IDIOPATHIC RECURRENT CALCIUM UROLITHIASIS (IRCU): AN ACID MEAL CHALLENGE UNCOVERS INAPPROPRIATE pH OF POSTPRANDIAL, FASTING AND DAILY URINE

A CROSS-SECTIONAL STUDY OF MALE PATIENTS PROVIDING INSIGHT INTO POST- AND PRE-LOAD URINARY STONE SUBSTANCES, CRYSTALLIZATION RISK, PRESENCE OF STONES, RENAL TRANSPORT AND SYSTEMIC METABOLIC FACTORS

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Abstract

Background: In IRCU the possible role of urinary pH (U-pH) as risk factor of calcium (Ca) stones is poorly understood.

Objectives: To evaluate in IRCU the response to an oral acid load, focussing on post- and pre-load U-pH, other urinary, renal and extra-renal factors, and linkage with Ca stones.

Methods: 237 male patients, either Ca stone-free (SF) or -bearing (SB), but without overt signs of systemic metabolic acidosis underwent a standardized laboratory programme that included, besides collection of urine and blood, the intake of an oxalate-free acid test meal (proton content 120 mM). Established analytical methods were used.

Results: In 79 patients the post-meal load U-pH was ≤5.30 (in healthy individuals accepted as the upper limit after the same proton load), but >5.30 in 158; in these two subsets the mean fasting pre-load U-pH was 5.84 and 6.37 (p <0.001), the mean U-pH in 24 h urine 5.70 and 6.03 (p <0.001), the mean score of stone formation activity 32 and 42 (p = 0.12), the SF/SB ratio 35/44 and 76/82 (not significant). However, when in pre-load urine undissociated uric acid concentration was low due to the high pH, the SF/SB ratio was 53/66 (p = 0.038), whereas isolated increase of U-pH with SF/SB ratio 54/65 (p = 0.059), urinary supersaturation with Ca phosphate (hydroxyapatite), Ca oxalate, uric acid, and isolated decrease of concentration of total protein, total uric acid and the crystallization inhibitors magnesium and citrate failed to affect significantly the frequency distribution of SF and SB patients. Pre-load U-pH was positively associated with urinary ratio sodium/proton excretion, renal reclaim of sodium and protein, negatively associated with body mass index, fasting insulinemia and uricemia, urinary protein concentration, renal reclaim of phosphate.

Conclusions: In IRCU 1) inappropriately high U-pH combined with increase of proteinuria and alteration of renal-tubular transport are frequent; 2) disturbed interactions of renal proton generation with sodium handling, urinary physico-chemical and systemic metabolic factors may initiate the development of Ca-containing concretions, presumably Ca phosphate, at some yet unknown renal anatomic site.

Key words: Idiopathic calcium urolithiasis; Acid test meal; Post- and pre-load urine pH; Stone substance crystallization risk; Absence and presence of stones

INTRODUCTION

In the pathophysiology of IRCU (for full definition see Material and methods) urinary excess of calcium (Ca) and oxalate (Ox), and deficit of crystallization inhibitors (citrate, magnesium, proteinaceous macromolecules) are considered as crystallization risk factors, eventually followed by stone development. Interestingly, except in patients with suspicion on systemic metabolic acidosis of renal-tubular origin (RTA) [1, 2], U-pH has received little attention as stone risk factor per se, or studies were restricted to smaller subsets of patients. From physical-chemical grounds U-pH is key for development of supersaturation of fluids containing stone substances such as inorganic Ca phosphate (CaPi) and uric acid. Ca stone formation therefore is interpreted to mean that it is secondary to crystallization in urinary tract fluids (for more details see ref. 3 – 6). However, although in the majority of Ca stones the dominant constituent is CaOx, mostly with admixture of small amounts of CaPi [7, 8], in both IRCU patients and healthy humans the prevailing urinary crystal type is CaPi, not CaOx [9 – 11], and IRCU patients exhibiting urinary excess of Ox are the exception rather than rule, a finding apparently depending on the definition of the upper limit of Ox in healthy normals [12]. It follows that better understanding of IRCU pathophysioloy (see ref. 3 – 5, 13, 14) requires more information on variation of U-pH, the accompanying urinary state of stone and other substances, and whether the presence of stones

(the "crystallization end-products") can be brought into connection with those factors. In the past we had the opportunity to examine a larger series of kidney stone patients using a standardized laboratory program (see below) that included the intake of an acid test meal. Upon screening of data there was the impression that when in IRCU postprandial U-pH was low, the pH of fasting and daily urine was low too, and vice versa.

In the present work a larger spectrum of variables than is usually practiced was set up in IRCU patients. One aim was to demonstrate U-pH, urinary excretion of sodium, protein and other substances in response to acid meal challenge, the same parameters in fasting and, within limits, daily urine, together with several general features. Another aim was to demonstrate the variation of urinary crystallization risk of stone substance(s) along the observed U-pH, and whether the number of patients bearing stones varies too. Finally, we wanted to learn whether U-pH can be related to changes of renal-tubular function, parameters in blood, and general factors.

MATERIAL AND METHODS

STUDY PARTICIPANTS

For 237 consecutively examined adult male stone patients, age 24 – 66 years, the term IRCU was coined on the basis of the following: During the past 4 – 5 years all had at least one stone recurrence (for quantitation of stone formation activity see section Calculations), with the last dating back more than 1 month; the diagnosis "Ca stone" was made using polarization microscopy or X-ray diffractometry exclusively, showing no component other than phases of CaOx and CaPi; when at the time of laboratory investigation (see below) one stone or more, including unilateral "stone nests", were detectable by clinical techniques in the renal pelvis, calyces, papillae or further upstream parenchyma, these patients were designated stone-bearing (SB), all others stone-free (SF). Excluded were cases with bilateral nephrocalcinosis (an accepted sign of major renal-tubular dysfunction, such as in classic RTA [1, 2, 15] and a number of other rare disorders), patients with non-European ethnology, residence outside North Bavaria, females (for reasons see ref. 16), gastrointestinal abnormalities, essential hypertension, diabetes mellitus, primary hyperparathyroidism, oxaluria >0.5 mmol in daily urine (precluding the possibility that urinary Ox excess could have induced abnormal oxidative metabolism [17]), hematuria (dipstick-positive urine), signs of urinary tract obstruction, urinary tract infection with urease-producing germs, struvite (magnesium-ammonium phosphate) as stone component, post-renal sources of protein release (cystitis, prostatitis, etc.), spontaneous stone passage or surgical stone removal dating back less than 4 weeks. 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 are given. Upon written information, all subjects gave their consent to the envisaged laboratory investigations. The study was approved by the Ethics Committee of the medical school and carried out in accordance with the principles of the Declaration of Helsinki.

