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ORIGINAL PAPER

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Vitamin B6 metabolites in idiopathic calcium stone formers: no evidence for a link to hyperoxaluria

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Abstract Vitamin B6 metabolites and their potential correlates to urinary oxalate excretion in idiopathic calcium stone formers (ICSF) compared with healthy subjects were investigated. This clinical study was performed in a population of male ICSF with (Hyperoxalurics, n = 55) or without hyperoxaluria (Normooxalurics, n = 57) as well as in 100 healthy male control subjects. Pyridoxal 5'-phosphate serum concentration (S-pyridoxal 5'P) and 24-h urinary excretion of 4-pyridoxic acid (U-4pyridoxic acid) were measured using HPLC; 24-h urinary excretion of oxalate (U-oxalate) was measured concurrently. A subgroup of subjects (40 Hyperoxalurics, 15 Normooxalurics and 50 controls) underwent the same measurements before and after 7-day pyridoxine loading per os (pyridoxine hydrochloride, 300 mg/d). Under usual conditions, U-4pyridoxic acid was similar in the three groups, whereas mean S-pyridoxal 5'P was significantly lower ($p < 0.0001$) in the Hyperoxalurics (59.6 ± 21.2 nmol/L) and in the Normooxalurics (64.9 ± 19.7 nmol/L) than in the controls (86.0 ± 31.0 nmol/L). No correlation could be found between U-oxalate and U-4pyridoxic acid or S-pyridoxal 5'P. After B6 loading, S-pyridoxal 5'P was still significantly lower in the Hyperoxalurics (415 ± 180 nmol/L, $p < 0.001$) and in the Normooxalurics (429 ± 115 nmol/L, $p = 0.036$) than in the controls (546 ± 180 nmol/L), although there was no difference between groups for U-4pyridoxic acid. No correlation in any group could be found between changes in U-oxalate and changes in U-4pyridoxic acid or S-pyridoxal 5'P. Although there is no vitamin B6 deficiency in ICSF with or without hyperoxaluria, these patients, on average, have lower levels of S-pyridoxal 5'P than healthy subjects. However, this slight decrease does not seem to account for idiopathic hyperoxaluria.

Keywords Nephrolithiasis · Pyridoxin · Hyperoxaluria

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Introduction

Nephrolithiasis is a common medical problem, affecting 5–10% of the western population over lifetime [1]. Eighty to 90% of stones are composed of calcium salts, mainly calcium oxalate monohydrate (whewellite), calcium oxalate dihydrate (weddellite) and less commonly calcium phosphate [1]. Idiopathic stone disease is characterized by recurrent formation of calcium oxalate or calcium phosphate stones without any apparent underlying cause.

The origin of so-called idiopathic hyperoxaluria is still unclear. Mild to moderate hyperoxaluria (450–670 μmol/24 h) may result from consumption of oxalate-rich food (spinach, nuts, chocolate) [1]. The absorption of oxalate is highly variable between individuals and can contribute up to 52.6 ± 8.6% of urinary oxalate [2] depending, for example, on the concurrent calcium content of the diet. The major part, however, is believed to have a metabolic origin (e.g., from meat protein), which may be either direct from tryptophan or indirect from hydroxyproline via glyoxylate. The latter is to a large extent transaminated into glycine, thus preventing excessive production of oxalate (Fig. 1).

