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Postprandial hyperinsulinaemia, insulin resistance and inappropriately high phosphaturia are features of younger males with idiopathic calcium urolithiasis: attenuation by ascorbic acid supplementation of a test meal

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Abstract In idiopathic recurrent calcium urolithiasis (RCU) the state of insulin and carbohydrate metabolism, and relationships to minerals such as phosphate, are insufficiently understood. Therefore, in two groups of males with RCU (n = 30) and healthy controls (n = 8) the response to an oral carbohydrate- and calcium-rich test meal was studied with respect to glucose, insulin, and C-peptide in peripheral venous blood (taken before and up to 180 min post-load), and phosphate and glucose in fasting and post-load urine. In one RCU group (n = 16) the meal was supplemented with ascorbic acid (ASC; 5 mg/kg body weight). The mean age (RCU 29, RCU + ASC 30, controls 27 years) and mean body mass index [RCU 24.4, RCU + ASC 25.0, controls 24.0 kg/m^2] were similar. Insulin resistance (synonymous sensitivity of peripheral organs to insulin) was calculated from insulin serum concentration, as was also integrated insulin, C-peptide, and glucose. Untreated stone patients (RCU) developed hyperinsulinaemia between 60 and 120 min post-load, increased integrated insulin, and insulin resistance ($P \leq 0.05$ vs controls), whereas the rise of C-peptide and glycaemia (absolute and integrated values) was only of borderline significance. Fasting phosphaturia was low in both RCU subgroups vs controls; however, phosphaturia in untreated RCU rose in response to the meal, contrasting sharply with a decrease in controls. ASC supplementation of the meal (in the RCU + ASC subgroup) normalized insulin, failed to normalize post-load phosphaturia, but reduced post-load glucosuria and urinary pH significantly (mean pH values 5.55 vs 5.93 in untreated RCU, controls 5.50). Postprandial urinary oxalate, calcium, protein, and supersaturation products were not changed. The postprandial changes in phosphaturia and insulin sensitivity were inversely correlated (n = 38, r = –0.44, P = 0.007). It was concluded that in younger RCU males: (1) postprandial hyperinsulinaemia, the failure to reduce phosphaturia and – within limits – glucosuria, appropriately, as well as poor urine acidification are important features of the metabolism; (2) these phenomena are probably caused by insulin resistance of organs, the kidney included; and (3) the addition of a supraphysiological dose of ASC to a meal, the subsequent abolition of hyperinsulinaemia, and the restoration of normal urine acidification suggest that this antioxidant is capable of counteracting some pre-existing basic abnormality of cell metabolism in RCU.

Key words Idiopathic calcium urolithiasis · Test meal · Hyperinsulinaemia · Insulin resistance · Inappropriate phosphaturia · Ascorbic acid effects

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Introduction

In the pathophysiology of idiopathic recurrent calcium urolithiasis (RCU) in humans, nutrition-related factors play an important role (for details see refs. [8, 9]). For example, it was long believed that calcium-containing stones are simply a result of an overconsumption of animal proteins, as shown by the so-called stone waves after each of the two World Wars. This concept has since been challenged by studies that failed to identify nutrient affluence as a regular feature in the history of stone patients (for overview see refs. [9, 15]). We [52], and others [9, 38], have therefore postulated the existence in RCU of some intrinsic factor(s) leading to an inappropriate response of metabolic processes, including, perhaps, those controlling nutrient fluxes from the gut lumen into the blood, the degradation of nutrients to intermediary substances, and the elimination of waste substances via urine. With respect to glycaemia and insulinaemia the nature of such interrelationships is not well understood.

In RCU, hyperinsulinaemia and glucose intolerance have been observed following glucose challenge [46, 51], as has also hypersecretion of pancreatic B-cell-connecting (C-)peptide [51]; the latter indicates that the hyperinsulinaemia is due, at least in part, to hypersecretion. Other investigators have reported that RCU patients are maladapted to refined carbohydrate, as reflected by the high level of calciuria following oral glucose intake [8, 38]. On the other hand, the levels of β-carotene and α-amino-nitrogen, marker substances in peripheral blood reflecting the degree of intestinal digestion and absorption of lipids and proteins, respectively, remain unremarkable, whereas the associated postprandial phosphaturia tends toward high values [51]. In healthy humans exogenous insulin reduces phosphaturia [11], in contrast to our finding in RCU with hyperinsulinaemia [51]. From this we hypothesized that RCU might be associated with some resistance of peripheral tissues – including the kidney – to the actions of endogenous insulin; if so, insulin resistance might not only help explain the cause of hyperinsulinaemia, but might also provide insight into the nature and status of phosphaturia. During the early part of the present study we came to realize that RCU and non-stone-forming individuals in fact differed substantially with regard to urinary phosphate and insulin studied during fasting and in response to a meal.

