to three sets of patients, with each set subdivided into the strata Low and High: Fasting urinary MDA excretion, fasting plasma uric acid concentration, the ratio urinary MDA/plasma uric acid (as crude measure of imbalance of oxidative metabolism); several additional parameters served as complementary data. For the sake of transparency and space a considerable body of data is presented in APPENDIX.

ANALYSES

For analyzing blood, plasma and urine routine methods or well-established techniques [5, 17] were utilized, including those 14 analytes required for estimation of urinary supersaturation (see below) with stone substances. Exceptions were: urinary pH (glass electrode); colorimetric determination of plasma TAS (using commercial reagents, supplied by Randox, Krefeld, Germany) which follows the principle outlined by Miller et al. [28]; high-performance liquid chromatography measurement of MDA and oxypurines in native urine [24], Ox in plasma ultrafiltrate [29], thawed and acidified (pH ≤1.5) urine [29]; urinary total protein by colorimetry [30], urinary Alb by immuno-nephelometry (using antibody OSAL 10, BN II analyzer, Dade Behring, Marburg, Germany); plasma vasopressin and insulin by radioimmunoassay (kit from Immuno-Biological Laboratory, Hamburg, Germany, and inhouse assay, respectively). Crystal nucleation was assessed by the earlier described small-scale (0.5 ml undiluted urine) light microscopy and image analysis-guided technique (for details see ref. [31]), using addition of microliter amounts of analytical grade sodium oxalate stock solution at original pH (for T-Ox), or addition of microliter amounts of analytical grade Ca chloride stock solution at prefixed pH 6.0 (for T-Ca). The morphology and nature of formed solids were documented.
by scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) elemental analysis (for details, techniques and equipment see ref. [18]).

**Calculations, Statistics**

N-Alb-P in plasma and urine was taken as difference between total protein and Alb (note that in present work urinary Alb was not further considered). FE and creatinine clearance were calculated conventionally. From EQUIL-2 [32] the free energy (DG) that drives urine supersaturation with CaOx, HAP and uric acid toward nucleation and crystal growth in aqueous solution was calculated, and from the same procedure the concentration of soluble Mg and Cit complexes, F-Mg and F-Cit, were obtained. The molar Ca/Pi ratio relative to concentration of Na, F-Mg, F-Cit, and urinary volume was given to illustrate possible interdependencies. Despite the wide scatter of individual values the decadic logarithm of numerical values mostly gave symmetric data, allowing application of a two-tailed Student's t-test, otherwise the Wilcoxon signed-rank test was used. Categorical data were examined by the Chi² test. When p ≤ 0.05, differences between strata were considered significant. For practical reasons results are given as mean values (with SE or range). Simple and multiple regression analyses (with adjustment for confounding variables) were established. The software STATISTICA (Statsoft, Tulsa, OK, USA) was used.

**Results**

**Uriney N-Alb-P, Stones in situ (Part 1)**

According to Table 1-A, in the High vs. Low patients urinary N-Alb-P excretion was approx. 6 times and total protein approx. 4 times higher, whereas the plasma levels of total protein, Alb and N-Alb-P were unchanged (data not shown); there was in addition increase of urinary volume, Na, K, Mg, Cit, creatinine clearance, and FE-N-Alb-P; in contrast, excretion of Ox, Ca and Pi was unchanged. Also in the High patients, there was slightly but significantly higher systolic and diastolic blood pressure, increase of urinary pH, excretion of the uric acid precursor oxypurines hypoxanthine and xanthine, uric acid, FE-Uric acid and MDA, but decrease of plasma concentration of uric acid and TAS, this indicating deficiency of antioxidants [5, 28] not studied here; BMI, ASFP and the number of SB (stones present) and SF (stones absent) patients were statistically indistinguishable among strata, as was plasma K and vasopressin (mean values 4.4 pm/l and 4.6 pm/l, assumed molecular mass 1084 Da). Table 1-B, giving data from the same variables as shown in Table 1-A separately for SF (column Absent) and SB (column Present) patients, shows that in the latter group urine pH was elevated, but that other variables differed only insignificantly. Significant (p ≤ 0.05) simple correlations were listed in APPENDIX I (Table, code 1 – 10), resulting in summed r² 0.74 (code 1 – 7, without FE-N-Alb-P) and r² 0.63 (code 8 – 10). Using log N-Alb-P excretion as outcome measure, multivariate regression analysis identified log MDA and log FE-N-Alb-P (R² 0.91, p < 0.00001 after adjustment for covariates) and log hypoxanthine (adjusted R² 0.42, p < 0.000001) as the dominant predictors; together these variables can explain more than the full range of variation of N-Alb-P excretion rate.