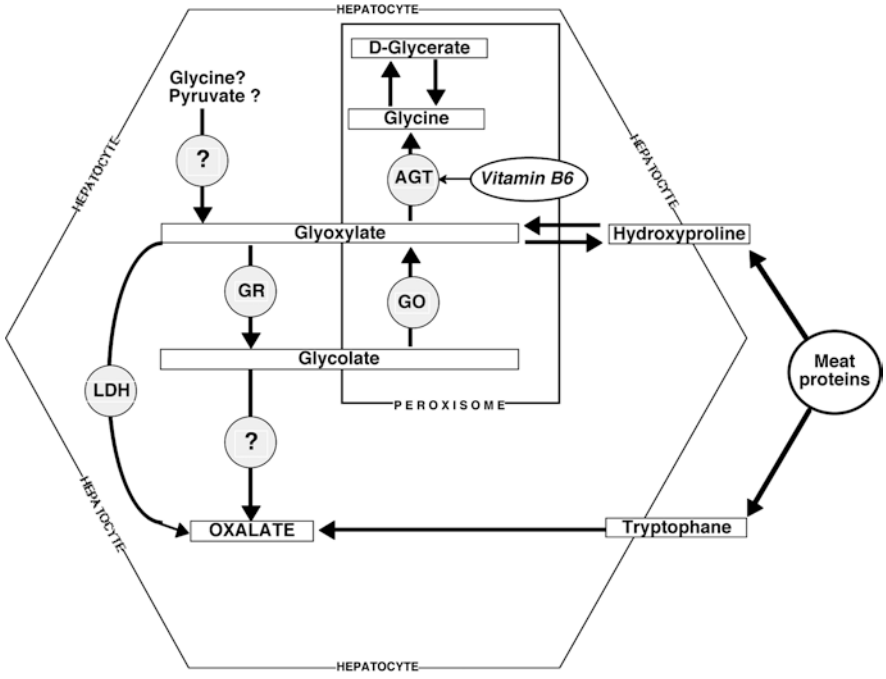

Fig. 1 Metabolic pathways from meat protein to oxalate. *Shaded circles* correspond to the various enzyme: *AGT* Alanine-glyoxylate aminotransferase, *GR* glyoxylate reductase, *GO* glycolate oxidase, *LDH* lactate dehydrogenase. AGT deficiency causes type 1 primary hyperoxaluria, where glyoxylate cannot be metabolized to glycine, leading both glycolate and oxalate to accumulate. Vitamin B6 supplementation may partially or completely correct the metabolic disorder

This transamination requires vitamin B6 as a cofactor in the alanine-glyoxylate transaminase enzyme pathway [3]. It is well established that nutritional pyridoxine deficiency leads to a marked increase in urinary oxalate excretion in rats [4] and to the production of urinary calculi [4]. Several studies both in animals [5] and in man [6, 7] suggest that pyridoxine and magnesium may prevent calcium oxalate urolithiasis and that pyridoxine administration leads to a decrease in urinary oxalate in patients with recurrent urolithiasis [8, 9] and in about 30% of patients with primary hyperoxaluria [10, 11, 12].

It is generally accepted that excessive intake of meat protein or oxalate, as well as insufficient hydration, confers a significant risk of renal stone formation, whereas the effect of intake of vegetable fibers has been a matter of debate for a long time [13, 14]. Data gleaned after World War II revealed that when a country undergoes protein deprivation, the prevalence of renal stones decreases, whereas the converse is true when protein intake resumes. Excessive ingestion of animal protein (meat, poultry and fish) also leads to an increase in urinary uric acid and calcium and to a decrease in urinary pH and citrate. These changes increase the risk of idiopathic calcium urolithiasis. Some patients, but not all, even develop mild hyperoxaluria on high protein intake, which may be due to the fact that vitamin B6 requirement increases with dietary protein intake [15].

The present study has been designed to look for alterations of vitamin B6 metabolites and their potential link to urinary oxalate excretion in idiopathic calcium stone formers (ICSF) with or without hyperoxaluria compared with healthy subjects taken as controls.

Methods

A. Cross-sectional study = investigation under free-choice diet

Calcium stone formers

From 271 consecutive male renal stone formers routinely examined at our clinic between January 1993 and March 1999, we selected two populations of idiopathic calcium stone formers (ICSF) as defined by their urinary oxalate excretion, i.e.:

- "the Hyperoxalurics" ICSF (n = 55) with consistent mild hyperoxaluria defined by a urinary excretion of oxalate above 450 μmol/day [13] on free-choice diet in at least two of three urine collections
- "the Normooxalurics" ICSF (n = 57) defined by a urinary excretion of oxalate below 450 μmol/day on a free-choice diet in all three urine collections