With the aim of throwing more light on these pathologies, we decided to investigate ascorbic acid (ASC) – for the following reasons. ASC has long been known to be a modulator of pancreatic B-cell function [24, 35]. In addition, ASC improves the disposal of glucose from blood in healthy persons and individuals with adult-onset diabetes, so-called type II diabetes [34], in which both hyperinsulinaemia and insulin resistance are well documented. It has also been postulated that ASC might modulate the composition of urine.

In the present study, we can demonstrate that in relatively young males with RCU ingestion of a test meal is frequently followed by hyperinsulinaemia, decreased insulin sensitivity, and inappropriately high phosphaturia. ASC supplementation of the meal prevented the hyperinsulinaemia and ameliorated insulin sensitivity, but was unable to restore the normally occurring meal-induced reduction of phosphaturia.

Material and methods

Study participants

The study included 38 males, comprising two groups of RCU patients and one group of healthy controls (see below), the latter recruited from our hospital personnel. Males were chosen because of the sex differences in insulin sensitivity and glucose metabolism [59], and informed consent was obtained from all participants. When attending the laboratory, 14 of the 30 RCU patients had stones in situ; previous stone analysis revealed calcium oxalate in 2 cases, a mixture of calcium oxalate and calcium phosphate in 12, whereas in the remaining 16 cases (without stone analysis) the clinical course, radiopaque concretions visible on the antecedent plain X-ray of the kidney-ureter-bladder region, and the biochemical findings in urine were consistent with RCU as the final diagnosis. The great majority of calcium stones contain some calcium phosphate, as demonstrated with the use of a sufficiently sensitive analytical technique [23]; this led us to reject splitting RCU further into subgroups with varying amounts of calcium phosphate(s) in stone(s), although on the grounds of recognition of early pathophysiological events this would have been highly desirable.

Owing to the lower age, the average metabolic activity of stone disease was also lower than that reported for older RCU populations [52]. The absence of overt diabetes mellitus was verified by normal fasting blood glucose (< 95 mg/dl), normal glycated haemoglobin $A1_C$ (<6%), and unremarkable glucose levels in fasting urine. Disorders such as primary hyperparathyroidism, essential hypertension, renal tubular acidosis, hyperoxaluria, and urinary tract infection were excluded. No patient was receiving long-term anti-stone medication, and none of them regularly supplemented home food with vitamins or minerals. Renal function was within normal limits (serum creatinine < 1.3 mg/dl). Blood acid-base status, serum sodium, potassium, total calcium and magnesium, phosphate, and parathyroid hormone concentrations were unremarkable. Three RCU patients had fasting hypercalciuria (calcium/creatinine > 0.12 mg/mg); the remainder were normocalciuric. The study was ambulatory, and the participants made no changes in their customary diet until the evening preceding the investigation. Although this uncontrolled variability in diet composition could constitute a confounding factor especially for blood glucose regulation, the design appeared justified, since food tables showed that the carbohydrate content of ingested nutrients was always in excess of 150 g/day, thereby meeting the nutritional criteria required for an oral glucose tolerance test [31].

Laboratory procedures, standard test meal

The examination in the clinical laboratory was part of a standardized programme described in detail elsewhere [51, 44]. Originally developed for diagnosing disorders of mineral metabolism, this programme is also applicable to the evaluation of the concomitant state of glucose metabolism and related hormones, since the test meal is rich in carbohydrates (see below). In brief: an overnight 12- to 14-h fasting period was followed by bladder voiding at 7:30 a.m., stimulation of mild diuresis by having the subject drink two 300-ml amounts of demineralized water, and insertion of a forearm venule for blood sampling at 8:00, 10:00, 10:30, and 11:00 a.m., noon, and 1:00 p.m. The balanced test meal (Vivasorb, Pfrimmer, Erlangen; FRG[1]) contains 71 g carbohydrates (25.5 g glucose, 45.5 g oligomeric amylose hydrolysates), 6.5 g free amino acids, 0.222 g essential fatty acids, 1000 mg elemental calcium (as gluconate), 66 mg elemental phosphorus, and 10 mg ASC; the total energy content was 300 kcal (1264 kJ), the fibre content was < 1%, and osmolarity was 500 mOsm/l. For one group of stone patients (coded RCU + ASC; see below), 5 mg/kg body weight water-free analytical grade ASC (Merck, Darmstadt, FRG) was added. The meal was taken in the form of a suspension in 300 ml deionized water, shortly after the 10:00 a.m. blood sampling and collection of fasting urine (8:00–10:00 a.m.). Urine from the fasting and postprandial period (10:00 a.m.–1:00 p.m.) was processed immediately, with an aliquot being frozen in liquid nitrogen and stored at –80 °C (for analysis of oxalate). Serum and plasma [1.2 mg ethylenediaminetetraacetate (EDTA), 500 kallikrein inhibitor units (KIU) aprotinin/ml blood] were stored at –30 °C.

Provided reliable analytical methodology [27] is used, the ASC-fortified meal results in a rise of ASC in peripheral venous plasma, e.g. from baseline (mg/l) 11, to 13 (30 min post-load), 15 (60 min)

[1]An equivalent preparation is available as Vivonex standard (Friesche Vlag, Leeuworden, The Netherlands).

and 20 (120 min); these levels indicate that ASC ingested with the meal reaches the peripheral tissues.

