Fresh lemon juice supplementation for the prevention of recurrent stones in calcium oxalate nephrolithiasis: A pragmatic, prospective, randomised, open, blinded endpoint *(PROBE)* trial

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Summary

Background Standard diet with normal calcium and reduced animal proteins and salt content reduces stone recurrence in calcium oxalate nephrolithiasis. Whether lemon juice supplementation further reduces recurrence rate is unknown.

Methods In this single-centre, prospective, randomised, open, blinded endpoint trial (Clinical Trials gov NCT01217372) we evaluated the effects of fresh lemon juice supplementation (60 mL twice daily) versus no supplementation, on time to stone recurrence in 203 patients with recurrent idiopathic calcium oxalate nephrolithiasis who were all prescribed a standard diet. Patients were included between July 2009 and March 2017 at the Nephrology Unit of the Papa Giovanni XXIII hospital in Bergamo, Italy. Time to stone recurrence at 2 years of follow-up was the primary outcome. Analyses were by intention-to-treat.

Findings During two years of follow-up 21 of 100 patients randomised to lemon juice supplementation and 32 of 103 controls randomised to no supplementation had stone recurrence [HR (95% CI): 0·62 (0·35–1·07), p = 0·089]. Patient adherence to lemon juice supplementation, however, progressively decreased from 68% at one-year to 48% at two-year follow-up. At explorative analyses restricted at one-year follow-up, ten patients with supplementation versus 22 controls had stone recurrence [0·43 (0·20–0·89), p = 0·028]. After adjustment by age, sex and normo or hypocitraturia, the HR (95%) was still significant [0·45 (0·20–0·93), p = 0·036]. At six months, 24 hour urinary sodium excretion decreased by 8·60±65·68 mEq/24 h in patients receiving lemon juice supplementation and increased by 3·88±64·78 mEq/24 h in controls. Changes significantly differed between groups (p = 0·031). This difference was subsequently lost. Treatment was safe. In patients with lemon juice supplementation gastrointestinal disorders were more frequent (p<0·001). Renal and urinary tract disorders were similar between groups (p = 0·103).

Interpretation Explorative analyses suggest that fresh lemon juice supplementation to standard diet might prevent stone recurrence in patients with calcium-oxalate nephrolithiasis. However, treatment effect was likely reduced by progressively declining adherence to lemon juice supplementation.

Funding This study received no funding.

Keywords: Nephrolithiasis; Kidney stones; Calcium oxalate; Juice supplementation

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Research in context

Evidence before this study

We searched PubMed for all English-language articles published between January 1, 1990, and June, 30, 2021, with the search terms "calcium oxalate", "nephrolithiasis", "stone", "recurrence", "citraturia", "lemon juice", "randomised controlled trial" and failed to detect any controlled study evaluating the role of lemon juice supplementation on long-term prevention of recurrent nephrolithiasis. A few studies suggested that lemon juice may reduce the risk of stone recurrence, but the findings appeared to be flawed by the retrospective, observational design and the small sample size that limited the power of statistical analyses. Thus we conducted a phase III prospective randomised open, blinded endpoint (PROBE) trial to evaluate the effects of fresh lemon juice supplementation, as compared to no supplementation, on long-term risk of stone recurrence in patients with recurrent idiopathic calcium oxalate nephrolithiasis who were all prescribed a diet based on restricted intake of animal proteins and salt, combined with a normal calcium intake (standard diet).

Added value of this study

Results of the present study showed that recommended standard diet integrated with 60 mL twice daily of fresh lemon juice and a standard diet without lemon juice supplementation are similarly effective in reducing the risk of recurrent stones in patients with calcium oxalate nephrolithiasis. Results were most probably biased by the progressively declining patient adherence to recommended lemon juice supplementation during the two-year follow-up period. Indeed, when the follow-up was closed at one year after randomisation when adherence was still largely superior to 50 per cent, standard diet with lemon juice supplementation was found to be more protective against stone recurrence than standard diet without supplementation.