All patients fulfilled the following criteria: (1) they were Caucasian patients with at least two episodes of renal stones; (2) they had an examination consisting of a general case history and a dietary case history focused on past and current intake of dairy products, oxalate sources, flesh protein, vegetable fibers, salt and water. Clinical status included body size, body weight, lumbar palpation, and blood pressure measurement. Classic blood and urine chemistry profiles were obtained, and any previously performed intravenous pyelography was read for disclosing medullary sponge kidneys; (3) they did not have any established cause of calcium stone formation such as primary hyperparathyroidism, renal tubular acidosis, sarcoidosis or primary hyperoxaluria; (4) they had no history of urinary tract infection or of any renal disease other than urolithiasis; (5) they had no history of

gastrointestinal disorders that could affect the intestinal absorption of vitamin B6; (6) they were not taking any vitamin B6 supplements or medications that might interfere with vitamin B6 metabolism; (7) they had deep-frozen serum samples (−80°C); and (8) they had three 24-h urine collections on a free-choice diet with frozen samples (−20°C).

The stones had been analyzed by infrared spectrophotometry at our institution in the 55 patients included in the prospective study and chemically in most of the remaining ones.

From frozen samples 24-h urine was assayed for 4-pyridoxic acid, the catabolite of B6 and serum for pyridoxal 5' phosphate, the active moiety of B6.

Healthy subjects

One hundred healthy caucasian male volunteers were recruited for the study through advertising. The subjects were selected on the basis of a general and medical history questionnaire. A past history of renal stone disease in the subject or his parents led to exclusion from the study.

The subjects were asked to remain on a free-choice diet. No nutrition diary was obtained. Urine and blood samples were collected 4 weeks after recruitment.

B. Intervention study = vitamin B6 loading

Among ICSF patients studied cross-sectionally, 55 volunteered to participate in the vitamin B6 loading study. From those, only 15 had a consistently normal rate of oxalate excretion. In addition, 50 healthy subjects were recruited through advertisement in the newspapers. They were specifically instructed to refrain from taking any mineral, vitamin, or protein supplements as well as sweeteners during the 4 weeks before vitamin B6 treatment. The subjects were asked to maintain their normal lifestyle and their usual level of activity and free-choice diet. They were encouraged to drink at least 2 l of fluid each day. The subjects kept a diary of tablet intake and potential side effects. The actual pill count was not performed, but the compliance of both patients and controls was assessed by measuring the urinary excretion of 4-pyridoxic acid.

At baseline (day 0), a 24-h urine was collected and a fasting blood sample was drawn at the end of the urine collection period (day 1). Pyridoxine supplementation was carried out with one tablet of 300 mg of pyridoxine hydrochloride (Benadon; Roche Pharma AG, Reinach, Switzerland) taken daily for 7 days before breakfast. The subjects collected a second 24-h urine on day 7 (last day on vitamin B6) and returned to the laboratory in the morning of day 8 for a second fasting blood sample.

Sample collection and preparation

Urine was collected over 24 h in 3-l plastic bottles containing 10 g of boric acid as preservative. Because of the sensitivity to light of vitamin B6 derivatives, the subjects were instructed to store the urine bottle in a dark and cool place during the collection period. After shaking, urine samples were taken from each bottle and placed into six 10-ml tubes. Three of them were acidified with concentrated hydrochloric acid to prevent oxalate crystallization and conversion of ascorbate to oxalate [3]. Samples were frozen immediately and stored at −20°C until assay.

After a 10-h fast, venous blood samples (approximately 50 ml) were obtained in the morning after completion of the 24-h urine collection. Serum and plasma samples were kept from light, frozen immediately and stored at −80°C until assay.

Analytical methods

Urine 4-pyridoxic acid (U-4pyridoxic acid) and serum pyridoxal 5'-phosphate (S-pyridoxal 5'P) were measured by HPLC with fluorescence detection. Fluorescence was enhanced through addition of a post-column buffer containing sodium bisulfite (No. S-9000; Sigma Chemical, St. Louis, MO). The columns consisted of a Nova-Pak C18, 4.6×250 mm, with 4-μm particles (WATO52840; Waters, Rupperswil, Switzerland), a μBondapak C18, 3.9×300 mm, with 10-μm particles (WATO27324), followed by a RCM 8×10 module with a μBondapak C18 column, 8×100 mm, with 10-μm particles (WATO85721). For the isocratic eluant, 10 ml of acetic acid 100% (No. 1.00063; Merck KGaA, Darmstadt, Germany) were dissolved in 900 ml water. Then, pH was adjusted to 3.20 with triethylamine 99% and diluted with water to 1 l. Hexanesulfonic acid (825 mg) was added just before analysis. Samples were measured by fluorescene with excitation and emission, wavelength being set at 320 and 420 nm for urine and at 335 and 405 nm for serum. The recovery of both vitamin B6 metabolites ranged from 97 to 102%. The intraassay coefficient of variation ranged from 3.6 to 6.0% for P5'P and from 1.7 to 2.0 for 4PA. The interassay variation coefficient ranged from 2.9 to 7.7% for P5'P and from 6.3 to 6.9% for 4PA.