**Uriney pH (Part 2)**

In the High vs. Low stratum (Table 2) the number of patients with BMI >25.0 was uneven (High: n = 54; Low: n = 67), and there was decrease of unclassified BMI. Also according to Table 2, the stratum High patients exhibited decrease of plasma insulin and uric acid; plasma TAS of 34 out of 57 patients studied was <1.35 mM/l (which is the lower limit of normalcy [5]); unchanged were systolic and diastolic blood pressure, urinary N-Alb-P concentration and the plasma levels of total protein, Alb, N-Alb-P (data not shown); there was increase of urinary hypoxanthine, xanthine and MDA excretion, FE-N-Alb-P and volume, but decrease of urinary concentration of Na, total Mg and F-Mg, total Cit and complexed Cit, uric acid, Ox, supersaturation with uric acid; most importantly, there was unchanged urinary Ca/Pi with dramatic increase of HAP in association with borderline significant decrease of CaOx supersaturation and significant increase of the number of SB patients. Urinary pH failed to correlate with MDA (n = 176, r² 0.01). Significant (p < 0.05) correlations were listed in APPENDIX I (Table), resulting in summed r² 0.49 (code 11 – 17), and summed r² 0.46 (code 18 – 21). Using urinary pH as outcome, multivariate regression analysis identified BMI and plasma log insulin as negative predictors (adjusted R² 0.14, p < 0.000006), log plasma uric acid as negative, log xanthine excretion and log FE-N-Alb-P (adjusted R² 0.28, p < 0.000001) as positive predictors. Together these variables can explain approx. 42% of variation of urinary pH.

In analogy to in vitro work, showing that the higher the pH, the higher is HAP supersaturation [27], from data of Table 2 a possible sequence of events in urine has been derived: Fig. 1, a – b shows that in the patients with high urine pH (stratum High) the increase of urinary volume and decreasing concentration of Na and F-Mg are central to the increase of (Ca/Pi)/ Na © and (Ca/Pi)/F-Mg ©, whereas (Ca/Pi)/F-Cit was resistant to the change of volume ©; volume correlated directly with FE-N-Alb-P (Fig. 1, B), (Ca/Pi)/Na (Fig 1, C), indirectly with Na concentration (Fig. 1, D) and CaOx DG (Fig. 1, E); conversely, HAP DG correlated directly with (Ca/Pi)/F-Mg (Fig. 1, F). In these plots segregation of SB from SF patients was not recognizable (Fig 1 B-F), but the data allow to postulate that a rise of HAP crystalization risk is characteristic for urine with higher pH, occurring in association with high urine volume and low small-molecular crystallization inhibitors (see also below).

Broadening this thinking, APPENDIX II (a – i) gives further information from additional significant (p < 0.05 - p < 0.001) simple correlations (n = 172 – 181 paired observations): positively correlated were HAP DG and log N-Alb-P concentration (a), log volume and (Ca/Pi)/Total Mg (b), (Ca/Pi)/Total Cit (c); negatively correlated were log volume and log N-Alb-
<p>| Table 1. Characteristics of IRCU patients with low and high N-Alb-P excretion in fasting urine (A), absence or presence of renal stones in situ (B), and of all patients (C). All excretions rates are per 2 h. If not otherwise indicated, data are mean values (SE). For further informations see text. |</p>
<table>
<thead>
<tr>
<th>N*</th>
<th>A. N-Alb-P; median 4.3 mg Low &gt;Median</th>
<th>B. Stones Absent Present</th>
<th>C. All Absent Mean</th>
<th>Range</th>
<th>Normal1</th>
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<tbody>
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<td><strong>Urine</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Total protein; mg</td>
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<td>3.3 (0.2); 94</td>
<td>13 (2); 93</td>
<td>&lt;0.001</td>
<td>6.2 (0.5); 94</td>
</tr>
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<td>N-Alb-P; mg</td>
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<td>1.7 (0.4); 94</td>
<td>11 (1.9); 93</td>
<td>&lt;0.001</td>
<td>5.1 (0.4); 94</td>
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<td>Cr* Clearance; ml/min</td>
<td>187</td>
<td>108 (2); 94</td>
<td>115 (3); 93</td>
<td>0.03</td>
<td>113 (2); 94</td>
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<td>103 x FE-N-Alb-P; %</td>
<td>187</td>
<td>0.90 (0.0); 94</td>
<td>3.4 (0.5); 93</td>
<td>&lt;0.001</td>
<td>1.8 (0.0); 94</td>
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<td>Volume; ml</td>
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<td>290 (17); 93</td>
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<td>229 (15); 94</td>
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<td>187</td>
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<td>15 (0.8); 93</td>
<td>&lt;0.001</td>
<td>13 (0.6); 94</td>
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<td>K; mM</td>
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<td>9.0 (3.3); 93</td>
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<td>Ca; µM</td>
<td>187</td>
<td>298 (19); 93</td>
<td>334 (16); 94</td>
<td>&lt;0.07</td>
<td>307 (17); 94</td>
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<td>Mg; µM</td>
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<td>231 (10); 94</td>
<td>277 (10); 93</td>
<td>&lt;0.002</td>
<td>257 (10); 93</td>
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<td>Pi; mM</td>
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<td>1.15 (0.06); 94</td>
<td>1.23 (0.07); 93</td>
<td>&lt;0.22*</td>
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<td>OX; µM</td>
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<td>23 (2.1); 91</td>
<td>0.34</td>
<td>23 (1); 89</td>
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<td>Cit; µM</td>
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<td>253 (13); 93</td>
<td>311 (14); 92</td>
<td>&lt;0.001</td>
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<td>pH</td>
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<td>5.92 (0.09); 94</td>
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<td>&lt;0.001</td>
<td>5.95 (0.08); 94</td>
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<td>Age; y</td>
<td>187</td>
<td>42 (1.2); 94</td>
<td>41 (1.2); 93</td>
<td>0.25</td>
<td>41 (1.2); 94</td>
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<td>ASFP; score</td>
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<td>35 (4); 94</td>
<td>40 (4); 93</td>
<td>0.18*</td>
<td>34 (4); 94</td>
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<td>Renal stones; Absent/ Present</td>
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<td>47/24; 94</td>
<td>46/73; 93</td>
<td>nd</td>
<td>94/0</td>
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<td>Systolic blood pressure; mmHg</td>
<td>152</td>
<td>126 (2); 78</td>
<td>132 (3); 74</td>
<td>0.004</td>
<td>129 (2); 75</td>
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<td>Diastolic blood pressure; mmHg</td>
<td>152</td>
<td>82 (1); 78</td>
<td>86 (2); 74</td>
<td>0.03</td>
<td>83 (1); 75</td>
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<td>BMI; kg/(m)²</td>
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<td>26.4 (0.3); 94</td>
<td>26.2 (0.4); 93</td>
<td>0.39</td>
<td>26.2 (0.3); 94</td>
</tr>
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<td>P** Insulin; µU/ml</td>
<td>184</td>
<td>17 (1); 92</td>
<td>16 (1); 92</td>
<td>0.26</td>
<td>16 (1); 91</td>
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<td>P-K; mM/L</td>
<td>106</td>
<td>4.2 (0.03); 62</td>
<td>4.2 (0.05); 44</td>
<td>0.46</td>
<td>4.2 (0.04); 49</td>
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<td>U** Hypoxanthine; µM</td>
<td>75</td>
<td>9.9 (0.8); 29</td>
<td>13 (0.7); 46</td>
<td>&lt;0.001</td>
<td>11.5 (0.8); 39</td>
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<td>U-Xanthine; µM</td>
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<td>4.0 (0.4); 29</td>
<td>7.8 (0.5); 36</td>
<td>&lt;0.001</td>
<td>6.4 (0.6); 39</td>
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<td>U-Uric acid; µM</td>
<td>187</td>
<td>357 (16); 94</td>
<td>433 (14); 93</td>
<td>&lt;0.001</td>
<td>403 (12); 94</td>
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<td>U-FE-Uric acid; %</td>
<td>186</td>
<td>7.4 (0.3); 94</td>
<td>8.8 (0.4); 92</td>
<td>&lt;0.001</td>
<td>8.3 (0.3); 94</td>
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<td>P-Uric acid; µM/l</td>
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<td>374 (7); 94</td>
<td>342 (7); 93</td>
<td>&lt;0.001</td>
<td>353 (7); 94</td>
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<td>P-TAS; mM/l</td>
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<td>1.37 (0.02); 21</td>
<td>1.31 (0.01); 36</td>
<td>0.007</td>
<td>1.34 (0.02); 30</td>
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<td>U-MDA; mM</td>
<td>176</td>
<td>116 (5); 89</td>
<td>148 (7); 87</td>
<td>&lt;0.001</td>
<td>135 (6); 88</td>
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<tr>
<td>U-MDA/P-Uric acid; mM/mM x 1</td>
<td>176</td>
<td>316 (14); 89</td>
<td>486 (22); 87</td>
<td>&lt;0.001</td>
<td>373 (20); 88</td>
</tr>
</tbody>
</table>

