Urol Res (1997) 25:417–426

ORIGINAL PAPER

P. O. Schwille · U. Herrmann · A. Schmiedl · H. Kissler · J. Wipplinger · M. Manoharan

Urinary phosphate excretion in the pathophysiology of idiopathic recurrent calcium urolithiasis: hormonal interactions and lipid metabolism

Received: 17 December 1996 / Accepted: 23 April 1997

Abstract Previous work in younger males with recurrent idiopathic calcium urolithiasis (RCU) demonstrated inappropriately high postprandial phosphaturia, hyperinsulinemia and insulin resistance, but normal glycemia. To investigate further whether these abnormalities occur also in RCU patients with a mean age corresponding to the life period with peak formation of calcium-containing stones, two trials were carried out in 155 males of comparable age and body mass index. All participants underwent a standardized laboratory examination, including collection of urine and blood before and following a test meal rich in carbohydrate and calcium but low in phosphorus. In trial 1, comprising control subjects (n = 12, mean age 42 years) and RCU patients (n = 24, mean age 41 years), phosphate (Pi) excretion and fractional Pi excretion in postprandial urine of controls did not change compared with the values in fasting urine, but were significantly increased in RCU, despite the fact that there was almost equal suppression of serum parathyroid hormone (PTH) and increase in serum calcitonin. Postprandially, RCU patients were hyperinsulinemic but still normoglycemic versus controls. In trial 2, carried out in unclassified (in terms of calciuria) RCU patients (n = 119, mean age 40 years) only, the post-load Pi-uria was similar in magnitude to Pi-uria of RCU patients in trial 1; increased postprandial Pi-uria was a phenomenon also of normocalciuria but was slightly more pronounced in hypercalciuria, while changes in calcium phosphate (brushite) and calcium oxalate supersaturation of urine were unrelated to calciuria. In RCU patients, but not controls, there was a tendency toward higher urinary glucose in post-load as compared with fasting urine. When urinary Pi and fractional Pi excretion in trial 2 were considered as dependent variables in multivariate regression analysis, they appeared unrelated to age, but positively associated with postprandial glycemia as the best predictor, followed by insulinemia, insulin resistance, to a lesser degree fasting serum PTH and the metabolic activity of stone disease, negatively associated with blood total lipids and very low density lipoprotein (VLDL) cholesterol. It was concluded that RCU males (1) show low Pi-uria during fasting but impaired renal Pi conservation in response to a mixed meal, a situation carrying the risk of Pi deficiency over the long term; (2) represent a population developing hyperPi-uria despite suppressed PTH; (3) exhibit insulin resistance but are still able to maintain normoglycemia at the expense of hyperinsulinemia. It is suggested that calcium-containing renal stones are related to impaired Pi and glucose translocation across cell membranes, and that the role of lipids in this setting deserves further investigation.

Key words Phosphaturia · Glucose · Insulin · lipids · Idiopathic recurrent calcium urolithiasis

P. O. Schwille (✉)
University Hospital, Departments of Surgery and Urology, Mineral Metabolism and Endocrine Research Laboratory, Maximiliansplatz, D-91054 Erlangen, Germany

Introduction

In recurrent idiopathic calcium urolithiasis (RCU), a disorder generating considerable health costs worldwide, the homeostasis of phosphorus [in this work synonymous inorganic phosphate (Pi)] metabolism is still incompletely understood. The great majority of renal calcium-containing stones formed in RCU contain Pi in the core [22], implying that Pi is somehow involved in the early events of stone development. It is not known whether the initiation of stones occurs in the renal tubular lumen or renal tissue, or reflects some combination of events. A better understanding of a possible pacemaker role of altered Pi metabolism in stone formation necessitates prior clarification of the state of Pi-uria under standardized conditions and in a sufficiently large number of RCU individuals for valid conclusions to be drawn.

It has been shown that in comparison with non-stone forming controls both Pi excretion in fasting urine and urinary Pi concentration is decreased in RCU patients [43, 34]. In contrast, Pi in postprandial urine was found to be increased in both hypercalciuric and unclassified (in terms of calciuria) RCU [41, 44]. More specifically, in younger adults (mean age 29 years), post-test meal Pi-uria was not only high compared with controls, but was also accompanied by hyperinsulinemia and resistance of organs to the actions of insulin (synonymous insulin sensitivity). In contrast, blood glucose dynamics were unremarkable, as was urinary glucose in postprandial urine [44]. Insulin, when administered acutely in metabolically healthy subjects matched for age, reduces Pi-uria and natriuresis, but stimulates calciuria [10]. Similar observations have been made in healthy humans under hyperinsulinemic euglycemic conditions [48]. In younger RCU patients, therefore, failure to reduce Pi-uria despite the presence of excess insulin [44] is evidence for an intrinsic abnormality linking the metabolism of Pi, glucose and insulin in some, as yet unknown, way. In an attempt to gain a better understanding of this situation, we hypothesized that the absence of the expected inverse correlation between the gradient (postprandial – fasting) Pi-uria and insulin sensitivity may not be restricted to younger [44], but may also occur in older RCU patients. If so, the insufficient postprandial renal Pi conservation should have important repercussions on the homeostasis of extracellular Pi, glucose and insulin, and to some extent also on urinary calcium and sodium excretion. Hypophosphatemia has long been known to occur in RCU (45), and impaired glucose metabolism and insulin sensitivity during long-term Pi deficit in animal species and humans were also reported [11, 18, 49], but the situation is still not clear in RCU patients with a mean age above 30 years. The frequency of RCU is greater in males, peaking at around 40 years of age [40]. With increasing age the homeostasis of minerals, Pi included, is maintained largely at the expense of regulatory hyperparathyroidism [1, 19], making it necessary for studies in these areas to include monitoring of age and parathyroid gland activity.

The present work comprises two cross-sectional trials, one carried out in male RCU patients and male controls, and the other in RCU patients without controls. The major aim was to elicit differences in Pi-uria, glucose and insulin, another being to evaluate additional variables possibly of relevance to the state of Pi metabolism, and to detect possible interrelations.

The information obtained provides evidence that enhanced postprandial Pi-uria is also a characteristic feature of RCU patients with a mean age of about 40 years, and that this disturbance indicates the existence of altered Pi translocation across renal cell membranes.