The plasma and urine concentration of creatinine was measured using an autoanalyzer. Urine oxalate was measured according to Buttery et al. [16] using oxalate oxidase and HPLC in the same assay; glycolate was determined using spinach glycolic acid oxidase. Urine concentration of urea was measured by using a kinetic test with urease and glutamate dehydrogenase (Cobas Integra 700).

Statistics

Statistical analyses were performed with StatView for Windows (version 5.0.1; SAS Institute, Cary, NC). All the values in the text, tables and illustrations are presented as mean ± SD. The normal range for the healthy volunteers was used as the 95% confidence interval. Statistical comparisons were considered to be significant at $P \leq 0.05$, using non parametric tests, i.e. Mann-Whitney U test for the comparison between two groups, Kruskal-Wallis test for comparison among three groups, Wilcoxon paired test for intragroup after/before comparison and the Spearman rank test for correlation between two parameters.

Ethics

Experimental plans, procedure, and consent forms of the study had been approved by the Committee for Medical and Ethical Questions of the University of Berne, and written informed consent was obtained from each participant.

Results

Cross-sectional study

The composition of renal stone was similar in the normooxaluric and the hyperoxaluric stone formers. By definition, U-oxalate was lower in the Normooxalurics than in the Hyperoxalurics. The control subjects had intermediate values. The daily urinary output of creatinine was higher in both ICSF groups than in the controls and higher in the Hyperoxalurics than in the

Table 1 Characteristics of the subjects (cross-sectional study)

	Group			P values			
	Controls (n = 100)	Normo Oxalurics (n = 57)	Hyper Oxalurics (n = 55)	Normo- vs. Hyper-Oxalurics	Normo-Oxalurics vs. controls	Hyper-Oxalurics vs. controls	All three groups
Age (y)	38.0 ± 10.3	45.8 ± 11.5	44.7 ± 12.7	NS	< 0.0001	0.0006	< 0.0001
Height (m)	1.78 ± 0.07	1.74 ± 0.08	1.75 ± 0.07	NS	0.003	0.006	0.002
Weight (kg)	74.4 ± 10.6	79.1 ± 13.7	82.1 ± 12.0	NS	0.02	< 0.0001	0.0002
BMI (kg/m^2)	23.4 ± 3.3	25.9 ± 3.5	26.9 ± 3.8	NS	< 0.0001	< 0.0001	< 0.0001
Stone Analysis (IR spectro)							
Oxalate monohydrate ≥ 80%	–	15/34	7/23	NS	–	–	NS
Other oxalate stones	–	12/34	12/23	NS	–	–	NS
Non predominant Oxalate	–	7/34	4/23	NS	–	–	NS
Urine parameters							
U-Volume (ml)	1843 ± 752	1737 ± 726	2313 ± 813	< 0.0001	NS	0.0006	0.0001
U-Creatinine (μmol/d)	14000 ± 2650	15159 ± 3504	17043 ± 4060	0.03	0.005	< 0.001	< 0.001
U-4 pyridoxic acid (μmol/d)	7.6 ± 3.3	7.2 ± 2.7	7.4 ± 3.7	NS	NS	NS	NS
U-Oxalate (μmol/d)	413 ± 134	300 ± 91	540 ± 165	by definition	< 0.0001	< 0.0001	< 0.0001
U-Glycolate (μmol/d)	363 ± 174	487 ± 219	564 ± 263	NS	< 0.0001	< 0.0001	< 0.0001
U-Urea (mmol/d)	400 ± 114	420 ± 103	476 ± 151	NS	NS	0.002	0.007
Blood parameters							
Creatinine (μmol/l)	93.0 ± 10.7	97.5 ± 10.9	96.8 ± 9.1	NS	0.01	0.015	0.008
S-pyridoxal 5'P (nmol/l)	86.0 ± 31.0	64.9 ± 19.7	59.6 ± 21.2	NS	< 0.0001	< 0.0001	< 0.001

