Kidney International, Vol. 57 (2000), pp. 1115–1123

Effects of low animal protein or high-fiber diets on urine composition in calcium nephrolithiasis

Michel Rotily, Françoise Léonetti, Cecilia Iovanna, Patrice Berthezene, Patricia Dupuy, Alain Vazi, and Yvon Berland

Centre d'Investigations Clinique, Sainte Marguerite, APHM-INSERM; ORS PACA-INSERM U379; Service de Néphrologie, Hôpital Sainte-Marguerite; and INSERM U315, Marseille, France

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Background. The purpose of this article is to evaluate the impact of low protein and high fiber intakes on risk factors of stone recurrence in idiopathic calcium stone formers (ICSFs).

Methods. Ninety-six ICSFs were randomly assigned a low animal protein diet (<10% of total energy), a high-fiber diet (>25 g/day), or a usual diet (control group); all patients were recommended to increase their fluid intake. Their daily urine compositions were analyzed at baseline and at four months. Compliance with dietary recommendations was checked by validated food frequency questionnaires. Compliance with total and animal protein intakes was assessed by 24-hour urea and sulfate outputs, respectively. The nutritional intervention (oral instructions, written leaflet, phoning) and food assessment were carried out by a research dietitian.

Results. At baseline, diets and the daily urine composition did not differ between the three groups. At four months, while diets differed significantly, the 24-hour output of calcium and oxalate did not differ significantly within and between groups after adjustment for potential confounders (age, sex, and personal and family history of calcium stones) and baseline values. However, as many as 12 out of 31 ICSFs (95% CI, 22 to 58%) assigned to a low animal protein diet achieved a reduction in the urine urea excretion rate of more than 50 mmol/day and also exhibited a significant decrease in urinary calcium excretion that averaged 1.8 mmol/day. A significant correlation between urea and calcium outputs was observed only among patients with hypercalciuria.

Conclusions. These results show that only ICSFs who markedly decrease their animal protein intake, especially those with hypercalciuria, can expect to benefit from dietary recommendations.

Nephrolithiasis has increased dramatically since the Second World War [1] and is recognized as a public health problem [2, 3]. Estimates of its prevalence range from 1% to as high as 5% in the general population of industrialized countries [4, 5]. The annual incidence was estimated to be between 1 and 3 per 1000 inhabitants [4, 6], with 50% of relapses every four years [7]. Calcium stones, the most prevalent type of nephrolithiasis, result from several metabolic defects. The combined role of genetic and nutritional factors, especially protein and dietary fiber, in the formation of calcium oxalate kidney stones has been suggested in metabolic investigations [8–12], case control studies [3, 13–17], and international ecologic correlation studies [18–20]. Only one prospective study has been published so far, and it showed that dietary intakes of animal protein and, above all, calcium were directly and independently associated with the risk of stone formation [21]. However, although idiopathic calcium stone formers (ICSFs) are encouraged to reduce animal protein in their diet [6, 22, 23], the protective effect of such a diet has not been demonstrated by controlled clinical trials. One trial showed that the risk of stone recurrence in the dietary intervention group was 5.6 [95% confidence interval (CI), 1.2 to 26.1], relative to the control group, after adjustment for possible confounding effects of age, sex, education, and baseline protein and fluid intake [24]. The authors concluded that the advice for ICSFs to follow a low animal protein, high-fiber, high-fluid diet has no advantage over advice to increase only fluid intake. However, in that study, the decrease in 24-hour output of urea was not significant, which makes it unlikely that the diet had any impact. Also, the incidence of a recurrent stone was not significantly different among the 32 ICSFs who reported a mean protein intake lower than 70 g/day (6.6 vs. 3 out of 100 person years, $P = 0.24$); patients did not significantly increase their dietary fiber intake, which did not differ significantly between the intervention group and the control group. Furthermore, the authors did not take into account calcium intake, which is considered a protective factor by Curhan et al [21].

Key words: renal stones, urinary lithiasis, fiber diet, kidney calculi, idiopathic stone formers.

Received for publication February 4, 1999
and in revised form October 7, 1999
Accepted for publication October 11, 1999

Consequently, further research, including statistical modeling, is needed to re-examine the validity and the effectiveness of current dietary recommendations [1]. We thus sought to determine whether a decrease in protein intake or an increase in total dietary fiber can prevent recurrence in ICSFs during the four years following an episode of urinary calcium lithiasis. We analyzed the influence of a low animal protein diet, a high-fiber diet (HFD), and a control diet on urinary solute excretion at four months.

METHODS

Participant selection

Participants were recruited among patients attending our nephrology outpatient clinic between January 1996 and January 1998. ICSFs were included regardless of the number of stone episodes. The diagnosis of calcium stones was based on spontaneous or surgical stone elimination or abdominal radiograph and ultrasound examination. All patients were evaluated in our center for clinical investigations to exclude those with a metabolic or urological cause of stone formation. Persons eligible for the trial had to be over 18 and less than 70 years of age, speak French, understand dietary instructions, live close to our center, and have no plan to move in the next months. Patients on a diet for other metabolic disorders or for urinary lithiasis were also excluded. Written informed consent was obtained from each participant, and the ethics committee approved the trial.

