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Sensitivity to meat protein intake and hyperoxaluria in idiopathic calcium stone formers

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Background. High protein intake is an accepted risk factor for renal stone disease. Whether meat protein intake affects oxaluria, however, remains controversial in healthy subjects and in stone formers. This study was designed (*1*) to test the oxaluric response to a meat protein load in male recurrent idiopathic calcium stone formers (ICSFs) with and without mild metabolic hyperoxaluria (MMH and non-MMH, respectively), as well as in healthy controls, and (*2*) to seek for possible disturbed vitamin B_6 metabolism in MMH, in analogy with primary hyperoxaluria.

Methods. Twelve MMH, 8 non-MMH, and 13 healthy males were studied after five days on a high meat protein diet (HPD; 700 g meat/fish daily) following a run-in phase of five days on a moderate protein diet (MPD; 160 g meat/fish daily). In both diets, oxalate-rich nutrients were avoided, as well as sweeteners and vitamin C-containing medicines. Twenty-four–hour urinary excretion of oxalate was measured on the last day of each period, along with 4-pyridoxic acid (U_{4PA}) and markers of protein intake, that is, urea, phosphate, uric acid, and sulfate. Serum pyridoxal 5′ phosphate (S_{P5P}) was measured after protein loading.

Results. Switching from MPD (0.97 ± 0.18 g protein/kg/day) to HPD (2.26 ± 0.38 g protein/kg/day) led to the expected rise in the urinary excretion rates of all markers of protein intake in all subjects. Concurrently, the mean urinary excretion of oxalate increased in ICSFs taken as a whole (+73 ± 134 μmol/24 h, $P = 0.024$) as well as in the MMH subgroup (+100 ± 144 μmol/24 h, $P = 0.034$) but not in controls (−17 ± 63 μmol/24 h). In seven ICSFs (4 MMH and 3 non-MMH) but in none of the healthy controls ($P = 0.016$, chi square), an increment in oxaluria was observed and considered as significant based on the intra-assay coefficient of variation at our laboratory (8.5%). There was no difference in S_{P5P} and U_{4PA} between the groups after protein loading.

Conclusion. Approximately one third of ICSFs with or without so-called MMH are sensitive to meat protein in terms of oxalate excretion, as opposed to healthy subjects. Mechanisms underlying this sensitivity to meat protein remain to be elucidated and do not seem to involve vitamin B_6 deficiency.

Key words: renal stone disease, oxaluria, urinary excretion, vitamin B_6 deficiency, metabolic hyperoxaluria, nephrolithiasis.

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Nephrolithiasis is a common source of morbidity in industrialized countries. Annual incidence is estimated at 1.3 per 1000 persons with a lifetime risk of 10 to 12% in men and 3 to 5% in women [1, 2]. The disease is at least twice as frequent in whites than in blacks [3, 4] and most commonly appears between the fourth and the sixth decade [1, 4].

Since Andersen's observations [5], several studies have confirmed a strong relationship between stone prevalence and affluence [6–9], thus linking nephrolithiasis to dietary factors [9–12]. Several authors observed a strong correlation between intake of dietary animal protein and nephrolithiasis [10, 13–15]. While it is generally accepted that excess of dietary protein leads urinary excretion of lithogenic substances such as calcium [16–18] and uric acid [18, 19] to rise and excretion of citrate to fall [17], the role of animal protein intake on oxalate excretion remains controversial. Evidence in some interventional studies providing a protein load show an increase in urinary oxalate excretion [18, 20] or no increase [17, 21], or an increase only in healthy females [22]. Similar discrepancies were found in protein restricted patients [23, 24]. The controversy may reside in the fact that the populations studied were heterogeneous and included various types of renal stone formers as well as healthy subjects. We hypothesized that only specific groups of stone formers such as patients with so-called mild metabolic hyperoxaluria (MMH) might be sensitive to meat protein in terms of urinary oxalate excretion. The present study has been carried out to test this hypothesis. It is an intervention study to compare the effect of a meat protein load on urinary oxalate in three groups of subjects, that is, healthy controls and idiopathic calcium stone formers (ICSFs) with and without MMH. We also postulated that, should this hypothesis be correct, an alteration in vitamin B_6 metabolism might underlie the observation, in analogy with primary hyperoxaluria.

METHODS

From 191 male kidney stone formers investigated at our outpatient renal stone clinic between January 1996

and December 1998, 73 recurrent ICSFs (defined by documented calcium-containing stones at infrared spectroscopy) were identified as potential candidates for the study, that is, 43 with MMH and 30 with normal oxaluria, this on the basis of three consecutive 24-hour urine collections. MMH was defined as a urinary excretion rate of both oxalate and glycolate above 450 μmol/24 hours on at least two of the three urine collections. Non-MMH had to have an oxalate excretion below 450 μmol/24 hours in all three 24-hour urine collections. At that point, a letter was sent to these patients inviting them to participate in the study that was conducted between December 1998 and April 1999. Male healthy subjects were invited to serve as controls by public announcement in newspapers and internal posting.

Twenty-one stone formers volunteered for the study: 13 MMH and 8 non-MMH. From 30 healthy subjects who also volunteered, 13 were finally selected after matching for MMH patients' age (±5 years) and body mass index (±2 kg/m^2) to serve as controls. One MMH withdrew before starting the trial because of self-limited unspecific abdominal discomfort.

Potential causes of urolithiasis (such as primary hyperparathyroidism, renal tubular acidosis, inflammatory bowel disease, medullary sponge kidney, sarcoidosis, vitamin D intoxication, and urinary infection) had been excluded in previous work-ups. For control subjects, a known history of renal stone or any of the aforementioned conditions were exclusion criteria. All participants were otherwise in good health, for example, they had a normal clinical examination, transaminase activity, and serum creatinine level, as well as no recent or present episodes of diarrhea. All gave written informed consent to participate in this study, which had been approved by the Ethical Committee of the University of Berne, School of Medicine (Berne, Switzerland).