In serum, the total calcium concentration does not exceed the upper limit of normalcy (in our laboratory 10.4 mg/dl, irrespective of serum protein content), calcitonin increases and parathyroid hormone declines [49], as does urinary pH, whereas glucose and insulin in peripheral venous blood reach levels comparable to those seen after the standard 75-g oral glucose load recommended for diagnosing diabetes [31].

Experimental groups

The three groups did not differ statistically with respect to mean age (standard error) and mean body mass index (body weight in kg, divided by height, in m^2; BMI): controls, $n = 8$, 27 (0.7) years, BMI 24.0 (0.7); RCU, $n = 14$, 29 (1.0) years, BMI 24.4 (0.8); RCU + ASC, $n = 16$, 30 (1.0) years, BMI 25.0 (0.8).

Analyses

Routine methods were employed for the measurement of serum and urine creatinine, total calcium, and phosphorus (as phosphate) concentrations. Glucose in plasma and urine was measured enzymatically (Gluco-Analyzer, Beckman, Fullerton, USA), and plasma insulin and C-peptide by radioimmunoassay. Other determinations included urinary pH (glass electrode), oxalate (high-performance liquid chromatography (HPLC); [50]), total protein [colorimetry (reagents from Bio-Rad Laboratories, Munich, Germany)], albumin [nephelometry (using the monoclonal antibody OSAL 14, Behring, Marburg, Germany)], α_1-microglobulin [nephelometry (using monoclonal antibody OWLA 10, Behring, Marburg,Germany), and sensitivity 2.5 mg/l].

Calculations and statistics

For plasma glucose, insulin, and C-peptide, the respective mean value of the two pre-load [8:00, 10:00 a.m.] samples was adopted as baseline. Peripheral insulin activity [A value] was derived from post-load plasma glucose and insulin:

$$A = \frac{10^4}{\text{peak glucose} \times \text{peak insulin}}$$

Decreased A is considered to indicate peripheral insulin resistance [56]. Integrated (area) glucose, insulin, and C-peptide were derived as follows: area = 0.25 (baseline) + 0.5 (value at 10:30 a.m.) + 0.75 (value at 11:00 a.m.) + 0.5 (value at noon) [19]. Supersaturation of urine with two calcium phosphates and calcium oxalate was expressed in terms of activity products (relative to the solubility of substances in water; for details see ref. [28]). Using the software Statistica (Stat Soft, Tulsa, USA), data were subjected to analysis of variance, or the Kruskal-Wallis test; in the case of a significant F- or H-value the t- or U-test was applied, as appropriate. Several variables were correlated (Spearman's procedure).

Results

Insulin, C-peptide, and glucose concentrations in fasting and post-load blood

The baseline total calcium and calciotropic hormones and their course in response to the test meal have not been presented here, because they closely followed the pattern demonstrated in an earlier study [49]. As a brief reminder: calcium and calcitonin increased above basal levels (peak at 120–180 min), and parathyroid hormone decreased below basal levels (nadir between 60 and 120 min). The changes observed with glucose, insulin, phosphaturia, and other variables (see below) therefore need to be understood and interpreted against this background.

Data in Table 1 indicate that the mean baseline glucose, insulin, and C-peptide levels were not different statistically; the highest glucose or insulin levels were still lower than the accepted upper limit in non-diabetics [31]. The post-load glucose peak in the control group occurred earlier (30 min) than in either of the two groups of patients (RCU, RCU + ASC, 60 min), but the final value (180 min) was close to baseline values in all three groups. C-peptide peaked at observation point 60 min post-load (all groups), but also remained above baseline at 180 min in all groups. Neither glucose nor C-peptide differed statistically among groups at any of the five post-load observation points. In contrast, insulin, al-

Table 1 Baseline and post-load (30–180 min) values of plasma glucose, C-peptide, and insulin in stone patients without (RCU) and with ascorbic acid supplementation (RCU + ASC) of the test meal, and in controls. The load was applied at time point 0 min. For details see text. Data are means (range) (*SE* overall standard error)