Among the 108 idiopathic ICSF included in our study, 12 (11%) left the trial during the four months of follow-up (3 moved and 9 did not succeed in staying on the diet). The proportion of patients who dropped out of the study did not differ significantly between the three groups (5 in the control group, 4 in the LAPD, and 3 in the HFD group, $P = 0.56$). These 12 patients did not differ in age, sex, education, personal and family history of calcium stones, and daily alcohol consumption. However, they had a higher body mass index (25.5 vs., 23.5, $P = 0.03$) and lower outputs of calcium (4.94 vs. 6.26, $P = 0.04$), oxalate (0.20 vs. 0.31, $P = 0.03$), and urea (305.9 vs. 357.1, $P = 0.02$). Hence, 96 ICSFs were followed up.

Data collection

All patients recruited with a diagnosis of idiopathic calcium stone disease were invited in our center for clinical investigations, where food questionnaires were given, urine was collected, and biochemical parameters were analyzed by a trained staff at baseline and after four months for the three diets. The initial interview included family and personal history of stone formation, education, and alcohol and tobacco consumption.

We collected the 24-hour urinary output of urine at baseline and at four months. Thymol was the urine preservative. The samples were stored at −80°C, and all measurements were carried out at the same time by two trained technicians. Samples were all measured by a DAX autoanalyzer (Bayer Diagnosis®) for sodium, potassium, phosphate, calcium, urea, creatinine, and urate. Oxalate was measured by an enzymatic method (Biorea®) [25, 26], citrate by spectrophotometer (Boehringer-Mannheim® kit) [27]; sulfate was measured turbidometrically [28]. Urinary solutes were expressed in mmol/day. The accuracy of the individual daily urine collection was evaluated on the basis of their creatinine content in relationship to body weight [29] and to creatinine content predicted on the basis of age, sex, and body weight through the Cockroft and Gault formulas [30]. The net gastrointestinal absorption of alkali (expressed in mEq/24 h), subsequently referred to as "GI-alkali," was derived from 24-hour urine excretions of noncombustible cations and anions according to the formula: $(Na + K + Ca + Mg) - (Cl + 1.8 \times P)$, where electrolyte excretions are in mEq/day, except for P, which is in mmol/day, with an average valence of 1.8 [9]. The relative CaOx saturation ratio was calculated with EQUIL93 software [31].

Dietary assessment

All participants were interviewed about their diet over the last month by a research dietitian using a standardized semiquantitative food frequency questionnaire [32, 33]. Nutrients were calculated from the national food composition table [34, 35]. Questionnaire results were compared with seven-day diet records collected simultaneously with the baseline evaluation and at four months. The two methods were significantly correlated for the nutrient measures: energy, total protein, animal protein, total fiber, and calcium intake. Correlation coefficients ranged from 0.47 for calcium to 0.63 for dietary fiber ($P < 10^{-3}$). In this analysis, we used nutrient intakes evaluated by the food frequency questionnaires.

Nutritional intervention

After giving informed consent, participants were randomized from a list of random numbers to receive instruction either on a low animal protein diet (LAPD group) or on a high fiber diet (HFD group). No dietary recommendation was given to persons included in the control group.

The LAPD group was instructed to decrease their intake of animal protein by limiting their consumption of meat, fish, and cooked pork to three servings per week and milk products (milk, cheese, and yogurt) to 100 g per day (milk and cheese). The target was to obtain a daily contribution of animal protein to energy of less than 10%. Instead of protein, they were counseled to eat refined cereals (that is, pasta and rice).

The HFD group was instructed to increase their intake

of raw and dried fruit, vegetables, and to substitute their usual cereals with whole grain dietary products in order to limit the increase in energy. The target was to obtain a daily total fiber intake of more than 25 g.

The dietitian instructed all participants in the LAPD and HFD groups and, for men, their spouses or family, by describing allowable animal protein intake or sources of fiber and how to follow the diet. Leaflets with detailed information on calcium stone disease, dietary recommendations, and appropriate recipes were given to these patients. The same dietitian called them four times during the four months to help them change their dietary habits, to counsel them, and to check the compliance with dietary instructions.

All participants, including those in the control group, were recommended to maintain a high-water intake. They were given a leaflet with information about the benefits of daily water intake and how to increase it by drinking eight glasses of tap water (2 L per day) at fixed times; the calcium concentration of tap water available in Marseille is 100 mg/L. The attending physician did not recommend any modification in dietary habits but did insist on an increase in fluid intake.

Statistical analysis

Results are presented as group means and standard deviations. Proportions were compared by the χ^2 Pearson statistic. The citrate output and the time elapsed since the last episode of urinary lithiasis, neither of which were normally distributed, were natural-log transformed for Pearson correlation analyses and significance testing of differences between groups. Analysis of variance was used to compare nutrient intakes and urinary parameters at baseline and at four months among the three groups. Analysis of covariance that took into account the baseline values was used to compare nutrient intakes and urinary parameters at four months only in controls and patients who complied with either of the diets (less than 10% of total energy brought by animal proteins in the LAPD group; dietary fiber intake greater than 25 per day in the HFD group). Paired *t*-test and Wilcoxon test were used to compare the differences in urinary parameters within intervention groups between baseline and at four months. Multiple regression analyses were performed to explain the variance of urinary parameters by nutrient intake and personal characteristics. *P* values of lower than 0.05 were considered significant. All analyses were done with SPSS® software (version 8.0).

