

Original Article

Risk factors for low urinary citrate in calcium nephrolithiasis: low vegetable fibre intake and low urine volume to be added to the list

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Abstract. Risk factors for low urinary citrate excretion were assessed in 34 consecutive male recurrent idiopathic calcium stone formers (RCSF) who collected two 24-h urines while on free-choice diet. Overt hypocitraturia (hypo-cit) was defined as $U_{Cit} \times V < 1.70$ mmol/day, and 'low' citraturia (low-cit) as $U_{Cit} \times V$ between 1.70 and 2.11 mmol/day. Twenty-three RCSF had normocitraturia (normo-cit), six low-cit and five hypo-cit. $U_{Cit} \times V$ positively correlated with urine volume (VOLUME, $r=0.44$, $P=0.009$), vegetable fibre intake (fibers, $r=0.46$, $P=0.009$) and GI-alkali absorption (alkali, $r=0.47$, $P=0.006$), and volume, fibres and alkali tended to be lower among RCSF with low-/hypo-cit. A 3-day NH_4Cl loading test (0.95 mEq/kg BW daily in 3 doses) was performed in RCSF as well as in 14 age-matched healthy male controls (C). On a plot of urine pH versus serum bicarbonate, 10 of 11 RCSF with low-/hypo-cit, but only six of 23 with normo-cit ($P=0.0004$) fell off the normal range, indicating incomplete RTA. Two or more risk factors simultaneously occurred in only four of 23 RCSF with normo-cit, but in eight of 11 with low-/hypo-cit ($P=0.002$). In conclusion, incomplete RTA is the most prevalent risk factor for low-/hypo-cit in RCSF, and decreases in vegetable fibres and urine volume emerge as two new risk factors for low urinary CIT.

Key words: calcium nephrolithiasis; citrate; gastrointestinal alkali absorption; renal tubular acidosis; urine volume; vegetable fibres

Introduction

Low urinary citrate excretion is an accepted pathogenic factor for calcium renal stone formation [1,2]. Variations of systemic acid-base status have been shown for a long time to influence urinary citrate excretion [3]: for instance, metabolic alkalosis increases urinary citrate [4], whereas ammonium-chloride-

induced acidosis lowers its excretion by 20–50% within a few hours [5]. Owing to the fact that cytosolic acidification activates citrate uptake by the proximal tubular cell [3], intracellular rather than systemic acidosis has been postulated as the denominator common to most patients with 'idiopathic' hypocitraturia [6].

Indeed, overt metabolic acidosis is usually absent in recurrent calcium renal stone formers with 'idiopathic' hypocitraturia. With the help of the classic ammonium chloride loading test, however, we [6] and others [7] have provided evidence for incomplete renal tubular acidosis (RTA) in a substantial fraction of patients with 'idiopathic' hypocitraturia. Such a reduced capability of renal acid excretion may favour intracellular acidosis and thus cause hypocitraturia in these patients.

Dietary factors also modulate the urinary excretion rate of citrate. Excess animal protein intake with the acid load that it conveys lowers urinary citrate [1,8], whereas dietary protein restriction does the opposite [9]. Urinary citrate positively correlates with net gastrointestinal absorption of alkali, which seems to be reduced in many idiopathic calcium stone formers [1]. Potassium depletion also triggers hypocitraturia [5,10]: it appears to do so via stimulation of the sodium-citrate cotransport system at the brush-border level of the proximal tubular cell, and intracellular acidosis, again, might provide the key signal [11]. Additionally, sodium chloride loading depresses urinary citrate; this effect may, again, be governed by intracellular acidosis, due to sodium-induced volume expansion with subsequent rise in urinary bicarbonate excretion [1].

Finally, primary intestinal malabsorption of citrate has been postulated to account for 'idiopathic' hypocitraturia in some patients with idiopathic calcium nephrolithiasis [1].

Given a population of patients with idiopathic calcium nephrolithiasis studied on their free-choice diet [12], the present study aimed to address the following issues: (1) what is the prevalence of 'idiopathic' hypocitraturia?; (2) among patients who turn out to have low urinary citrate, how often does the disorder have a metabolic origin, and how often a nutritional one?; (3) are there other mechanisms to account for low urinary citrate than those alluded to above?

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Subjects and methods

From the patients routinely seen for metabolic work-up at our renal stone clinic, 34 consecutive male recurrent idiopathic calcium stone formers (RCSF) meeting the following criteria were studied: (1) passage or removal of at least two calcium-containing stones, defined either by stone analysis (X-ray diffraction) or disappearance of opaque material on conventional radiographs or excretory urograms; (2) no established cause of calcium stone formation such as primary hyperparathyroidism, medullary sponge kidney, overt distal renal tubular acidosis, sarcoidosis, excessive vitamin D intake, and hypercalciuria due to hypercalcaemia of malignancy or immobilization; (3) absence of obvious causes of hypocitraturia such as malabsorption with steatorrhoea [13], hypokalaemia (serum K < 3.5 mmol/l) due to acetazolamide or thiazide treatment, or urinary tract infection [14]; and (4) $C_{\text{crea}} > 70 \text{ ml/min/1.73 m}^2$.