	Baseline	30	60	90	120	180 min	SE
RCU ($n = 14$)							
Glucose (mg/dl)	78 (67–86)	118 (93–145)	122 (86–156)	109 (82–146)	97 (73–142)	76 (48–118)	2.8
C-peptide (ng/ml)	1.3 (0.9–2.7)	6.0 (1.4–14.5)	10.3 (4.7–20.0)	8.1 (4.0–19)	5.9 (2.0–18)	4.5 (1.0–17)	0.6
Insulin (μU/ml)	7 (2–16)	103 (13–500)	134[a] (60–359)	111[a] (46–266)	87[a] (20–228)	61 (2–265)	10
RCU + ASC ($n = 16$)							
Glucose (mg/dl)	77 (64–91)	114 (91–150)	120 (85–157)	107 (79–153)	95 (56–149)	69 (43–101)	3.0
C-peptide (ng/ml)	1.3 (0.7–2.3)	3.9 (0.6–7.1)	8.7 (1.2–20)	8.2 (3–18)	7.8 (1.8–20)	4.1 (0.8–15)	0.8
Insulin (μU/ml)	4 (2–13)	58 (6–150)	78[b] (36–146)	65[b] (19–106)	52[c] (2–161)	19 (2–74)	4.0
Controls ($n = 8$)							
Glucose (mg/dl)	78 (72–89)	122 (98–160)	103 (79–114)	94 (76–107)	86 (72–103)	72 (48–98)	3.0
C-peptide (ng/ml)	1.0 (0.8–1.3)	4.3 (2.1–8.8)	5.7 (2.2–16)	4.5 (2.0–12.4)	3.4 (1.6–8.8)	1.7 (0.8–5.0)	0.4
Insulin (μU/ml)	6 (2–16)	69 (25–151)	71 (28–212)	53 (21–151)	35 (13–58)	13 (2–58)	6.4

[a] $P < 0.05$ vs Controls
[b] $P < 0.05$ vs RCU
[c] $P < 0.10$ vs RCU

though peaking at 60 min in all groups, was increased in both RCU and RCU + ASC versus controls, at time points 60, 90, and 120 min post-load; at 180 min the highest value was observed in untreated RCU.

Integrated insulin, C-peptide, glucose, and insulin sensitivity (Fig. 1)

The insulin area was significantly higher in RCU (approximately 2.1-fold) than in controls; also the associated areas of C-peptide and glucose were higher in the patients, but the respective mean value did not reach the level of significance in comparison with controls. In contrast, in the RCU + ASC group, i.e. stone patients that took the ASC-supplemented test meal, the insulin excess was abolished; the areas of each C-peptide and glucose in this group did not differ significantly from those of controls. For RCU, RCU + ASC, and controls, the absolute figures for the areas [mean value (SE)] were, respectively, insulin 200 (60), 115 (18), 107 (27), C-peptide 14.0 (1.7), 12.6 (1.7), 8.4 (2.0), glucose 218 (8), 214 (6), 200 (7).

Insulin sensitivity (synonymous in this work with peripheral insulin activity and insulin resistance) was markedly reduced in untreated RCU as compared with controls (Fig. 1, top left). ASC supplementation of the test meal improved insulin sensitivity slightly but non-significantly (RCU + ASC vs RCU; $P < 0.12$), and there remained a significant difference between untreated RCU and controls. For RCU, RCU + ASC, and controls, the absolute figures for insulin sensitivity [mean value (SE)] were, respectively, 0.66 (0.10), 0.98 (0.09), 1.50 (0.35).

Urinary phosphate and glucose in fasting and postprandial urine

For substance excretion (in milligrams) as factorized for urinary creatinine (in grams), the values [mean (range)] are given. Fasting phosphaturia – RCU 201(27-344), RCU + ASC 234(57-420), controls 482 (223-1053); the two groups of stone patients differed significantly from controls ($P = 0.003$), thus confirming the previously reported decrease in urinary phosphate in fasting RCU [41, 47]. Postprandial phosphaturia – RCU 350 (109-628), RCU + ASC 353 (23-564), controls 364 (95-639); both patient groups were statistically indistinguishable from controls. A plot of changes in postprandial phosphaturia against fasting phosphaturia (postprandial minus fasting) revealed practically no overlap between untreated RCU and controls. There were negative differences in six out of eight controls, i.e. phosphaturia decreased in response to the meal; in contrast, all untreated RCU, and 13 out of 16 ASC-treated RCU, failed to show negative differences. In other words, in the great majority of RCU there is a significant postprandial loss of phosphate via the urine (Fig. 2, top). This change in phosphaturia was negatively correlated with insulin sensitivity ($n = 38$, $r = -0.44$, $P < 0.01$; Fig. 3).

The respective data for glucose in the urine [mean (range)] were: fasting glucosuria – RCU 91 (51-216), RCU + ASC 149 (38-435), controls 114 (23-231); postprandial glucosuria – RCU 188 (9-745), RCU + ASC 108 (38-321), controls 106 (34-176); the differences between RCU (untreated, ASC-treated) and controls were not significant. The plot of individual changes (see above) reveals that in the majority of healthy controls and untreated RCU there was no suppression of postprandial