All subjects were given guidelines by a dietitian to prepare their meals at home and were encouraged to follow five different specified menus for both diets. They first underwent a five-day run-in period with moderate animal protein intake (MPD), based on 160 g of lean meat or fish providing about 15% of total energy intake. Then they were switched for five days to a high protein diet (HPD), based on 700 g of lean meat or fish providing approximately 35% of total energy intake. As a consequence of increased meat intake, the HPD contained 15% more fat and was designed to provide 42% less carbohydrates than MPD in order to secure identical energy intakes. Both diets were restricted in oxalate and had a similar calcium content. During the entire test period, participants were advised to abstain from taking ascorbic acid-containing medicines or sweeteners, and were encouraged to drink more than usually in order to produce at least 2000 mL urine/day. Diets were designed to be taken at home, and instructions were given both orally and in written form. Daily dietary records were kept during the entire study to insure control of good compliance, and the records were assessed with the help of PRODI 4.4, a software developed by the Section of Nutrition and Dietetics of the Department of Medicine at the University of Freiburg (Freiburg, Germany).

Table 1. Subject characteristics

	Controls (N = 13)	All ICSF (N = 20)	MMH (N = 12)	Non-MMH (N = 8)
Age *years*	53.2 ± 8.7	54.3 ± 9.6	52.8 ± 10.8	56.5 ± 7.8
BMI *kg/m^2*	26.6 ± 2.9	26.8 ± 3.4	27.7 ± 3.7	25.5 ± 2.4
Number of stone passings	0	4.8 ± 4.7[a]	5.3 ± 5.6[a]	4.0 ± 3.0[a]

Abbreviations are in the Appendix.
[a] $P < 0.0001$ vs. controls

All participants were instructed to collect 24-hour urines (from 6 a.m. to 6 a.m.) over the last day on both MPD and HPD into one or two plastic bottles (3 liters each) containing 10 g of boric acid as a preservative agent. Blood was drawn in fasting condition at the end of the study.

Measurements

Oxalate was measured using the oxalate oxidase method [25]. In our hands, the mean intra-assay coefficient of variation (CV) for oxalate concentration, based on duplicate measurements of two reference samples (Sigma Diagnostics, St. Louis, MO, USA), performed on 16 different days was 8.5% for the low oxalate reference urine and 6.0% for the high oxalate reference urine. Accuracy was good, with a mean value of 329 μmol/L for the low oxalate reference urine (reference range from manufacturer = 250 to 370 μmol/L) and 925 μmol/L for the high oxalate reference urine (reference range from manufacturer = 850 to 1120 μmol/L).

Urinary glycolate was measured by an enzymatic assay using glycolic acid oxidase extracted from spinach leaves [26], urinary citrate using the citrate lyase method [27], and sulfate using the high-performance liquid chromatography (HPLC) technique. Urea, calcium, phosphorus, magnesium, sodium, potassium, chloride, and uric acid were measured by autoanalyzer techniques. Vitamin B_6 metabolites were assayed using HPLC in serum (pyridoxal 5′ phosphate, that is, the active metabolite of vitamin B_6) and urine (4-pyridoxic acid, that is, the excretion product of B_6).

Statistics

Data are presented as mean ± SD. Intergroup differences at baseline were tested using either nonparametric tests or analysis of variance (ANOVA) according to the distribution. Threshold value to accept a significant difference between two consecutive values of a parameter,

Table 2. Composition of the diets

Nutrient intake *mean of 5 days*	Diet	Controls ($N = 13$)	All ICSF ($N = 20$)	MMH ($N = 12$)	Non-MMH ($N = 8$)
Protein *g/day*	MPD	75 ± 14	81 ± 13	84 ± 13	77 ± 12
	HPD	184 ± 31[c]	191 ± 29[c]	201 ± 27[c]	172 ± 20[bc]
Protein energy/total energy %	MPD	16.4 ± 2.3	16.7 ± 2.1	16.5 ± 2.6	16.4 ± 1.0
	HPD	31.0 ± 5.2[c]	30.5 ± 3.4[c]	31.2 ± 3.6[c]	29.3 ± 3.0[c]
Fat *g/day*	MPD	57 ± 8	64 ± 17	64 ± 16	63 ± 19
	HPD	90 ± 21[c]	95 ± 13[c]	96 ± 15[c]	93 ± 10[c]
Carbohydrate *g/day*	MPD	250 ± 59	265 ± 71	273 ± 77	251 ± 57
	HPD	209 ± 81[c]	217 ± 55[c]	227 ± 53[c]	200 ± 57
Energy/body weight *KCal/kg*	MPD	23 ± 3	25 ± 5	25 ± 6	25 ± 5
	HPD	31 ± 6[c]	32 ± 5[c]	32 ± 6[c]	32 ± 4[c]
Calcium *mg/day*	MPD	1028 ± 317	1245 ± 443	1336 ± 525	1090 ± 192
	HPD	1185 ± 481	1312 ± 560[c]	1331 ± 582	1279 ± 562
Vitamin C *mg/day*	MPD	198 ± 155	115 ± 64[a]	124 ± 70	100 ± 54
	HPD	137 ± 69	107 ± 57	121 ± 55	82 ± 56
Vitamin B_6 *mg/day*	MPD	1.6 ± 0.5	1.4 ± 0.5	1.4 ± 0.5	1.3 ± 0.4
	HPD	2.9 ± 0.5[c]	2.9 ± 0.8[c]	3.1 ± 0.9[c]	2.7 ± 0.8[c]

[a] Significant vs. controls ($P \leq 0.05$)
[b] Significant vs. MMH ($P \leq 0.05$)
[c] Significant vs. MPD ($P \leq 0.05$)

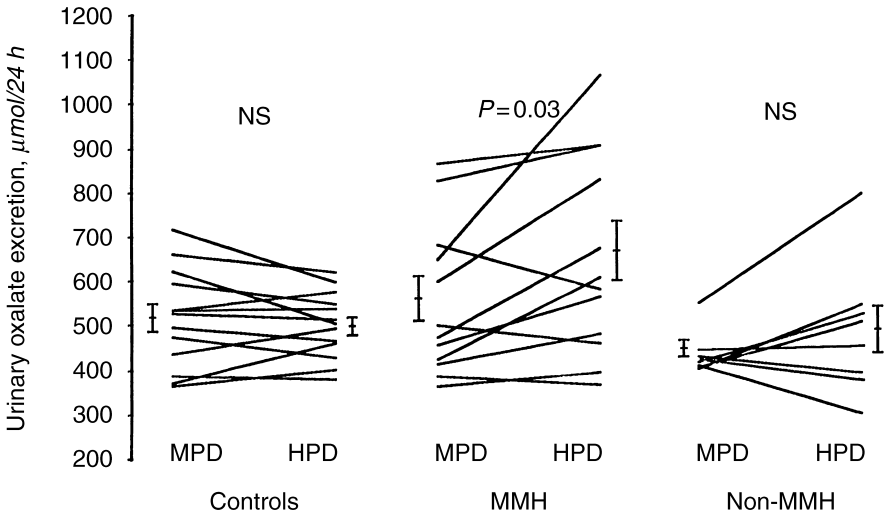

Fig. 1. Individual and mean changes in urinary oxalate excretion in controls and in idiopathic calcium stone formers (ICSFs) with or without mild metabolic hyperoxaluria (MMH), switching from a moderate protein (MPD) to a high protein diet (HPD).