LABORATORY PROGRAM, TEST MEAL

Details of the standardized clinical examination and the 4-stages laboratory program [collection of 24 h urine at home, fasting venous blood and timed 2 h pre-acid meal load (PRAML) urine, intake of a meal, and collection of 3 h post-acid meal load (POAML) urine] have been described [18]. In short: 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 an ear-lobe punctured for blood gas analysis. Aliquots of plasma and paper-filtered (Whatman no. 3) urine were prepared and either analysed on the same day or stored at -80 °C. The basic mixture of the Ox- and purine-free carbohydrate-rich meal was commercially available (trade name Vivonex, supplied by Friesche Vlag, MA Leeuwarden, The Netherlands), its components were earlier reported [18]. After addition of calcium lacto-bionate (9.34 g) the calcium content was 25 mM (1000 mg), with a suspension in 300 ml demineralized water (approx. 500 mOsm/l) delivering approx. 120 milliequivalent proton (in this work synonymous with hydrogen (H)) according to in vitro hydrochloric acid dissolution and back-titration to pH 7.40 by sodium hydroxide. Routine application of this expanded laboratory program offered advantages: 1) unlike intake of nonmetabolizable ammonium chloride (0.1 g/kg body weight), traditionally in use for probing urine acidification [15], the intake of a metabolizable acid meal of constant composition allows to study the response of the kidney under physiological gastrointestinal conditions; 2) investigation of pH and other urinary parameters in the pertinent baseline PRAML period helps unmask pre-existing factors that might be able to influence the risk of crystallization of stone substances and stone formation.

DATA COMPILATION, STUDY DESIGN

Based on study objectives, data were compiled to give several parts. Part 1: The POAML U-pH was stratified (values ≤5.30: stratum Low; values >5.30: stratum High); additionally given were substance excretory rates and the ratio of excretion of sodium and hydrogen (Na_e/H_e). The same spectrum was given for PRAML urine, and a similar spectrum, including urea nitrogen excretion as a marker of dietary protein intake, was obtained for 24 h urine that was collected during eating free-choice home food. Part 2: The risk of crystallization of stone substances and stone formation was illustrated in several ways for POAML and PRAML urine (not 24 h urine): 1) the free energy that drives supersaturation toward homogeneous nucleation of uric acid, CaOx, hydroxyapatite (HAP) [19]; 2) the concentration of total (T) and undissociated (UD) uric acid, because CaOx nucleation has been reported to occur via "salting out effect" of the former, and because of epitaxial growth of CaOx upon pre-formed uric acid crystals (for details see ref. 14); 3) the

Table 1. pH and other variables in urine, general features of IRCU patients. Data are mean values (SE or range). For further information see footnotes and text.

	POAML U-pH Low	High	High vs. Low p-value	All	Normal†
	≤5.30	>5.30			
N^+	79	158		237	
a. POAML urine (excretion rates are per 3 h)					
pH	5.02 (4.46 – 5.30)	6.0 (5.30 – 7.48)	<0.001	5.67 (4.46 – 7.48)	<5.6
Sodium; mM	13.5 (0.8)	15.6 (0.7)	0.02^X	14.9 (1.3-86)	<30
Na_e/H_e; µM/nM	8 (0.5)	196 (37)	$<0.001^X$	133 (1 – 3140)	85 (40 – 1600)
Ca; mM	0.99 (0.05) [74]	1.08 (0.04) [144]	0.08	1.0 (0.16 – 2.8) [218]	<0.7
Pi; mM	2.77 (0.2)	2.50 (0.07)	0.03	2.59 (0.4 – 9.8)	2 (0.6 – 4)
Citrate; mM	0.39 (0.03) [70]	0.38 (0.02) [133]	0.43	0.38 (0.04 – 1.1) [203]	>0.30
Magnesium; mM	0.61 (0.02)	0.77 (0.03)	<0.001	0.72 (0.08 – 4.0)	>0.24
Potassium; mM	6.6 (0.7) [49]	7.2 (0.45) [99]	0.25	6.9 (0.5 – 27) [148]	<12
Oxalate; µM	30 (3) [77]	29 (1) [151]	0.39	29 (4 – 151) [228]	<50
Uric acid; mM	0.49 (0.03) [49]	0.52 (0.2) [99]	0.16	0.51 (0.1 – 1.1) [148]	<0.60
Protein; mg	5.2 (1.7) [48]	6.4 (0.8) [70]	0.005^X	5.9 (1 – 76) [118]	<16
Volume; ml	201 (16)	233 (12)	0.03^X	222 (48 – 720)	<310
b. PRAML urine (excretion rates are per 2 h)					
pH	5.84 (4.41 – 7.58)	6.37 (4.88 – 7. 60)	<0.001	6.19 (4.41 – 7.60)	<6.9
Sodium; mM	12.1 (0.7)	13.6 (0.5)	0.02^X	13.1 (2.3 – 47)	<12
Na_e/H_e; µM/nM	97 (27)	617 (95)	$<0.001^X$	445 (1 – 10570)	200 (50 – 4050)
Ca; mM	0.31 (0.02)	0.32 (0.01)	0.37	0.32 (0.03 – 1.3)	<0.5
Pi; mM	1.28 (0.08)	1.20 (0.07)	0.47	1.20 (0.1 – 3.9)	2.3 (1 – 5)
Citrate; mM	0.28 (0.02) [70]	0.27 (0.01)[133]	0.22	0.28 (0.04 – 1.5) [203]	>0.21
Magnesium; mM	0.23 (0.01)	0.27 (0.01)	0.01	0.25 (0.05 – 0.78)	>0.17
Potassium; mM	9.4 (0.5)	10.3 (0.4)	0.09	10 (0.6 – 36)	<14
Oxalate; µM	22 (2) [77]	24 (2) [151]	0.17	23 (1.3 – 193) [228]	<35
Uric acid; mM	0.36 (0.01)	0.41 (0.01)	0.01	0.39 (0.03 – 1.5)	<0.40
Protein; mg	4.4 (0.3) [74]	9.8 (1.9) [144]	$<0.001^X$	7.9 (0.8 – 16.2) [218]	<11
Volume; ml	224 (15)	265 (11)	0.02	251 (32 – 890)	<450
c. Daily urine (excretion rates are per 24 h)					
pH	5.7 (4.8 – 7.1)	6. 03 (4.9 – 7.2)	<0.001	5.93 (4.8 – 7.2)	>5.0
Sodium; mM	188 (9) [44]	176 (6) [94]	0.12	180 (42 – 390) [138]	<280
Na_e/H_e; µM/nM	107 (15) [47]	199 (37) [94]	0.02^X	168 (4 – 2707) [141]	nd
Citrate; mM	2.68 (0.17)	2.50 (0.11)	0.17	2.56 (0.24 – 10.1)	>2.1
Magnesium; mM	3.9 (0.12) [74]	4.1 (0.11) [144]	0.18	4.1 (0.12 – 9.2) [218]	>3.1
Potassium; mM	65 (4) [44]	64 (3) [84]	0.37	64 (15 – 198) [128]	<120
Uric acid; mM	3.7 (0.16)	3.8 (0.09)	0.43	3.7 (30 – 1310)	<4.2
Volume; ml	1586 (72)	1772 (49)	0.16	1743 (480 – 4200)	<2000
Urea-nitrogen; mM	458 (17) [78]	399 (11) [158]	<0.001	418 (82 – 989) [236]	<700
d. General features					
Age; y	44 (1)	42. 4 (0.8)	0.21	42.7 (24 – 66)	<60
Weight	85.4 (1.3)	80.9 (0.8)	<0.001	57-130	nd
Height; cm	177 (0.8)	175 (0.5)	0.05	160 - 200	nd
BMI; kg/(m)2	27.2 (0.3)	26.2 (0.2)	0.01	26.5 (19 – 40)	≤25.0††
MABP; mm Hg	108 (2) [67]	106 (1) [127]	0.30	107 (75) [194]	<105
ASFP; score	32 (3)	42 (3)	0.12	39 (1 – 271)	1
SB/SF	35/44	76/82	nd	111/126	