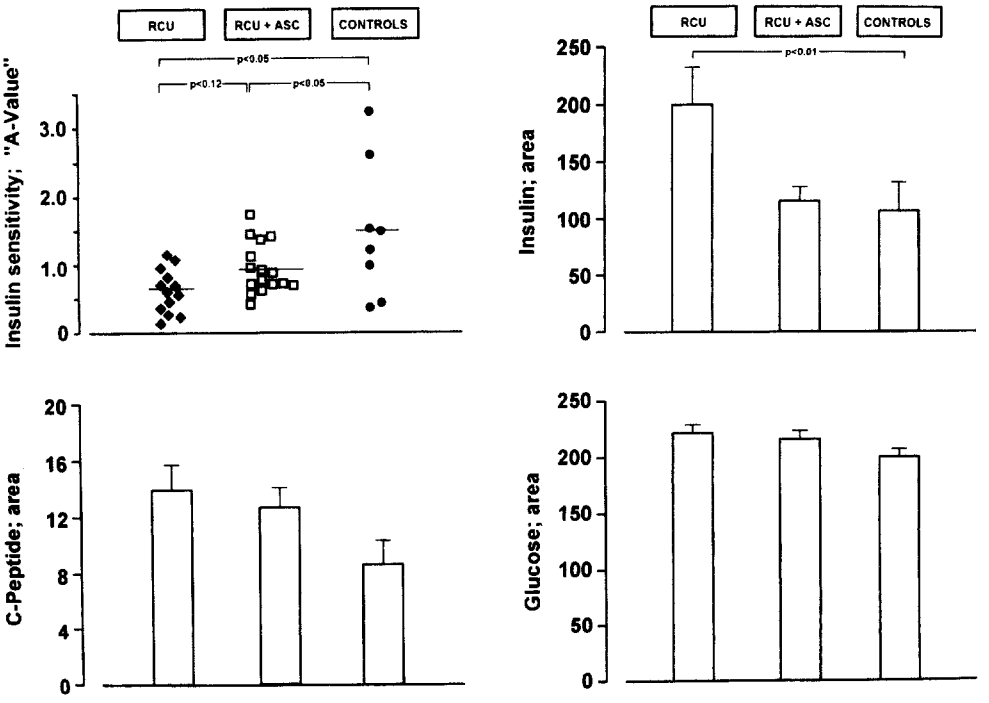

Fig. 1 Insulin sensitivity of tissues, and integrated (area) insulin, C-peptide, and glucose, in the three study groups (for abbreviations and other explanations see text and Table 1). *Horizontal lines* indicate mean values

glucosuria to below the values in fasting urine, but that in RCU with ASC supplementation of the test meal there was a marked suppression (Fig. 2, bottom).

pH, other substances, and supersaturation in postprandial urine (Table 2)

In untreated RCU, but not in the RCU + ASC group, the mean pH was significantly higher than in the controls. Also in RCU, there was a trend toward higher excretion of calcium, oxalate, and total protein, which was somewhat weaker in the RCU + ASC group. In all groups, the mean albumin excretion was < 3 mg/g creatinine, and the mean α_1-microglobulin excretion was < 2.75 mg/l (below the detection limit). In comparison with controls, the relative supersaturation with respect to the major crystal- and stone-forming urine constituents in untreated RCU shows no increase, but rather a significant uric acid undersaturation due to the less acidic pH. Other differences between study groups were insignificant.

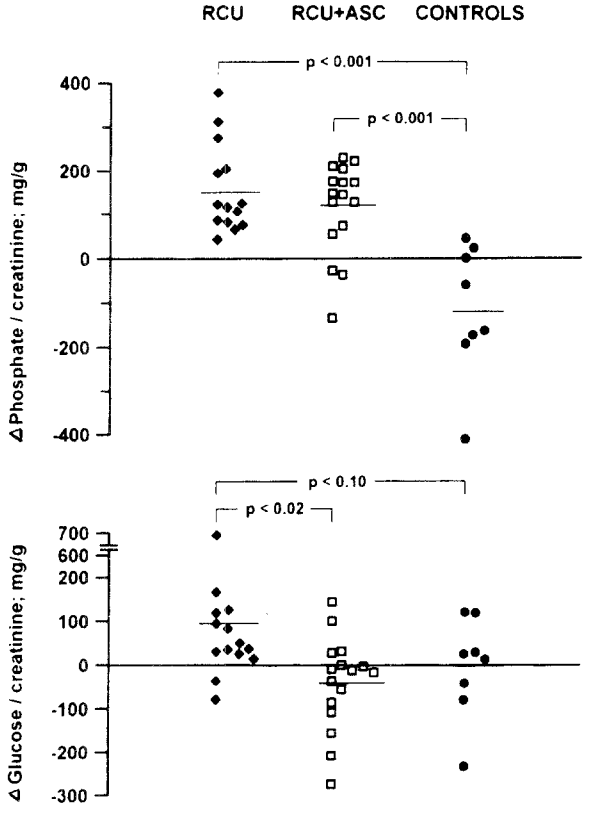

Fig. 2 Change (Δ) of phosphate/creatinine and glucose/creatinine in postprandial versus fasting urine. *Horizontal lines* indicate mean values: phosphate—RCU 149, RCU + ASC 118, controls 118; glucose—RCU 97, RCU + ASC 40, controls 7

Discussion

We report on excess of blood insulin and, within limitations, urinary glucose and phosphate in RCU – issues that so far have received only limited attention in urolithiasis research [38, 43, 46, 47, 51] – and on ASC as a potential agent for their normalization. Our data were obtained with the use of a carbohydrate-rich mixed meal of constant composition, but despite this they should not be extrapolated to those that may be obtained by the use of monomeric glucose. In contrast to insulin, there was no excess of post-load blood glucose in untreated RCU, which means that glucose utilization may be largely unimpaired at the expense of accumulation of insulin. ASC appears to enhance insulin removal from blood possibly via cellular degradation. Thus, comments on actions of insulin and ASC appear justified. The important question arises: is the observed insulin excess an epiphenomenon of RCU, or does it contribute to stone formation; if the latter is true, what are the pathways?