RESULTS

Sample characteristics

The LAPD, HFD, and control groups did not differ in age, education, weight, height, body mass index, personal and family history of calcium stones, time elapsed since the last episode of urinary lithiasis, and daily alcohol consumption (Table 1). Only the proportion of men was higher in the LAPD group than in the control and in the HFD groups. Eighty eight percent of men were married; 46% of men and 45% of women reported that they had eaten at least twice a week outside. When they had eaten outside their homes, 27% had prepared their meal at home. The others had eaten in a staff cafeteria (30%) or a restaurant (35%), or they had eaten a snack (8%). Of the 96 participants, 37% had hypercalciuria (>0.1 mmol/kg/day); that proportion did not differ among the three groups. The proportion of patients recruited during summer time (June through September) did not differ significantly between the control, LAPD, and HFD groups (29, 29, and 44%, respectively). In the same way, the proportion of patients examined at four months during the summer months did not differ significantly (48, 28, and 25%, respectively, $P = 0.22$).

Dietary intake assessments

Dietary intake assessment at baseline did not differ significantly between the three groups (Table 2). After four months, the nutrient intake did not change significantly in the control group, but it changed dramatically in the LAPD and HFD groups. In the LAPD group, total and animal protein intake decreased significantly; 31 out of 34 subjects achieved the intervention target (<10% of calories from animal proteins). In the HFD group, energy and total fiber and calcium intakes increased significantly; 27 out of 31 subjects achieved the intervention target (total fiber intake >25 g/day). Total protein intake did not change after four months in the HFD group. In contrast, their animal protein intake decreased significantly, but that decrease was significantly greater in the LAPD group.

Daily urine composition at baseline and at four months

Both at baseline and at four months, the creatinine excretion rates for all urine collected by the 96 patients were significantly correlated with their body weights ($r = 0.64$, $P < 10^{-4}$) and with predicted urinary creatinine content ($r = 0.55$, $P < 10^{-4}$). These data indicate that the participants collected their urine samples with reasonable accuracy.

The means of daily urine composition for each group are shown in Table 3. On the whole, there was no significant difference in urinary solute excretion between groups both at baseline and at four months among subjects who complied with the nutritional recommendations (Table 3).

Whereas 24-hour urinary volume did not change in the LAPD group, it decreased significantly in the control and the HFD groups. As expected, the LAPD group showed a significant decrease in urea and sulfate excretion and to some extent in creatininuria. Citraturia also

Table 1. Demographic characteristics and history of study subjects with at least one documented kidney stone

Variable	Control group	LAPD group	HFD group	Total	*P*
Sample size	31	34	31	96	
Men %	58	82	58	67	0.05
Age *years* (mean ± SD)	44.9 ± 12.0	45.3 ± 13.0	43.7 ± 13.7	44.7 ± 12.8	0.87
Education *years;* %					
<12 y	32	53	48	45	0.50
12 y	23	15	16	18	
>12 y	45	32	36	37	
Weight *kg* (mean ± SD)	68.2 ± 13.6	71.6 ± 10.4	65.9 ± 13.2	69.0 ± 12.5	0.60[a]
Height *cm* (mean ± SD)	168 ± 9	173 ± 7	169 ± 7	170 ± 8	0.50[a]
Body mass index kg/m^2 (mean ± SD)	23.8 ± 3.4	23.8 ± 3	23 ± 3.4	23.5 ± 3.5	0.54
Family history of kidney stones %					
No	52	44	48	48	0.25
One parent	26	44	23	31	
More than one parent	22	12	29	21	
Personal history of kidney stones (%)					
1	29	18	26	24	0.82
2–3	36	35	32	34	
4	35	47	42	42	
Hypercalciuria >0.1 mmol/kg/d %	32	32	48	38	0.31
Elapsed time since the last episode of urinary lithiasis					
<2 months	30	15	32	25	0.40
2–6 months	37	53	45	45	
>6 months	33	32	23	30	
Smokers %	32	18	32	28	0.20
Daily alcohol consumption *g/d* (mean ± SD)	8.1 ± 10.5	11.2 ± 12.7	8.3 ± 9.0	9.3 ± 10.9	0.43

Abbreviations are: LAPD, low animal protein diet; HFD, high fiber diet; SD, standard deviation.
[a]Statistical significance of the analysis of variance after adjustment for sex

decreased significantly in this group. For the three groups, we observed no change in calcium, oxalate, sodium, and potassium urinary excretion, and CaOx saturation index. However, patients with hypercalciuria in the LAPD group showed a slight but not significant decrease in calcium output from 8.7 to 7.4 mmol/day (Table 4). Such a decrease was not observed among patients with normocalciuria.