according to the CV of the method (CV%) is $1.96 \times \sqrt{2} \times$ CV%. Thus, based on a CV of oxalate measurement of 8.5%, the minimum difference between two consecutive values of oxaluria in a patient to be significant is 23.6%. This does not mean, however, that such a difference is biologically relevant on an individual basis, due to the large biological variability of oxaluria.

RESULTS

Table 1 summarizes the basal characteristics of all study participants. There was no difference in age and body mass index between the three groups.

Table 2 summarizes the actual composition of both diets, expressed as the mean value of the respective parameters recorded over five days. There was no difference in energy/body weight, protein energy/total energy, carbohydrate, fat, calcium, and vitamin B_6 intake between the three groups, neither on MPD nor on HPD. Protein intake was slightly lower on HPD in non-MMH than in MMH; for those on a MPD, vitamin C intake was larger in controls than in ICSFs. Switching from MPD to HPD led to an expectedly large increase in protein intake and protein energy in all groups. Fat intake increased by 50%, that is, much more than the expected 15%, whereas carbohydrate decreased by only 17% compared with the planned 42%. As a consequence, total energy intake increased by 28%. These changes in protein, fat, and carbohydrate intake were similar in all groups. Such was also the case for the increase in vitamin B_6 intake in those switching from a MPD to HPD. Calcium intake also increased slightly from the MPD to HPD subjects, the change being significant in ICSFs only.

Figure 1 depicts individual and mean changes in oxalate excretion in the three groups. No significant individual change was observed in controls, whereas four MMH

Table 3. Changes of urinary parameters switching from MPD to HPD

Urinary excretion	Diet	Controls ($N = 13$)	All ICSF ($N = 20$)	MMH ($N = 12$)	Non-MMH ($N = 8$)
Oxalate *μmol/24h*	MPD	515 ± 112	511 ± 147	557 ± 173	442 ± 55
	HPD	498 ± 74	584 ± 216[c]	657 ± 229[ac]	476 ± 147
Glycolate *μmol/24h*	MPD	499 ± 154	576 ± 168	633 ± 160[a]	490 ± 149[b]
	HPD	804 ± 250[c]	883 ± 30[c]	991 ± 288[c]	721 ± 255[bc]
Urea *mmol/24h*	MPD	452 ± 101	494 ± 119	533 ± 124	435 ± 86
	HPD	851 ± 176[c]	813 ± 203[c]	885 ± 139[c]	706 ± 244[bc]
Calcium *mmol/24h*	MPD	5.6 ± 3.7	8.7 ± 4.0[a]	10.7 ± 3.7[a]	5.7 ± 1.9[b]
	HPD	6.1 ± 3.6	10.0 ± 5.5[ac]	12.0 ± 5.7[a]	7.1 ± 3.8[b]
Sodium *mmol/24h*	MPD	210 ± 49	216 ± 67	237 ± 66	186 ± 60
	HPD	276 ± 87[c]	271 ± 71[c]	285 ± 63[c]	250 ± 80[c]
Sulfate *mmol/24h*	MPD	23.5 ± 7.7	25.5 ± 9.9	28.5 ± 11.0	21.0 ± 5.8
	HPD	43.0 ± 9.0[c]	39.6 ± 13.9[c]	44.0 ± 14.7[c]	33.0 ± 10.3[bc]
Phosphorus *mmol/24h*	MPD	32.3 ± 10.3	37.6 ± 10.6	41.8 ± 10.9[a]	31.2 ± 6.7[b]
	HPD	45.4 ± 15.7[c]	47.2 ± 12.6[c]	51.5 ± 12.3[c]	40.8 ± 10.8[c]
Uric acid *μmol/24h*	MPD	4297 ± 963	3901 ± 741	4192 ± 721	3464 ± 557[a]
	HPD	6557 ± 1797[c]	6093 ± 1448[c]	6739 ± 1052[c]	5123 ± 1470[abc]
Citrate *mmol/24h*	MPD	4.3 ± 1.4	4.2 ± 1.4	4.4 ± 1.4	4.0 ± 1.5
	HPD	4.0 ± 1.3	3.8 ± 1.4[c]	4.0 ± 1.2[c]	3.4 ± 1.7
Magnesium *mmol/24h*	MPD	6.5 ± 1.6	6.7 ± 2.3	7.5 ± 2.1	5.4 ± 2.0[b]
	HPD	6.3 ± 1.7	6.8 ± 2.8	7.7 ± 3.0	5.6 ± 2.2
4-PA *μmol/24h*	MPD	10.1 ± 3.4	9.7 ± 4.8	9.6 ± 3.9	9.9 ± 6.2
	HPD	13.3 ± 3.8[c]	11.3 ± 4.1	12.0 ± 4.0	10.3 ± 4.2
Urine volume *mL/24h*	MPD	3069 ± 584	2698 ± 989	3142 ± 884	2031 ± 762[ab]
	HPD	3208 ± 515	2940 ± 1165	3425 ± 1141	2213 ± 792[ab]

[a] Significant vs. controls ($P \leq 0.05$)
[b] Significant vs. MMH ($P \leq 0.05$)
[c] Significant vs. MPD ($P \leq 0.05$)

and three non-MMH experienced a significant rise in urinary oxalate as defined in the **Methods** section. It must be pointed out that 9 out of 13 controls and 2 out of 8 non-MMH on a MPD had 24-hour oxaluria levels over the normal range, whereas on that diet, 4 out of 12 MMH had normal oxaluria.