Materials and methods

Participants and study design

The two trials comprised a total of 155 adult men. Both trials were organized as cross-sectional field studies, i.e. they were performed in outpatients. In trial 1, 24 RCU patients and 12 healthy controls participated. In trial 2, aiming additionally to clarify the specific influence of the degree of calciuria on Pi and glucose in RCU, 119 patients participated. None of the participants had been included in previous work of our laboratory [44]. Apart from the parameters Pi, glucose, and insulin metabolism, a number of additional clinical chemistry parameters were considered in trial 2. Formation of calcium-containing stones was verified on the basis of the clinical course in combination with plain kidney and upper bladder (KUB) X-ray films, the last one having been obtained within the 4 weeks preceding the laboratory examination. Analysis revealed pure or ≥80% calcium oxalate for most stones, only six having ≥80% calcium phosphate. Thus, the term RCU applies to mixed stones, in which oxalate predominates. Preliminary work showed that with respect to the state of Pi metabolism there was no dependence on the type of calcium stone formed. The metabolic activity of RCU during the 24 months preceding the laboratory examination was assessed by increasingly weighting (by multiplication) the following: the number of stones present, the growth of stone(s) present, the number of newly formed stone(s), spontaneous stone passage(s), and intervention(s) for stone removal; the sum was taken as score. Among the exclusion criteria for RCU were several diseases, in particular primary hyperparathyroidism and diabetes mellitus (synonymous fasting blood glucose >95 mg/dl). RCU patients were off any specific anti-stone medication for at least 2 weeks. None of the participants regularly supplemented home food with vitamins or minerals, and all were asked to avoid substantial changes in the composition of their home diet up to and including the evening before the investigation. The average carbohydrate content of food in this geographic area is ≥150 g per day, and the amount of carbohydrate in the test meal was appropriate for an oral glucose tolerance test [30].

Laboratory examination and analyses

All individuals participated in our standardized laboratory programme [39], of which a test meal of fixed composition is an essential part. The meal (formula diet) is fibre free, balanced, presents a carbohydrate (71 g) and calcium (1000 mg) challenge, but is low in elemental phosphorus (44.5 mg) (for details see [44]). The pH in postprandial urine declines (this work, [44]) due to the only weak buffering capacity of the meal for protons, and prevention of the alkaline tide that is normally developed in response to intake of natural food.

The programme also included a fasting (12–15 h overnight) period, stimulation of diuresis to approximately 1 ml/min by having the subject drink two 300-ml amounts of demineralized water, collection of fasting (2 h) and postprandial (3 h) urine, withdrawal of fasting and postprandial venous blood from a catheter in an antecubital vein. In both trials pre-test meal blood was obtained at 8:00 and 10:00 a.m. (see also below). In trial 1 post-test meal blood was obtained at 10:30, and 11:00 a.m., 12 noon, and 1:00 p.m., in trial 2 also at 11:30 a.m.. Aliquots of serum, plasma (1.2 mg EDTA, 500 KIU aprotinin per ml blood) and urine were stored at −30°C until analysis. During the laboratory examination, physical activity of participants was restricted to walking inside the hospital.

Analyses were carried out in accordance with generally accepted methodologies (for glucose, insulin, C-peptide and others see [41, 44]). Pi (serum, urine) was measured using a micromethod [7], magnesium in urine by atomic absorption spectrophotometry, calcium (serum, urine) by EGTA-complexometry, urinary pH by glass electrode, plasma intact human parathyroid hormone (PTH) and calcitonin by commercial radioimmunoassay kits (Nichols, Bad Nauheim, Germany), urinary cyclic adenosine monophos-

phate (AMP) by an in-house protein-binding radioassay, plasma cholesterol, triglycerides (kits from Boehringer, Mannheim, Germany), and phospholipids (kits from Bio-Merrieux, Nürtingen, Germany) by colorimetry.

Calculations and statistics

Results from the two pre-load blood samples were averaged to give one baseline value. The threshold concentration for renal-tubular Pi reabsorption was read from a nomogram [4]. Fractional urinary Pi excretion (FE) was calculated using the mean value of serum Pi measured at 11:00 a.m. and noon (trial 1), and 11:30 a.m. (trial 2), respectively. Integrated variables (area) were calculated using the trapezoid rule, with baseline subtracted (trial 1), or from a combination of baseline and post-load values (trial 2) [17]. Pancreatic β-cell function was approximated from fasting glucose and insulin [26]. Insulin sensitivity was calculated from post-load glucose and insulin ("A-value") [44], very low density lipoprotein-bound (VLDL) cholesterol using the standard formula [12]. Pi, calcium, magnesium and pH in urine are given for the fasting and postprandial periods, and as the difference between them (postprandial minus fasting). Because of the wide scatter of several variables, logarithmic transformation of data was used, sometimes after prior conversion into a positive value (addition of a fixed amount). Supersaturation products of calcium oxalate and acid calcium phosphate (brushite) in urine were computed from activity products [25]; using instead EQUIL-2 software for calculation of the free energy there would be high correlations for calcium oxalate and brushite (r = 0.95–0.99), the stone substances of relevance in this study. For the sake of clarity, results are given as means and standard error. Differences between groups were tested for significance ($P \leq 0.05$) by one- or two-sided t-test, or U-test, as appropriate. In trial 2, a product-moment correlation matrix was constructed; in addition, to identify predictors of postprandial Pi-uria (excretion; fractional excretion), the database was used for multiple regression analysis, with casewise forward and backward logistic (oriented to pathophysiology of RCU) deletion of individual variables. The software STATISTICA (Stat Soft, Tulsa, Okla.) was used.

Results

General data from participants in trials 1 and 2 (Table 1)

In controls and RCU patients the mean age and body mass index were comparable; in about one third of both controls and RCU patients there was stage I generalized obesity [body mass index 25–29 kg/(M)^2, in accordance with existing guidelines of the German Society of Nutrition]. Serum creatinine (for urinary creatinine clearance see Table 2), parathyroid gland activity (PTH, urinary cyclic AMP), and threshold concentration for maximal renal-tubular Pi reabsorption during fasting were statistically indistinguishable, but the latter tended towards low values in trial 2. The number of RCU patients showing normo- or hypercalciuria was selected so as to give a ratio of 1:1 in trial 1, thereby minimizing factors that are more specifically related to calciuria and which bias the interpretation of phosphaturia and the dynamics of blood Pi, glucose, and hormones (for further details see below); in trial 2 the ratio normo- to hypercalciuria was 3:2, as is observed in many stone clinics. The mean score of metabolic activity of stone

Table 1 General data on study participants, and baseline clinical chemistry in fasting serum (S) and urine (U). Mean values [(SE); except age (range)]. *BMI* body mass index, *MA* metabolic activity, *Cr* creatinine, *TmPi* renal phosphate threshold concentration [synonymous tubular phosphate transport maximum (per 100 ml creatinine clearance; see Table 2)], *P/A* present/absent

Trial		Age (years)	BMI (kg/M^2)	MA (score)	Stones P/A	NC/IHC*	S-Cr (mg/dl)	S-Ca (mg/dl)	S-Pi (mg/dl)	TmPi (mg/dl)	S-PTH (pg/dl)	U-cyclic AMP (μmol/g Cr)
1	RCU group (n = 24)	41 (24–58)	27.1 (0.6)	35 (2)	8/16	12/12	1.12 (0.03)	9.43 (0.06)	3.36 (0.10)	3.71 (0.25)	26 (2)	3.83 (0.33)
1	Control group (n = 12)	42 (25–61)	26.7 (1.1)		0/12	12/0	1.05 (0.04)	9.68 (0.05)	3.46 (0.18)	3.52 (0.33)	30 (4)	3.65 (0.40)
2	RCU group (n = 119)	40 (19–68)	26 (0.29	38 (3)	67/52	71/48	1.04 (0.01)	9.38 (0.03)	2.98 (0.04)	3.20 (0.07)	27 (1)	3.31 (0.10)