$^+$: number of patients, except [ ]; ^{X}: based on log data; †: data refer to limits or mean (range) of normalcy as observed in similarly aged healthy male volunteers (n = 8 – 13) in the authors' laboratory, or from literature; ††: generally accepted as upper limit; nd: not determined.

molar Ca/Pi ratio (the volume-independent marker of precipitation of Ca-poor CaPi, beginning at Ca/Pi values as low as 0.01) [20] which in biological systems is considered as Ca sink that in turn drives the transformation of amorphous CaPi to Ca-rich HAP crystals [21]; 4) the concentration of total protein (in this work conceived as a pool of proteinaceous modifiers (promoters and inhibitors) of both heterogeneous nucleation and aggregation of CaOx crystals [22, 23]); 5) the concentration of citrate and magnesium, well-doc-

umented small-molecular inhibitors of Ca salt crystallization [24]; 6) presentation of the effect of variation of the aforementioned crystallization risk factors, U-pH, Na_e/H_e upon the associated number of SF and SB patients as observed on the day of laboratory investigation (with the underlying idea being that Ca stones represent the end-products of all stone-forming processes (see ref. 3, 4)). Part 3: From the PRAML period of the two groups of patients as in part 1 complementary data are given, emphasizing renal-tubular net reabsorption of substances (ratio filtered/excreted; FE) and concentration of several substances in blood. Part 4: Interrelationships of variables are given.

The design of the study was cross-sectional (comparison of strata Low and High of POAML U-pH) and correlative. The overlap of patients in present and previous studies, using different strategies and outcomes [16, 25, 26], was $80 - 90\%$.

ANALYSES

Routine methods or well-established techniques (see ref. 18, 27) were utilized, including the 14 analytes required for estimation of urinary supersaturation (for details see ref. 19) with stone substances. Exceptions were U-pH (by glass electrode), high-performance liquid chromatography measurement of oxypurines [28], blood pH and bicarbonate (by Blood Gas Analyzer), preparation of plasma ultrafiltrate (using a <10 kD pore size cellulose nitrate membrane and N_2 pressure), Ox in plasma ultrafiltrate, thawed and acidified (pH ≤1.5) urine [29], bone collagen crosslinks in urine [30], urinary total protein by colorimetry [31], plasma osmolarity by freezing point depression (Osmometer; Knauer, Berlin, FRG), urinary 3,5-cyclic adenosine monophosphate (cAMP) and plasma insulin by in-house radioassays.

CALCULATIONS, STATISTICS

From both the stone events reported in disease history and the situation as demonstrated in the laboratory (see above) a score was calculated as previously reported [28], approximating the actual activity of stone-forming processes (ASFP). FE and the conversion of U-pH to H concentration were conventionally calculated, UD Uric acid was derived from urinary T-Uric acid, using pK 5.35 [32]. The renal threshold Pi concentration relative to glomerular filtration rate (Tm-Pi) was read from nomogram [33]. Urinary supersaturation was calculated by EQUIL-2, with the free energy expressed as DG [19]. Non-Gaussian distribution of data was frequent, but in numerous instances log_{10} of numerical values gave symmetric data, allowing application of an unpaired or paired Student's t-test, otherwise the Wilcoxon test was used. For simplicity, results were given as mean values (SE and range), except in Fig. 1 A and B (medians and ranges), Table 2 (medians). Categorical data were examined by χ^2-test. Possible linkages of variables were tested by simple correlations (Pearson) and logistic multivariate (with stepwise deletion) regression analysis. The level of significance was set as p ≤0.05. The STATISTICA software was used (Statsoft, Tulsa, OK, USA).

RESULTS

pH, VOLUME AND SUBSTANCE EXCRETORY RATES OF URINE, GENERAL FEATURES (PART 1)

According to Table 1 (strata Low and High, All) out of 237 patients only 79 were able to achieve POAML U-pH ≤5.30, but surprizingly more than 60 per cent failed to decrease U-pH below this limit (in contrast, in 2 of 12 healthy volunteers the pH response to the meal was >5.3 but <5.6; see column Normal). The High patients exhibited, in addition to inappropriately high POAML U-pH, increase of volume, sodium, magnesium, protein, elevation of Na_e/H_e and decrease of Pi. PRAML U-pH was also elevated in the High vs. Low patients, and there was concomitant increase of volume, sodium, magnesium, Na_e/H_e, uric acid and protein. Worthy of note, in the strata Low and High a total of 15 patients exhibited PRAML U-pH of $5.14 \pm SE\ 0.03$ (range 4.88 – 5.30) which in POAML urine paradoxically rose to 5.96 SE $\pm$ 0.11 (range 5.32 – 6.57), showing in addition a marked increase of excretion per 1 hour of Pi (fasting: 0.74 ± 0.1 mM; postprandial: 1.02 ± 0.11 mM; p = 0.01) and magnesium (fasting: 0.13 ± 0.01 mM; postprandial: 0.24 ± 0.02 mM; p <0.001), but only insignificant change of excretion of sodium, citrate, oxalate, protein, and Na_e/H_e. In daily urine of the High patients urea-nitrogen was decreased but pH and Na_e/H_e were increased; also, they were significantly less overweight and obese (BMI >25.0 in 99 out of 158 (63%)) vis-à-vis the Low patients (BMI >25.0 in 61 out of 79 (77%)), but age, mean ((systolic + diastolic)/2) blood pressure (MABP) and the ASFP score were statistically indistinguishable in both strata.