Insulin as a primary messenger in RCU

Target organs like the kidney can develop a normal response, resistance, or hypersensitivity to the biological effects of hormones. The latter two are well-known

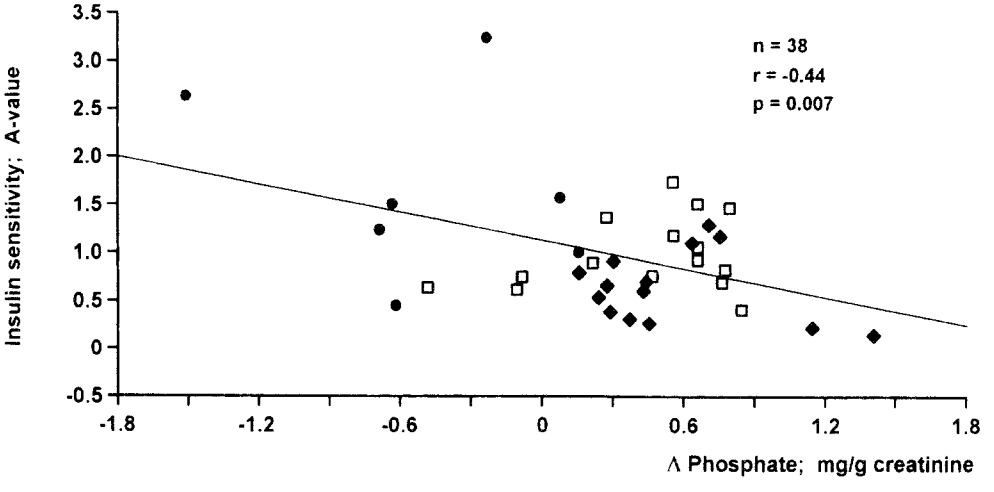

Fig. 3 Interrelationship of "gradient" (Δ; postprandial fasting) phosphaturia and insulin sensitivity of peripheral organs, in controls (●), RCU (■), and RCU + ASC (□). Note that in one individual in each of the control and RCU + ASC groups both variables were identical. For further explanation see text

Table 2 pH, substances (in milligrams per (gram creatinine), and relative supersaturation products (*RSP*) in postprandial urine. For abbreviations and other details see text. Data are means (range)
[a]Statistics based on $\log_{10}$ values
[b]Values > 1.0 mean spontaneous precipitation, 0–1 metastable supersaturation, < 0 undersaturation (see ref. [28])

	RCU (n = 14)		RCU + ASC (n = 16)		Controls (n = 8)	
pH	5.93***	(5.07–7.07)	5.55	(4.80–7.07)	5.50	(5.06–6.00)
Calcium	254	(116–402)	248	(107–443)	192	(113–396)
Oxalate	14	(4–28)	13	(8–24)	12	(6–20)
Protein[a]	23	(10–66)	20	(3–137)	18	(7–49)
RSP[b] – brushite	−0.01	(−0.97–0.92)	0.13*	(−1.24–1.02)	−0.27	(−0.82–0.20)
RSP – octacalcium phosphate	−0.38	(−1.97–1.24)	−0.41*	(−2.18–1.30)	−0.93	(−1.79)–0.33)
RSP – calcium oxalate	0.64	(0.12–0.88)	0.89	(0.66–1.3)	0.61	(0.0–1.0)
RSP – uric acid	−0.68**	(−3.24–0.65)	0.22	(−2.85–1.86)	0.34	(−1.58–1.41)