Implications of all the available evidence

Fresh lemon juice supplemented to standard diet appeared to exert some protective effect against stone recurrence in patients with calcium oxalate nephrolithiasis. Treatment effect, however, was largely mitigated by progressively declining patient adherence and became evident only at explorative, post-hoc analyses closed at one year of follow-up, when patient adherence was still acceptable. Thus, our findings are not robust enough to recommend oral juice supplementation in everyday clinical practice, but provide useful information to inspire more effective strategies to improve patient adherence in future trials designed to test the hypothesis that oral juice supplementation might decrease the risk of stone recurrence in patients with calcium oxalate nephrolithiasis.

Introduction

Calcium oxalate nephrolithiasis is the most common form of nephrolithiasis. It may affect two to 15 per cent of the general population, with an incidence rate ranging from 114 to 720 new cases reported every year per 100,000 individuals.[1] Without specific pharmacological therapy, 27 to 50 per cent of patients may have stone recurrence over five years.[2] Of note, in 15 per cent of cases recurrence of stones is associated with clinical symptoms and many symptomatic patients require surgical intervention annually.

Kidney stone formation requires crystals to form, grow, aggregate and interact with a non-crystalline organic matrix. The pathogenesis is multifactorial and includes an increased excretion of urinary lithogenic solutes including calcium, oxalate, phosphate, uric acid, and sodium, along with a reduced urinary excretion of inhibitors of crystallization such as citrate and magnesium. Moreover, a reduction or an increase in urinary pH may favour the precipitation of uric acid or calcium phosphate salts, respectively.[1]

In addition to genetic and metabolic factors,[3] also environmental factors including diet play an important role in the development of nephrolithiasis.[4] Diet based on restricted intake of animal proteins and salt, combined with a normal calcium intake, may reduce the risk of stone formation.[5] However, the efficacy in preventing stone recurrences is limited, in particular in chronic stone formers. Potassium citrate is effective in preventing stone recurrence in patients with calcium oxalate nephrolithiasis, but approximately one third of treated patients stops the medication because of gastrointestinal disorders such as eructation, bloating or gaseousness, and diarrhoea.[6] Citrus fruits are a natural rich source of citrate and diet supplementation with fresh juice of citrus fruits may represent a valuable alternative option to citrate supply. Amongst the most commonly consumed citrus fruits, lemons contain the greatest concentrations of citric acid. Indeed, citric acid concentration in lemon juice (49·2 g/Kg) exceeds by approximately five-folds the concentration in orange juice.[7] The citrate supplied with the juice that escapes metabolic degradation in vivo is excreted unchanged in the urine where it may prevent the tendency of calcium oxalate salts to precipitate. Moreover, urinary oxalate excretion is not affected by lemon juice, but is increased by orange juice supplementation.[8] This difference probably reflects the greater ascorbic acid concentration in orange compared to lemon juice.

Thus, fresh lemon juice has been suggested as a possible alternative to potassium citrate for the treatment of patients with calcium oxalate nephrolithiasis, in particular those with hypocitraturia.[9] However, results of the few studies designed to evaluate the effects of lemon juice supplementation in patients with calcium oxalate

nephrolithiasis are flawed by the retrospective, observational design and the too small sample size to test treatment effect with an adequate power of statistical analyses.[10–13] Thus, we designed a pragmatic Prospective, Randomised, Open, Blinded Endpoint *(PROBE)* Trial [14] to evaluate the effects of fresh lemon juice supplementation, as compared to no supplementation, on long-term risk of stone recurrence in patients with recurrent idiopathic calcium oxalate nephrolithiasis who were all prescribed a standard diet based on restricted intake of animal proteins and salt, combined with a normal calcium intake.[5]