* total number of patients; ++ number of patients in subgroups; * creatinine; ** U and P indicate urine and plasma, respectively; * based on log10; nd: not determined; 1 limits or range observed in 10 – 20 healthy adult male subjects in the authors’ laboratory, and from literature.
Table 2. Characteristics of ICU patients with either low or high pH in fasting urine, and of all patients. Excretion rates are per 2 h. N: number of participating patients; for the number of patients in strata, data for a and b and other information, see Table 1 and text.

<table>
<thead>
<tr>
<th>General features</th>
<th>pH; median 6.14 (Low)</th>
<th>High</th>
<th>High vs. Low</th>
<th>All</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>≤Median</td>
<td>&gt;Median</td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASFP; score</td>
<td>187</td>
<td>40 (5); 93</td>
<td>35 (3); 94</td>
<td>0.49*</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Renal stones; Absent/ Present</td>
<td>187</td>
<td>57/36</td>
<td>37/37</td>
<td>0.03*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI; kg/(m)^2</td>
<td>187</td>
<td>27.0 (0.4); 93</td>
<td>25.6 (0.3); 94</td>
<td>0.001</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>P*-Insulin; µU/ml</td>
<td>184</td>
<td>20 (1); 90</td>
<td>13 (1); 94</td>
<td>&lt;0.001</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Systolic blood pressure; mm Hg</td>
<td>152</td>
<td>130 (3); 74</td>
<td>128 (2); 78</td>
<td>0.24</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Diastolic blood pressure; mm Hg</td>
<td>152</td>
<td>84 (2); 74</td>
<td>83 (1); 78</td>
<td>0.29</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>U*-Hyponxanthine; µM</td>
<td>75</td>
<td>11 (0.9); 40</td>
<td>13 (0.6); 35</td>
<td>0.03*</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>U-Xanthine; µM</td>
<td>75</td>
<td>5.2 (0.5); 40</td>
<td>7.7 (0.5); 35</td>
<td>&lt;0.001*</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>P-Uric acid; µM/l</td>
<td>187</td>
<td>374 (7); 93</td>
<td>342 (7); 94</td>
<td>0.001</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>P-TAS; mM/l</td>
<td>57</td>
<td>1.35 (0.02); 29</td>
<td>1.32 (0.02); 28</td>
<td>0.14</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>P-Oxalate; µM</td>
<td>61</td>
<td>1.72 (0.09); 32</td>
<td>1.71 (0.08); 29</td>
<td>0.46</td>
<td>1.9</td>
<td>1.1-3.8</td>
</tr>
<tr>
<td>U-MDA; mM</td>
<td>176</td>
<td>124 (5); 89</td>
<td>140 (7); 87</td>
<td>0.03</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>B*-pH</td>
<td>185</td>
<td>7.40 (0.00); 91</td>
<td>7.41 (0.00); 94</td>
<td>0.02</td>
<td>7.40*</td>
<td>7.35-7.49</td>
</tr>
<tr>
<td>B-Bicarbonate; mM/l</td>
<td>185</td>
<td>23.2 (0.2); 91</td>
<td>23.7 (0.2); 94</td>
<td>0.07</td>
<td>23.5*</td>
<td>18-31</td>
</tr>
<tr>
<td>U-MDA/P-Uric acid; nM/M/m x 1^1</td>
<td>176</td>
<td>345 (17); 90</td>
<td>426 (22); 86</td>
<td>0.002</td>
<td>a</td>
<td>b</td>
</tr>
</tbody>
</table>