Results expressed as mean ± SD. Statistics using Mann-Whitney U test for the comparison of two groups and Kruskal-Wallis for the comparison of three groups. *NS* not significant

Normooxalurics. U-glycolate was significantly higher in the Hyperoxalurics and the Normooxalurics than in the controls, and U-urea was higher in the Hyperoxalurics than in the controls (see Table 1).

Individual values of U-4pyridoxic acid excretion as well as S-pyridoxal 5'P concentrations are plotted in Fig. 2. The normal range was 3.5–14.5 μmol/24 h for U-4 pyridoxic acid excretion and 42–165 nmol/L for S-pyridoxal 5' P. Although there were no intergroup differences in terms of U-4 pyridoxic acid excretion, S-pyridoxal 5'P concentrations, on average, were significantly lower in the NormoOxaluric and Hyperoxalurics than in the controls.

U-4pyridoxic acid and S-pyridoxal 5'P concentrations correlated positively in the controls and in Normooxalurics but not in the Hyperoxalurics. In all three groups U-4 pyridoxic acid was positively correlated with U-urea. In the controls only, there were weak positive correlations between S-pyridoxal 5'P concentration vs. U-urea, U-4 pyridoxic acid vs. U-glycolate and U-4pyridoxic acid vs. U-oxalate, respectively. No correlation in any group could be found between S-pyridoxal 5'P and U-oxalate (see Table 2).

Pyridoxine loading

Characteristics of the 105 subjects who underwent pyridoxine loading on free-choice diet are presented in Table 3. After vitamin B6 loading S-pyridoxal 5'P was found elevated far above the normal range. In all three groups, U-4 pyridoxic acid also increased similarly among the three groups.

The individual values of vitamin B6 metabolites after pyridoxine loading are plotted in Fig. 3 showing a large natural variability. One Hyperoxaluric and one control subject had U-4pyridoxic acid levels lower than the other subjects, suggesting either poorer absorption of pyridoxine or inadequate compliance in the last days of treatment. After 7 days on vitamin B6, S-pyridoxal 5'P was elevated in all subjects (inclusively in those two subjects with low U-4pyridoxic acid). The mean post-loading values of S-pyridoxal 5'P were lower in the Normooxalurics or the Hyperoxalurics than in the controls.

On average, the urinary excretion rate of oxalate did not change significantly after pyridoxine loading either in the controls or in ICSF and there was no correlation between the changes in oxalate excretion and the