We divided the HFD group into three subsets of equal size, according to the statistical distribution of increase in total dietary fiber (<14 g/day, 14 to 23 g/day, >23 g/day). No change in urinary calcium and oxalate outputs was observed within each subset. We also divided the LAPD group into three subsets according to the statistical distribution of the decrease in urine urea: no decrease, decrease <50 mmol/day, decrease >50 mmol/day (50 mmol/day was the higher 33rd percentile). Among patients with a decrease in urine urea of more than 50 mmol/day, there was a significant decrease in urine calcium, together with a significant decrease in creatinine, sulfate, urate, and citrate (Tables 4 and 5). Such decreases were not observed in the other two subsets (no decrease or decrease <50 mmol/day). In the entire series of 96 patients, 31 had a decrease in urine urea of more than 50 mmol/day; among these 31 patients, there was a marked decrease in urine calcium (7.12 to 5.13 mmol/day, $P < 10^{-3}$), together with a significant decrease in creatinine, sulfate, and urate. Twenty-four–hour urinary volume and citrate also decreased significantly ($P < 10^{-3}$), 2.11 to 1.65 L/day and 3.74 to 1.84 mmol/day, respectively. Decreases in urine calcium were not observed in the other two subsets, with no decrease in urine urea or a decrease of less than 50 mmol/day.

Relationships between dietary intake and urinary solute excretion at baseline

The baseline 24-hour urinary volume did not vary according to age, sex, personal and family history of urinary lithiasis, and time elapsed since the last episode of urinary lithiasis. Although the baseline 24-hour urinary volume was not correlated with the outputs of creatinine, calcium, urea, and urate, it was significantly correlated with the outputs of sulfate ($r = 0.25$, $P = 0.04$) and citrate ($r = 0.27$, $P = 0.03$; Table 6). Baseline urinary concentrations of calcium ($r = -0.62$, $P < 10^{-4}$), oxalate ($r = -0.37$, $P < 10^{-3}$), and urate ($r = -0.71$, $P < 10^{-4}$) were significantly and negatively correlated with the 24-hour urinary volume. However, urinary concentrations of citrate, urea, and creatinine were not correlated with the 24-hour urinary volume.

After the outputs of creatinine, citrate, sulfate, and urate were controlled for, the correlation between the output of calcium and that of urea was significant among patients with hypercalciuria ($r = 0.83$, $P = 0.003$) but not among those with normocalciuria ($r = 0.21$, $P = 0.31$).

Many urinary parameters were associated at baseline with individual characteristics and nutrient intake, but none with the time elapsed since the last episode of

Table 2. Dietary intake at Baseline (M0) and at 4 months (M4) reported by the food frequency questionnaire in the three nutritional intervention groups

Variable	Control group ($N = 31$)					LAPD group ($N = 34$)					HFD group ($N = 31$)					Contrasts between intervention groups (P value)[b]				
	M0		M4			M0		M4			M0		M4			M4				
	mean	SD	mean	SD	P[a]	mean	SD	mean	SD	P[a]	mean	SD	mean	SD	P[a]	M0	M4	C vs. HFD	C vs. LAPD	HFD vs. LAPD
Energy *Kj/24 h*	1909	440	1905	520	0.9	1998	535	1933	434	0.5	2021	468	2301	681	0.02	0.6	$<10^{-3}$	$<10^{-2}$	0.9	$<10^{-4}$
Protein *g/24 h*	82.9	21.0	83.3	26.5	0.9	87.3	24.8	70.2	18.8	$<10^{-3}$	92.0	22.1	93.2	28.5	0.9	0.3	$<10^{-4}$	0.10	$<10^{-2}$	$<10^{-4}$
Animal protein *g/24 h*	57.2	18.6	58.8	21.8	0.7	61.3	21.4	40.7	13.4	$<10^{-3}$	64.9	19.0	56.1	20.8	0.04	0.3	$<10^{-4}$	0.53	$<10^{-4}$	$<10^{-3}$
Total fiber *g/24 h*	17.0	8.7	16.3	6.2	0.6	16.4	5.5	18.4	7.2	0.2	17.9	6.9	35.9	13.4	$<10^{-3}$	0.7	$<10^{-4}$	$<10^{-4}$	0.9	$<10^{-4}$
Calcium *mg/24 h*	771	334	799	315	0.6	783	369	763	265	0.7	804	347	955	351	0.04	0.9	0.01	0.05	0.3	$<10^{-2}$

Abbreviations are: LAPD, low animal protein diet; HFD, high fiber diet; SD, standard deviation.

[a] P = statistical significance of paired t-test between M0 and M4

[b] P = statistical significance of the inter-group comparison test; analysis of variance after adjustment for sex and age

urinary lithiasis (Table 6). Multiple regression models explained a small part of variance of urinary parameters (between 6 and 24%). Citraturia was significantly lower among people who reported previous episodes of urinary lithiasis. Energy intake was negatively correlated with urinary oxalate and GI-alkali. Urinary potassium, phosphate, creatinine, oxalate, urea, uratem, and GI-alkali were significantly and positively correlated with animal protein intake. Total fiber intake was not significantly correlated with any urinary parameter. CaOx saturation was independently associated with animal protein intake and was negatively associated with energy.