Urinary compounds

- pH
  - 187 measurements: 5.46 (0.04); 93 (median: 93); 6.81 (0.04); 94; <0.001; a; b
- Volume: ml
  - 187 measurements: 194 (14); 93; 256 (17); 94; 0.003; a; b
- Cr**/Creatinine; ml/min
  - 187 measurements: 127 (4); 93; 119 (3); 94; 0.08; a; b
- N-Alb-P; mg/l
  - 181 measurements: 32 (5); 89; 34 (5); 92; 0.24*; 33; 2-435
- 10^x FE-N-Alb-P; %
  - 187 measurements: 1.9 (0.3); 91; 2.5 (0.5); 96; 0.002*; a; b
- FE-Uric acid; %
  - 186 measurements: 7.7 (0.3); 92; 8.5 (0.4); 94; 0.07; a; b
- FE-Oxalate; %
  - 61 measurements: 108 (8); 32; 120 (9); 29; 0.16; 113; 40-245
- Na; mM/l
  - 187 measurements: 77 (4); 93; 66 (4); 94; 0.03; 72; 13-201
- Ca; mM/l
  - 187 measurements: 2.1 (0.2); 93; 1.9 (0.2); 94; 0.18*; 2.0; 0.2-11
- Pi; mM/l
  - 187 measurements: 8.6 (0.7); 93; 7.7 (0.8); 94; 0.20*; 8.2; 0.4-66
- Ox; µM/l
  - 179 measurements: 144 (10); 87; 110 (8); 92; 0.003*; 130; 3-470
- Uric acid; µM/l
  - 187 measurements: 2.7 (0.1); 93; 2.2 (0.1); 94; 0.005; 2.4; 0.39-6.1
- Uric acid; DG
  - 187 measurements: 5.26 (0.16); 85; 4.45 (0.17); 87; <0.001; 0.82; 8.13
- Total Mg; mM/l
  - 187 measurements: 1.75 (0.13); 93; 1.38 (0.08); 94; 0.005; 1.6; 0.21-4.58
- Complexed Mg; mM/l
  - 172 measurements: 0.83 (0.06); 85; 0.71 (0.06); 87; 0.08; 0.77; 0.1-2.8
- F-Mg; mM/l
  - 172 measurements: 0.95 (0.06); 85; 0.70 (0.05); 87; 0.001; 0.82; 0.1-2.8
- Total Cit; mM/l
  - 185 measurements: 1.93 (0.13); 93; 1.61 (0.13); 92; 0.03*; 1.8; 0.18-5.9
- Complexed Cit; mM/l
  - 172 measurements: 1.63 (0.12); 85; 1.33 (0.11); 87; 0.03; 1.5; 0.2-5.2
- F-Cit; mM/l
  - 172 measurements: 0.29 (0.03); 85; 0.31 (0.03); 87; 0.31; 0.30; 0.02-1.5
- CaOx; DG
  - 172 measurements: 1.0 (0.11); 85; 0.78 (0.13); 87; 0.06; 0.91; -2.7-3.5
- HAP; DG
  - 172 measurements: 1.9 (0.32); 85; 3.7 (0.28); 87; <0.001; 2.8; -4.6-8.6

* P, U and B indicate plasma, urine and blood, respectively; **: creatinine; *: based on log_{10} x**; Chi^2 9.0 (3 degrees of freedom); ^1, ^2: limits of normalcy in the authors' laboratory are ≥7.35, ≥18, respectively.

**P** concentration (d), insulin and HAP DG (c), blood bicarbonate (f), blood pH (g), BMI and blood bicarbonate (h); BMI and plasma uric acid correlated positively (i) (for correlation of BMI and insulin see Table 4); note that in h and i some of the patients exhibited BMI >25.0 (dashed line), and that modest clustering of SB patients was restricted to those with insulin in the low-normal range (for example in e: n = 39 SB and n = 27 SF; the dashed line in e, f, g marks the upper limit of normal insulin (20 µU/ml [33]), the stippled line mean insulin within the normal range).