* Number of patients with normocalciuria (NC) or idiopathic hypercalciuria (IHC), as based on the upper limit in this laboratory of the calcium/creatinine ratio (mg/mg) in fasting (< 0.12 = NC) and postprandial (< 0.27 = IHC) urine

Table 2 Fasting, postprandial, and postprandial minus fasting urinary creatinine clearance (C-Cr), phosphate (Pi), glucose, magnesium, calcium, all expressed as excretion per unit creatinine (Cr), fractional phosphate excretion (FE-Pi). Mean values (SE), *n.s.* not significant

Trial	Variable	Fasting	Postprandial	$P \leq$	Postprandial − fasting
1	*RCU group* (n = 24)				
	C-Cr; ml/min	106 (5)	102* (4)	n.s.	−5 (5)
	Pi/Cr; mg/g	251** (24)	362 (40)	0.005	111^{+} (36)
	FE-Pi; %	8.0** (0.8)	10.3 (1.0)	0.008	2.2^{++} (0.8)
	pH	6.22 (0.20)	5.64 (0.13)	0.0001	−0.88 (0.09)
	Glucose/Cr; mg/g	90 (14)	101 (25)	n.s.	9** (18)
	Magnesium/Cr; mg/g	45 (4)	114 (13)	0.0005	69 (14)
	Calcium/Cr; mg/mg	0.10* (0.009)	0.28 (0.03)	0.0001	0.18 (0.03)
1	*Control group* (n = 12)				
	C-Cr; ml/min	94 (5)	87 (5)	n.s.	−8 (4)
	Pi/Cr; mg/g	429 (46)	458 (42)	n.s.	30 (35)
	FE-Pi; %	13.3 (1.7)	12.4 (1.2)	n.s.	0.8 (1.4)
	pH	6.32 (0.18)	5.44 (0.08)	0.005	−0.87 (0.17)
	Glucose/Cr; mg/g	78 (15)	50 (7)	n.s.	−28 (20)
	Magnesium/Cr; mg/g	48 (4)	112 (13)	0.005	64 (11)
	Calcium/Cr; mg/mg	0.07 (0.01)	0.19 (0.03)	0.005	0.12 (0.02)
2	*RCU group* (n = 119)				
	C-Cr; ml/min	114 (3)	109 (3)	n.s.	−5 (3)
	Pi/Cr; mg/g	250 (10)	390 (20)	0.0001	134 (10)
	FE-Pi; %	8.8 (0.4)	13.6 (0.6)	0.0001	4.9 (0.4)
	pH	6.18 (0.07)	5.73 (0.07)	0.0001	−0.45 (0.05)
	Glucose/Cr[b]; mg/g	118 (10)	408 (110)	0.065[a]	287 (110)
	Magnesium/Cr; mg/g	50 (2)	90 (5)	0.001	44 (5)
	Calcium/Cr; mg/mg	0.10 (0.01)	0.22 (0.01)	0.0001	0.12 (0.01)

[a] Based on $\log_{10}$ of values (see Materials and methods)
[b] Omitting the 10 patients with highest urinary glucose would yield mean values of 96 (SE 10) and 127 (10) mg/g for fasting and postprandial urine, respectively (difference not significant; see also Results section on phosphaturia in normocalciuric versus hypercalciuric trial 2 patients
* $P < 0.05$, ** $P < 0.01$, $^{+}$ $P = 0..057$, $^{++}$ $P = 0.095$ versus controls in same trial

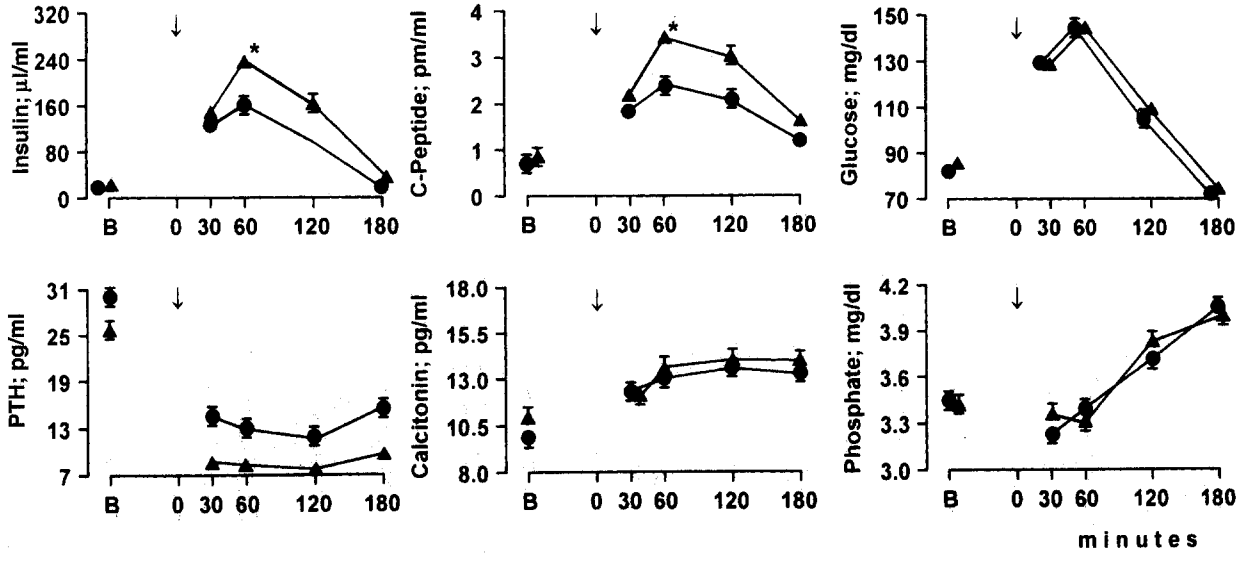

Fig. 1 Patterns of mean values of blood glucose, phosphate and related hormones, before (*B*) and in response to a test meal. Note that scatter values (standard error) frequently fell within symbols. *Arrow* load, *filled circles* controls, *triangles* RCU. For further details see Materials and methods and text. * $P < 0.05$ versus controls at the same observation point

disease was comparable in both trials, but showed wide scatter (range 1 to >200).

Phosphaturia, other data from urine and blood in unclassified (in terms of calciuria) RCU

In trial 1 (Table 2, upper part; Fig. 1) there was a higher mean creatinine clearance in RCU, but the fasting Pi excretion per unit creatinine was decreased versus controls. In contrast, postprandial versus fasting Pi excretion was dramatically increased in RCU, while the controls were able to maintain a degree of Pi-uria similar to that seen during fasting; creatinine clearance was again moderately elevated in RCU. Also FE-Pi in RCU was decreased versus controls during fasting, but was significantly elevated postprandially, whereas postprandial FE-Pi of controls remained unchanged. This constellation indicates that the more pronounced Pi-uria of RCU was not due to the higher creatinine clearance and thus the higher filtered load of Pi, but was caused mainly by diminished tubular Pi reabsorption.