URINARY CRYSTALLIZATION RISK, RENAL STONES IN SITU (PART 2)

The propensity of urine to crystallize stone substances is shown in Fig. 1: In POAML urine, with pH ≤5.30, the order of median pressure toward homogeneous nucleation was Uric acid-DG > CaOx-DG > HAP-DG; in contrast, when POAML U- pH was >5.30, the order was Uric acid-DG > HAP-DG > CaOx-DG (left panel, A-1 – A-3). The corresponding PRAML urine showed a similar order (left panel, B-1 – B-3), although this urine was not subject to biassing by ingestion and metabolism of nutrients. In highly vs. less acidic POAML urine the concentration of T-Uric acid, UD-Uric acid and citrate was increased, magnesium unchanged, Ca/Pi ratio and protein decreased (Fig. 1, right panel, C-1 – C-6). In contrast, in the corresponding PRAML urine the concentration of T-Uric acid was unchanged and UD-Uric acid low (due to the higher U-pH), magnesium was dramatically lower than postprandially, and protein was increased (Fig. 1, right panel, D-1 – D-6).

The impact of the above criteria on the presence of stones was as follows: In POAML urine the frequency distribution of SF and SB patients was only insignificantly modulated (data not shown). Table 2 shows that in PRAML urine the crystallization risk factors (see Fig. 1, B-1 – B-3 and D-1 – D-6), pH and Na_e/H_e failed to modulate significantly the SF/SB ratio (note that the observed was contrasted with the expected
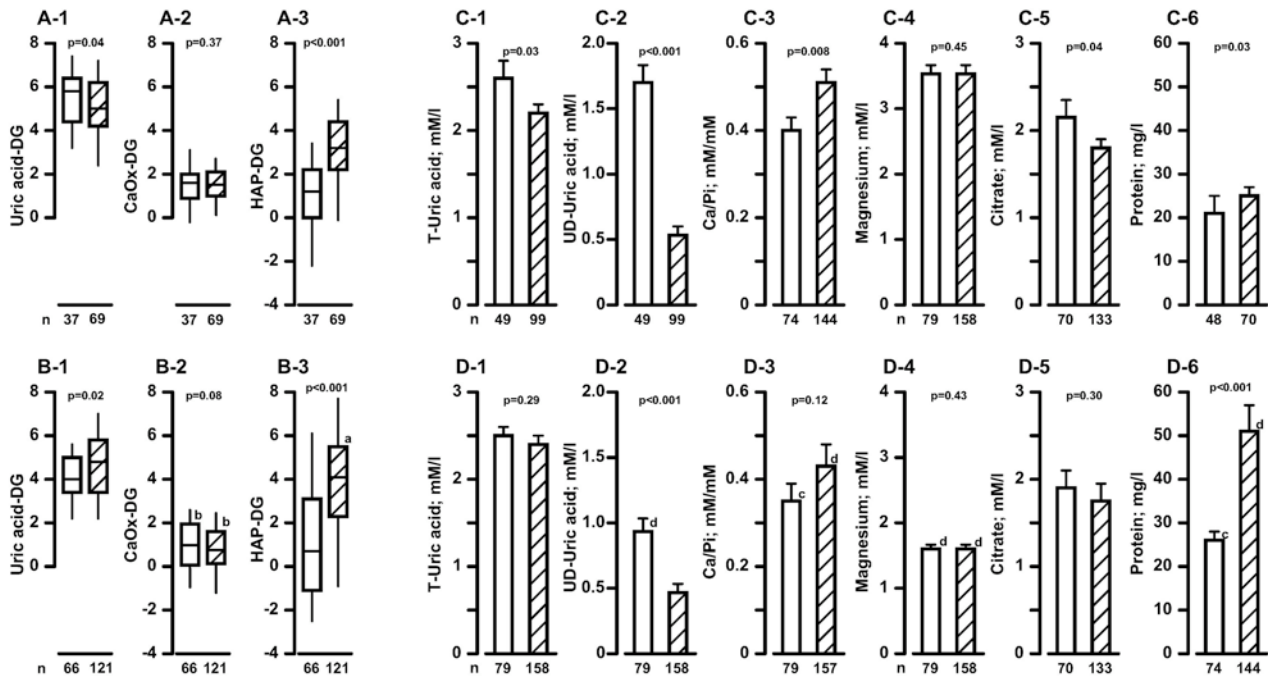
Fig. 1. Urine supersaturation with several stone substances and crystallization modifiers. *Left:* Drive towards crystallization of urinary uric acid, CaOx and HAP expressed as free energy (DG) in POAML (A-1 – A-3) and corresponding PRAML (B-1 – B-3) urine. Note that DG = 0 equals saturation, positive and negative DG equal pressure toward nucleation and undersaturation (synonymous dissolution), respectively (19). Light symbols indicate POAML U-pH ≤5.30, hatched symbols POAML U-pH >5.30, boxes the central 50%, vertical lines 90% (range 5 – 95%), thin horizontal lines the median of all observed values (n); p-values relate to differences in the two types of urine in series A and B (for numerical median values see Table 2); [a, b]: denote that log data in B and corresponding log data in A differ at p <0.05 and p <0.001, respectively. *Right:* Urinary concentration of total (T) and undissociated (UD) uric acid, magnesium, citrate, total protein and Ca/Pi ratio in POAML (C-1 – C-6) and corresponding PRAML (D-1 – D-6) urine. Columns indicate mean values, bars standard error. For light and hatched symbols and p-values see above; n: number of observations; [c, d]: denote that log data in D and corresponding log data in C differ at p <0.002 and p <0.001, respectively.
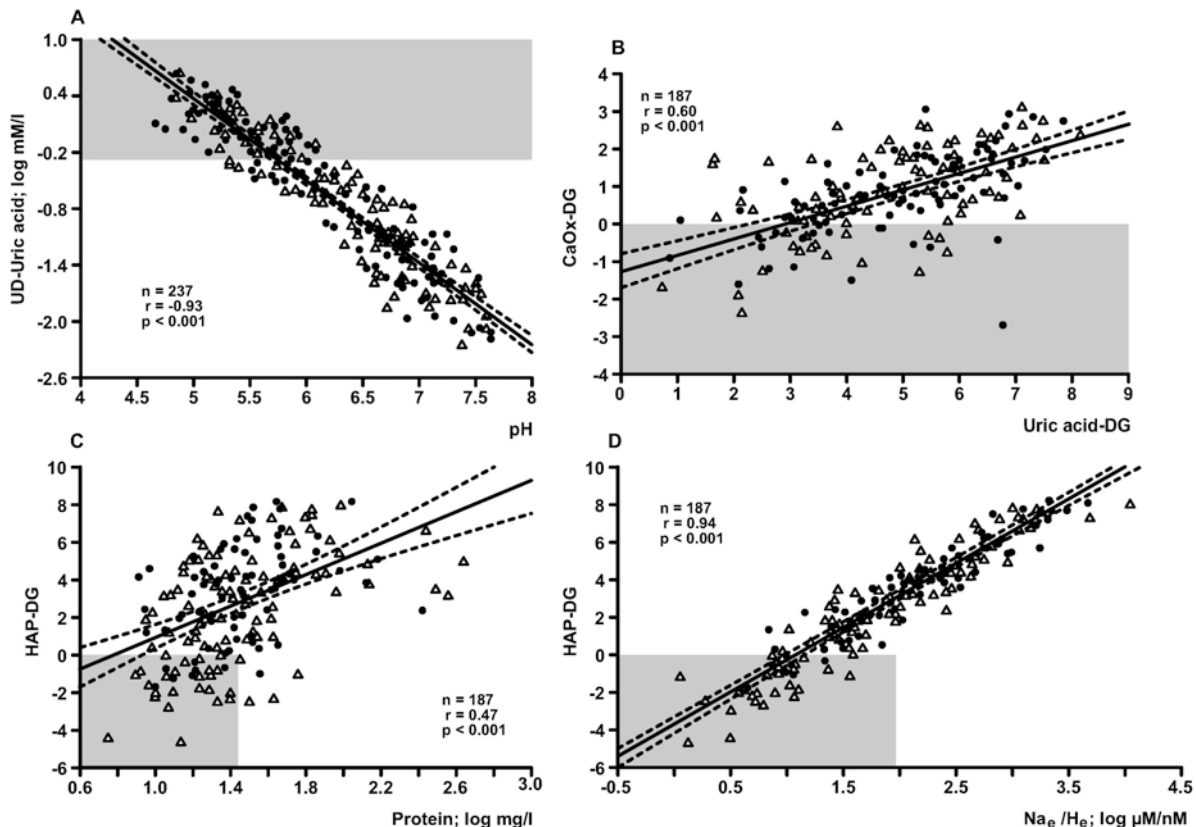
Table 2. Frequency distribution of SF and SB patients in the strata Low and High according to median of pH in PRAML urine. For further information and abbreviations see text and Fig. 1.