**Nucleation of Ca Salts (Part 3)**

Table 3 represents low and high (below and above the median) fasting urinary MDA, plasma uric acid and the ratio of these parameters, their association with postprandial urine T-Ox, T-Ca, T-Ca/10^2×T-Ox, pH, su-
persaturation with CaOx and HAP, and with several complementary parameters from the fasting 2 h period (see Tables 1 and 2). Despite the presence of high MDA and high MDA/Ur acid, both signalling oxidant excess (7, 13), T-Ox was unchanged. Conversely, when plasma uric acid, signalling antioxidant deficiency [21], was low, T-Ca and T-Ca/10²xT-Ox were decreased too; this latter constellation was associated with elevation of FE-N-Alb-P, volume, Na in fasting urine, and decrease of fasting plasma insulin and BMI.

Fig. 2 shows the morphology (A-1 – C-1) and mineral nature (A-2 – C-2) of the formed solids. In the majority of the 49 samples studied at prefixed urine pH 6.0 the first appearing crystal was spheroidal CaPi (molar ratio approx. 1:6, synonymous HAP, see A-1; n = 31), followed by rhomboidal CaOx dihydrate crystals (weddelite) together with small amounts of CaPi (molar ratio approx. 1:0, synonymous amorphous and poorly crystallized CaPi, see B-1; n = 18); note that in only one of the latter samples plate-like brushite (Ca/P 1:0) was formed (not depicted). At original urine pH, weddelite was exclusively detected (C-1) but no phosphorus peak (C-2). Potassium and chloride peaks were detectable in A-2 – C-2, but both peaks were sizable in B-2. APPENDIX, I codes 22-24 shows that Log T-Ox and Log T-Ca tended to correlate negatively, whereas Log T-Ca and Log plasma uric acid, Log (T-Ca/10² x T-Ox) and Log plasma uric acid approached the level of significance. Neither correlated
**Table 3.** A – Low and High fasting urinary (U) MDA excretion, plasma (P) concentration of uric acid, ratio U-MDA/P-Uric acid; B – Ca salt crystal nucleation and other data in postprandial urine; C – complementary data from fasting period. Mean values (SE), followed by number of observations. For further information see Table 2 and text.

<table>
<thead>
<tr>
<th></th>
<th>Fasting Low</th>
<th>Fasting High</th>
<th>Postprandial</th>
<th>T-Ox</th>
<th>T-Ca</th>
<th>T-Ca/10^5 x T-Ox</th>
<th>pH</th>
<th>CaOx</th>
<th>HAP</th>
<th>% x 10^5</th>
<th>ml</th>
<th>mM</th>
<th>µU/ml</th>
<th>kg/(m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>B</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>U-MDA; nM</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3)</td>
<td>89(2):88</td>
<td>0.52(0.03):25</td>
<td>24(3):20</td>
<td>0.51(0.08):20</td>
<td>5.46(0.09):29</td>
<td>1.3(0.1):84</td>
<td>3.1(0.3):84</td>
<td>1.5(0.1):85</td>
<td>167(13):88</td>
<td>11(0.5):88</td>
<td>16(1):86</td>
<td>26.0(0.3):88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>0.41 d</td>
<td>0.18 d</td>
<td>0.23 d</td>
<td>0.17</td>
<td>&lt;0.001</td>
<td>0.17</td>
<td>0.002 d</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.15</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-Uric acid; µM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2)</td>
<td>301(10):94</td>
<td>0.52(0.03):30</td>
<td>16(2):25</td>
<td>0.34(0.06):25</td>
<td>5.72(0.1):36</td>
<td>0.8(0.1):88</td>
<td>3.5(0.3):88</td>
<td>2.9(0.6):91</td>
<td>252(17):94</td>
<td>13(0.6):94</td>
<td>14(1):93</td>
<td>25.4(0.3):94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>0.29 d</td>
<td>0.007 d</td>
<td>0.007 d</td>
<td>0.01</td>
<td>0.18</td>
<td>0.01</td>
<td>&lt;0.008 d</td>
<td>0.07</td>
<td>0.03</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-MDA/P-Uric acid; nM/mM</td>
<td>244(6):88</td>
<td>0.52(0.03):27</td>
<td>25(3):20</td>
<td>0.55(0.09):20</td>
<td>5.41(0.08):33</td>
<td>1.3(0.1):78</td>
<td>2.8(0.4):78</td>
<td>1.4(0.1):85</td>
<td>157(12):88</td>
<td>11(0.5):88</td>
<td>17(1):86</td>
<td>26.6(0.3):88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>0.26 d</td>
<td>0.09 d</td>
<td>0.13 d</td>
<td>0.04</td>
<td>&lt;0.001</td>
<td>0.38</td>
<td>&lt;0.001 d</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.18</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a, xx indicate ≤ and > median, respectively; 1, 2, 3: medians are 123, 349 and 337, respectively; a: at original pH; b: at prefixed urine pH 6.9; c: original urine pH; d: based on log₁₀ data;

**Table 4.** Simple correlations of parameters of oxidative metabolism, precursor oxypurines of uric acid, several complementary data from Table 3, and blood pressure (BP). U-urine; P-plasma. Coefficients in bold (p ≤ 0.05) are followed by number of pairs.