The urinary pH and magnesium were of a similar order of magnitude, whereas glucose tended to be higher

in both fasting and postprandial urine of RCU patients (see also below, trial 2). Calciuria of controls and RCU was of the order of magnitude observed in many stone clinics, in which a similar laboratory programme is used. These changes in the composition of postprandial urine of RCU versus controls occurred when absorption and utilization of nutrients from the test meal modulated the homeostasis of blood glucose, calcium and hormones, in a manner illustrated in Fig. 1. In RCU there was obvious hypersecretion of C-peptide, hence of insulin, by pancreatic β-cells during the post-load period, while glycemia remained unremarkable; in contrast, PTH declined continuously and almost in parallel with PTH of controls, while calcitonin increased in both groups in a similar fashion (data for the virtually equal increase in calcemia in RCU and healthy controls are not shown [42]. Phosphate declined only initially (until 60 min post-load; both groups) but then rose steadily (values at 180 min post-load being higher than during baseline). The integrated (area) variables were as follows: insulin (mIU/ml) 23.6 (SE 2.2) in RCU and 16.3 (8.6) in controls ($P < 0.005$; based on $\log_{10}$ of data); in the same order C-peptide (pM/ml) was 334 (26) and 211 (31) ($P < 0.01$), glucose (g/dl) 5.2 (0.5) and 5.3 (1.1) (n.s.), PTH (ng/ml) −2.7 (0.3) and −2.7 (0.5) (n.s.), calcitonin (ng/ml) 0.50 (0.09) and 0.56 (0.16) (n.s.), phosphate (mg/dl) 36 (15) and 24 (15) (n.s.).

In trial 2 (Table 2, lower part) creatinine clearance, pre- and post- load Pi-uria (excretion, fractional excretion), urinary pH, magnesium, and calcium were similar to those found in RCU patients in trial 1 (see above). Urinary glucose excretion, especially in postprandial urine, was exaggerated; in approximately 5–10% of the patients glucosuria was clearly higher than the upper limit of glucosuria reported for normals [27], but it is worth noting that none of these patients exhibited fasting blood glucose >95 mg/dl. Therefore, a hitherto unrecognized subset of RCU probably represents a preclinical symptom of adult-onset type 2 (synonymous non-insulin-dependent) diabetes mellitus. Omitting these individuals from evaluation would result in a fasting urinary glucose of 96 (SE 10) and a postprandial urinary glucose of 127 (10) mg/g creatinine (difference not significant), values more comparable to those seen in the RCU patients of trial 1 (Table 2, upper part).

Phosphaturia, other data from urine and blood in normocalciuric versus hypercalciuric RCU patients in trial 2

To clarify further whether abnormal renal processing of Pi is a feature of RCU in general, or is merely a symptom of the hypercalciuric group [23] that masks the true situation in the normocalciuric group, urinary Pi and glucose were given separately for normo- and hypercalciuria. Elevated postprandial Pi-uria (excretion, fractional excretion) was not restricted to hypercalciuric RCU, and the increased postprandial over fasting glucosuria was characteristic for both calciuria types (Fig. 2). However, the difference in postprandial minus fasting Pi-uria, but not glucosuria, was somewhat greater in hypercalciuria than in normocalciuria.

Table 3 Data from RCU patients of trial 2. *All* unclassified, *NC* normocalciuria, *IHC* idiopathic hypercalciuria. Note that in NC and IHC age was 39 and 40 years, respectively. *F* fasting, *P* postprandial, *RSP* relative supersaturation product. For further details see Materials and methods

	All (n = 119[a])		NC (n = 71[a])		IHC (n = 48[a])		Limits of normalcy[b]
	Mean	SE	Mean	SE	Mean	SE	
Serum							
F-Beta cell activity (%)	56	(4) [118]	59	(5) [70]	51	(6)	[see 26]
P-Integrated glucose (area)	214	(3) [118]	215	(4) [70]	212	(5)	(<250)
P-Integrated insulin (area)	246	(14) [118]	243	(17) [70]	251	(22)	(<200)
Insulin sensitivity (A-value)	0.57	(0.03) [118]	0.56	(0.04) [70]	0.59	(0.06)	(>0.50)
F-Total cholesterol (mg/dl)	196	(4)	201	(5)	188	(5)*	<250
F-Triglycerides (mg/dl)	106	(6)	113	(9)	93	(7)**	<170
F-Total lipids[d] (mg/dl)	302	(8)	315	(11)	282	(11)*	<420
F-VLDL (mg/dl)	21	(1)	23	(2)	19	(1)**	<25
F-Total phospholipids (mg/dl)	181	(3)	185	(4)	175	(5)*	<250
Urine							
F-Sodium/Cr (mg/mg)	2.07	(0.08)	1.90	(0.11)	2.32	(0.12)*	<2.30
F-RSP calcium oxalate	0.49	(0.05)	0.45	(0.06)	0.56	(0.07)	<1.0[c]
F-RSP brushite	−0.24	(0.06)	−0.38	(0.08)	−0.03	(0.09)*	<1.0[c]
P-Sodium/Cr (mg/mg)	1.82	(0.08)	1.71	(0.11)	1.98	(0.13)	<3.50
P-RSP calcium oxalate	0.75	(0.03)	0.72	(0.04)	0.78	(0.05)	<1.0[c]
P-RSP brushite	0.10	(0.03)	0.03	(0.08)	0.21	(0.08)*	<1.0[c]

[a] Except [ ]
[b] In the author's laboratory, and from literature
[c] Metastable limit of solubility [25]
[d] Total cholesterol plus triglycerides
* $P \leq 0.05$, ** $P = 0.06$ versus NC