	Dimension	n	Median	Low (< median) Expected[1] SF/SB	Σ	Observed SF/SB	Σ	p-value[2]	High (≥ median) Expected[1] SF/SB	Σ	Observed SF/SB	Σ	p-value[2]
pH		237	6.22	64/54	118	72/46	118	0.13	64/55	119	54/65	119	0.059
Na_e/H_e	Log μM/nM	236	1.97	64/54	118	66/52	118	0.67	64/54	118	59/59	118	0.38
Uric acid	DG	187	5.0	52/44	96	49/47	96	0.56	49/42	91	48/43	91	0.81
CaOx	DG	187	0.84	51/43	94	51/43	94	0.96	50/43	93	46/47	93	0.38
HAP	DG	187	3.23	51/43	94	52/42	94	0.80	50/43	93	45/48	93	0.28
T-Uric acid	mM/l	237	2.25	70/60	130	72/58	130	0.75	58/49	107	59/53	107	0.46
UD-Uric acid	mM/l	237	0.26	64/55	119	53/66	119	0.038	64/54	118	73/45	118	0.087
Ca/Pi	Log mM/mM	236	-0.59	64/54	118	67/51	118	0.54	64/54	118	59/59	118	0.38
Magnesium	mM/l	237	1.29	64/55	119	66/53	119	0.75	64/54	118	60/58	118	0.49
Citrate	mM/l	203	1.31	55/47	102	49/53	102	0.23	55/46	101	52/49	101	0.61
T-Protein	Log mg/l	218	1.43	58/50	108	56/52	108	0.65	59/51	110	52/58	110	0.16

n: number of observations; [1]: on the basis of SF (n = 126) and SB (n = 111) patients at entry into study (see table 1, column All); [2]: by χ^2 test; Σ: SF + SB.

SF/SB ratio). However, when UD-Uric acid concentration was low (mainly due to high U-pH; 32), there was a significant (p = 0.038) shift toward more SB patients, and a similar tendency existed for high pH (p = 0.059); conversely, high UD-Uric acid tended to have the opposite effect (p = 0.087). Also in the PRAML period there were correlations of urinary UD-Uric acid with pH (negative, $r^2 = 0.59$), T-Uric acid concentration (positive, $r^2 = 0.28$) and HAP-DG (negative, $r^2 = 0.11$), together explaining most of the variation of crystal-forming UD-Uric acid. Furthermore, SF outnumber SB patients by far when the solubility limit of UD-Uric acid was exceeded (Fig. 2 A, shaded area, SF/SB 55/29); log UD-Uric acid correlated positively with CaOx-DG (r = 0.34, n = 187, p <0.001), but CaOx-DG correlated stronger with Uric acid-DG (Fig. 2 B). Notably, while the latter was always positive, CaOx-DG was often negative (indicating CaOx undersaturation and dissolution [19]) despite the fact that numerous patients bore stones (Fig. 2 B, shaded area, SF/SB 21/18) and the correlation of CaOx-DG and HAP-DG was only borderline (r = 0.14, n = 187, p = 0.06). In contrast, HAP-DG correlated with log protein concentration (Fig. 2 C), stronger with log Na_e/H_e (Fig. 2 D); worthy of note, despite often negative HAP-DG the SF/SB ratio in the latter two situations was dramatically decreased to 7/24 and 7/26, respectively (Fig. 2 C and D, shaded areas).

COMPLEMENTARY VARIABLES IN PRAML PERIOD (PART 3)

According to Table 3, Low and High patients (conforming to those in the strata Low and High of Table 1 a) did not differ with respect to urinary creatinine clearance (assumed measure of glomerular filtration rate). However, the High patients exhibited significant increase of FE-Protein, FE-Sodium, FE-Magnesium, FE-Uric acid, whereas there was no change of Tm-Pi (the renal threshold concentration for plasma Pi), urinary cAMP, deoxypyridinium and hypoxanthine (markers of parathyroid hormone bioactivity at the level of kidney and bone, respectively [30], and tissue hypoxia [28]; all data not shown for the sake of space). In the Low patients plasma uric acid and insulin were increased, glucose, triglycerides (and other lipids such as cholesterols, free fatty acids; data not shown) unchanged, whereas the glucose/insulin ratio (a crude surrogate marker of resistance of peripheral organs to the actions of insulin [34]) and blood bicarbonate were decreased.