***$P < 0.01$, **$P < 0.05$, *$P < 0.10$, vs Controls

phenomena in endocrinology. Phylogenetically, insulin is an old hormone. During evolution its actions could have been modulated to counteract incipient damage; but the opposite is also true, i.e. the body could have deployed countermeasures to curb its actions. When considering insulin's possible role in kidney function in RCU, it is important to keep in mind that the hormone must first leave the pancreatic B-cell, circulate in the blood, cross the glomerular basement membrane, capillary endothelium, and interstitial space to reach the proximal tubular lumen, and reach the basolateral membranes of the peritubular cells. Processes that limit the delivery of insulin and glucose to target tissues potentially cause in vivo insulin resistance and hyperinsulinaemia. Hence, in the presence of apparent normoglycaemia but clear hyperinsulinaemia in RCU patients in this work, insulin might theoretically have interacted abnormally with the glomerular morphology and function, as well as with the apical and basolateral membrane of proximal and distal tubular epithelium. An earlier publication reported that insulin enhances calcium accumulation in tissues, but failed to specify the underlying mechanisms [17]. More recently, insulin, in physiological concentrations, was found to inhibit specifically plasma membrane Ca^{2+}-ATPase in adipocytes [36], and to decrease cell membrane fluidity due to its effects on membrane ultrastructure, especially the cooperativity of Na^{+}-K^{+}-ATPase (see ref. [29]). The insulin dependency of the Ca^{2+}-Mg^{2+}-ATPase in basolateral membranes of proximal renal tubules has been documented [25], and this enzyme helps keep intracellular calcium levels low (10^{-7} *M*). Alteration of membrane dynamics, known to contribute to insulin resistance in non-insulin-dependent diabetics [57], in whom hyperinsulinaemia is common, may also apply to RCU [51; this work]. The assumption is therefore justified that via such cell-bound pathways, insulin itself, the state of insulin resistance, or both, are linked to the stone-forming processes occurring during the postprandial period. This interpretation is further substantiated by the absence of increased physicochemical supersaturation of post-glucose load urine with stone substances, including proteins (Table 2). In contrast, independent work on the composition of fasting urine of RCU patients has revealed hyperexcretion of total protein [43] and non-albumin protein (P.O. Schwille, unpublished data), indicating that the time course of cellular events, and the appearance in urine of stone matrix materials, crystals, and microliths, may differ during the various periods of a daily cycle.

Insulin sensitivity and phosphaturia in RCU

The participants in the present study were matched for age and body mass, factors that had been largely neglected in previously published studies on the response of stone patients to the ingestion of nutrients. As a result of this neglect, such studies may have been heavily biased in terms of, for example, blood glucose and insulin, and phosphaturia. Obesity is one of the determinants of postprandial glycaemia and insulinaemia, and investigators of RCU have been reminded of this situation in a recent report [58]. Furthermore, RCU patients are often heavier than non-stone-forming controls of the same gender and age [26], a fact that necessitates the matching of study participants for BMI also. RCU in males has a maximum incidence between 36 and 55 (peak 42) years [45], whereas our stone patients and controls were markedly younger (average 30 years). The finding of unimpaired postprandial glycaemia in combination with hyperinsulinaemia and insulin resistance in relatively young RCU patients therefore allows us to exclude age as a causative factor [39], and to add RCU to the list of disorders in which insulin resistance and hyperinsulinaemia have a high prevalence [16]. For the development of impaired insulin sensitivity in association with hyperinsulinaemia, an initially abnormal regional accumulation of fat or obesity in general may be crucial, but opinions on this differ [1, 7]. Although, on a BMI basis, our RCU patients were somewhat more obese than controls, this degree of obesity ought not to be sufficient to cause insulin resistance. Nevertheless, more detailed studies on obesity and lipids in RCU are necessary, as also are studies on a possible genetic fixation of insulin resistance [37] and specific glucose transporter molecules.

An alternative cause of impaired insulin sensitivity might be cellular magnesium deficiency. In RCU patients of similar age we found lowered total magnesium

in red blood cells [43], while others have shown low free ionized magnesium in erythrocytes to be associated with decreased insulin sensitivity in a non-stone-forming population [33]. In a magnesium-deficient metabolic environment, not only are insulin release from B cells [20] and insulinaemia [33] high, but there is also parathyroid-hormone-independent hyperphosphaturia [12], the glucose phosphorylation in the hexokinase reaction is impaired (see ref. [13]), and an insufficiently low urinary pH might result from insufficient proton generation by distal tubules [21]; such magnesium-dependent disorders strongly resemble those seen in RCU in the present work. Furthermore, the combination of obesity, insulin resistance, and magnesium deficiency carries the risk of pathological calcification with enhanced calcium phosphate deposition in the arterial vessels [2], as has been shown for diabetics [40]. Certainly, these events occur in lesioned vascular tissue. Similarly, fasting hyperproteinuria [43] and postprandially impaired insulin sensitivity may arise from lesioned renal tissue. Indicative of this may be the fact that in our present study postprandial glucosuria was somewhat high in untreated RCU patients, but in the RCU + ASC group was still susceptible to the antiglucosuric effect of ASC (Fig. 2), an agent that counteracts tissue damage induced by oxygen free radicals, and possibly repairs tissue by improving glucose utilization [24, 35, 42; see also below].