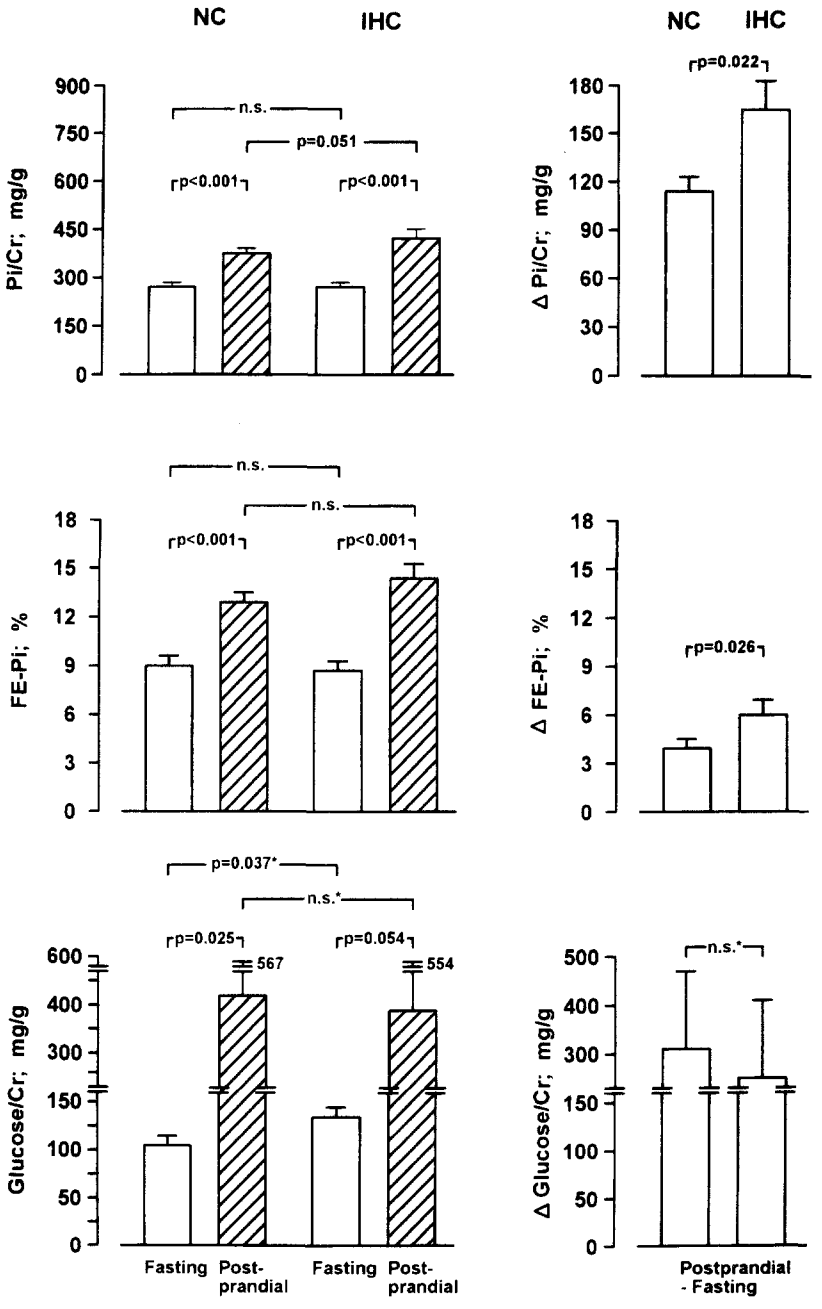

Fig. 2 Pi-uria (excretion, fractional excretion) and glucosuria in normocalciuric (*NC*, $n = 71$) and hypercalciuric (*IHC*, $n = 48$) stone patients. *Cr* creatinine (in same urine); *triangles* difference postprandial – fasting urine. Data are mean values and SE. Note the large scatter of glucose in postprandial urine. *Based on $\log_{10}$ of values

Table 4 Correlation coefficients (r) from trial 2 for several independent variables, postprandial phosphate excretion (Pi/Cr; mg/mg creatinine) and fractional phosphate excretion (FE-Pi%) as dependent variable ($n = 117$ observations). *F* fasting, *P* postprandial, *S* serum, *U* urine. For additional information see Materials and methods

Variables X[a]	Pi/Cr = Y		Variables X[a]	FE-Pi = Y	
	r	P		r	P
PU-Glucose/Creatinine (mg/mg)[b]	0.294	0.001	PU-Glucose/Creatinine (mg/mg)[b]	0.330	0.001
PS-Integrated glucose	0.268	0.005	PS-Integrated glucose	0.316	0.001
FS-PTH	0.242	0.011	FS-PTH	0.277	0.003
Age	0.215	0.024	Age	0.255	0.007
PS-Integrated insulin	0.172	0.073	PS-Integrated insulin	0.174	0.069
Metabolic activity[b]	0.146	0.128	Metabolic activity[b]	0.120	0.211
FS-B-cell activity	0.132	0.170	FS-B-cell activity	0.048	0.379
Insulin sensitivity	−0.082	0.385	Insulin sensitivity	−0.044	0.644
FS-Total phospholipids	−0.122	0.204	FS-Total phospholipids	−0.092	0.399
FS-Total lipids	−0.197	0.039	FS-Total lipids	−0.160	0.094

[a] For dimensions see Tables 1–3
[b] on the basis of $\log_{10}$ data

Table 3 provides an overview of variables that appear of relevance for further elucidation of the state of metabolism and the propensity of urine to form stones in normocalciuric and hypercalciuric RCU. In addition, lipid levels in fasting blood are given because cholesterol was found to be elevated in renal stone patients [58], and we also found a positive association of cholesterol with the metabolic activity of RCU [38]. In atherosclerosis which, like RCU, is associated with hyperinsulinemia, insulin resistance, and the precipitation of calcium phosphate, increased total and VLDL cholesterol are regularly found, as also is increased pancreatic β-cell activity, another increasingly accepted predictor [52] that can be readily assessed [26].

The two calciurias did not differ statistically with respect to the dynamics of blood glucose, insulin, etc. (data not shown; see Fig. 2 and [41, 44]), and integrated glucose, insulin, and insulin resistance (Table 3). The same was valid for urinary sodium, considered to parallel urinary calcium to a certain degree [10], and urinary supersaturation with calcium oxalate, whereas in postprandial urine of hypercalciurics the brushite supersaturation was moderately increased within the metastable range. In RCU patients as a whole the fasting serum total lipids, total cholesterol, its VLDL fraction, and total phospholipids were in the upper range of normalcy (Table 3); however, lipids were generally higher in normo- than hypercalciuria, and total lipids and total phospholipids were significantly elevated.

Interrelationships of variables in trial 2

The coefficients from the bivariate correlation matrix, constructed for anthropometric and chemical parameters as the influential variables, and urinary Pi (excretion, fractional excretion) as a dependent variable, are given in order of size, from positive to negative (Table 4). Pi-uria (excretion, fractional excretion) correlated directly and significantly with postprandial urinary glucose (see also below), integrated serum glucose, fasting serum PTH, and age. Pi-uria was inversely and only insignificantly correlated with insulin sensitivity, integrated postprandial serum insulin, β-cell activity and total phospholipids. However, Pi-uria correlated inversely and significantly with total lipids.

The variables listed in Table 4, as well as several others assumed to be of relevance in the pathophysiology of RCU [pH, calcium, magnesium, glucose, all in postprandial urine, low density lipoprotein-bound cholesterol (LDL, data not shown), VLDL-cholesterol, all in fasting serum], were included in multivariate regression analyses. Table 5 shows that Pi-uria (excretion rate) was best predicted by integrated glucose, followed by integrated insulin, PTH, metabolic activity, and lipids; for fractional Pi-excretion a similar order of associations was found. In these models insulin sensitivity was a predictor of fractional Pi, but not of Pi excretion; postprandial urinary glucose excretion, age and calciuria had no influence on Pi-uria. The significant statistical predictors explain about 38% and 53% of the total variation of Pi excretion and fractional Pi excretion, respectively, indicating that additional variables, unidentified in the present work, contribute to postprandial hyperPi-uria of RCU.