At baseline, both in the sample including all patients and in the LAPD group, the correlation between citrate and urea was not significant (coeficient = -0.02, $P = 0.84$; coeficient = 0.11, $P = 0.32$). At four months, in the LAPD group, the correlation between citrate and urea was highly significant (coeficient = 0.62, $P < 10^{-3}$). In the control and the HFD group, that relationship was not significant.

DISCUSSION

The main result of our randomized and controlled nutritional intervention is that a decrease in animal protein intake, with a urine urea output of more than 50 mmol/day, among ICSFs can lead to a decrease in daily urine calcium output. Our study has also shown that an increase in dietary fiber intake did not succeed in modifying the main predictive factors of calcium oxalate stone formation, calcium, and oxalate outputs.

Our patients were very similar to those evaluated in previous studies for urinary output of urea, calcium, and oxalate [3, 9, 14, 16] and for the proportion of patients with hypercalciuria (about a third) [3]. The mean protein intake in our sample was also very close to that reported for ICSFs dwelling in the same geographic area, Marseilles [3], and those in other studies [21].

The proportion of patients who left the trial between baseline and at four months was 11% (12 out of 108) and did not vary between groups. This shows how difficult it is for these patients to stay on a diet in spite of the strong support given by the dietitian. In the same way, a large increase in diuresis, recognized as a basic recommendation in ICSFs [11, 22, 23], was not obtained in spite of recommendations by both the dietitian and the attending physician. It has been shown that ICSFs with low urine volumes have altered thirst sensitivity and vasopressin release (*abstract*; Hess et al, *J Am Soc Nephrol* 7:1802, 1996).

The dramatic decrease in animal protein intake (about a third of the initial intake) between baseline and at four months and in the outputs of urea and sulfate and to some extent the decrease in creatinine show that dietary habits were drastically modified in the LAPD group. However, these modifications were not homogeneous

Table 3. Urinary excretion at baseline (M0) and at 4 months (M4) in the three nutritional intervention groups

	Control ($N = 31$)					LAPD group ($N = 31$)					HFD group ($N = 27$)					Comparisons between groups	
	M0		M4			M0		M4			M0		M4			M0	M4
Variable	mean	SD	mean	SD	P[a]	mean	SD	mean	SD	P[a]	mean	SD	mean	SD	P[a]	P[b]	P[c]
Volume *L/d*	1.8	0.6	1.6	0.6	0.02	1.8	0.8	1.9	0.6	0.60	2.2	0.7	1.7	0.6	0.002	0.06	0.07
pH	6.3	0.4	6.2	0.4	0.11	6.3	0.4	6.3	0.5	0.44	6.3	0.4	6.3	0.6	0.96	0.57	0.70
Sodium *mmol/d*	153	52	132	47	0.06	169	50.4	174	61	0.91	159	60	143	58	0.19	0.97	0.13
Potassium *mmol/d*	55.8	22.6	47.1	14.5	0.10	63.4	20.1	58.0	18.5	0.06	58.0	22.6	56.2	22.6	0.79	0.80	0.29
Phosphate *mmol/d*	26.0	8.4	27.3	9.5	0.41	31.0	11	26.1	9.3	0.005	26.6	8.5	28.1	10	0.49	0.62	0.04
Creatinine *mmol/d*	11.1	3.4	10.2	3.7	0.24	12.7	2.7	11.9	3.2	0.11	11.9	3.9	11.7	3.4	0.73	0.78	0.49
Calcium *mmol/d*	5.9	2.8	6.1	3.8	0.62	6.4	2.4	6.2	2.7	0.57	6.6	2.5	6.9	3.2	0.68	0.45	0.46
Oxalate *mmol/d*	0.29	0.15	0.28	0.13	0.62	0.30	0.19	0.30	0.15	0.92	0.33	0.18	0.31	0.19	0.76	0.71	0.79
Sulphate *mmol/d*	12.0	5.3	9.4	4.8	0.14	13.8	5.5	9.3	3.5	0.003	11.2	4.8	9.0	3.9	0.10	0.47	0.65
Urea *mmol/d*	345	98	348	136	0.77	387	99	361	102	0.03	348	95	370	114	0.24	0.82	0.17
Urate *mmol/d*	3.2	1.0	3.0	1.3	0.47	3.6	1.0	3.4	1.3	0.29	3.2	1.0	3.3	1.3	0.56	0.85	0.58
Citrate *mmol/d*	3.1	2.6	2.3	1.3	0.09	3.5	2.2	2.3	1.2	0.04	3.6	3.8	2.3	1.5	0.16	0.95	0.30
GI-alkali[d]	29.1	25.5	22.2	21.6	0.20	37.1	43.9	30.5	23.1	0.50	39.5	24.0	32.5	27.9	0.39	0.72	0.33
CaOx saturation	11.4	6.2	11.5	5.6	0.93	12.0	7.9	11.4	5.2	0.61	13.46	6.9	13.26	8.2	0.92	0.51	0.55