Methods

Study design and participants

In this Phase III, pragmatic PROBE trial we included patients with recurrent idiopathic calcium oxalate nephrolithiasis referred to the outpatient Renal Stone Clinic of the Unit of Nephrology of the Azienda Socio-Sanitaria Territoriale (ASST) Papa Giovanni XXIII in Bergamo (Italy). Consenting adult (≥ 18-year-old) males or females with history of recurrent nephrolithiasis, with one or more calcium oxalate stone - or mixed calcium oxalate and phosphate, or calcium oxalate and uric acid stone - formation over the last five years, and at least one kidney stone at baseline documented by renal echography at inclusion were eligible for study participation. We excluded patients with obstructive uropathy, chronic urosepsis, serum creatinine >1·8 mg/dL, renal tubular acidosis, primary hyperparathyroidism, primary hyperoxaluria, pure uric acid and cystine stones, medullary sponge kidney; lithotripsy treatment within the last six months; active peptic ulcer disease, gastric oesophageal reflux; systemic disease or cancer; treatment with thiazide diuretics; drug or alcohol abuse or any chronic clinical conditions that could affect completion of the trial or confound data interpretation. We also excluded patients who were unable to provide informed consent, and pregnant, lactating, or potentially childbearing women without effective contraception (see Study Protocol and https://clinicaltrials.gov/ct2/show/NCT01217372?term=lemon+juice&cond=Nephrolithiasis&cntry=IT&city=bergamo&draw=2&rank=1).

The study protocol was approved by the Ethics Committee of the ASST Papa Giovanni XXIII and written informed consent was obtained from all patients in compliance with the Declaration of Helsinki. The study was coordinated and monitored by the Department of Renal Medicine of the Clinical Research Centre (CRC) for Rare Diseases "Aldo e Cele Daccò" Villa Camozzi-Ranica of the Istituto di Ricerche Farmacologiche Mario Negri IRCCS in Bergamo (Italy) according to Good Clinical Practice guidelines. Data were recorded locally on dedicated paper Case Report Forms and centralised into the database at the CRC. Consolidated Statement of Reporting Trials (CONSORT) guidelines were adhered to. This trial is registered with ClinicalTrials.gov Identifier: NCT01217372.

Stratification, randomisation and masking

According to urinary citrate excretion at baseline evaluation, patients satisfying the selection criteria were stratified into two strata: hypocitraturia if urinary citrate excretion was ≤ 320 mg/24 h or normocitraturia if urinary citrate excretion was > 320 mg/24 h.

All patients were recommended to drink 2 to 2.5 litres of water per day and were prescribed the same standard diet (Table S1) based on restricted intake of animal proteins and salt, combined with a normal calcium intake.[5] Then, within each stratum, patients were randomised on a 1:1 basis to two-year treatment with standard recommended diet with fresh lemon juice supplementation or to standard diet without lemon juice supplementation. All patients in the hypocitraturia stratum were also prescribed 2 g of potassium citrate twice daily. Randomisation was centralised at the Laboratory of Biostatistics of the CRC under the responsibility of an independent investigator (G.A.G.) according to a web-based, computer-generated randomisation list created using SAS (version 9.2). Blocking was used to ensure balance in the number of patients in each group at any time during the trial. A block size of eight was used. According to the PROBE design neither patients nor care providers were masked to group assignment, whereas investigators involved in data handling and analyses were blinded to patient treatment allocation. A blinded-to-treatment adjudicating group reviewed the data to determine which patients had reached study endpoints and to evaluate safety.

Procedures

All patients satisfying the selection criteria were asked to submit after one and four months respectively a 24-hour urine collection for the measurement of 24-hour urine output, urinary excretion of calcium, oxalate, magnesium, phosphate, citrate, urea, uric acid, sodium, sulphate, creatinine and evaluation of urinary pH. The averages of the two measures were taken as baseline. In the occasion of the second urine submission, patients had a complete physical evaluation with measurement of blood pressure, heart rate, body weight, body mass index (BMI), abdominal circumference and a blood sample was collected for routine hematochemistry (baseline evaluation). Baseline evaluation also included a visit with a Dietician who submitted the diet (Table S1) and a questionnaire to assess patient compliance to the diet. Patients allocated to lemon juice supplementation were recommended to drink 60 mL of fresh lemon juice twice daily (an amount expected to provide six grams or 92 mEq of citric acid per day). All patients - regardless of treatment randomisation - were

maintained on their standardised diet and were recommended not to reduce daily fluid intake.