RELATIONSHIP OF PRAML U-pH AND OTHER VARIABLES (PART 4)

These data are presented because U-pH is conceived as integral of extrarenal and renal-cellular H-generat-

◄ *Fig. 2.* Relationship of PRAML U-pH and urinary log UD-Uric acid (A), uric acid-DG and CaOx-DG (B), log protein concentration and HAP-DG (C), log Na_e/H_e and HAP-DG (D). • and Δ are SF and SB patients, respectively. The shaded areas include: in A the patients above log solubility limit of UD-Uric acid (0.54 mM/l; [32]), in B the patients with negative CaOx-DG (indicating undersaturation), in C the patients with negative HAP-DG and low (< median) log protein concentration, in D patients with negative HAP-DG and low (< median) log Na_e/H_e. Note that within the shaded areas the SF/SB ratio is 55/29 (A), 21/18 (B), 7/24 (C), 7/26 (D), and that numerous symbols stand for more than one patient. For abbreviations and further informations see text and Table 2.

Table 3. Complementary data from the PRAML period of patients in the subgroups Low and High of table 1 (see there for number of patients, pH, excretion rates of substances, symbols, and other informations).

	Low	High	High vs. Low p-value	All	Normal
Renal transport					
Creatinine clearance; ml/min	120 (4)	120 (3)	0.47	120 (50 – 313)	>50
FE-Protein x 1000; %	0.46 (0.04) [74]	1.1 (0.14) [144]	<0.001	0.9 (0.1 – 16) [218]	<0.5
FE-Sodium; %	0.63 (0.04) [79]	0.72 (0.03) [157]	0.03	0.69 (0.1 – 2.1) [236]	<0.5
FE-Ca; %	1.5 (0.1) [79]	1.6 (0.1) [157]	0.35	1.6 (0.3 – 5.1) [236]	<2
FE-Pi; %	9.7 (0.5) [79]	8.9 (0.4) [157]	0.12	9.2 (1.0 – 28) [236]	<20
FE-Citrate; %	19 (1) [69]	21 (1) [129]	0.21	21 (2 – 110) [198]	<20
FE-Magnesium; %	2.5 (0.1) [79]	2.9 (0.1) [157]	0.02	2.7 (0.7 – 7.5) [236]	<4
FE-Potassium; %	13 (0.5) [78]	14 (0.5)[155]	0.10	14 (3 – 36) [233]	<20
FE-Ox; %	117 (10) [29]	114 (6) [51]	0.41	115 (33 – 245) [80]	<150
FE-Uric acid; %	7.3 (0.3) [79]	8.5 (0.3)[157]	0.003	8 (0.7 – 24) [236]	<11
Tm-Pi†; mM/l	1.01 (0.03) [79]	1.04 (0.02) [158]	0.15	1.03 (0.58 – 1.7) [237]	>1.2
Blood (B), plasma (P), plasma ultrafiltrate (PU)					
P-Total protein; g/l	71 (0.5) [79]	71 (0.3) [158]	0.43	71 (61 – 82) [237]	
P-Osmolarity; mOsm/l	294 (1) [44]	297 (2) [83]	0.16	296 (200 – 351) [127]	<315
P-Sodium; mM/l	143 (0.4) [79]	143 (0.2) [158]	0.10	143 (133 – 152) [237]	<145
PU-Ca; mM/l	1.47 (0.01) [74]	1.47 (0.01) [144]	0.37	1.47 (1.29 – 1.70) [218]	<1.5
P-Pi; mM/l	0.97 (0.02) [79]	0.99 (0.01) [158]	0.28	0.98 (0.62 – 1.6) [237]	>1.0
P-Citrate; mM/l	0.12 (0.01) [74]	0.11 (0.0) [141]	0.06	0.11 (0.02 – 0.65) [215]	>0.11
PU-Magnesium; mM/l	0.66 (0.01) [79]	0.66 (0.0) [158]	0.39	0.66 (0.3 – 0.91) [237]	>0.54
P-Potassium; mM/l	4.21 (0.03) [78]	4.21 (0.02) [156]	0.49	4.21 (3.5 – 5.6) [234]	<5.0
PU-Oxalate; μM/l	1.77 (0.08) [33]	1.75 (0.06)[57]	0.44	1.76 (0.9 – 3.3) [90]	<3
P-Uric acid; μM/l	377 (9) [79]	354 (5) [158]	0.009	361 (159 – 632) [237]	<390
B-Bicarbonate; mM/l	23.3 (0.2) [77]	23.8 (0.2) [153]	0.03	23.6 (18 – 31) [230]	>18
B-pH	7.40 (0.0) [77]	7.41 (0.0) [153]	0.19	7.40 (7.34 – 7.49) [230]	>7.34
P-Triglycerides; mM/l	1.6 (0.14) [69]	1.6 (0.08) [133]	0.41	1.6 (0.3 – 7.3) [202]	<1.8
P-Insulin; pM/l	143 (13) [72]	105 (7) [143]	0.007[x]	118 (9 – 644) [215]	<145
P-Glucose; mM/l	5.0 (0.08) [74]	4.9 (0.05) [142]	0.11	4.9 (3.6 – 6.8) [216]	<5.0
P-Glucose/P-Insulin; μM/pM	68 (10) [72]	90 (8) [142]	0.008[x]	83 (8 – 523) [214]	>40

†: renal Pi threshold relative to creatinine clearance (33)

ing and -buffering processes (including the exchange of sodium for H (NHE) [35], as a possible proxy of which Na_e/H_e was tentatively monitored in present work). Three blocks (models) of correlations are given (Table 4): model 1 – systemic metabolic factors; model 2 – net renal-tubular transport (FE) of substances; model 3 – urinary Na_e/H_e, protein concentration. U-pH was significantly negatively correlated with BMI, insulinemia and uricemia, significantly positively correlated with FE of protein, sodium, Ca, potassium, uric acid, Na_e/H_e and blood bicarbonate (Table 4 A). Multivariate regression analysis, restricted to simple correlations with p-values ≤0.15, identified insulinemia and uricemia (model 1), FE-Protein, FE-Sodium, FE-Pi (model 2), urinary Na_e/H_e and protein concentration (model 3) as remaining significant determinants, together accounting for appox. 60% of the variation of PRAML U-pH (Table 4 B).

DISCUSSION

We can show that when in IRCU the pH of POAML and PRAML urine is low, this was associated with a lower risk for HAP crystallization and Ca stones, higher body weight, BMI and insulinemia, but when U-pH is high the reverse seems to develop. Several comments appear justified.