Table 3 summarizes biochemical urinary parameters: in groups on the MPD, urinary calcium and phosphorus levels were significantly higher in MMH than in controls and non-MMH, respectively; as expected, glycolate was higher in MMH than in non-MMH and controls. For those on a HPD, urea, sulfate, uric acid, phosphorus, and glycolate levels increased similarly in all groups versus MPD. Citrate fell on HPD in ISCFs as a whole and in MMH; a significance level, however, was not reached in controls and in non-MMH. As a consequence of increased intake of vitamin B_6 in subjects on a HPD versus MPD, urinary 4-pyridoxic acid increased slightly, the change being significant in controls only. On both diets, urine volume was in the order of 3 L/day in controls as well as in ICSFs, which is substantially more than under normal conditions, as a consequence of encouragement to increase fluid intake during the study. Urine volume was similar on both diets in the three groups. However, non-MMH subjects had a smaller urine volume than MMH or controls.

Figure 2 shows the regression analysis between changes in urinary oxalate versus changes in urinary glycolate and sulfate. For Δ-glycolate, there was a positive correlation for both ICSFs and controls. For Δ-sulfate, however, a significant correlation was found with Δ-oxalate in ICSFs only, as was the case for Δ-urea ($r = 0.53$, $P = 0.02$) and Δ-uric acid ($r = 0.59$, $P = 0.005$). There were trends for similar correlations when separately considering MMH and non-MMH.

Figure 3 depicts the individual values of serum pyridoxal-5′-phosphate and urine 4-pyridoxic acid on HPD. The pyridoxal 5′ phosphate concentration was normal in all subjects (normal range, 50 to 142 nmol/L) without a significant difference between the groups on average. However, subjects with the lowest four serum concentrations of pyridoxal-5′-phosphate and the lowest five urinary outputs of 4-pyridoxic acid were all ICSFs.

In ICSF patients, there was a positive correlation between serum concentration of pyridoxal-5′-phosphate and urinary excretion rate of 4-pyridoxic acid. Such was not the case in controls (Fig. 4). On the other hand, in controls, there was a strong negative correlation between oxaluria and serum concentrations of pyridoxal-5′-phosphate, but this was not the case in ICSFs (Fig. 5).

DISCUSSION

This interventional study demonstrates that a diet providing high amounts of protein derived from meat induces an increased urinary oxalate excretion in recurrent

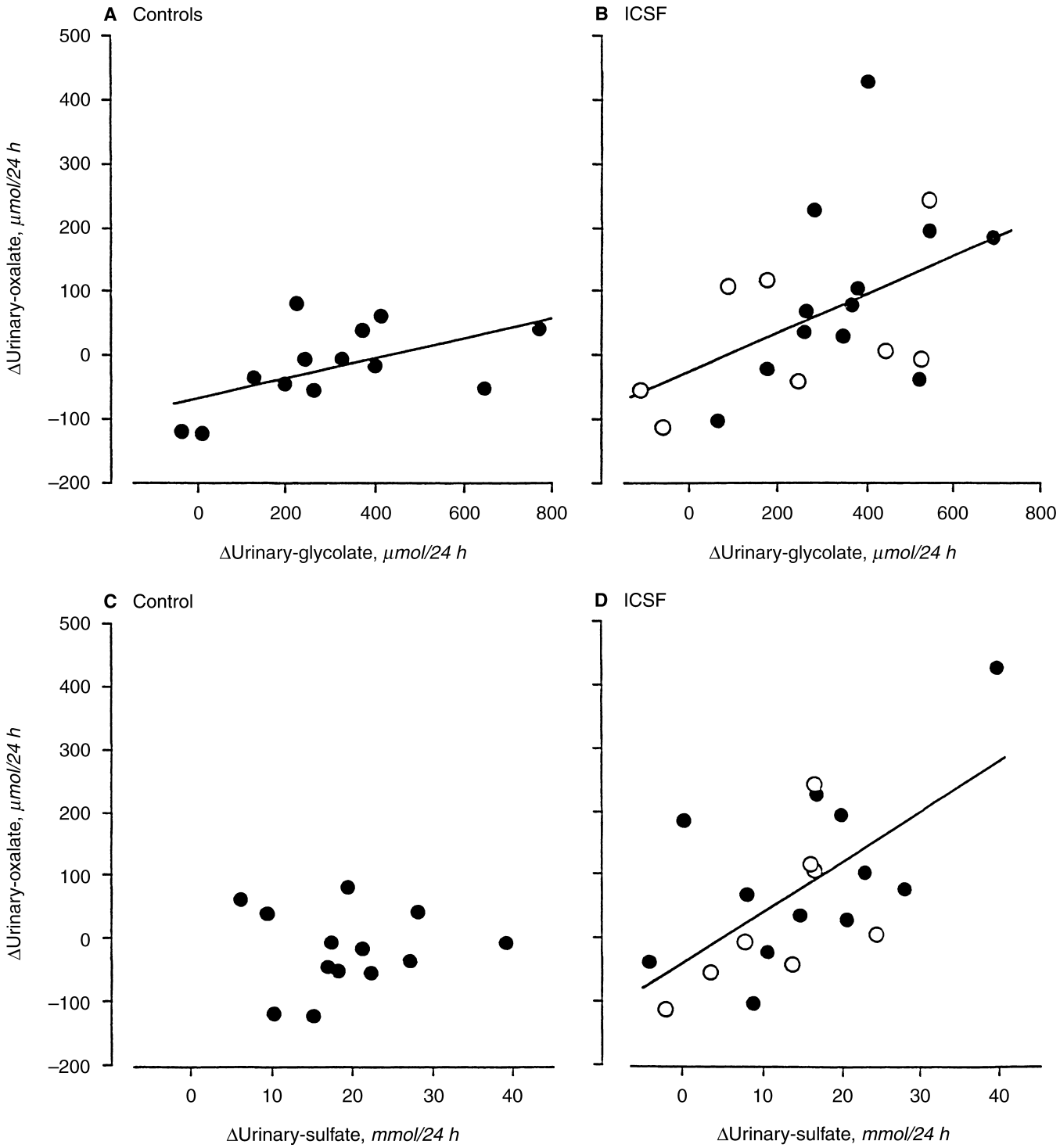

Fig. 2. Correlations between changes in urinary excretion of oxalate and changes in urinary excretion of glycolate or sulfate in controls and in ICSFs. Symbols are: (○) ICSF subjects without MMH; (●) ICSF with MMH. In (*A*), $r = 0.55$, $P = 0.05$; in (*B*), $r = 0.50$, $P = 0.03$; in (*C*) *NS* (not significant); in (*D*), $r = 0.62$, $P = 0.003$.

calcium stone formers with or without mild MMH, but not in healthy subjects.