The bulk of tubular phosphate reabsorption occurs via sodium cotransport in which the apical entry step is the rate-limiting process, and which can be affected by numerous factors, including insulin and parathyroid hormone [30]. The fact that the intake of the ASC-supplemented test meal improves phosphate reabsorption only marginally (Fig. 2), but glucose reabsorption markedly, suggests that the situation in the stone-forming kidney may be complex. Owing to the low levels of circulating parathyroid hormone – induced by the calcium content of the test meal – one would expect the regulation of intracellular signals, dependent on PTH suppression, to be appropriate, especially those signals linking phosphate reabsorption with the adenylate cyclase-cyclic AMP second messenger system. Since phosphate escapes reabsorption, and only peripheral steps of the cyclic AMP system are involved in transduction of insulin-sensitive signals [5], any intracellular dysfunction related to signals elicited by these two hormones should be sought elsewhere. Support for this view is provided by the fact that suppression of urinary and nephrogenous cyclic AMP by calcium supplementation of the meal in RCU is adequate [48, 49, 51]. Worthy of note is the fact that calcitonin is stimulated by the calcium content of the test meal [49], and that exaggerated hyperphosphaturia can be triggered by injection of calcitonin, reflecting hypersensitivity of tubular cells to this calciotropic peptide hormone [6]. Finally, phosphaturia in RCU may be abnormally programmed by a change in the rhythmicity of renal excretory functions during a daily cycle [54]. Whatever the determinants of phosphaturia in RCU are, inappropriate postprandial hyperphosphaturia in the long-term probably results in phosphate deficiency. Evidence of this may be the low fasting serum phosphate level found in the numerous studies on the pathophysiology of RCU done in the past. A low, not high, phosphate level in fasting urine was originally described by our laboratory [47] and has now been confirmed by other investigators [41]. It may be that a low phosphate level under fasting conditions indicates a need for phosphorus conservation.

Actions of ASC in RCU

Vitamin C deficiency has been considered to be associated with abnormal B-cell function including hypo- and hypersecretion of insulin [24, 35]. In the present work ASC normalized insulinaemia and glucosuria of RCU. Although the mechanisms underlying the effect of ASC cannot be reconstructed from available data, they may be related to some pre-existing intrinsic disorder of cell metabolism inherent in the tissues of RCU. For instance, increased lipid peroxidation, due to accumulation of oxygen free radicals, has been observed in an animal model of calcium oxalate stones [22], and in erythrocytes of a rather inhomogeneous group of stone-forming humans [3]. A scavenger role of supraphysiological doses of ASC against oxygen free radicals, especially superoxide anions, is well documented [4, 32, 42]. In our work, therefore, prevention of radical generation, or radical neutralization, by even lower doses of ASC than those used for amelioration of non-insulin-dependent diabetics [14], could have eliminated tissue damage pre-existing in pancreatic B cells – and probably the kidneys – of untreated RCU patients. The normalization of insulin (Fig. 1) and normal proteinuria (Table 2) would fit into this concept.

In the past, the role of ASC as a possible risk factor for renal calcium stones, for example via stimulation of oxaluria, has been variously assessed. One group stated that high intake of vitamins, especially vitamin C, is a habit regularly practiced by calcium stone patients [18], and another group found that ASC is an abettor of calcium urolithiasis in animals [55]. These reports can be interpreted to indicate that, since high doses of ASC enhance oxaluria, this would stimulate intrarenal and urinary calcium oxalate and possibly calcium phosphate crystallization. However, measurement of urinary oxalate is reliable only if the analytical method used is error free, a criterion not met by colorimetric methods [10]. Normo-oxaluria, developed in response to our test meal containing alkali (sodium, potassium) and ascorbic acid, also militates against the possibility that, given the alkaline pH of the gut lumen, enhanced non-enzymatic conversion of ASC to oxalate occurred. Therefore, even with a supraphysiological oral dose of ASC (5 mg/kg body weight; normal intake per day with nutrients is approximately 70 mg) oxaluria remains unchanged – confirming observations of others [10]. ASC creates a more acidic urinary pH (Table 2), probably due to the

renal proton load resulting from the dissociation of the dicarboxylic acid under the in vivo alkaline conditions in the blood. Hence, at a mean urinary pH < 6.0 crystallization of calcium oxalate and calcium phosphate should have been delayed, corroborating the data on supersaturation (Table 2). Finally, although direct ASC effects at the level of crystallization of stone-forming substances in urine have not been reported, preliminary unpublished work done in our laboratory reveals that after intake of the ASC-supplemented test meal the aggregation time of calcium oxalate crystals in undiluted urine is prolonged.

Outlook

The fact that renal tissue of humans is not directly amenable to investigation of processes leading to stones, especially not the bidirectional transport characteristics developed by tubular epithelium, forces more indirect approaches to the early metabolic events of RCU to be used. Among these may be the measurements of the increasingly specific markers for glomerular and tubular functions, and conceptual in vitro models, involving cultured human renal tubular cells, able to reflect the end points of impaired systemic metabolism as shown in this work. Targets of such modelling work could be the overall viability of cells and their proneness to calcification in response to effectors in varying concentration, primarily insulin and ASC, with their multifacetted actions brought to the basolateral and luminal border, and the interior, of cells. Finally, the standardized diagnostic work-up of RCU patients, in the presented or a modified version, may offer opportunities for modelling procedures directed at thermodynamics and kinetics of effectors in urine or tubular fluid during selected periods at greater risk for stones, effectors in the systemic blood circulation, or some combination.

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