RENAL CA STONE FORMATION REFLECTS VARIATION OF U-pH AND PHYSICAL-CHEMICAL PROPENSITY TO FORM CRYSTALS?

Given stones evolve from crystals formed in tubular fluid and urine with high supersaturation and/or deficit of crystallization inhibitors [4 – 6], one would expect that in present work such characteristics are mainly exhibited by SB patients. According to data, this

Table 4. Relationships of U-pH (= dependent) with other variables in the PRAML period.

A. Univariate					B. Multivariate		
Influential	Dimension	n^+	r	p-value	Beta	SE	p-value
Model 1:							
BMI	kg/(m)^2	237	-0.28	<0.001	ni-1		
P-Insulin	pM/l†	214	-0.30	<0.001	-0.21	0.07	0.004
P-Uric acid	µM/l	237	-0.23	<0.001	-0.15	0.07	0.04
B-Bicarbonate	mM/l	230	0.23	<0.001	0.12	0.07	0.07
					N^{++} = 207; R^2 = 0.11 (0.05 x 10^{-3})		
Model 2:							
FE-Protein x 1000	%†	218	0.33	<0.001	0.26	0.07	<0.001
FE-Sodium	%†	236	0.22	<0.001	0.16	0.07	0.02
FE-Ca	%†	236	0.14	0.03	0.06	0.08	0.44
FE-Pi	%†	236	-0.09	0.15	-0.20	0.08	0.01
FE-Citrate	%†	198	0.11	0.14			
FE-Magnesium	%†	236	0.11	0.09	0.08	0.07	0.25
FE-Potassium	%†	233	0.25	<0.001	ni-2		
FE-Ox	%†	80	0.11	0.34			
FE-Uric acid	%†	236	0.23	<0.001	0.12	0.08	0.11
					N = 217; R^2 = 0.13 (0.05 x 10^{-3})		
Model 3:							
U-Na$_e$/H$_e$	µM/nM†	236	0.62	<0.001	0.64	0.06	<0.001
U-Protein	mg/l†	218	0.13	0.07	-0.13	0.06	0.03
					N = 217; R^2 = 0.36 (0.05 x 10^{-3})		

+: number of pairs; r: coefficient of simple correlation; ni-1, ni-2: Beta (= partial regression coefficient) not calculated (inclusion would cancel the influence of P-Insulin and FE-Sodium, respectively); †: $\log_{10}$ data; ++: number of pairs in the models 1 – 3; R^2: squared coefficient of multivariate models (after adjustment for confounders); ( ): level of significance of the model; U: urine; P: plasma; B: blood

is not the case, however. After all, low CaOx-DG vis-à-vis high Uric acid-DG and high HAP-DG (see Fig. 1) renders unrealistic that CaOx crystallization was the primary stone-initiating event. Conversely, in PRAML urine, the combination of low UD-Uric acid (hence low uric acid crystalluria) with high pH and increase of SB patients (Table 2) would agree with facilitation of pairing of Pi and Ca ions via increasing deprotonation of Pi anion when ambient pH is only slightly acid or even alkaline [19]. In urine of IRCU patients with pH 6.0 the induction of HAP crystallization by Ca excess was regularly associated with co-development of CaOx crystals, compatible with heterogeneous CaOx nucleation [26]; HAP crystals have been demonstrated to serve as template for CaOx crystallization [36]. In present work, it appears that along decreasing H export via urine Ca-poor CaPi phases (starting at molar Ca/Pi 0.01 [20]) accumulate Ca (Fig. 1 C-3 and D-3), ending up in a rise of HAP-DG (Fig. 1 A-3 and B-3). It follows that Ca stone formation should be mainly due to a scenario in which renal H export steadily decreases but urinary HAP-DG steadily rises (present work, ref. 19), followed by Ca-rich urinary crystals [10, 11]. If true, the corollary should be that at low U-pH (and assumed abundance of uric acid crystals) less or no Ca stones but instead uric acid or mixed stones were formed. However, cases with uric acid as stone component were excluded from present work. Besides, we are not aware of reports demonstrating that in IRCU low

U-pH and abundance of uric acid crystalluria are prerequisites for the development of pure Ca stones, although from experiments in vitro uric acid crystal-induced formation of CaOx crystals via epitaxy has been reported, i.e., due to similarities of the crystal lattice of the two substances [37]. Therefore, the numerous SB patients exhibiting UD-Uric acid concentration above the solubility limit (Fig. 2 A) may have formed their Ca stone(s) independent of U-pH and the state of urinary uric acid, and at a site remote from urine and/or tubular fluid (see below).

IRCU PATHOPHYSIOLOGY – KEY TO UNDERSTANDING ARE BMI, INSULINEMIA, PROTEINURIA?

Ca-containing stones were reported to be more frequent when BMI is <25 (synonymous normal) than when BMI is ≥25 - 30 (synonymous overweight) and >30 (synonymous obesity) [38], and in idiopathic nephrolithiasis body weight was found to vary inversely with pH of 24 h urine [39]. Unfortunately, those reports did not communicate additional clinical chemistry and biochemistry data, obscuring which weight- and BMI-related anomaly of metabolism varies together with urinary stone components [38] and pH [39]. Weight gain and obesity reduce the sensitivity of tissues to the actions of insulin, with the consequence of hypersecretion of insulin to counterbalance insulin resistance [40]. In present work the correlation of weight

and plasma insulin (r = 0.50, n = 214, p <0.001) indicates tight coupling of these variables, and in the PRAML period the combination of low urinary sodium excretion and pH (Table 1), unchanged plasma osmolarity and elevated insulinemia (Table 3) would agree with involvement of insulin in renal H release regulation, reclaim of sodium [41] and its deposition at osmotically inactive sites [42]. The origin of increase of proteinuria is unclear; it may be not a consequence of the presence of stones, i.e., mechanical abrasion of tissue and protein shedding (P.O. Schwille and J. Wipplinger, manuscript in preparation), and based on present work not due to post-renal protein sources (see Material and methods) or impairment of glomerular sieving function. Therefore, in the majority of patients the higher degree of protein excretion (Table 1, stratum High), protein concentration (Fig. 1, C-6 and D-6) and FE-Protein (Table 3, High) should reflect increase of protein manufacturing by renal tissue.

Malregulation of U-pH and Tubular Transport – Manifestation of Renal Damage?