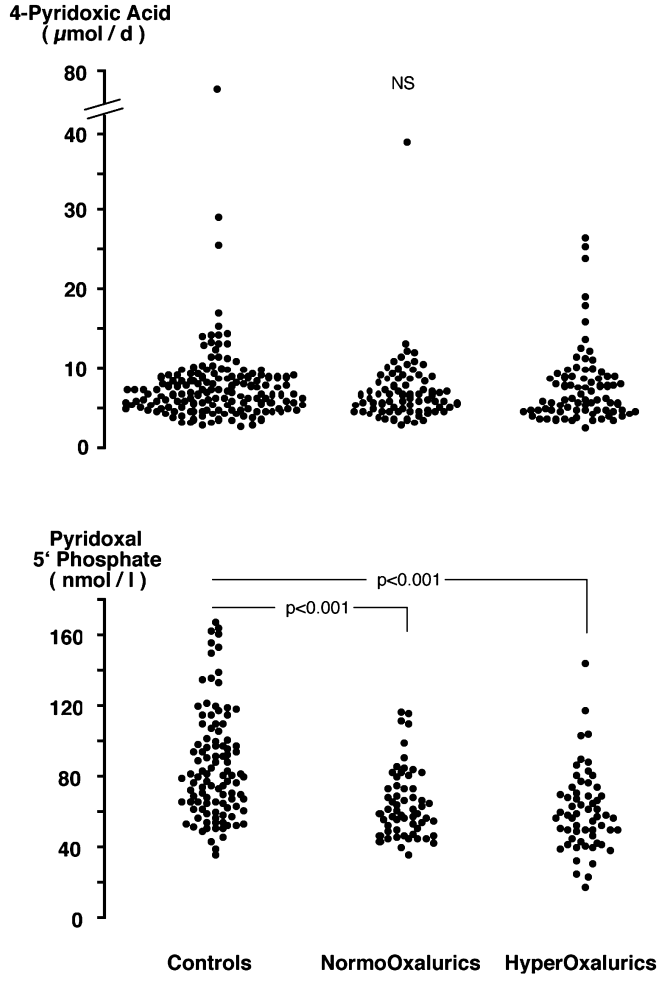

Fig. 2 Urinary 4-pyridoxic acid and serum pyridoxal 5'-phosphate in a population of healthy subjects (controls, n = 100) and recurrent calcium stone formers without hyperoxaluria (Normooxalurics, n = 57) or with hyperoxaluria (Hyperoxalurics, n = 55). Statistics were performed using the Mann-Whitney U-test

changes in S-pyridoxal 5'P or U-4pyridoxic acid. A small but significant decrease in mean urinary excretion rate of glycolate after pyridoxine loading was found in the Hyperoxalurics.

Discussion

A link between vitamin B6 and oxalate metabolism was discovered many years ago. Primary hyperoxaluria of type I, for instance, a genetic disease characterized by nephrolithiasis, nephrocalcinosis, renal insufficiency as well as systemic deposition of oxalate in various tissues and organs other than the kidney, sometimes responds to pharmacologic administration of vitamin B6 [10, 11, 12, 17]. Conversely, experimental depletion in vitamin B6 also leads to hyperoxaluria and nephrocalcinosis, in cats [18] and rats [5], where it has been amply studied. Both observations are related to the fact that pyridoxine is the cofactor of hepatic alanine-glyoxylate-aminotransferase (AGT) that transaminates glyoxylate into glycine, thus curtailing the pathway from glyoxylate to oxalate (Fig. 1).

Idiopathic hyperoxaluria is a completely different disease, regarded as a nutritional disorder derived either from an excessive consumption of oxalate-containing nutrients or from excessive endogenous oxalate production from meat protein, via high hydroxyproline and tryptophan intake. Idiopathic hyperoxaluria is classically viewed as non-responsive to vitamin B6, at least on a short-term basis (< 3 weeks) although several studies have suggested that pyridoxine given for longer periods of time (6 weeks to 6 months) might be beneficial to ICSF [11]. In the present study, we observed a large scatter in the response to vitamin B6 loading, with significant individual decreases in oxaluria (i.e.

Table 2 Correlation between 4-pyridoxic acid, pyridoxal 5'-phosphate and various parameters

	U-4-Pyridoxic Acid					
	Controls		NormoOxalurics		HyperOxalurics	
	r	p	r	p	r	p
U-Creatinine	+0.38	0.0001	+0.17	NS	+0.42	0.002
U-Oxalate	+0.21	0.03	+0.21	NS	+0.25	NS
U-Glycolate	+0.21	0.03	−0.09	NS	+0.20	NS
Protein intake (U-Urea)	+0.54	< 0.0001	+0.30	0.02	+0.50	0.0002
	S-Pyridoxal 5'-Phosphate					
	Controls		NormoOxalurics		HyperOxalurics	
	r	p	r	p	r	p
U-4 pyridoxic acid	+0.41	< 0.0001	+0.38	0.005	+0.26	NS
U-Creatinine	+0.11	NS	+0.15	NS	+0.33	0.01
U-Oxalate	+0.19	NS	+0.20	NS	+0.16	NS
U-Glycolate	+0.05	NS	+0.01	NS	+0.36	0.01
Protein intake (U-Urea)	+0.22	0.05	+0.10	NS	+0.24	NS

Statistics using Spearman rank correlation test

Table 3 Characteristics, blood and urinary data of the subjects before and after pyridoxine loading