All RCSF were referred after ESWL or endourological treatment of their stone disease, and they were asked to keep unchanged the free-choice diet they used to have before stone treatment. They were never studied until at least 2 months had elapsed after urological intervention. Other medications known to interfere with calcium and citrate metabolism were discontinued at least 2 weeks prior to evaluation.

The normal values for 24-h urinary excretion rates currently used in our stone clinic are based on 107 healthy male volunteers living in the area of Berne, Switzerland, while on a free-choice diet. Upper normal levels (95th percentile) are 9.00 mmol/d for calcium, 440 $\mu\text{mol/day}$ for oxalate, and 5.00 mmol/day for uric acid; the lower normal level of $U_{\text{Cit}} \times V$ (5th percentile) is 1.70 mmol/day (320 mg/d). In an early report by Hodgkinson [15], hypocitraturia in men had been defined as $U_{\text{Cit}} \times V < 2.12 \text{ mmol/day}$ (<400 mg/day); accordingly we subclassified our RCSF into those with overt hypocitraturia (hypo-cit), i.e. $U_{\text{Cit}} \times V < 1.70 \text{ mmol/day}$ (<320 mg/day), and those with 'low citraturia' (low-cit), i.e. $U_{\text{Cit}} \times V$ between 1.70 and 2.11 mmol/d (320–399 mg/day). Since the risk of calcium stone formation has been demonstrated to increase exponentially with decreasing 24-h urine volumes below 1200 ml [16], 'low volume' was defined as <1200 ml/day. All RCSF collected two 24-h urine specimens according to our standard protocol [12]; 24-h urine values are expressed as the means of both collections.

Twenty-four-hour urines were collected in 3-litre plastic bottles containing 10 g of boric acid as preservative agent. Specimens were analysed for creatinine (Crea), calcium (Ca), phosphate (P), sodium (Na), potassium (K), chloride (Cl), magnesium (Mg), uric acid (UA) and urea by autoanalyser techniques. Citrate (Cit) was measured using the citrate lyase method [17]. Oxalate (Ox) was measured after enzymatic oxidation of oxalate by oxalate oxidase [18] as previously described [12]. Urinary sulphate (Sulph) was measured by an HPLC system utilizing an anion column to separate sulphate from other urinary anions, where sulphate is detected by conductivity change as eluent flows through a conductivity cell [19].

Urinary excretion rates of urea, UA and Sulph were taken as markers of animal protein consumption, and $U_{\text{Na}} \times V$ as a measure of sodium intake. Since fibre-rich foods such as vegetables, grain products, and fruits are sources of alkali [20], alkali consumption was estimated from the daily ingestion of vegetable fibres, subsequently referred to as 'dietary fibres'. The latter ones were assessed from the patients' diet protocols filled out during collections of 24-h urines, by means of food composition and nutrition tables [20], i.e.

two 24-h diet protocols were retrospectively analysed from each patient; direct measurements of food compositions were not performed. Since dietary fibre contributes 15–25 g to the average US diet [21], ingestion of less than 15 g/day was defined as low dietary fibre intake, indicating lower alkali consumption. Net gastrointestinal absorption of alkali (expressed in mEq/24 h), subsequently referred to as 'GI-alkali', was derived from 24-h urine excretions of non-combustible cations and anions according to the formula

$$(\text{Na} + \text{K} + \text{Ca} + \text{Mg}) - (\text{Cl} + 1.8 \times \text{P}) \quad [1,22],$$

where electrolyte excretions are in mEq/day except for P which is in mmol/day with an average valence of 1.8 [1,22]. Low GI-alkali was defined as <15 mEq/day, since the lower normal limit (5th percentile) among our male healthy volunteers was 15 mEq/day.

An ammonium chloride loading study was performed in all RCSF and additionally in 14 of our 107 healthy male volunteers, aged 44.2 ± 2.6 years (range 28–62) and with normokalaemia (fasting serum K concentration between 3.5 and 4.1 mmol/l). It consisted of sampling venous blood and 2-h urine after 12 h without eating and 8 h without drinking (only tap water allowed after the last meal) on day 0 (indicated as 0). From then on, ammonium chloride (NH_4Cl) was added to the free-choice diet of each subject for 3 days and given in three divided doses 20 min before each meal (total 0.05 g/kg/day, or 0.95 mEq/kg/day). On day 3, sampling of fasting venous blood and 2-h urine was repeated (indicated as 3). For the nomogram of fasting venous bicarbonate concentration versus urine pH after acid loading only (Figure 2), nine additional male healthy volunteers were included from a previous study with NH_4Cl loading [6].

Plasma concentrations of Crea, total Ca, P, Mg, Na, K, Cl, UA, and total protein were measured by autoanalyser techniques. Serum anion gap, an indirect index for unmeasured anions, was defined as $[\text{Na}^+] - [\text{Cl}^- + \text{HCO}_3^-]$; normal values were 8–16 mmol/l. In whole blood, ionized calcium (Ca^{2+}) was measured by an ion-selective electrode (Ciba-Corning Diagnostics Corp., Medfield, Massachusetts, USA) and bicarbonate by a Ciba-Corning 278 blood gas system (Ciba-Corning Diagnostics Corp.) immediately after sampling without compression. Serum intact parathyroid hormone (PTH) concentration was measured by a radioimmunoassay kit (Nichols Institute, San Juan Capistrano, California, USA); normal values are 10–65 pg/ml. Serum 25 (OH) vitamin D (normal range 6–40 ng/ml) and serum 1,25 (OH) $_2$ vitamin D $_3$ (calcitriol, normal range 25–79 pg/ml) were measured by radioimmunoassay (Nichols Institute, San Juan Capistrano, California, USA).