Abbreviations are: LAPD, low animal protein diet; HFD, high fiber diet; SD, standard deviation.
[a]P = statistical significance of paired *t*-test between M0 and M4
[b]P = Statistical significance of inter-groups comparison tests; analysis of variance after adjustment for sex
[c]P = Statistical significance of inter-groups comparison tests; analysis of covariance (covariate = sex and value at baseline M0)
[d]GI-alkali is the net gastrointestinal absorption of alkali: $(Na + K + Ca + Mg) + [Cl + (1.8 \times P)]$

Table 4. Urinary excretion at baseline (M0) and at 4 months (M4) according to the decrease in 24-h urine urea excretion in idiopathic calcium stone formers on a low animal protein diet

	No decrease in urine urea excretion ($N = 11$)			Decrease $<$ 50 mmol/d ($N = 8$)			Decrease $>$ 50 mmol/d ($N = 12$)		
Variable	M0 mean	M4 mean	P[a]	M0 mean	M4 mean	P[a]	M0 mean	M4 mean	P[a]
Volume *L/d*	1.7	1.7	0.78	1.3	1.8	0.08	2.3	2.0	0.24
pH	6.2	6.2	0.59	6.3	6.4	0.58	6.2	6.2	0.93
Sodium *mmol/d*	147	177	0.15	169	181	0.46	189	163	0.12
Potassium *mmol/d*	55.6	53.9	0.38	57.6	56.8	0.92	73.8	60.2	0.03
Phosphate *mmol/d*	27.4	27.8	0.81	31.7	25.1	0.14	34.6	26.0	0.02
Creatinine *mmol/d*	11.7	12.8	0.09	12.2	11.5	0.14	13.6	11.3	0.02
Calcium *mmol/d*	6.0	7.0	0.02	6.4	7.2	0.89	7.0	5.2	0.004
Oxalate *mmol/d*	0.33	0.34	0.92	0.20	0.22	0.35	0.31	0.30	0.94
Sulphate *mmol/d*	12.5	10.8	0.72	11.4	8.4	0.08	16.8	8.9	0.03
Urea *mmol/d*	337	389	0.001	374	344	0.03	449	337	0.002
Urate *mmol/d*	3.26	3.73	0.10	3.7	3.1	0.23	3.8	3.2	0.008
Citrate *mmol/d*	3.3	2.9	0.72	1.7	1.7	0.75	4.5	1.9	0.04
GI-alkali[b]	23.5	25.5	0.35	37.3	29.2	0.69	49.5	29.2	0.42
CaOx saturation	13.1	12.8	0.65	8.4	9.2	0.46	12.2	10.7	0.69

[a]P = statistical significance of Wilcoxon paired test between M0 and M4
[b]GI-alkali is the net gastrointestinal absorption of alkali: $(Na + K + Ca + Mg) + [Cl + (1.8 \times P)]$

within the LAPD group, and only 12 of 34 patients succeeded in decreasing their urea output by more than 50 mmol/day and their calcium output. In the HFD group, fiber intake assessments from food frequency questionnaires showed a dramatic increase at four months. Unlike for protein intake, biomarkers of fiber intake are not currently available, and we cannot ascertain the compliance of participants with the high-fiber intake. The measurement of fecal weight as a check of dietary fiber intake is fraught with problems and is unacceptable to the general public [32]. In our study, the research dietitian noticed that the increase in total dietary fiber intake through increased fruit, vegetable, and cereal consumption was easier to achieve than the decrease in animal protein consumption. Thus, it is unlikely that patients in the HFD group falsified their answers to the dietitian to show their compliance with the given recommendations.

Some studies reported no difference in daily protein intake between ICSF and healthy subjects [3, 13–15, 36], whereas others found a significant positive correlation

Table 5. Urinary excretion at baseline (M0) and at four months (M4) among idiopathic calcium stone formers on low animal protein diet according to calciuria

Variable	Normocalciuric ($N = 22$)			Hypercalciuric[a] ($N = 9$)		
	M0 mean	M4 mean	P[b]	M0 mean	M4 mean	P[b]
Volume *L/d*	1.8	1.8	0.88	1.9	2.1	0.34
pH	6.3	6.2	0.95	6.2	6.5	0.14
Sodium *mmol/d*	164	168	0.66	181	188	0.52
Potassium *mmol/d*	62.8	56.5	0.05	64.6	61.3	0.62
Phosphate *mmol/d*	30.7	25.4	0.03	31.7	27.5	0.13
Creatinine *mmol/d*	12.3	11.6	0.15	13.4	12.8	0.59
Calcium *mmol/d*	5.4	5.7	0.81	8.7	7.4	0.08
Oxalate *mmol/d*	0.34	0.32	0.58	0.20	0.26	0.26
Sulphate *mmol/d*	13.3	9	0.04	14.0	10.0	0.07
Urea *mmol/d*	367	353	0.34	438	377	0.02
Urate *mmol/d*	3.4	3.4	0.87	3.8	3.3	0.04
Citrate *mmol/d*	3.4	2.2	0.01	3.3	2.5	0.87
GI-alkali[c]	38.8	28.7	0.15	31.9	31.5	0.59
CaOx saturation	13	11.4	0.51	9.8	11.3	0.37