Table 5 Multiple stepwise (forward and backward) regression analysis of variables predicting postprandial urinary phosphate in RCU (n = 117). For dimensions and further explanations see Tables 1–4 and text. R^2 (adjusted to non-included variables): 0.179, $P < 0.00009$ (for the model phosphate excretion, Pi/Cr); 0.227, $P < 0.000001$ (for the model fractional phosphate excretion, FE-Pi). Beta: standardized regression coefficient. R^2 (%): the portion of total variation explained by a given predicting variable

	Beta	R^2 (%)	P-value
Pi/Cr			
Integrated glucose	0.335	11.2	0.000798
Insulin sensitivity	0.265	7.0	0.060149
Integrated insulin	0.264	6.9	0.043078
PTH	0.199	4.0	0.024221
Metabolic activity[a]	0.185	3.4	0.032889
Total lipids	−0.227	5.2	0.032889
FE-Pi			
Integrated glucose	0.404	16.3	0.000034
Insulin sensitivity	0.385	14.8	0.005517
Integrated insulin	0.295	8.7	0.020589
PTH	0.245	6.0	0.004491
Metabolic activity[a]	0.177	3.2	0.035776
VLDL	−0.171	2.9	0.046182

[a] on the basis of $\log_{10}$ data

Discussion

In RCU patients with a mean age near the maximum of stone incidence the spectrum of post-test meal load Pi-uria in conjunction with normoglycemia, hyperinsulinemia, and insulin resistance, was found independent of the underlying calciuria; this confirms and extends previous findings in younger adults with RCU [44]. Theoretically, the older age of the participants in the present work – a factor known to impair glucose utilization and Pi-uria [33, 36, 53] – could have manifested similarly in RCU and controls, yet disturbed insulinemia and Pi-uria were restricted to stone patients. Thus, over a wide age range males with RCU exhibit impairment of vitally important hormonal (insulin) and micronutritional (phosphorus) processes. The long-term effects of excess insulin in the blood of RCU patients cannot be sufficiently well assessed from the data in the present work, but there is abundant information in the literature suggesting that among the sequelae are atherosclerosis, dyslipidemia, hyperuricemia, and anomalous regulation of intracellular calcium, to mention but a few [17, 48, 52].

Urinary Pi loss in RCU and its potential impact on metabolic regulatory processes, i.e., apart from its contribution to urine supersaturation with stone-forming substances (see below), has remained largely uninvesti-

gated. Some of the reasons for this may be differences in study design, age of participants, dietary regimens, etc., that have masked Pi abnormalities. In a more recent report, fractional Pi excretion in calcium phosphate and calcium oxalate stone patients, calculated for 24-h urine collected from patients on a free home diet, was significantly higher (on average 54%) than in non-stone-forming controls [13]. In another report focusing on Pi in calcium stones, Pi was not mentioned among the substances excreted in daily urine of urolithiasis patients [31]. Exaggerated Pi loss via the urine should theoretically constitute a risk factor for higher urine supersaturation with calcium phosphate, homogeneous calcium phosphate nucleation and, possibly, heterogeneous nucleation of calcium oxalate [28]. The achieved degree of supersaturation for acid calcium phosphate was insufficient for inducing homogeneous nucleation (Table 3). Also, our work failed to detect differences in urinary pH (Table 2), allowing us to rule out the possibility that the renal tubules reabsorbed less monovalent versus divalent Pi, and that a renally mediated acid-base disturbance was followed by enhanced Pi-uria. On the other hand, enhanced calcium phosphate crystalluria in postprandial urine with an undefined state of Pi has repeatedly been observed [20, 51]. Therefore further work in this area should not only include urinary pH, Pi-uria, and calcium phosphate supersaturation, but also pyrophosphate, which increases with increasing Pi-uria, but inhibits growth of crystals [5]. There are other consequences of Pi losses deserving comments.

Long-term Pi losses cause Pi deficiency and, once manifest should lead to impaired metabolism of insulin and glucose [49], a characteristic form of osteopathy [3, 24], and hypercalciuria [15]. Several symptoms of RCU, as found in the present work (e.g., insulin resistance, hyperinsulinemia, higher-than-normal calciuria; Tables 2, 3 [15]) or reported by others (e.g., osteopathy [2, 55]), would mesh with the concept of a manifest Pi deficit, but in the absence of specific investigations on cellular Pi interpretation needs to be cautious. While phosphorus malnutrition is an unlikely cause, previous work has shown enhanced Pi-uria in idiopathic (i.e., renal plus absorptive) and absorptive hypercalciuria, but not normocalciuria [6, 56, 57]. Intracellular Pi deficiency in the kidney may be followed by enhanced production of 1,25-dihydroxyvitamin D [6], which stimulates intestinal calcium absorption thereby completing the chain of events so far considered to apply in idiopathic hypercalciuria among RCU. There is further circumstantial evidence of low cellular Pi in RCU. Fasting Pi-uria is low (Table 2) [34, 43, 44], fasting serum 1,25-dihydroxyvitamin D of normocalciuric RCU tends to be high [46, additional unpublished data], calciuria is, on average, higher than in non-stone-forming controls albeit still within normal limits (Table 2) [41], hypophosphatemia and calciuria in RCU patients of similar age to those in the present study were found to be positively correlated [56, 57], and insulin resistance of organs has long been known to be a sign of Pi deficiency [11]. Finally, the divergent findings of a positive correlation of postprandial Pi-uria with insulin in RCU of present work (Tables 4, 5), but a negative correlation of the two variables in healthy controls [10, 44] strengthen the contention that in RCU Pi is insufficiently conserved during such periods. Unexpectedly, PTH in postprandial serum of the RCU patients of trial 1 was suppressed by the calcium content of the test meal (Fig. 1) [42], although there was a positive association of fasting serum PTH with postprandial hyperPi-uria in trial 2. Whether this statistical linkage reflects reality or is merely fortuitous awaits clarification from investigation of the integrated postprandial PTH response (see also below).

Owing to the low postprandial PTH (Fig. 1) its Pi-uria-stimulating effect, mediated via intracellular accumulation of cyclic AMP, should have been scaled down. Cyclic AMP degradation occurs through phosphodiesterase, but the activity of this enzyme is under partial control of insulin-dependent intracellular messengers such as inositol phosphate glycan [35]. Speculatively, this pathway may be impaired in the insulin resistance exhibited by RCU; as a result, intracellular accumulation of cyclic AMP may have been paradoxically facilitated, as well as (apparently PTH-related) hyperPi-uria. The existence of some PTH-insensitive mechanism for Pi reabsorption was invoked for the first time in inherited X-linked hypophosphatemia in humans [47]. In vitro studies showed that the brush border-lined surface of proximal tubules accomplishes the bulk of Pi and glucose reabsorption; on the other hand, insulin is involved in the control of Pi but not glucose reabsorption [8, 29, 55]. In the present work Pi-uria and glucosuria in RCU do not change quantitatively in parallel (Table 2), and multiple regression analysis revealed that glycosuria is not a predictor of Pi-uria (Table 5); therefore, renal Pi transport in ICU may occur at sites remote from those for glucose. The transepithelial Pi transport, sodium-dependent to maintain electroneutrality, necessitates an ATP-driven sodium pump in the basolateral membrane, a structure also equipped with a sodium-independent outward (blood)-directed Pi transport system [29]. Simply put, impairment of the sodium pump may be followed by uncoupling of the Pi and sodium co-transport, decreased Pi efflux, intracellular Pi accumulation, and subsequent arrest of Pi uptake by brush-border membrane vesicles, i.e., net Pi reabsorption is diminished. Such a sequence of events would leave open the possibility of an intracellular redistribution of Pi, namely Pi deficiency in some, but Pi accumulation in another compartment. In a Pi-deficient compartment the availability of ATP becomes rate-limiting for enzyme activity. Formation of calcium phosphate(s), brushite included, inside cells characterized by different ATP content has been demonstrated [59], as well as calcium phosphate deposition outside the tubular lumen of stone patients [32].