In freshly voided 2-h fasting urines, pH was measured by a Metrohm 654 pH-Meter (Metrohm, Herisau, Switzerland). The fasting urines were analysed for Ca, P, Na, K, Cl, urea, and crea by autoanalyser techniques. Hydroxyproline (OHP) was assayed using a kit (Hypronosticon[®], Organon Teknika, Bostel, The Netherlands), where OHP is coloured by the Ehrlich's reagent and quantified photometrically after binding to an acid cation exchange resin, hydrolysis (16 h) and oxidation. Urinary anion gap (AG), an indirect measure of urinary ammonium concentration [23], was calculated as $[\text{Na}] + [\text{K}] - [\text{Cl}]$. All 2-h fasting urine values are presented in relation to urine creatinine concentration (mmol/mmol ratio), except renal phosphate threshold (TmP/GFR), which was derived from the nomogram of Walton and Bijvoet [24], and OHP-E ($\mu\text{mol/l GF}$), which was calculated using the formula $\text{OHP}_U/\text{Crea}_U \times \text{Crea}_S$.

All values are presented as means \pm SEM. Non-parametric Mann-Whitney U-test for comparisons between and

Wilcoxon signed-rank test for comparisons within groups were used. Simple and multiple linear regression analysis was performed for correlation studies.

Results

The 34 male RCSF participating in the study were 42.7 ± 1.6 years old (range 23–64, not different from controls). None of them had hypokalaemia (<3.5 mmol/l), their fasting serum K concentrations ranging between 3.5 and 4.5 mmol/l. Twenty-three RCSF (67.6%) were normocitraturic (normo-cit), whereas $U_{Cit} \times V$ was below 2.12 mmol/day in 32.4% (combined low-/hypo-cit), i.e. low-cit (1.70–2.11 mmol/day) in six (17.6%) and hypo-cit (<1.70 mmol/day) in five RCSF (14.8%). Among patients classified as normo-cit, two of 23 had $U_{Cit} \times V < 2.12$ mmol/day in one of their two urine collections. Four of six RCSF with low-cit had urinary citrate < 2.12 mmol/day in only one urine collection, whereas four of five RCSF classified as hypo-cit had $U_{Cit} \times V < 1.70$ mmol/day in both 24-h urines. Among healthy volunteers, urinary citrate < 2.12 mmol/day occurred in 19 of 107 normal controls (17.8%), i.e. 12 (11.2%) had hypo-cit and 7 (6.5%) low-cit. Twelve RCSF had hypercalciuria (HCSF), and 22 were normocalciuric (NCSF). Dietary protocols from 31 RCSF (91.2%) were available to us for further analysis; three of them (in 3 RCSF with normo-cit) were inappropriately filled out.

Twenty-four-hour urine data

Table 1 summarizes the main parameters derived from 24-h urine collections in RCSF. With the exception of GI-alkali, which was only 14 ± 4 mEq/day in hypo-cit

compared with 36 ± 5 mEq/day in normo- and 39 ± 7 mEq/day in low-cit, $P < 0.05$ and < 0.025 respectively, none of the parameters was different between subgroups. When comparing the combined low-/hypo-cit group ($U_{Cit} \times V < 2.12$ mmol/day, $n = 11$) with normo-cit, however, dietary fibre intake was lower in the former ones (11.9 ± 1.6 g/day versus 18.3 ± 1.8 , $P = 0.037$), and so was $U_{Ca} \times V$ (6.51 ± 0.84 mmol/day versus 9.41 ± 0.81 , $P = 0.029$).

Table 2 summarizes important correlations between $U_{Cit} \times V$ and various other parameters. Positive correlations between $U_{Cit} \times V$ and S_{crea} , C_{crea} , $U_{Ca} \times V$, $U_{Mg} \times V$ and GI-alkali as well urine volume and dietary fibre intake were noted. Among the 107 male controls, citrate excretion was also positively, though less strongly, correlated with urine volume ($r = 0.240$, $P = 0.014$). Figure 1 demonstrates that fibre consumption below 10 g/day was always associated with low- or hypo-cit, whereas RCSF whose fibre consumption was > 22.5 g/d all were normocitraturic. In RCSF whose fibre consumption was between 10 and 22.5 g/day, an

Table 2. Simple linear correlations between $U_{Cit} \times V$ and other parameters in all 34 RCSF

y axis	x axis	r value	slope	P
$U_{Cit} \times V$	S-Crea	0.490	-0.04	0.003
	C_{crea}	0.440	0.02	0.010
	Volume	0.440	0.0001	0.009
	$U_{Ca} \times V$	0.570	0.15	0.0004
	$U_{Mg} \times V$	0.370	0.24	0.032
	$U_K \times V$	0.280	0.01	0.111, NS
	GI-alkali	0.470	0.02	0.006
	Fibre intake	0.460	0.02	0.009

S-Crea, fasting serum creatinine before NH_4Cl loading. NS, not significant.