		Group			p values		
		Controls (n = 50)	NormoOxalurics (n = 15)	HyperOxalurics (n = 40)	Normo- vs. HyperOxalurics	Normo-Oxalurics vs. controls	HyperOxalurics vs. controls
Age (y)		37.4 ± 10.9	55.0 ± 8.8				
Height (m)		1.79 ± 0.07	1.75 ± 0.06	50.8 ± 12.5	NS	< 0.0001	< 0.0001
Weight (kg)		75.2 ± 11.3	79.7 ± 11.6	1.75 ± 0.07	NS	NS	0.02
BMI (kg/m^2)		23.6 ± 3.6	26.2 ± 3.9	82.2 ± 11.6	NS	NS	0.003
				27.1 ± 3.9	NS	0.006	< 0.0001
U-Volume (ml/d)	baseline	1826 ± 673	2285 ± 631	1961 ± 681	NS	0.02	NS
	after B6	1887 ± 791	2205 ± 687	2112 ± 672	NS	NS	NS
U-Creatinine (μmol/d)	baseline	14122 ± 2817	13137 ± 2495	15012 ± 3225	NS	NS	NS
	after B6	13434 ± 3354	14066 ± 2685	14924 ± 3492	NS	NS	NS
U-Oxalate (μmol/d)	baseline	385 ± 107	419 ± 92	501 ± 182	NS	NS	0.0001
	after B6 loading	412 ± 125	419 ± 103	508 ± 191	NS	NS	0.01
U-Glycolate (μmol/d)	baseline	415 ± 186	440 ± 103	521 ± 218	NS	NS	0.007
	after B6	409 ± 205	427 ± 102	483 ± 196*	NS	NS	NS
U-4 pyridoxic acid (μmol/d)	baseline	9.0 ± 10.5	6.4 ± 2.3	8.4 ± 5.3	NS	NS	NS
	after B6	862 ± 323**	1003 ± 226**	857 ± 354**	NS	NS	NS
S-pyridoxal 5'P (nmol/l)	baseline	80.0 ± 27.4	63.3 ± 20.6	60.1 ± 21.8	NS	0.04	0.004
	after B6	546 ± 180**	429 ± 115**	415 ± 180**	NS	0.04	0.001

Results expressed as mean ± SD. Statistics using Mann-Whitney U test for intergroup comparison and Wilcoxon paired test for intragroup comparison.
*$p \leq 0.03$ vs. baseline, **$p \leq 0.001$ vs. baseline

≥20%) in some subjects (three Normooxalurics, nine Hyperoxalurics, 12 controls), a significant increase in some others (four Normooxalurics, eight Hyperoxalurics, 18 controls) and no change in most individuals; part of this is probably due to the well-known day to day variability of the urinary excretion rate of oxalate on free-choice diet.

The issue as to whether or not idiopathic calcium stone formers might have a disordered metabolism of vitamin B6 which might account for their hyperoxaluria has not been systematically studied. In the early 1990s Edwards and Rose carried out a preliminary study in eight patients selected for their high urinary excretion rates of oxalate and glycolate. They observed lower values of serum pyridoxal 5'-phosphate after vitamin B6 loading in some patients than in a control population, particularly in patients who had a good response of urinary oxalate to vitamin B6. For some reason these patients became very rare and the study was therefore interrupted (A. Rose, personal communication). To the best of our knowledge, these are the only data available on this aspect of the subject so far.

Most idiopathic calcium stone formers have serum levels of pyridoxal 5' phosphate, the active moiety of vitamin B6, in the normal range. However, the present study shows that mean serum levels are lower in patients than in controls, not only due to very low values in some patients but also to an overall shift in the distribution of that parameter in the patient population. In other words, ICSF do not activate pyridoxine into pyridoxal 5'-phosphate to the same extent as healthy subjects do, either on a physiological or on a pharmacological intake of pyridoxine, and this is observed independently of the oxaluric status of the patients. There was no relationship between serum levels of pyridoxal 5'-phosphate and urinary oxalate excretion.

It has been postulated in the past [19] that this lack of activation of pyridoxine might simply reflect an insufficient intake and/or absorption of the vitamin. However, the fact that the excretion rate of 4-pyridoxic acid is similar in patients and in controls, both before and after vitamin B6 loading, rules out this hypothesis. This is in agreement with Tiselius et al., who measured the urinary excretion of 4-Pyridoxic acid and oxalic acid in 75 patients with urinary calculi and in 50 normal subjects on regular diet and who found that most patients had a normal 4-Pyridoxic acid excretion [20].