[a]Hypercalciuria = (24-h calcium urine excretion >0.1 mmol/kg/d)
[b]P = statistical significance of Wilcoxon paired test between M0 and M4
[c]GI-alkali is the net gastrointestinal absorption of alkali: $(Na + K + Ca + Mg) + [Cl + (1.8 \times P)]$

Table 6. Multiple regression analyses for 24-hour urinary solutes according to nutrient intake, 24-hour urinary volume, and personal and family history of lithiasis at baseline [standardized coefficients (statistical significance); $N = 96$]

	Personal history of lithiasis	Family history of lithiasis	Energy	Animal protein	Total fiber	Calcium	24-hour urinary volume	R^2 (percent)[a]
pH	−0.06 (0.60)	−0.15 (0.21)	0.14 (0.39)	−0.24 (0.16)	−0.15 (0.28)	0.08 (0.56)	−0.19 (0.12)	12.0
Sodium	−0.04 (0.73)	0.11 (0.39)	0.12 (0.49)	0.11 (0.51)	−0.14 (0.30)	−0.01 (0.97)	0.08 (0.54)	6.4
Potassium	−0.27 (0.02)	0.03 (0.81)	−0.10 (0.52)	0.44 (0.008)	0.15 (0.23)	0.12 (0.38)	0.03 (0.80)	11.9
Phosphate	−0.15 (0.20)	0.06 (0.62)	−0.06 (0.73)	0.38 (0.02)	0.07 (0.62)	0.04 (0.76)	−0.18 (0.12)	9.5
Creatinine	0.09 (0.41)	0.00 (0.99)	0.07 (0.65)	0.50 (0.003)	−0.02 (0.87)	−0.23 (0.10)	−0.02 (0.84)	19.8
Calcium	−0.01 (0.93)	0.19 (0.10)	0.12 (0.47)	0.30 (0.08)	−0.13 (0.36)	−0.17 (0.23)	−0.03 (0.83)	12.5
Oxalate	0.04 (0.72)	−0.05 (0.69)	−0.43 (0.009)	0.45 (0.007)	0.21 (0.10)	−0.16 (0.25)	0.18 (0.11)	18.4
Sulphate	−0.02 (0.89)	−0.19 (0.11)	−0.25 (0.13)	0.10 (0.56)	−0.02 (0.90)	0.24 (0.10)	0.25 (0.04)	15.9
Urea	0.09 (0.44)	0.01 (0.92)	0.07 (0.68)	0.41 (0.01)	−0.13 (0.31)	0.04 (0.77)	−0.08 (0.51)	18.3
Urate	−0.14 (0.23)	0.14 (0.23)	−0.10 (0.54)	0.43 (0.01)	−0.07 (0.62)	−0.22 (0.13)	−0.14 (0.24)	13.4
Citraturia	−0.24 (0.04)	0.03 (0.83)	−0.04 (0.80)	0.07 (0.66)	0.03 (0.85)	−0.02 (0.90)	0.27 (0.03)	15.3
GI-alkali[b]	−0.31 (0.007)	−0.03 (0.77)	−0.39 (0.01)	0.54 (0.001)	0.05 (0.68)	−0.03 (0.80)	−0.09 (0.41)	24.0
CaOx saturation	0.05 (0.69)	−0.02 (0.88)	−0.34 (0.04)	0.44 (0.008)	0.18 (0.18)	−0.22 (0.13)	0.18 (0.12)	16.8

[a]R^2 is the percentage of variance of the dependent variable (that is, sodium) explained by the multiple regression model
[b]GI-alkali is the net gastrointestinal absorption of alkali: $(Na + K + Ca + Mg) + [Cl + (1.8 \times P)]$

between the protein consumption and the risk of stone disease [16, 37, 38]. These discrepancies were discussed by Robertson, who noted that most studies matched for socioeconomic background failed to find any consistent difference between the dietary intake of the two groups, whereas the unmatched studies showed several significant differences [22]. We could add that in most studies, the risk factors were not adjusted for confounding factors, and samples were small. Furthermore, even if case control studies were to show significant relationships between animal protein intake and calcium stones, they would not demonstrate that a restriction in animal protein intake would prevent recurrent stones among ICSF. In support of a role of nutrient intake in calcium stone formation is the prospective study of Curhan et al, who found a higher relative risk of stone episodes in persons reporting low calcium intakes and to some extent high protein intakes [21]. Rightly, that study did not conclude that advice to decrease protein intake or increase calcium intake could reduce the stone recurrence rate.

Results about the protective role of dietary fiber in calcium stone formation, established mainly from metabolic and retrospective studies, are also discordant [13, 20, 37]. Barker et al found a significant correlation between protein intake and emergency rates for renal stones and colic in 72 areas in England and Wales [20]. However, they did not adjust for protein intake when analyzing the correlation between fiber intake and the emergency rate. Furthermore, since it was an ecologic study, the results of their study cannot be considered as establishing a causal relationship between protein intake and renal stone formation.