Table 1. Twenty-four-hour urine data (mean \pm SEM) from 34 RCSF.

Measurement	Normo-cit (n=23)	Low-cit (n=6)	Hypo-cit (n=5)
$U_{Cit} \times V$	3.07 ± 0.15	$1.94 \pm 0.05^{**}$	1.18 ± 0.23^{oo}
$U_{Ca} \times V$	9.41 ± 0.81	6.67 ± 0.8	6.31 ± 1.65
$U_{Ox} \times V$ (μ mol/day)	334 ± 27	358 ± 17	310 ± 80
$U_P \times V$	32.9 ± 1.7	31.7 ± 2.8	33.1 ± 8.0
$U_{Urea} \times V$	415 ± 16	397 ± 17	387 ± 45
$U_{UA} \times V$	3.60 ± 0.15	3.40 ± 0.22	3.50 ± 0.59
$U_{Salph} \times V$	21.9 ± 1.3	21.3 ± 1.0	21.7 ± 2.3
$U_{Na} \times V$	233 ± 17	193 ± 14	177 ± 41
$U_K \times V$	63 ± 4	73 ± 7	55 ± 10
$U_{Cl} \times V$	229 ± 17	191 ± 14	178 ± 41
$U_{Mg} \times V$	4.64 ± 0.34	3.77 ± 0.42	3.67 ± 0.61
Volume (ml)	2051 ± 158	1654 ± 214	1399 ± 332
$U_{crea} \times V$	15.5 ± 0.5	15.1 ± 0.9	15.3 ± 1.7
C_{crea} (ml/min/1.73 m ²)	109.7 ± 4.4	103.6 ± 8.0	114.4 ± 17.2
GI-alkali (mEq/day)	36 ± 5	39 ± 7	14 ± 4^o
Fibre intake (g/day)	18.3 ± 1.8	12.6 ± 2.5	11.0 ± 2.0

All values except $U_{Ox} \times V$ (μ mol/day), volume (ml), GI-alkali (mEq/day) and fibre intake (g/day) are mmol/day. For abbreviations, see 'Methods'. Cit, urinary citrate excretion rate; Hypo-cit, < 1.70 mmol/day; Low-cit, 1.70–2.11 mmol/day. ⁺ $P < 0.05$ versus Normo-cit; ^o $P < 0.025$ versus Low-cit; ^{oo} $P < 0.01$ versus low-cit; ^{**} $P < 0.001$ versus normo-cit. In the combined low-/hypo-cit group, fibre intake was lower than in normo-cit (11.9 ± 1.6 versus 18.3 ± 1.8 g/day, $P = 0.037$).

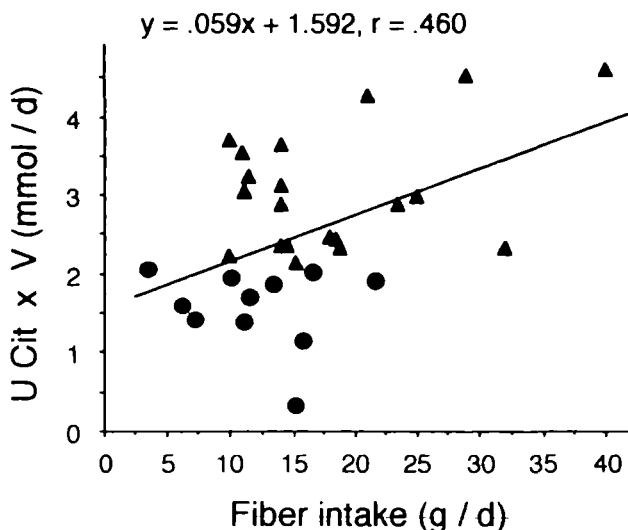


Fig. 1. 24-h urinary citrate excretion rate (y axis) in relation to daily intake of vegetable fibres calculated from dietary protocols (x axis). Circles, RCSF with $U_{Cit} \times V < 2.12$ mmol/day (<400 mg/day) ($n = 11$); triangles, RCSF with normocitraturia ($n = 20$; three RCSF missing due to inappropriately returned dietary protocols).

abnormality of urine acidification (see paragraph on NH_4Cl loading) occurred in six of seven with low-/hypo-cit, but only in three of 14 with normo-cit ($P = 0.034$, Chi-square). There was only a trend for a positive correlation between urinary excretion rates of citrate and potassium, whereas no correlations were found between $U_{Cit} \times V$ and urinary markers of protein intake (Urea, UA, Sulph) as well as of sodium consumption ($r = 0.280$, $P = 0.105$).

In a multiple regression analysis, $U_{Cit} \times V$ in RCSF was positively correlated ($r = 0.700$, $P = 0.004$) with a combination of the four factors C_{crea} (partial $F = 2.9$), urine volume (partial $F = 0.1$), GI-alkali (partial $F = 3.2$) and dietary fibre consumption (partial $F = 6.5$). When correlating urinary citrate with only GI-alkali and dietary fibres combined, a positive correlation was again noted ($r = 0.600$, $P = 0.002$); partial F values were 6.5 for GI-alkali and 6.0 for dietary fibre intake respectively.