The link between dietary factors and nephrolithiasis was first suspected by Andersen back in the late 1960s [5] and this finding was confirmed subsequently by several studies [6–9]. Excessive protein intake was rapidly postulated to play a key role in this process [10, 13–15] because it leads to urinary excretion of lithogenic substances such as calcium [16–18] and uric acid [18, 19] to rise, and urinary excretion of citrate to fall [17].

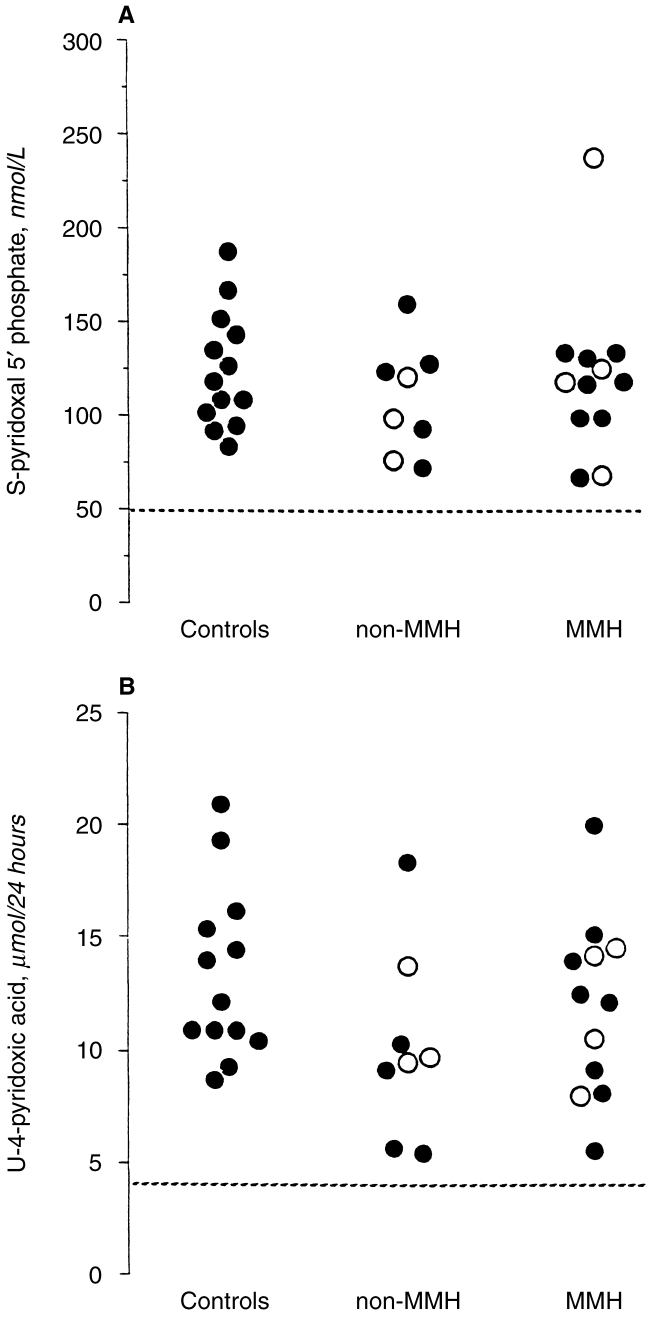

Fig. 3. Urinary excretion of 4-pyridoxic acid (*B*) and serum concentration of pyridoxal-5′-phosphate (*A*) in subjects on a high-protein diet (HPD). Symbols are: (○) ICSFs with a significant HPD-induced rise in urinary oxalate excretion; (●) ICSF with normal urinary oxalate levels. Horizontal lines are the lower normal limits (percentile 2.5).

Thus far, the role of high intake of protein on oxalate excretion remains controversial, be it in healthy subjects or in ICSFs. In six healthy male volunteers (not controlled for oxalate ingestion), Robertson et al had observed an increased excretion of oxalate following animal protein loading [18], results that were not confirmed by Kok et al in 8 healthy males [28] studied a decade later. Holmes et al also made such a negative observation in six healthy males studied under a high protein diet, whereas in six healthy females studied under similar conditions, the urinary excretion rate of oxalate actually rose by 20% [22]. In 12 ICSFs with absorptive hypercalciuria type II (7 males and 5 females), Urivetzky et al did not observe any oxaluric effect of protein loading [21], whereas a significant effect took place in 26 other stone formers, most of them being uric acid stone formers [20]. In eight ICSFs (7 males and 1 female), however, Fellström et al did not find any change in oxalate excretion after increasing their animal protein intake from 57 g to 142 g/day [17].

The present study was designed to test the hypothesis that only a subpopulation of ICSFs, that is, patients with MMH, might be sensitive to meat protein loading in terms of oxalate excretion. Actually, our evidence demonstrates that protein loading leads to an oxaluric response in one third of ICSFs, be they MMH or non-MMH, but not in healthy controls. In fact, these results raise the question of whether "MMH" would be an artificial entity, since we observed that not all control subjects on a MPD had a normal urinary oxalate despite their low oxalate intake, whereas 4 out of 12 MMH had oxaluria in the normal range and 2 out of 8 non-MMH had elevated oxaluria. Thus, the definition of hyperoxaluria may need to be revisited, especially since we are not the only ones to observe a large overlapping of oxalate excretion rates between healthy subjects and ICSFs [29]. In addition, oxaluria appears to vary widely over time, at least in patients, and so-called MMH may correspond to a transitory state. Urinary oxalate excretion and urinary volume observed in controls on MPD were 1.6- and 2.2-fold higher than the respective values observed in healthy subjects on a free-choice diet and usual fluid intake in a previous study [30]. Increased urine volume is interpreted as the consequence of encouragement to drink, and the apparent hyperoxaluria observed in many control subjects may just be the consequence of the high fluid intake, since increased urinary oxalate excretion has been shown to positively correlate to urinary volume [31]. If anything, one might argue that our findings should be generalized to standard drinking conditions with caution.