Disordered blood and tissue lipids in RCU, reported by others [58] and by us [38], can originate from over-

nutrition and malnutrition – frequent in Western and westernized civilizations – or from malregulation of lipid metabolism. Calcium oxalate stones of humans contain cholesterol and phospholipids [21]. Unimpaired lipids, especially phospholipids, of cell membranes are a prerequisite for the maintenance of membrane fluidity [14]. For instance, in situations with altered homeostasis of insulin and/or lipids, such as atherosclerosis and magnesium deficiency [9, 14, 16], membrane properties become disintegrated. In normocalciuric RCU a cellular magnesium deficit is detectable [37]. Thus, the high levels of circulating lipids in the normocalciuric majority as compared with the hypercalciuric minority of RCU, and the fact that both calciurias exhibit hyperPi-uria, suggest that defective membranes predominate in RCU; in hypercalciurics this damage may be attenuated at the cost of development of hypercalciuria. Alternatively, the statistical linkage between lipids and stones could reflect yet unknown pathomechanisms. In preliminary work we found that a rise of total cholesterol may be accompanied by an increase of metabolic activity of stone disease in a subset of RCU [38].

In conclusion, we were able to demonstrate disordered Pi, insulin and probably lipid metabolism in RCU patients, irrespective of calciuria. Despite hyperPi-uria substantially increased supersaturation of urine with renal calcium stone-forming substances was not detectable. It is therefore suggested that cellular events leading to insulin resistance, insulin excess, abnormal Pi and lipid status may initiate renal stone formation at a site remote from the tubular lumen.

Acknowledgements We are grateful to B. Schreiber, K. Schwille and G. Hoffmann for technical assistance and I. Goldberg for secretarial work. Supported by the University of Erlangen Hospital Research Funds, Erlangen, and the W. Sander Foundation for the Advancement of Medical Sciences, Munich.

References

1. Armbrecht J (1986) Age-related changes in calcium and phosphorus uptake by rat small intestine. Biochim Biophys Acta 882:281
2. Baskin J, Wilson DR, Manuel MA, Bailey A, Murray T, Harrison J (1985) Bone mineral content in idiopathic calcium nephrolithiasis. Miner Electrolyte Metab 11:19
3. Baylink D, Wergedal J, Staufer M (1971) Formation, mineralization and resorption of bone in hypophosphatemic rats. J Clin Invest 50:2519
4. Bijvoet OLM, Van der Sluys Veer J (1972) The interpretation of laboratory tests in bone disease. Clin Endocrinol Metab 1:217
5. Bisaz S, Felix R, Neuman WF, Fleisch H (1978) Quantitative determination of calcium phosphate precipitation in whole urine. Miner Electrolyte Metab 1:74
6. Broadus AE, Insogna KL, Lang R, Mallette LE, Oren DA, Gertner JM, Kliger AS, Ellison AF (1984) A consideration of the hormonal basis and phosphate leak hypothesis of absorptive hypercalciuria. J Clin Endocrinol Metab 58:161
7. Chen PS, Toribara TY, Warner H (1956) Microdetermination of phosphorus. Anal Chem 28:1756
8. Crabbé J (1982) Stimulation by insulin of transepithelial sodium transport. Ann NY Acad Sci 372:220
9. Darley-Usmar V, Hogg N, Kalyanaraman B, Moore K (1995) Free radicals in the vasculature: pathological and physiological significance. In: Ria-Evans C, Bruckdorfer KR (eds) Oxidative stress, lipoproteins and vascular dysfunction. Portland Press, London, p 81
10. DeFronzo RA, Cooke CR, Andres R, Faloona GR, Davis PJ (1975) The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. J Clin Invest 55:845
11. DeFronzo RA, Lang R (1980) Hypophosphatemia and glucose intolerance: evidence for tissue insensitivity to insulin. N Engl J Med 303:1259
12. Friedenwald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of LDL cholesterol in plasma without the use of the preparative ultracentrifuge. Clin Chem 18:499
13. Gault MH, Chafe LL, Morgan JM, Parfrey S, Harnett JD, Walsh EA, Prabhakaran VM, Dow D, Colpitts A (1991) Comparison of patients with idiopathic calcium phosphate and calcium oxalate stones. Medicine 70:345
14. Gomez-Fernandez JC, Villalain J (1992) The interaction of membrane-intrinsic proteins with phospholipids. In: Quinn PJ, Cherry RJ (eds) Structural and dynamic properties of lipids and membranes. Oxford University Press, Oxford, p 77
15. Grabie M, Lau K, Agus ZS, Goldberg M, Goldfarb S (1978) Role of parathyroid hormone in the hypercalciuria of phosphate depletion. Miner Electrolyte Metab 1:279
16. Gueux E, Azais-Braesco V, Bussière L, Grolier P, Mazur A, Rayssiguier Y (1995) Effect of magnesium deficiency on triacylglycerol-rich lipoprotein and tissue susceptibility to peroxidation in relation to vitamin E content. Br J Nutr 74:849
17. Haffner SM, Stern MP, Hazuda HP, Pugh JA, Patterson JK (1986) Hyperinsulinemia in a population at high risk for non-insulin-dependent diabetes mellitus. N Engl J Med 315:220
18. Harter HR, Santiago JV, Rutherford WE, Slatopolski E (1976) The relative roles of calcium, phosphorus, and parathyroid hormone on glucose- and tolbutamide-mediated insulin release. J Clin Invest 58:359
19. Heaney R (1996) Editorial. Calcium, parathyroid function, bone and aging. J Clin Endocrinol Metab 81:1697
20. Herrmann U, Schwille PO, Kuch P (1991) Crystalluria determined by polarization microscopy. Urol Res 19:151
21. Khan SR, Shevock PN, Hackett RL (1988) Lipids of calcium oxalate stones. In: Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (eds) Urolithiasis. Plenum Press, New York, p 125
22. Larsson L, Sörbo B, Tiselius HG, Öhman S (1984) A method for quantitative wet-chemical analysis of urinary calculi. Clin Chim Acta 140:9
23. Lau YK, Wasserstein A, Westley RG, Bosanac P, Grabie M, Mitnick P, Slatopolsky E, Goldfarb S, Agus ZS (1982) Proximal tubular defects in idiopathic hypercalciuria: resistance to phosphate administration. Miner Electrolyte Metab 7:237
24. Lotz M, Zisman E, Bartter FC (1968) Evidence for a phosphorus depletion sydrome in man. N Engl J Med 278:409
25. Marshall RW, Robertson WG (1976) Nomograms for the estimation of the saturation of urine with calcium oxalate, calcium phosphate, magnesium ammonium phosphate, uric acid, sodium acid urate, ammonium acid urate and cystine. Clin Chim Acta 72:253
26. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412
27. McIntosh IC (1984) The excretion of sugars in urine. Clin Chim Acta 143:169
28. Meyer JL, Bergert JH, Smith LH (1975) Epitaxial relationships in urolithiasis: the calcium oxalate monohydrate–hydroxyapatite system. Clin Sci Mol Med 49:369
29. Murer H, Malmström K (1987) How renal phosphate transport is regulated. News Physiol Sci 2:45
30. National Diabetes Data Group (1979) Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039