In healthy humans the renal regulation of H excretion is accomplished via, among others, glutaminase-mediated synthesis of ammonia and its excretion as ammonium, activity of the H-extruding tubular NHE and cell energy-consuming H-ATPases [35], with the latter be-

ing magnesium-dependent [43]. Given in IRCU as a whole, ammonium excretion is decreased [44] and renal reclaim of bicarbonate unimpaired (blood bicarbonate and U-pH are positively correlated; Table 4), in the majority of patients a fall, not a rise of POAML U-pH (Table 1, High) should have been developed. Thus, the cause of the inappropriately high U-pH in the said majority is unknown, but may be sought in impairment of H translocation by NHE, magnesium-sensitive H-ATPase (note that low magnesium status may be among the characteristics of IRCU [25]), or some combination. If true, alteration of intracellular ion milieu and impaired functionality of ion transport within the nephron should ensue. Indicative of the latter in present work could be the increase of sodium, magnesium and Pi in POAML urine of the subset of 15 patients with PRAML U-pH ≤5.30 (see Results, part 1). Urinary loss of magnesium and Pi may develop independent of activity of parathyroid hormone [45] as, for example, epiphenomenon of primary sodium loss. Thus, among the factors causing diminished renal H generation in IRCU may be low cellular magnesium [46], the often reported low Pi status (note that hypophosphatemia has deleterious effects upon vitally important cell functions [47]), overweight and obesity (in present work frequent (Table 1 d), and in numerous countries considered as epidemics [48] contributing to insulinemia and proteinuria [49]); finally, diminished renal uric acid reabsorp-
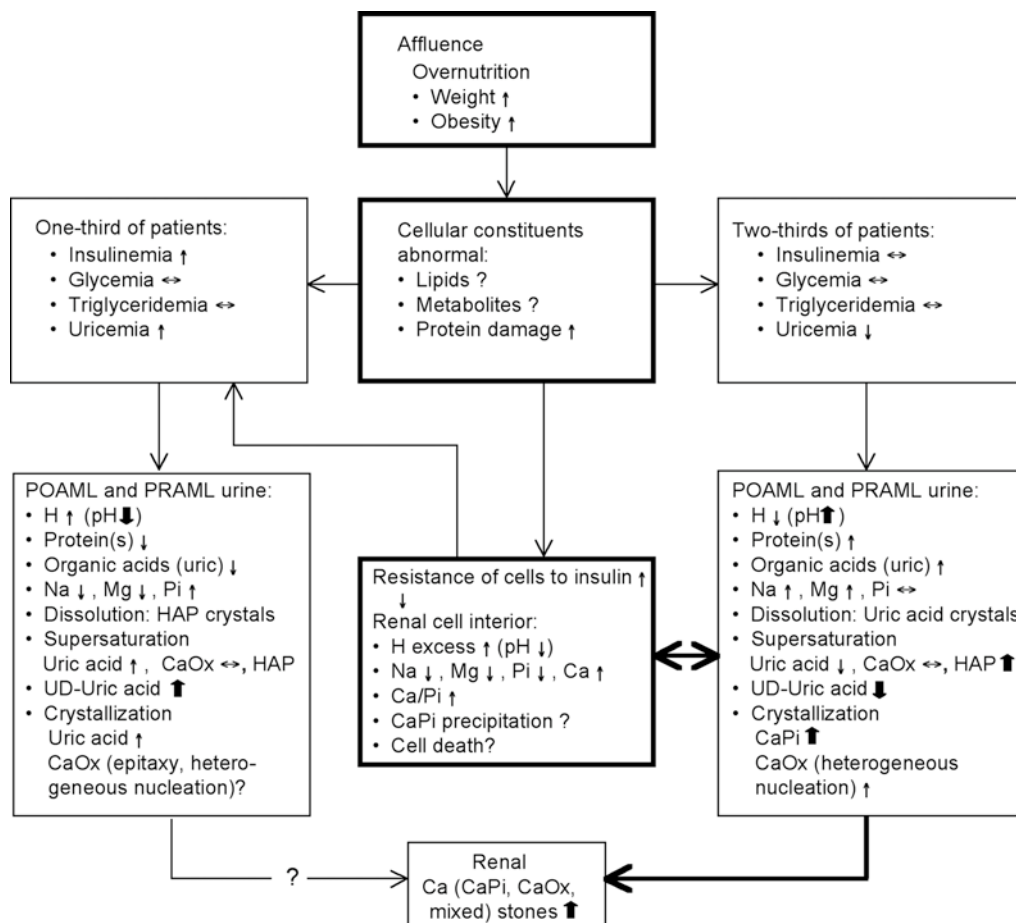

Fig. 3. Tentative schematic representation of IRCU pathophysiology, as based on data of present work and literature information; note the possible crucial role of overnutrition in the creation of abnormal cellular milieu [48]. Na: sodium; Mg: magnesium; ↑, ↓, ⟷, ?: increase, decrease, no change, undecided, respectively; thick frames and thick arrows, respectively: key events.

tion may underlie the low uricemia of the High patients (Table 3), in turn exposing renal tissue to antioxidant deficit [16, 50], causing damage via oxidative stress.

INHOMOGENEITY OF IRCU – INDICATOR THAT RENAL CA STONES ORIGINATE FROM CELLS?

Contrasting with the traditional concept of IRCU as a single disease entity, our data suggest that at least with respect to renal, not systemic, acid-base regulation two populations exist: one that can tolerate the intake of acid food, presumably via activation of H excretion via urine, and another showing defective H excretion. Interpretation of the sequence of events that lead to precipitation of Ca, Pi and Ox, and correct in vivo localization of the renal anatomic site, are not feasible with currently available technologies. However, the co-existence of Ca stones in situ with low PRAML urinary Na_e/H_e, HAP undersaturation and low protein concentration (Fig. 2 C, D) would be compatible with the view that for nascent Ca stones renal tissue may be the birthplace [51], rather than intrarenal and urinary tract fluids. In red blood cells of IRCU a type of mineral imbalance exists that theoretically favors HAP crystallization: a combined decrease of sodium and magnesium (an important inhibitor of HAP crystallization [52]) but rise of Ca/Pi [16]. Finally, in an animal model of transplanted renal tubular cells, development of HAP inside cells (cytoplasmic pH range is approx. 6.5 – 7.2) has been reported [53].

CONCLUSIONS

Despite limitations of present work (no assessment of nature and degree of crystalluria, no defined control group studied), the data allow to infer that in IRCU U-pH varies within a wide range, determined by renal capacity to generate H and regulate the transport of substances, systemic metabolism (insulinemia), anthropometric features such as body weight and nutritional factors (protein content of food). Insofar Ca stone development via low U-pH and increase of urinary uric acid supersaturation remains elusive, but inappropriately high U-pH together with increase of HAP supersaturation may indicate that a major risk for Ca stones exists. To illustrate the possible pathways of Ca stone formation, the now available information may be composed to give Fig. 3. Future controlled studies of SF and SB patients appear worthwhile, focussing on renal tissue and cell sampling and analysis, combined with interrelationships of U-pH and the dominant type of Ca concretion.

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