Because all participants were on concurrent nutritional oxalate restriction, increased oxalate excretion after protein loading observed in ICSFs most likely has a metabolic origin. Interference with vitamin C is unlikely, based on the dietary records. We cannot definitely exclude that the change in energy/carbohydrate/lipid content between HPD and MPD did not influence oxaluria. However, the slight decrement in carbohydrate intake observed when switching from a MPD to HPD, if anything, should have led oxaluria to decrease, not to increase, as discussed by a group of authors [32–34]. The large fat intake on HPD can be suspected to increase oxaluria, that is, by binding calcium in the intestine and

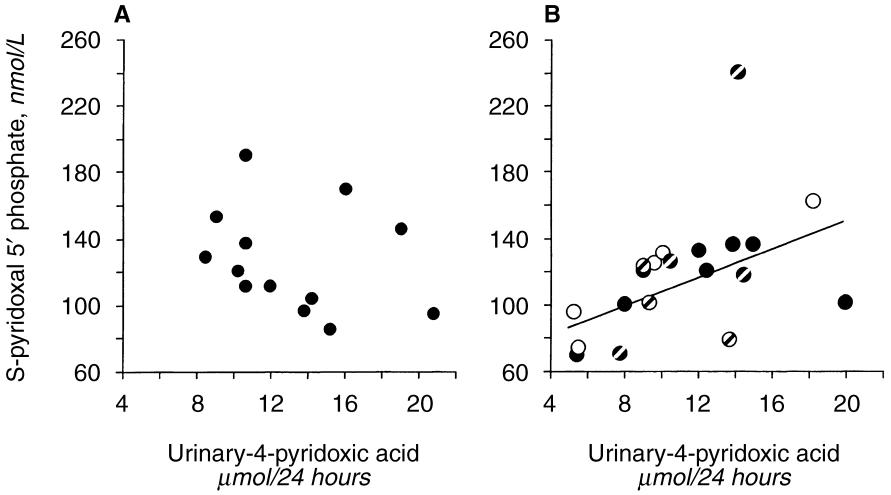

Fig. 4. Correlations between serum concentration of pyridoxal-5′-phosphate and urinary excretion of 4-pyridoxic acid in controls (*A*) and in ICSFs (*B*) on a high-protein diet (HPD). Open circles represent ICSF without MMH, and the bar across the circle indicates a significant HPD-induced rise in urinary oxalate excretion. In (A), $r = -0.26$, $P = 0.39$; in (B), $r = 0.48$, $P = 0.03$.

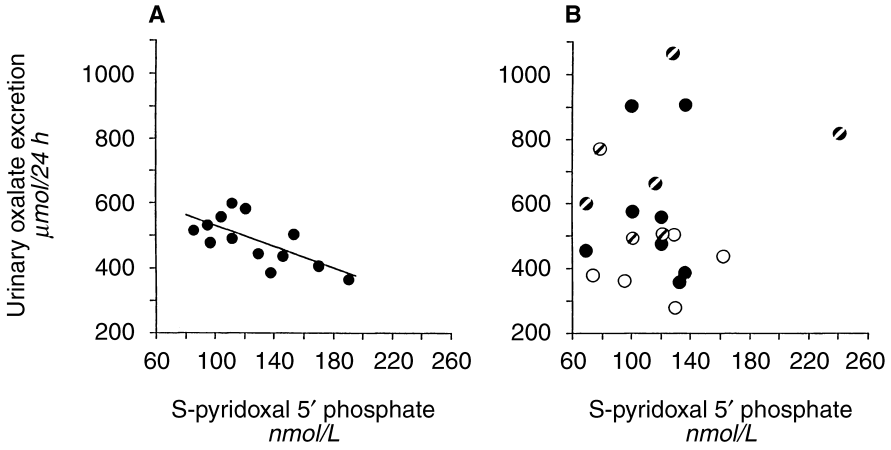

Fig. 5. Correlations between urinary oxalate excretion and serum concentration of pyridoxal-5′-phosphate in controls (*A*) and in ICSFs (*B*) on a high-protein diet (HPD). Open circles represent ICSF without MMH, and the bar across the circle indicates a significant HPD-induced rise in urinary oxalate excretion. In (A), $r = 0.69$, $P < 0.01$; in (B), *NS* (not significant).

thus favoring intestinal overabsorption of oxalate. Indeed, Masai, Ito, and Kotake found a positive correlation between fat intake and oxaluria in 60 patients [35]. However, Bailly, Norman, and Thompson could not find such a relationship in a much larger population of 476 calcium stone formers [36]. Moreover, it is unlikely that increasing fat intake would lead oxaluria to rise only in ICSFs and not in controls.

Other arguments for a protein related oxaluric effect are the correlations between changes in oxaluria and changes in excretion of the urinary markers of protein intake. The fact that these correlations were only present in ICSF actually supports the contention of a rather "exclusive" pro-oxaluric effect of proteins in ICSF. In this regard, it is noteworthy that urinary glycolate also appears to be an indirect marker of protein intake or, more specifically, a marker of meat protein intake, as this could have actually been anticipated from the glyoxylate pathway portrayed in Figure 6.

We could not find any specificity of the seven patients with positive oxaluric response to meat protein loading: serum concentration of pyridoxal 5′ phosphate (the active moiety of vitamin B_6) and urinary excretion rate of 4-pyridoxic acid (the excretion product of vitamin B_6) both were normal in these patients, thus at first glance in these cases excluding an impaired conversion of pyridoxin into its active metabolite, as well as a pyridoxin deficiency or malabsorption. Looking more carefully at the data, however, it appears that in 25% of ICSFs, urinary excretion of 4-pyridoxic acid was lower than the lowest value observed in the control population. Moreover, in these patients, there was a positive correlation between serum pyridoxal 5′ phosphate and urinary 4-pyridoxic acid, thus pointing to the functional significance of the relative vitamin B_6 deficiency seen in this fraction of ICSF. The fact that in controls a tight and negative correlation took place between urinary oxalate and serum pyridoxal 5′ phosphate further supports the relationship between the vitamin B_6 status of an individual and its oxalate excretion rate. That this relationship was abolished in ICSFs further illustrates the fact that vitamin B_6 metabolism is disturbed in the hyperoxaluric patients