<table>
<thead>
<tr>
<th>Dependent 1</th>
<th>U-Fe-N-Alb-P</th>
<th>U-Xanthine</th>
<th>U-Volume</th>
<th>U-Na</th>
<th>P-Insulin</th>
<th>Systolic BP</th>
<th>Diastolic BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>-0.20:181</td>
<td>-0.15:75</td>
<td>-0.10:187</td>
<td>-0.10:187</td>
<td>0.48:184</td>
<td>0.47:152</td>
<td>0.49:152</td>
</tr>
<tr>
<td>U-Hypoxanthine</td>
<td>0.26:75</td>
<td>0.69:75</td>
<td>0.15:75</td>
<td>-0.13:75</td>
<td>-0.01:73</td>
<td>0.43:54</td>
<td>0.27:54</td>
</tr>
<tr>
<td>U-MDA</td>
<td>0.30:170</td>
<td>0.35:74</td>
<td>0.54:176</td>
<td>0.34:176</td>
<td>0.06:177</td>
<td>0.23:144</td>
<td>0.22:144</td>
</tr>
<tr>
<td>P-Uric acid</td>
<td>-0.26:184</td>
<td>-0.35:75</td>
<td>-0.18:187</td>
<td>-0.19:187</td>
<td>0.28:184</td>
<td>0.22:152</td>
<td>0.21:152</td>
</tr>
<tr>
<td>U-MDA/P-Uric acid</td>
<td>0.38:170</td>
<td>0.46:74</td>
<td>0.57:176</td>
<td>0.39:176</td>
<td>0.07:176</td>
<td>0.13:144</td>
<td>0.12:144</td>
</tr>
<tr>
<td>P-TAS</td>
<td>-0.39:57</td>
<td>-0.24:57</td>
<td>-0.20:57</td>
<td>-0.21:57</td>
<td>0.37:57</td>
<td>0.08:41</td>
<td>0.18:41</td>
</tr>
</tbody>
</table>

1) For other abbreviations see text, for dimensions Tables 3 and 1 (log data are used, except BMI and TAS).
T-Ox, T-Ca, T-Ca/10² x T-Ox significantly with the original urinary pH and Ca salt supersaturation in postprandial urine, nor with any of the complementary parameters of the fasting period. On the other hand, upon rearrangement of data of Tables 2 and 3, numerous correlations were identified, linking BMI, hypoxanthine, TAS and the three parameters of oxidative metabolism to a series of dependent variables (Table 4); each of the former variables impacted significantly on FE-N-Alb-P (positively or negatively), but xanthine and uricemia correlated negatively, xanthine and hypoxanthine positively.

**DISCUSSION**

**GENERAL REMARKS**

The disclosure that Ox in the physiological range is nontoxic to renal tissue [34], with earlier reports on a role as oxidant [16], forces to uncover the true etiology of IRCU. Although the present uncontrolled study cannot elicit the latter, it raises questions as to how Ox-independently disturbed oxidative metabolism can be integrated with IRCU pathophysiology. We can show that above pH 6.14 of fasting urine SB outnumber SF patients significantly, contrasting with earlier work [1] showing that the correlation between fasting urine pH and the number of SB patients was only of borderline significance (p=0.059). We ascribe this discrepancy to the extremely careful clinical search for presence of stones; in addition, it indicates that, provided the urine pH is only weakly acid or alkaline, this will optimize renal crystal and stone forming processes. Thus, it would be unrealistic to assume that stones per se elicit the higher pH via, for example, diminished bicarbonate reabsorption by tubules [4]; even more important, reduction in size and total disappearance of stones did not improve the functional abnormality of tubules [35]. Therefore, the rise of urine pH, although etiologically insufficiently explicable by data presented in part 2, may be more complex and include diminished renal proton (H) generation, hyperfiltration of bicarbonate by glomeruli (see Tables 1, 2) and onset of defense mechanisms, together facilitating stone formation (see [36] and below). Because in the literature there is abundance of articles dealing with renal diseases as modified by oxidants and antioxidants, but virtually no reports with similar objectives as in present work, comments are made rather than discussing more in depth.