Since $U_{Cit} \times V$ was positively correlated with $U_{Ca} \times V$, RCSF were divided into hypercalciurics (HCSF, $U_{Ca} \times V > 9.00$ mmol/day, $n = 12$) and normocalciurics (NCSF, $n = 22$). Table 3 summarizes values of 24-h urine excretion rates, C_{crea} , GI-alkali and daily fibre intake in HCSF and NCSF. $U_{Cit} \times V$ was higher in HCSF than in NCSF; low-cit, however, occurred in two of 12 HCSF and nine of 22 NCSF ($P = 0.289$, Chi-square), and hypo-cit was found in one of 12 HCSF and four of 22 NCSF ($P = 0.788$, Chi-square). Also elevated in HCSF versus NCSF were excretion rates of Ox, P, urea, Sulph, Mg, urinary volume, C_{crea} and daily fibre consumption. Since values of C_{crea} were higher among HCSF, urinary parameters were also expressed relative to creatinine (mmol/mmol ratios); after this calculation, significant differences between HCSF and NCSF remained for Ca/Crea, P/Crea, and

Table 3. Differences between hypercalciuric (HCSF) and normocalciuric (NCSF) recurrent calcium stone formers

Measurement	HCSF (12)	NCSF (22)	P
$U_{Cit} \times V$	3.19 ± 0.29	2.27 ± 0.17	0.014
$U_{Ca} \times V$	13.00 ± 0.50	6.00 ± 0.35	0.0001
$U_{Ox} \times V$ ($\mu\text{mol/d}$)	394 ± 40	303 ± 23	0.046
$U_P \times V$	38.4 ± 2.8	29.5 ± 1.7	0.011
$U_{urea} \times V$	447 ± 21	386 ± 14	0.028
$U_{UA} \times V$	3.73 ± 0.17	3.45 ± 0.18	0.313, NS
$U_{sulph} \times V$	24.1 ± 1.6	20.5 ± 1.0	0.020
$U_{Na} \times V$	255 ± 27	197 ± 14	0.052, NS
$U_K \times V$	67 ± 5	62 ± 4	0.576, NS
$U_{Cl} \times V$	249 ± 28	196 ± 13	0.105, NS
$U_{Mg} \times V$	5.75 ± 0.40	3.63 ± 0.22	0.0003
Volume (ml)	2232 ± 178	1696 ± 161	0.019
$U_{crea} \times V$	16.4 ± 0.8	14.9 ± 0.5	0.171, NS
C_{crea} (ml/min/ 1.73 m ²)	120.3 ± 7.6	103.4 ± 4.2	0.040
GI-alkali (mEq/day)	42.4 ± 7.0	28.7 ± 3.9	0.109, NS
Fibre intake (g/day)	20.2 ± 2.8	13.9 ± 1.4	0.035

All values are means \pm SEM; P values are for differences between HCSF and NCSF (Mann-Whitney U test). For abbreviations, see 'Methods' and Table 1.

Mg/Crea, whereas they disappeared for Cit/Crea ($P = 0.066$), Ox/Crea, Urea/Crea, and Sulph/Crea. A positive correlation was also noted between daily fibre intake and $U_{Ca} \times V$ ($r = 0.440$, $P = 0.014$).

Fasting blood and urine samples before/after NH_4Cl loading

Table 4 summarizes the most important fasting blood and 2-h urine acid-base parameters in controls as well as in RCSF with normo-cit, low-cit and hypo-cit before and after NH_4Cl . Among stone formers with low-cit and hypo-cit, serum anion gap was higher than in controls and RCSF with normo-cit, both before and after NH_4Cl loading. During NH_4Cl loading there was a trend for venous bicarbonate concentration to slightly decrease in the four groups, limit of significance

Table 4. Fasting acid-base parameters and urinary cit/crea ratios (means \pm SEM) before and after NH_4Cl loading in 14 healthy male controls and 34 RCSF

Measurement	Controls (n=14)	Normo-cit (n=23)	Low-cit (n=6)	Hypo-cit (n=5)
AG ₀ (mM)	10.3 ± 0.7	12.2 ± 0.8	$13.4 \pm 0.7^\dagger$	$16.8 \pm 1.4^{**}$
AG ₃ (mM)	10.2 ± 1.0	11.8 ± 0.8	$17.3 \pm 2.3^\dagger$	$16.7 \pm 0.6^*$
Bic ₀ (mM)	25.9 ± 0.7	24.9 ± 0.5	26.7 ± 0.7	24.8 ± 1.4
Bic ₃ (mM)	<u>24.8 ± 0.5</u>	<u>23.8 ± 0.6</u>	<u>$19.7 \pm 2.0^\dagger$</u>	<u>21.8 ± 1.0</u>
Urine pH ₀	5.93 ± 0.23	6.07 ± 0.16	6.26 ± 0.18	5.78 ± 0.16
Urine pH ₃	<u>4.94 ± 0.05</u>	<u>$5.08 \pm 0.04^\dagger$</u>	<u>$5.27 \pm 0.07^*$</u>	<u>$5.28 \pm 0.10^\dagger$</u>
AG/Crea ₀	3.34 ± 0.80	3.53 ± 0.41	4.66 ± 0.95	3.21 ± 0.88
AG/Crea ₃	0.32 ± 0.43	<u>0.02 ± 0.31</u>	<u>1.05 ± 0.48</u>	1.26 ± 0.38
Cit/Crea ₀	0.18 ± 0.02	0.19 ± 0.02	$0.10 \pm 0.01^\dagger$	0.11 ± 0.04
Cit/Crea ₃	<u>0.10 ± 0.02</u>	<u>0.10 ± 0.01</u>	<u>$0.04 \pm 0.01^\dagger$</u>	<u>$0.04 \pm 0.01^\circ$</u>