Explanations to account for the present findings so far are either an insufficient activation of pyridoxine into Pyridoxal 5'P or an excessive catabolism of Pyridoxal 5'P. The exact site where the phenomenon occurs and the precise mechanism whereby it takes place remain to be elucidated. What is currently known is that

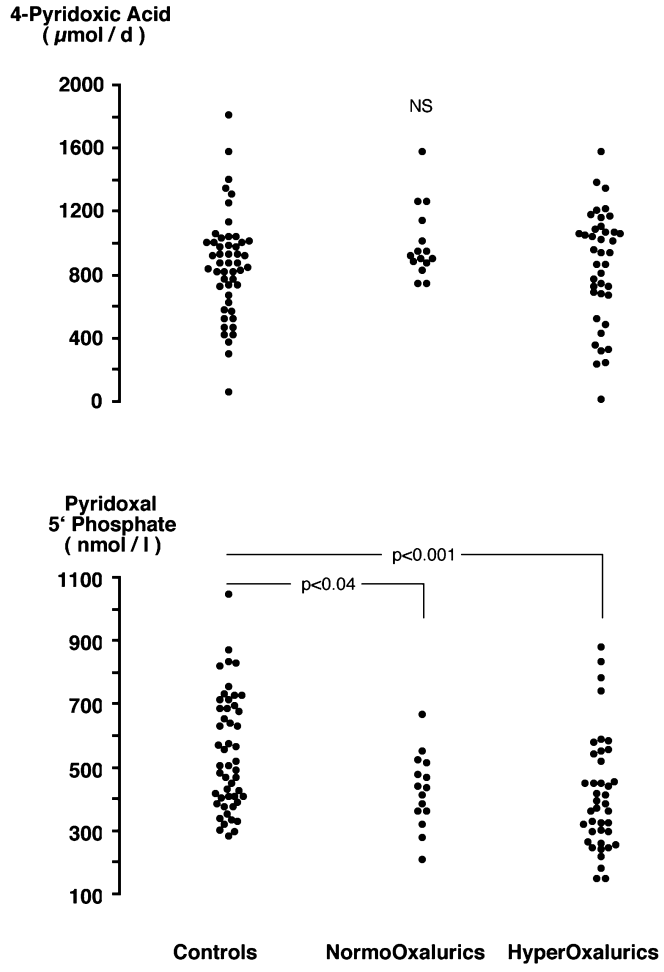

Fig. 3 Urinary 4-pyridoxic acid and serum pyridoxal 5'-phosphate levels after a 7-day loading with pyridoxine (300 mg/d) in healthy subjects (controls, n = 50) and recurrent calcium stone formers without hyperoxaluria (Normooxalurics, n = 15) or with hyperoxaluria (Hyperoxalurics, n = 40). Statistics were performed using the Mann-Whitney U-test

(1) the absorption of different dietary forms of vitamin B6 (i.e. pyridoxine, pyridoxine-glucosides and pyridoxamine) is influenced by microbial and mucosal enzymes at the level of intestine [21] and (2) transformation of pyridoxine into pyridoxal 5'-phosphate requires pyridoxine oxidase, pyridoxalkinase-phosphatase and vitamin B2 in the liver. It remains to be seen, however, whether one component of the biochemical pathway is altered.

Clearly, stone formers have a higher protein intake than healthy volunteers, thus having a large requirements for vitamin B6 [15]. This might have led S-pyridoxal 5'P to decrease via catabolism. In fact, this explanation does not hold true because of the observed positive, not negative, correlation between S-pyridoxal 5'P and U-urea, but in the controls only. This is probably due to the presence of significant amounts of vitamin B6 in meat, fish and poultry.

Finally, although low levels of the active metabolite of vitamin B6 are sometimes observed in idiopathic calcium stone formers, the present study does not provide evidence for a cause and effect relationship with hyperoxaluria, contrary to what had been postulated so far. This points to the fact that other important factors do influence urinary oxalate excretion, in particular the calcium to oxalate ratio in the diet.

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