Other arguments for a decrease in protein intake among ICSFs rely on correlations between animal pro-

tein intake and calcium output, mainly among healthy people, or rely on experimental studies in which nutrient intake levels were far from the usual ones among ICSF. Kok et al showed that a high animal protein intake decreased the ability of urine to inhibit the agglomeration of calcium oxalate crystals [10]. Those authors suggested that this impairment was a possible physicochemical explanation for the adverse effects of dietary habits on renal stone formation. However, that investigation was carried out among healthy male subjects, and the amount of protein intake was very high (2 g/kg/day) relative to usual diets among ICSFs (in our sample, 1.25 g/kg/day at baseline). Another clinical investigation has shown that a high bran intake, rich in phytic acid, significantly reduced renal calcium excretion [8]. First, in that trial, the fiber intake was very high (36 g rice bran per day) and would not be feasible in a long-term perspective. Furthermore, that investigation was performed among healthy women and not among ICSFs. Future studies should investigate the impact of different achievable recommendations on dietary fiber (that is, fiber brought by fruit and vegetables, usually soluble, or cereals, usually not soluble) among ICSFs.

Correlations between nutrients and urinary parameters are difficult to interpret. Whereas our dietary assessment reflects the nutrient intake during the last month, measured urinary parameters reflect the last 24-hour metabolism. This difference in timing can explain some potential discrepancies or lack of correlations between dietary assessment and urinary output. For urea, creatinine, and sulfate, the estimated correlations with animal protein intake concur with the current knowledge. We did not, however, observe the correlation between the output of citrate and fiber intake reported by Hess et al [9]. Their sample was small (34 patients), and above all, they did not adjust that correlation for other characteristics and nutrients, such as animal protein intake, which is often negatively correlated with fiber intake. It is equally possible that the type of dietary fiber consumed by our patients differed from theirs. That issue will remain unsolved because the French food composition table, as in many countries, does not make a distinction between soluble and nonsoluble dietary fibers.

Although Hiatt et al did not consider the citrate output in their risk analysis of recurrent calcium oxalate kidney stones [24], hypocitraturia has been considered by some authors as a risk factor for calcium stone formation [11]. In our study, a reduction of animal protein intake did not significantly increase the output of citrate. On the contrary, citrate output was decreased, and a trend toward reduction in mean citrate excretion was observed in the whole sample. No clear explanation for that reduction in mean citrate excretion can be proposed. The analysis of correlations at baseline showed that citraturia was not correlated with energy, animal protein, calcium, and total fiber intake, but citraturia was significantly and independently correlated with personal history of urinary lithiasis and the 24-hour urine volume. This correlation emphasizes the need for ICSFs to increase their water intake and highlights that giving dietary recommendations to ICSFs could be counterproductive: When the recommendation to these patients is to reduce their animal protein intake, they tended not to increase their fluid intake. That agrees with Hiatt's study, which showed that the stone recurrence was higher in the group on a dietary regimen in comparison with the control group [24].

As reported by other studies [3, 9], we found a significant correlation between the calcium and urea output. However, after we controlled for the output of creatinine, citrate, sulfate, and urate, that correlation was significant among patients with hypercalciuria but not those with normocalciuria. In the LAPD group, we also found a small but not significant decrease in 24-hour output of calcium among ICSFs with hypercalciuria. One could hypothesize that patients with hypercalciuria are metabolically more sensitive to animal protein intake. If this is confirmed, only ICSFs with hypercalciuria should be advised to reduce their animal protein intake. It is very likely that some genetically acquired characteristics play a more important role than nutrition in the recurrence of calcium stones, and that nutrition could be a stimulus only in metabolically sensitive subjects.

In conclusion, our randomized controlled trial using urinary risk factors as end points does not support recommendations to increase dietary fiber among idiopathic calcium stone formers. Among those patients who were recommended to be on a low animal protein diet, as many as 12 out of 31 (95% CI, 22 to 58%) of ICSFs assigned to a low animal protein diet achieved a reduction in the urine urea excretion rate of more than 50 mmol/day and also exhibited a significant decrease in urinary calcium excretion that averaged 1.8 mmol/day, and could expect to benefit from such a diet. Furthermore, patients with hypercalciuria could draw more benefit from a low animal protein diet. The four-year follow-up of patients included in that trial will confirm whether a low animal protein or HFD has an impact on the stone recurrence rate.

ACKNOWLEDGMENTS

We thank Dr. Barnay, G. Burkhart, Dr. Brunet, J. Casanova, Pr. Coulange, Pr. Dussol, Dr. Fabre-Monges, C. Gauthier, Dr. Gugliotta, Dr. Lechevallier, B.M. Margetts, Dr. Morange, C. Portelli, Dr. Purgus, Dr. Rossi, Dr. Roubicek, and Pr. Serment.

Reprint requests to Michel Rotily, M.D., Center d'Investigations Cliniques, Hôpital Sainte Marguerite, Boulevard Sainte Marguerite, 13009 Marseille, France.
E-mail: rotily@marseille.inserm.fr

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