AG, serum anion gap; bic., venous bicarbonate. $^\circ P < 0.05$; $^\dagger P < 0.025$; $^* P < 0.01$; $^{**} P < 0.005$ versus healthy controls. Underlined values were significantly different from corresponding values before NH_4Cl loading ($P < 0.05$, Wilcoxon signed-rank test).

being reached in controls and in RCSF with low-cit or hypo-cit. Urine pH was not significantly different between subgroups on day 0; it uniformly fell upon acid loading, but less markedly in RCSF than in controls. Urine anion gap/creatinine ratios also were similar between subgroups on day 0; after NH₄Cl they fell sharply in all subgroups, as did urine Cit/Crea ratios. The latter ones were abnormally low in RCSF with low-cit or hypo-cit, i.e. they had 'fasting hypocitraturia'.

Figure 2 is a scatter plot of fasting venous bicarbonate concentrations versus urine pH values after NH₄Cl loading; the shaded area is a nomogram obtained in 23 healthy volunteers. Urine pH decreased below 5.32 and venous bicarbonate remained above 20.5 mmol/l in all the normal subjects. However, 18 of the 34 RCSF that we studied fell out of the nomogram: two normocitraturics, with urine pH values of 4.84 and 5.03 in the face of blood bicarbonate concentrations of about 28 mM, were considered to be normal. In one RCSF with low-cit, venous bicarbonate after NH₄Cl loading was inadvertently not determined; however, since his urine pH decreased from 6.67 to 5.49 after acid loading while his serum chloride concentration rose from 104 to 106 mmol/l, he was considered abnormal, but not depicted in Figure 2. Thus, 16 of 34 RCSF (47.1%) had an abnormality in urine acidification, characterized either as insufficiently acidic urines in the presence of normal venous bicarbonate concentrations, or as sufficiently acidic urines at the expense of systemic acidosis (venous bicarbonate concentration < 20.5 mmol/l), or both. Abnormal urine acidification occurred in 10 of 11 RCSF with low-/hypo-cit, but only in six 23 with normo-cit ($P=0.0004$, Chi-square); $U_{\text{Cit}} \times V$ tended to be inversely correlated with fasting urine pH after NH₄Cl loading ($r=-0.300$, $P=0.064$). The prevalence of abnormal urinary acidification was similar amongst HCSF (7 of 12) and NCSF (9 of 22, $P=0.331$ versus HCSF, Chi-square).

Serum phosphate concentration was moderately

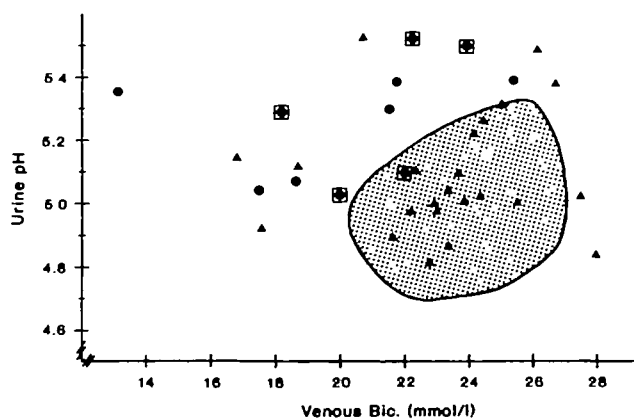


Fig. 2. Fasting urine pH (y axis) in relation to venous bicarbonate (x axis) after NH₄Cl (0.1 mEq/kg BW/day for 3 days) in 23 healthy men (shaded area) and 33 stone formers. Triangles, normo-cit; circles, low-cit; framed circles, hypo-cit; one RCSF with low-cit and urine pH of 5.49 not shown because of missing value of venous bicarbonate concentration.

lower (0.84 ± 0.03 versus 0.98 ± 0.04 mmol/l, $P < 0.05$) and serum intact PTH concentration higher (33.0 ± 3.2 versus 25.0 ± 2.1 pg/ml, $P < 0.05$) in RCSF with hypo-cit versus controls. Only before NH₄Cl loading were Tmp/GFR values lower in RCSF with normo-cit (0.76 ± 0.03 , $P=0.006$) and in those with low-cit (0.79 ± 0.03 mmol/l GF, $P=0.028$) than in controls (0.95 ± 0.04 mmol/l GF). All other blood and urine parameters of calcium phosphate and bone metabolism were similar in controls and the three subgroups of RCSF, and they were not differently affected by acid loading in controls and in the various subgroups of RCSF (data not shown).

Table 5 compares parameters of calcium phosphate metabolism between healthy controls and RCSF classified either as HCSF or NCSF, before and after acid loading. Compared with controls, Tmp/GFR was lower both in HCSF and NCSF, and HCSF had lower serum P and higher urine Ca/Crea both before and after acid loading. In comparison with NCSF, serum calcitriol and urine Ca/Crea were higher in HCSF. Serum parameters and urine Ca/crea were unaffected by acid loading, whereas Tmp/GFR fell slightly in NCSF and OHP-E rose in HCSF.