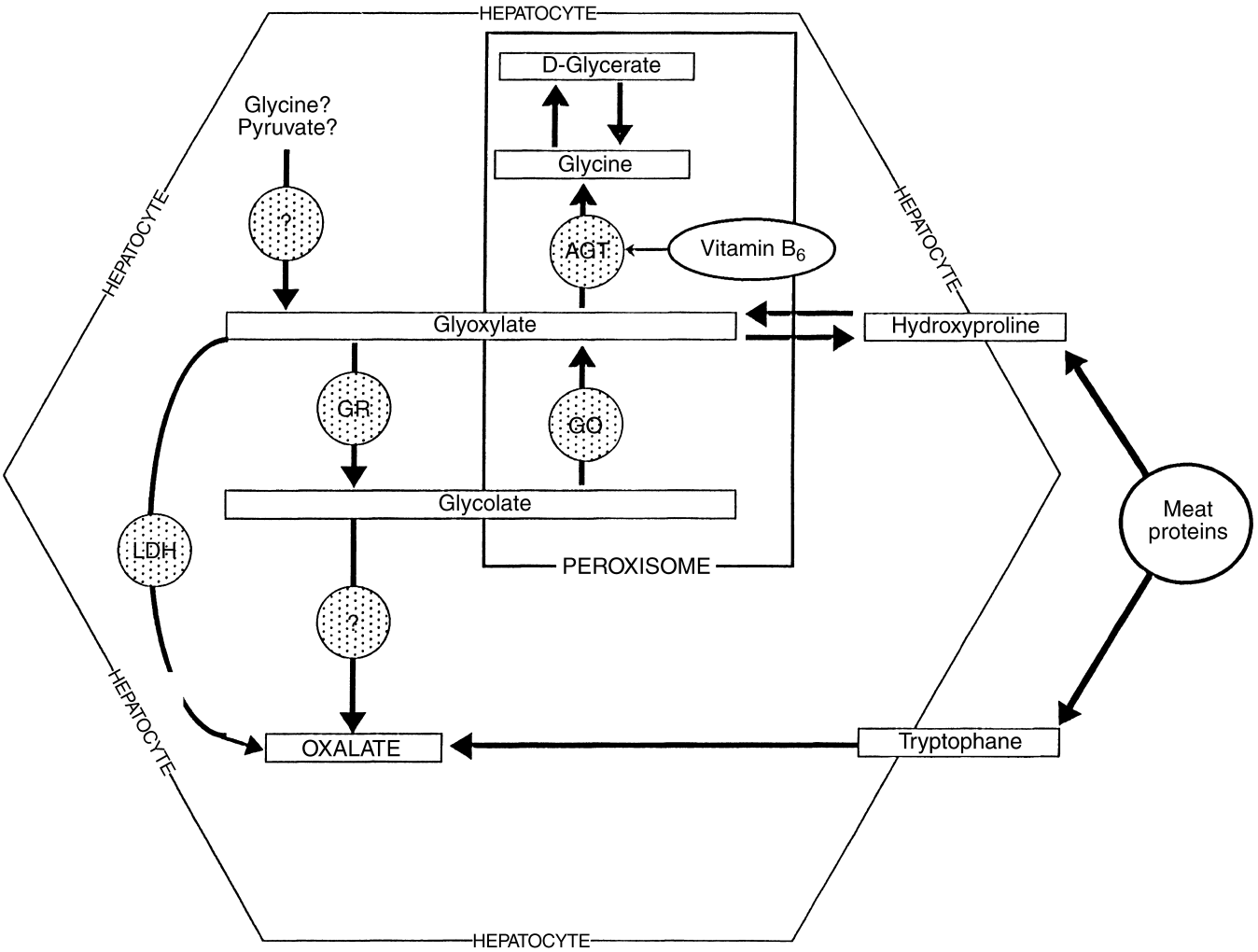

Fig. 6. Metabolic pathways from meat proteins to oxalate. Shaded circles correspond to various enzymes: AGT, alanine-glyoxylate aminotransferase; GR, glyoxylate reductase; GO, glycolate oxidase; LDH, lactate dehydrogenase. AGT deficiency causes type 1 primary hyperoxaluria, where glyoxylate cannot be metabolized to glycine, leading both glycolate and oxalate to accumulate. Vitamin B_6 supplementation may partially or completely correct the metabolic disorder.

taken collectively compared with the control population (unpublished observation). This, in turn, may account, at least in part, for protein sensitivity and/or hyperoxaluria observed in a substantial fraction of patients with so-called idiopathic calcium oxalate stone formation.

The present study suggests, however, that other mechanisms have to be evoked to account for this sensitivity to meat protein in terms of urinary oxalate. Some of them might be related to increased activity of the enzymes involved in the metabolic pathway from hydroxyproline or tryptophane to oxalate (Fig. 6). Indeed, these pathways are influenced by many factors. In rats, for instance, testosterone promotes the activity of glycolate oxidase [37], and insulin, glucagon, glucocorticosteroids, and thyroid hormone are possible inducers of oxalate synthesis [22]. Thus, an unsuspected endocrine disorder might underlie these observations and should require further investigation.

In conclusion, about one third of ICSFs, with or without MMH, are sensitive to meat protein in terms of oxalate excretion, and this response to meat protein could be a more meaningful criterion to define a subgroup of stone formers than so-called MMH. The precise mechanisms underlying this sensitivity remain to be elucidated and do not seem to involve vitamin B_6 deficiency.

In theory, protein load might be proposed as a test to identify patients with a clear oxaluric response who might optimally benefit of meat protein restriction. In fact, curtailing an excessive protein intake also confers the advantage of reducing uric acid and calcium excretion and of raising citrate excretion. Therefore, this dietary measure should still be widely recommended.

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APPENDIX

Abbreviations used in this article are: BMI, body mass index; CV, coefficient of variation; HPD, high protein diet; HPLC, high pressure liquid chromatography; ICSF, idiopathic calcium stone formers; LPD, low protein diet; MPD, moderate protein diet; MMH, mild metabolic hyperoxaluria; S_{P5P}, serum pyridoxal 5'-phosphate; U_{4PA}, urinary 4-pyridoxic acid.