**VARIATION OF URINARY N-Alb-P – GUIDE TO A ROLE OF ROS IN IRCU?**

In IRCU, known as a disorder of affluence and approx. 50% recurrence rate within 5 years from the onset, the consumption of food with high protein content, producing acid ash and lowering urine pH, is frequent; despite, repercussions on Ca stone forming
processes are poorly understood [1]. In animals fed a high protein diet increase of ROS production by mesangial cells has been interpreted as sequel of dis- creant oxygen demand and supply, but only incom- mensurate increase of antioxidant defense mecha- nisms, ending up in glomerulo-tubulo-interstitial tis- sue damage [37]. Amplification of chronic kidney dis- ease from obesity has been ascribed to association with oxidative stress [38], and overweight and obesity are frequent in IRCU (APPENDIX, I (h, i)). Lipid uptake and lipoprotein degradation by human prox- imal tubular cells cause proteinuric disease [39], and renal lipid accumulation underlies impairment of Na+/H+ exchange [40]. Peroxidation of cell mem- brane lipids, MDA accumulation and cell death of re- nal epithelial cells are sequela of prior increase of for- mation of hydrogen peroxide (H$_2$O$_2$) [41, 42]. Mem- bers of the ROS family also affect tubular enzymes with functions for the transport of Na and other ions, although the site(s) of action along the nephron [43, 44] and involvement of vasopressin-independently impaired re-uptake of water via aquaporins [45], lead- ing to enhanced diuresis, are uncertain. Kidney corti- cal cells exposed to hypoxia undergo ATP depletion [46], and H$_2$O$_2$ inhibits ATP-dependent proton extru- sion mechanisms of tubular epithelial cells [47], in turn contributing to cellular acidosis and K loss via urine (presumably in exchange for H$^+$ (Table 1)). The cells of ascending loop of Henle are most vulnerable due to limited receipt of blood and oxygen, but yet need to sustain high ATP production for electrolyte transport and synthesis of proteins [42] (note that among urinary N-Alb-P the quantitatively dominant fraction is Tamm-Horsfall protein, together with os- teopontin powerful inhibitors of calcification and stones [48], but unclarified susceptibility to ROS excess and resulting state of inhibitor and/or promoter function in crystal and stone formation of IRCU [49]). Similarly, whether a rise of blood pressure is caused by ox- idative stress (Tables 1, 4), hence becomes part of IRCU pathophysiology, is unsolved. In humans with chronic progressive kidney disease due to type 2 dia- betes mellitus (mostly in association with insulin resis- tance of organs and hyperinsulinemia), blockade of angiotensin II receptors reduces oxidative damage of proteins and lipids, and markers of inflammation and fibrosis [50]; in that setting [50] blood pressure re- mained unchanged, and urinary MDA and protein turned out to be superior markers vis-à-vis measure- ment in plasma. From experiments in normotensive obese rats it was concluded that oxidative stress trig- gers the onset of kidney lesions in the absence of hy- perglycemia, inflammation and hypertension [51]. Thus, if one accepts that increase of urinary hypox- anthine reflects transient, periodic or permanent tis- sue hypoxia, followed by deficient cellular ATP pro- duction, excess of ROS and MDA (as marker of dete- rioration of renal cell membrane function and exag- geration of release of N-Alb-P and probably other macromolecules into urine), then a good deal of our data (Tables 1 A and 3; APPENDIX, I, codes 1-10) would be in agreement with information from work in basic science and ROS markers in related clinical dis- orders [51, 52].

**CaPi Nucleation and the Making of Ca Stones — Key is an Oxidatively Modified Environment?**

Since the early reports that Ca stone patients, but not none-stone-forming humans, bear light microscopi- cally visible calcified interstitial areas close to renal papilla [53], and that the vast majority of analyzed stones harbours a CaPi core [54], an unsolved conun- drum exists as to whether CaPi or CaOx crystals ap- pear first and at which renal anatomical site (tubular fluid, urine, interstitium). With knowledge available to date, a more sound interpretation of the chain of events appears possible. In saturated urine-like solu- tions, containing Ca, Pi and macromolecules, hetero- geneous nucleation of CaPi in the form of (Ca-rich) octacalcium phosphate is the dominant mechanism, provided the pH is weakly acidic or alkaline and kept constant together with Ca and Pi concentration (for details see [23, 27]). Once in an oxidatively modified urinary environment the pH is high (Table 2) and there develops an additional need for dissipation of Ca ions from nephrons (note that with use of a closed crystallization system [31] the Ca concentra- tions tolerated by urine (Table 3) become unphysi- ologically high), either via excretion of Ca-poor CaPi or CaOx crystals in urine or, alternatively, via intersti- tial deposition as HAP (containing 10 moles Ca per mole), this latter appears as the preferred strategy and therefore may be fundamental for IRCU [53]. In this sense, Ca crystals in urine and Ca deposits in tissue may function as Ca sink. Fig. 2, A-1 – C-1, illustrates that at least in urine, with the pH varying within a broader range than in blood, heterogeneous nuclea- tion [23,27] may run in both directions, depending on pH and abundance of reaction partners: Ca ions

$\bigtriangledown$ Ca poor CaPi forms $\bigtriangledown$ Ca-richer CaPi forms

$\bigtriangledown$ HAP $\bigtriangledown$ CaOx. If correct, this interpretation means that the situation in Fig. 2 B-1 is pivotal for Ca crystal nucleation, because it leaves the possibility of HAP formation along a rise of pH (Fig. 2 A-1), and of virtually complete CaPi dissolution and isolated presence of CaOx crystals once urine pH becomes in- creasingly acidic (Fig. 2 C-1); on the other hand, this view is limited by the sensitivity of the instrumen- tation in use for the detection of CaPi solid. Regarding the metabolic environment in interstitium, bicarbon- ate enrichment of blood (Table 2) may hint towards leakage of basolateral cell membranes for this anion, mainly of distal tubular cells, owing to a genetic defect [55] or acquired insufficient ATPase-dependent H$^+$ generation. As mentioned above, clinically inap- parent (intracellular) acidosis may be a so far neglected fea- ture of IRCU, all the more as the elemental peaks of chloride and potassium (Fig. 2, A-2 – C-2) may – in the case of chloride – reflect malregulation of renal- tubular acid-base status, which is discussed as a possi- ble factor in Ca stone etiology [57], and – in the case of potassium – is reminiscent of the transition of amorphous CaPi to HAP, a process that in calcifying tissues is under the control of both cellular acid-base status [55] and matrix proteins [56]. In the light of present and other recent work the question "is ROS excess a primary event or secondary to interaction of renal epithelium with HAP [58]" deserves more con-
exclusive addressing: 1) In IRCU HAP was found within the basolateral membrane of, and juxtapositioned outside, the thin part of loop of Henle [59, 60], and alkalization of interstitium is pre-requisite for HAP formation [36]; 2) the inverse correlations of blood bicarbonate with insulin and BMI (APPENDIX, II, f and h), together with the observation that oxidatively modified metabolism in the form of a trend towards systemic alkaline tide (rise of blood bicarbonate and pH) coincides with a higher pressure to form stones (Table 2), are strong hints that ROS excess, extracellular bicarbonate accumulation and extratubular HAP development may be interrelated. Furthermore, once HAP invades epithelial cells and protrudes into tubular lumen [61] cellular particles serve as scaffolds and allow overgrowth of HAP either by CaPi, CaOx or both, depending on urine volume and the associated state of saturation of tubular fluid with these Ca salts [61, 62]. The latter would imply that urinary physicochemical equilibria in fact determine the predominance of minerals in Ca stones (CaOx or CaPi) [54, 62], but only indirectly. To reconcile this scenario with insufficient ROS neutralization, needs to consider the role of uric acid in this respect.