Frequencies of risk factors for low citraturia

When comparing normocitraturic RCSF with the combined group of those with low-/hypo-cit, i.e. all RCSF with $U_{\text{Cit}} \times V < 2.12$ mmol/day (< 400 mg/day), abnormal urine acidification was significantly more frequent among the latter ones (10 of 11 versus six of 23 in normo-cit, $P=0.0004$, Chi-square). Low urine volume was found in 27.3% of RCSF with low-/hypo-cit, compared with 8.7% in those with normo-cit (NS, Chi-square); 18.2% of RCSF with low-/hypo-cit and 17.4%

Table 5. Parameters of calcium phosphate and bone metabolism in fasting blood and urine samples of male controls, HCSF and NCSF before and after NH₄Cl loading

Measurement	Controls (n=14)	HCSF (n=12)	NCSF (n=22)	P
Ca ²⁺ ₀ (mM)	1.27 ± 0.01	1.27 ± 0.01	1.26 ± 0.01	NS
Ca ²⁺ ₃ (mM)	1.26 ± 0.01	1.26 ± 0.02	1.27 ± 0.01	NS
S-P ₀ (mM)	0.98 ± 0.04	0.82 ± 0.04†	0.88 ± 0.03	NS
S-P ₃ (mM)	0.95 ± 0.05	0.77 ± 0.04†	0.86 ± 0.03	NS
I-PTH ₀ (pg/ I-PTH ₃ ml)	25.0 ± 2.1	27.2 ± 3.3	27.8 ± 2.0	NS
1,25-D ₃ ₀ (pg/ 1,25-D ₃ ml)	50.5 ± 5.1	65.3 ± 4.5	49.4 ± 3.3	0.018
Ca/Crea ₀	0.25 ± 0.02	0.46 ± 0.06**	0.31 ± 0.03	0.021
Ca/Crea ₃	0.34 ± 0.05	0.53 ± 0.04*	0.37 ± 0.04	0.004
Tmp/GFR ₀ (mmol/ Tmp/GFR ₃ l GF)	0.95 ± 0.04	0.73 ± 0.05**	0.79 ± 0.03†	NS
OHP-E ₀ (μmol/ OHP-E ₃ l GF)	1.10 ± 0.13	1.66 ± 0.29	1.67 ± 0.13*	NS
	1.44 ± 0.30	2.28 ± 0.50	1.59 ± 0.27	NS

I-PTH, intact PTH; 1,25-D₃, calcitriol; Tmp/GFR, renal phosphate threshold; OHP, hydroxyproline. Indicated P values are for comparisons between HCSF and NCSF. † $P < 0.05$; ‡ $P < 0.025$; * $P < 0.01$; ** $P < 0.005$ versus healthy controls. Underlined values were significantly different from corresponding values before NH₄Cl loading ($P < 0.05$, Wilcoxon signed-rank test).

of those with normo-cit had GI-alkali below 15 mEq/day; low fibre intake was present in 63.4% of RCSF with low-/hypo-cit and in 50.0% of those with normo-cit (NS, Chi-square). As shown in Figure 3, two or more risk factors for decreased citruria occurred simultaneously in only 17.4% of RCSF with normo-cit (4 of 23), whereas it did so in 72.7% of RCSF with low-/hypo-cit (8 of 11, $P=0.002$ versus normo-cit, Chi-square).

Discussion

Among the 34 recurrent calcium stone formers studied herein under free-choice diet, overt hypocitraturia (<1.70 mmol/day) occurred in 15% and 'low citruria' (1.70–2.11 mmol/day) in another 18%. In the past 'idiopathic' hypocitraturia has been reported in 19–63% of patients with nephrolithiasis, either as an isolated disorder or in association with other urinary risk factors of stone formation [25]. Thus the prevalence obtained in the present study lies within the expected range inasmuch as patients with obvious causes of hypocitraturia, such as chronic diarrhoea, overt renal tubular acidosis, hypokalaemia, renal insufficiency and urinary tract infection [2] all had been carefully excluded from this study.

Four main risk factors, occurring separately or in combination, may be associated with low urinary citrate excretion and deserve further consideration: an abnormality in renal handling of acid, decreased net GI-alkali absorption, low dietary fibre consumption, and low urinary volume.

Previously, we had provided evidence for increased frequency of incomplete RTA among calcium stone formers with hypocitraturia [6]. The study, however, had been carried out under 'heavy' acid loading. In the present approach we wanted to evaluate acid-base metabolism under more 'physiological' conditions of acid loading than originally described [26]; therefore we applied only 0.05 g (0.95 mEq) NH_4Cl per kg BW daily in three divided doses given for 3 consecutive

days. Nevertheless our healthy controls lowered their urine pH below 5.32, about the same level as that originally described by Wrong and Davies [26], despite 3 days of lighter acid loading. Forty-one percent of all RCSF either were unable to do so, or they only did it at the cost of metabolic acidosis. Both patterns reflect diminished and/or retarded renal excretion of acid. That the latter predisposes to hypocitraturia (in that case via intracellular acidosis) is supported by the fact that the defect in urinary acidification was significantly more frequent among RCSF with low citruria or overt hypocitraturia (10 of 11) than among those with normocitraturia (4 of 23, $P=0.0004$, Chi-square).