REFERENCES

1. Johnson CM, Wilson DM, O'Fallon WM, *et al:* Renal stone epidemiology: A 25-year study in Rochester, Minnesota. *Kidney Int* 16:624–631, 1979
2. Coe FL, Parks JH, Asplin JR: The pathogenesis and treatment of kidney stones. *N Engl J Med* 327:1141–1152, 1992
3. Boyce WH, Garvey FK: Incidence of urinary calculi among patients in general hospitals. 1948 to 1952. *JAMA* 161:1437–1442, 1956
4. Hiatt RA, Friedman GD: The frequency of kidney and urinary tract diseases in a defined population. *Kidney Int* 22:63–68, 1982
5. Andersen DA: Historical and geographical differences in the pattern of incidence of urinary renal stones considered in relation to possible aetiological factors, in *Proceedings of the Renal Stone Research Symposium*, edited by Hodgkinson A, Nordin BEC, London, Churchill Livingstone, 1968, p 7
6. Ljunghall S: Family history of renal stone-formers and healthy subjects. *Br J Urol* 51:249–252, 1979
7. Robertson WG, Peacock M, Heyburn PJ, *et al:* The role of affluence and diet in the genesis of calcium containing stones. *Fortschr Urol Nephrol* 11:5–14, 1978
8. Boyce WH: Epidemiology of lithiasis in the United States, in *Proceedings of the 17th Congress of the International Society of Urology* (vol 1), Paris, Joly, 1979, pp 79–86
9. Robertson WG, Peacock M, Heyburn PJ, Hanes FA: Epidemiological risk factors in calcium stone disease. *Scand J Urol Nephrol* Suppl 53:15–28, 1980
10. Coe FL, Moran E, Kavalich AG: The contribution of dietary purine over-consumption to hyperpuricosuria in calcium oxalate stone formers. *J Chronic Dis* 29:793–800, 1976
11. Thomas WC, Malagodi MH: Dietary habits in patients with calcareous calculi, in *Idiopathic Urinary Bladder Stone Disease*, edited by Reen V, Washington D.C., U.S. Department of Health, Education and Welfare, 1977, pp 311–320
12. Zechner O, Latal D, Pfluger H, Schreiber V: Nutritional risk factors in urinary stone disease. *J Urol* 125:51–84, 1981
13. Robertson WG, Peacock M, Heyburn PJ, *et al:* Risk factors in calcium stone disease of the urinary tract. *Br J Urol* 50:449–454, 1978
14. Trinchieri A, Mandressi A, Luongo P, *et al:* The influence of diet on urinary risk factors for stones in healthy subjects and idiopathic renal calcium stone formers. *Br J Urol* 67:230–236, 1991
15. Curhan GC, Willett WC, Rimm EB, Stampfer MJ: A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *N Engl J Med* 328:833–838, 1993
16. Wasserstein AG, Stolley PD, Soper KA, *et al:* Case-control study of risk factors for idiopathic calcium nephrolithiasis. *Miner Electrolyte Metab* 13:85–95, 1987
17. Fellström B, Danielson BG, Karlstrom B, *et al:* Effects of high intake of dietary animal protein on mineral metabolism and urinary supersaturation of calcium oxalate in renal stone formers. *Br J Urol* 56:263–269, 1984
18. Robertson WG, Heyburn PJ, Peacock M, *et al:* The effect of high animal protein intake on the risk of calcium stone-formation in the urinary tract. *Clin Sci* 57:285–288, 1979
19. Breslau NA, Brinkley L, Hill KD, Pak CY: Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. *J Clin Endocrinol Metab* 66:140–146, 1988
20. Urivetzky M, Braverman S, Motola JA, Smith AD: Absence of effect of allopurinol on oxalate excretion by stone patients on random and controlled diets. *J Urol* 144:97–98, 1990
21. Urivetzky M, Motola J, Braverman S, Smith AD: Dietary protein levels affect the excretion of oxalate and calcium in patients with absorptive hypercalciuria type II. *J Urol* 137:690–692, 1987
22. Holmes RP, Goodman HO, Hart LJ, Assimos DG: Relationship of protein intake to urinary oxalate and glycolate excretion. *Kidney Int* 44:366–372, 1993
23. Marangella M, Bianco O, Martini C, *et al:* Effect of animal and vegetable protein intake on oxalate excretion in idiopathic calcium stone disease. *Br J Urol* 63:348–351, 1989
24. Giannini S, Nobile M, Sartori L, *et al:* Acute effects of moderate dietary protein restriction in patients with idiopathic hypercalciuria and calcium nephrolithiasis. *Am J Clin Nutr* 69:267–271, 1999
25. Chiriboga J: Some properties of an oxalic acid oxidase purified from barley seedlings. *Biochem Biophys Res Commun* 11:277–282, 1963
26. Kasidas GP, Rose GA: A new enzymatic method for the determination of glycollate in urine and plasma. *Clin Chim Acta* 96:25–36, 1979
27. Toftegaard-Nielsen T: A method for enzymatic determination of citrate in serum and urine. *Scand J Clin Lab Invest* 36:513–519, 1976
28. Kok DJ, Iestra JA, Doorenbos CJ, Papapoulos SE: The effects of dietary excesses in animal protein and in sodium on the composition and the crystallization kinetics of calcium oxalate monohydrate in urines of healthy men. *J Clin Endocrinol Metab* 71:861–867, 1990
29. Osther PJ: Hyperoxaluria in idiopathic calcium nephrolithiasis. *Scand J Urol Nephrol* 33:368–371, 1999
30. Hess B, Jost C, Zipperle L, *et al:* High-calcium intake abolishes hyperoxaluria and reduces urinary crystallisation during a 20-fold normal oxalate load in humans. *Nephrol Dial Transplant* 13:2241–2247, 1998
31. Klan R, Butz M: Effect of diuresis on the oxalate excretion and the calcium product in non-stone formers. *Urol Int* 42:19–22, 1987
32. Nguyen NU, Dumoulin G, Wolf JP, Berthelay S: Urinary calcium and oxalate excretion during oral fructose or glucose load in man. *Horm Metab Res* 21:96–99, 1989
33. Nguyen NU, Dumoulin GTHM, Regnard J: Increase in urinary calcium and oxalate after fructose infusion. *Horm Metab Res* 27:155–158, 1995
34. Nguyen NU, Dumoulin G, Henriet MT, Regnard J: Aspartame ingestion increases urinary calcium, but not oxalate excretion, in healthy subjects. *J Clin Endocrinol Metab* 83:165–168, 1998
35. Masai M, Ito H, Kotake T: Effect of dietary intake on urinary oxalate excretion in calcium renal stone formers. *Br J Urol* 76:692–696, 1995
36. Bailly GG, Norman RW, Thompson C: Effects of dietary fat on the urinary risk factors of calcium stone disease. *Urology* 56:40–44, 2000
37. Yoshihara H, Yamaguchi S, Yachiku S: Effect of sex hormones on oxalate-synthesizing enzymes in male and female rat livers. *J Urol* 161:668–673, 1999