31. Öhman S, Larsson L, Tiselius HG (1992) Clinical significance of phosphate in calcium oxalate renal stones. Ann Clin Biochem 29:59
32. Randall A (1937) The origin and growth of renal calculi. Ann Surg 105:1009
33. Reaven GM, Chen N, Hollenbeck C, Chen YDI (1989) Effect of age on glucose tolerance in healthy individuals. J Am Geriatr Soc 37:735
34. Rodgers AL, Barbour LJ, Pougnet BM, Lombard CJ, Ryall RL (1995) Re-evaluation of the "week-end effect" data: possible role of urinary copper and phosphorus in the pathogenesis of renal calculi. J Trace Elements Med Biol 9:150
35. Saltiel AR, Fox JA, Sherline P, Cuatrecasas P (1986) Insulin-stimulated hydrolysis of a novel glycolipid generates modulators of cAMP phophodiesterase. Science 233:967
36. Schellenberg B, Tschöpe W, Ritz E, Wesch H, Schlierf G (1980) Urinary sodium excretion in renal stone formers. Klin Wochenschr 58:575
37. Schmiedl A, Schwille PO (1996) Magnesium status in idiopathic calcium urolithiasis : An orientational study in younger males. Clin Chem Clin Biochem 34:393
38. Schmiedl A, Schwille PO, Manoharan M (1996) Lipids in fasting blood, metabolic activity and associated variables in idiopathic calcium urolithiasis (ICU) of males: a pilot study. In: Pak CYC, Resnick MI, Preminger GM (eds) Urolithiasis 1996. Millet The Printer, Dallas, p 58
39. Scholz D, Schwille PO (1981) Klinische Laboratoriumsdiagnostik der Urolithiasis. Dtsch Med Wochenschr 106:999
40. Scholz D, Schwille PO, Ulbrich D, Bausch WM, Sigel A (1979) Composition of renal stones and their frequency in a stone clinic: relationship to parameters of mineral metabolism in serum and urine. Urol Res 7:161
41. Schwille PO, Rümenapf G, Köhler R (1989) Blood levels of glucometabolic hormones and urinary saturation with stone forming phases after an oral test meal in male patients with recurrent idiopathic calcium urolithiasis and in healthy controls. J Am Coll Nutr 8:557
42. Schwille PO, Rümenapf G, Schmidtler J, Köhler R (1987) Fasting and post-calcium load serum calcium, parathyroid hormone, calcitonin, in male idiopathic calcium urolithiasis: evidence for a basic disturbance in calcium metabolism. Exp Clin Endocrinol 90:71
43. Schwille PO, Samberger NH, Wach B (1976) Fasting uric acid and phosphate in urine and plasma of renal calcium-stone formers. Nephron 16:116
44. Schwille PO, Schmiedl A, Herrmann U, Wipplinger J (1996) Postprandial hyperinsulinaemia, insulin resistance and inappropriately high phosphaturia are features of younger males with idiopathic calcium urolithiasis: attenuation by ascorbic acid supplementation of the test meal. Urol Res 25:49
45. Schwille PO, Schmiedl A, Rümenapf G (1996) Disorders of calcium metabolism: appraisal and management. In: Whitfield H, Hendry B, Kirby R, Duckett J (eds) Textbook of genitourinary surgery. Blackwell Scientific, Oxford
46. Schwille PO, Töpper K, Schwille K, Herrmann U (1991) Recurrent idiopathic calcium urolithiasis (RCU): blood levels of two vitamin D metabolites in males. In: Norman AW, Bouillon R, Thomasset M (eds) Vitamin D, gene regulation, structure-function analysis and clinical application. Walter de Gruyter, Berlin, p 932
47. Scriver CR (1980) Transepithelial transport of phosphate: perspectives from man and mouse. In: DeLuca HF, Anast CS (eds) Pediatric diseases related to calcium. Blackwell Scientific, Oxford, p 165
48. Shimamoto K, Higashiura K, Nakagawa M, Masuda A, Shiiki M, Miyazaki Y, Ise T, Fukuoka M, Hirata A, Imura O (1995) Effects of hyperinsulinemia under the euglycemic condition on calcium and phosphate metabolism in non-obese normotensive subjects. Tohoku J Exp Med 177:271
49. Simonson D, DeFronzo RA (1982) Hypophosphatemia and glucose intolerance. Adv Exp Biol Med 151:217
50. Sluiter WJ, Erkelens DW, Terpstra P, Reitsma WD, Doorenbos H (1975) Glucose tolerance and insulin release, a mathematical approach. II. Approximation of the peripheral insulin resistance after oral glucose loading. Diabetes 25:245
51. Smith LH, Werness PG, Erickson SD, Phillips SF (1984) Postprandial response to a normal diet in patients with idiopathic calcium urolithiasis. In: Ryall R, Brockis JG, Marshall V, Finlayson B (eds) Urinary stone. Churchill Livingstone, Edinburgh, p 47
52. Solymoss BC, Marcil M, Chaour M, Gilfix BM, Poitras AM, Campeau L (1995) Fasting hyperinsulinism, insulin resistance syndromes, and coronary artery disease in men and women. Am J Cardiol 76:1152
53. Sorribas V, Lötscher M, Loffing J, Biber J, Kaissling B, Murer H, Levi M (1996) Cellular mechanisms of the age-related decrease in renal phosphate reabsorption. Kidney Int 50:855
54. Steiniche T, Mosekilde L, Christensen MS, Melsen F (1989) A histomorphometric determination of iliac bone remodeling in patients with recurrent renal stone formation and idiopathic hypercalciuria. APMIS 97:309
55. Thierry J, Poujeol P, Ripoche P (1981) Interactions between Na^+-dependent uptake of D-glucose, phosphate and L-alanine in rat renal brush border vesicles. Biochim Biophys Acta 647:203
56. Tschöpe W, Ritz E, Schellenberg B (1980) Plasma phosphate and urinary calcium in recurrent stone formers. Miner Electrolyte Metab 4:237
57. Tschöpe W, Ritz E, Schmidt-Gayk H (1980) Is there a renal phosphorus leak in recurrent renal stone formers with absorptive hypercalciuria? Eur J Clin Invest 10:381
58. Varalakshmi G, Radha Shanmuga Sundaram K, Venugopal A (1977) Blood lipids in renal stone disorder. Indian J Med Res 66:840
59. Wuthier RE, Rice GS, Wallace jr. JEB Beaver RL, LeGeros RZ, Eanes ED (1985) In vitro precipitation of brushite from an amorphous precursor in the absence of ATP. Calcif Tissue Int 37:401