Our study confirms the finding by Pak [1] that urinary citrate excretion correlates with net G-I alkali absorption, when assessed indirectly from non-combustible urinary cations and anions [22]. In this study, GI-alkali absorption was particularly low in RCSF with overt hypocitraturia (14 ± 4 mEq/day). Since alkali intake, as estimated from dietary fibre consumption, was comparable to that observed in RCSF with low- and normo-cit, alkali absorption itself may have been reduced in RCSF with hypo-cit. Alternatively, hypocitraturia might have resulted—at least in some RCSF—from reduced intestinal absorption of citrate itself, as described by Cowley *et al.* [27].

Animal protein excess is often regarded as a risk factor for hypocitraturia [1,9], by virtue of the organic acid load that this type of diet conveys. In this study, however, we did not find any significant correlations between $U_{\text{Cit}} \times V$ and the urinary markers of protein intake, e.g. urea, uric acid, and sulphate. Reasons for that are unclear. However, it has to be noted that the studies usually referred to in the context of protein-induced hypocitraturia (quoted in [1] and [9]) actually describe increases in citruria within the normal range of daily urinary citrate excretion after dietary restriction of protein.

We also confirm our previous finding of higher $U_{\text{Cit}} \times V$ in HCSF, compared with NCSF [28]. This seems to be partially related to higher C_{TRR} values in HCSF. There is, however, another potential explanation for the finding: urinary excretion rate of citrate was positively correlated with urinary excretion rates of calcium, as previously demonstrated by others [7,29], as well as with that of magnesium. Citrate forms soluble complexes with both ions, and such complexes cannot be absorbed along the nephron [13]; thus the higher the filtered load of calcium or magnesium, the higher the urinary output of citrate.

Two original observations are provided by the present study. First, dietary intake of vegetable fibres is reduced in RCSF with low-/hypo-cit, and a positive correlation exists between urinary citrate excretion and fibre intake. The prevalence of calcium nephrolithiasis is known to be inversely related to dietary fibre intake [30], and stone formers have been reported to ingest less fibres than the normal population [31]. Until now, however, the mechanism governing this association had remained unknown, an intestinal hyperabsorption of calcium consecutive to insufficient binding of the

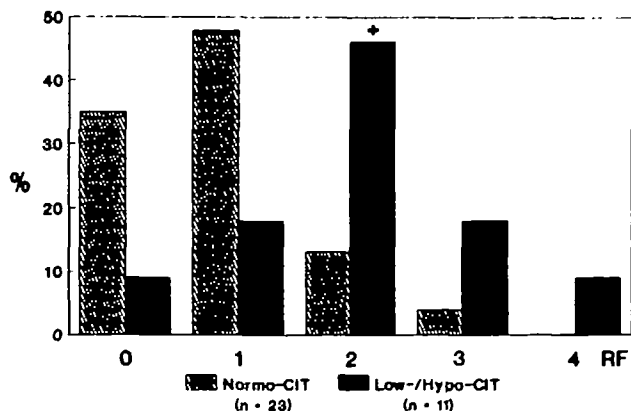


Fig. 3. Frequencies of occurrence of 0, 1, 2, 3, or 4 simultaneous risk factors (RF) for low citruria (<2.12 mmol/day) in RCSF. +, <0.05 versus normo-cit (Chi-square).

ion due to missing fibres with subsequent hypercalcaemia being the most widely postulated mechanism [30]. If such was the case, HCSF ought to have lower fibre intake than NCSF. The opposite, however, was observed in this study: fibre consumption was higher in HCSF, and urinary calcium and fibre consumption were positively, not negatively correlated. Therefore, the intestinal hyperabsorption of calcium that we must postulate in HCSF was not the consequence of missing fibres, but most probably of increased calcitriol levels, as generally accepted (reviewed in [32]) and observed also in the present study. Since dietary fibres emerge as a source of alkali [20], our finding rather suggests that increased fibre ingestion protects against calcium renal stone disease by raising urinary citrate excretion.

The other contribution of this study is the demonstration that a positive correlation exists between urinary citrate and urinary volume. The mechanism behind this association remains speculative but might involve the impact of the proximal luminal flow rate on bicarbonate reabsorption. Indeed, net bicarbonate reabsorption positively correlates with luminal flow rate along the proximal tubule [33–35], due to flow-dependent stimulation of proton secretion by proximal tubular cells [35]. Thus at higher flow rates, cytosolic pH might rise, which in turn is expected to lower citrate uptake by those cells and increase urinary citrate excretion [3].

In conclusion the present study allows to rank the most relevant causes for 'idiopathic' hypocitraturia: incomplete RTA, reduced net GI-alkali absorption, low vegetable fibre intake and low urinary volume. Whereas incomplete RTA, the most prevalent risk factor among RCSF with low citrate excretion, and reduced GI-alkali absorption had already been widely discussed in the past, low vegetable fibre intake and low urinary volume had gone unnoticed so far. Moreover in an individual stone former idiopathic hypocitraturia appears to be of multifactorial origin. Given the fact that most of these causes are readily accessible to dietary advices and medical treatment, they should be carefully looked for in recurrent idiopathic calcium stone formers.

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