**Plasma Uric Acid – Friend or Foe of IRCU?**

The degree of uricemia, when within normal limits (Table 1, [33]), in the past failed to attract the interest of most researchers of IRCU pathophysiology. Even more, the roles of uric acid synthesis catalyzing xanthine oxidase as source of ROS and of uric acid as ROS scavenger in renal diseases of humans are controversially judged [63 – 67]. In general, enzyme-substrate relationship dictates that uric acid biosynthesis is the higher, the greater the accumulation of precursor oxypurines. In present work, the negative correlation of urinary xanthine excretion and uricemia, but positive correlation of excretion of hypoxanthine and xanthine (Table 4) may be indirect proof of a primary defective xanthine oxidase activity. If yes, the nature of this finding is unknown. Therefore, once ROS are insufficiently buffered due to lowering of uric acid and possibly other scavengers [5, 6] as reflected by plasma TAS [28], oxidative damage of vitally important tissues, pancreatic B-cells included [68, 69], may enhance perturbations of acid-base [70] and mineral [71, 72, 73] homeostasis, ending up in renal stones.

**Conclusions**

Summarizing the data from the present uncontrolled tripartite study of constituents of urine and blood, and urinary crystallization risk, there is the impression that stone formation arises from disturbances of oxidative metabolism, secondary malregulation of functionality of nephrons and interaction with interstitial tissue, rather than from primarily disturbed physicochemical supersaturation of tubular fluid and urine. Therefore, re-evaluation of IRCU pathophysiology is recommended. In future controlled studies emphasis may be placed on 1) the nature of oxidative and impact on systemic metabolism; 2) the nature and function of proteins, especially N-Alb-P in urine and renal tissue (proteomics?); 3) the determinants of (in this order) urine volume, Na, pH, and the pH inside renal cells of Ca stone patients.

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APPENDIX

I: Simple correlation of variables in fasting urine (U) and plasma (P) of Results, parts 1-3; n: paired observations; r: coefficient; p: level of significance. For dimension of variables see Tables 1 (part 1), 2 (part 2), 3 (part 3); *: based on log data.

<table>
<thead>
<tr>
<th>Code</th>
<th>Variables</th>
<th>n</th>
<th>r</th>
<th>p</th>
<th>Code</th>
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<td>16</td>
<td>U-FE-Uric acid</td>
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<td></td>
<td>18</td>
<td>U-Hypoxanthine</td>
<td>75</td>
<td>0.29</td>
<td>0.01*</td>
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<td></td>
<td>19</td>
<td>U-Xanthine</td>
<td>75</td>
<td>0.50</td>
<td>&lt;0.001*</td>
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<td>20</td>
<td>P-Uric acid</td>
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<td></td>
<td>21</td>
<td>P-TAS</td>
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<td>-0.28</td>
<td>0.03</td>
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Part 2
Dependent: U-pH
Influential:
11   BMI           187| -0.27| <0.001 |
12   P-Insulin     184| -0.29| <0.001* |
13   U-Glu         185| -0.14| 0.05* |
14   U-Volume      187| -0.19| 0.01* |
15   U-FE-N-Alb-P  181| 0.26 | <0.001* |

Part 3
Dependent: Log T-Ox
Influential:
16   U-FE-Uric acid 186| 0.18 | 0.015* |
17   U-HAP          172| 0.42 | <0.001 |
18   U-Hypoxanthine 75 | 0.29 | 0.01* |
19   U-Xanthine     75 | 0.50 | <0.001* |
20   P-Uric acid    187| -0.23| 0.001 |
21   P-TAS          57 | -0.28| 0.03 |
22   T-Ca           49 | -0.26| 0.07* |

II: Extra relationships of parameters in urine (a-d), urine and plasma (c), blood, plasma and BMI (f-i) of part 2 (for abbreviations and symbols see Results section and Fig. 1).
NOTE ADDED IN PROOF

Since the submission of present work 4 articles were published [see ref. 74-77], highlighting several aspects of IRCU pathophysiology and able to substantiate the idea that this disease is of cellular origin (electrolytes, proteins, genes), and probably shares defects seen in other related clinical disorders. For clinical calcium stone research a change of paradigms appears worthwhile, encompassing a shift from physico-chemistry of urine to renal soft tissue and cell research at the level of biochemistry and molecular biology, as well as interrelationships with systemic metabolism.

REFERENCES


73. We apologize that for the sake of space further articles in this context cannot be cited, and that the list of references is but a selection out of many excellent publications in the field.


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