Baseline evaluations - including measurement of 24-hour urine output, urinary excretion of calcium, oxalate, magnesium, phosphate, citrate, urea, uric acid, sodium, sulphate, creatinine, evaluation of urinary pH, dietician evaluation and renal ultrasound - were repeated at six months and then at one and two years after randomisation. X-ray evaluations were not standardised and were performed as deemed clinically appropriate. The dietary questionnaire was administered and the importance of diet adherence reaffirmed by a dietician at the same time points. Routine hematochemistry was evaluated at baseline and at yearly intervals. Additional evaluations were planned whenever deemed appropriate on clinical ground. Patients were asked to report to the investigators any sign or symptom potentially associated with the underlying disease such as colic pain, haematuria, dysuria, and stone passage. They were also instructed to report these events in a diary that was submitted to the investigator in the occasion of any visit.

Outcome measures

The study was primarily aimed at evaluating whether fresh lemon juice added to standard diet recommendations compared to standard diet without lemon juice supplementation may reduce the risk of stone recurrence. The primary outcome was time to stone recurrence. Stone recurrence was defined by one of the following criteria: 1. A typical renal colic, or an episode of haematuria, with expulsion or removal of a previous undiscovered stone; 2. Echography detection of a previous undiscovered stone, even in the absence of symptoms; 3. More than 30 percent increase in pre-existing stone size. In all cases recurrence had to be confirmed by echography. All echography evaluations were performed by the same nephrologist (S.R.) who was blinded to treatment allocation. Secondarily, the study evaluated changes in urine volume, pH and solutes potentially associated with stone recurrence. All adverse events were recorded until study end and monitored up to complete resolution.

Sample size and statistical analyses

Primary outcome variable of the study was time to stone recurrence. Based on previous reports and regardless of baseline stratification, 40% of patients in the control arm on diet alone (with or without potassium citrate supplementation) were conservatively expected to have at least one new stone formation over two-year follow-up.[5] Over the same observation period, new stone formation was expected to be observed in 20% of patients on active treatment with fresh lemon juice. We considered that event rate reduction to 20% with fresh lemon juice supplementation would have been a clinically relevant effect. To give the trial an 80% power to detect as statistically significant (alpha=0.05, two-tailed test) the expected difference in event rate (40% vs. 20%) between the two treatment groups, 91 patients per group had to be available for the statistical evaluation. Assuming a 10% drop-out rate, 101 patients per group had to be included. Thus, a total of 202 patients had to be randomised.

All outcomes were assessed by intention-to-treat analyses. For survival data, the Kaplan–Meier method was used to plot the probability to achieve the end points of interest. Cox proportional hazard regression models were performed, and results were expressed as hazard ratios (HRs) and 95% confidence intervals (95% CIs). The primary analysis, i.e. time to stone recurrence, was assessed by the unadjusted Cox model. In a post-hoc analysis, an adjusted model which took into account age, sex, and baseline stratification (i.e. normocitraturia or hypocitraturia) was also considered. Proportionality assumptions were assessed using Schoenfeld residuals by means of the proportional hazards (PH) test. Non-pre-specified, post-hoc, explorative survival analyses were performed to assess the effects of fresh lemon juice supplementation on stone recurrence during the first year of follow-up. Changes in continuous efficacy variables listed in Table 2 were evaluated at different time points (i.e. 6 months, 1 year and 2 years) by analysis of covariance (ANCOVA), including treatment and baseline measurements. Adverse events were classified using the Medical Dictionary for Regulatory Activities system (version 17.1). Data were analysed by SAS (version 9.4) and STATA (version 15) and were presented as number (%), mean (SD), or median (interquartile range), as appropriate. All P values were two-sided.

Role of the funding source

This study received no funding.

Results

A total of 224 patients were screened for study participation: 14 of potentially eligible patients withdrew their consent to study participation, four did not fulfil the eligibility criteria, two were lost to follow-up and one had an adverse event. Thus, 21 of screened patients were excluded and 203 were randomised between July 2009 and March 2017. One-hundred patients were assigned to standard diet and fresh lemon juice supplementation, and 103 to standard diet (Fig. 1). One patient happened to be on concomitant therapy with a thiazide diuretic. She was considered as a protocol violator but was included in study analyses according to the intention-to-treat principle. In both groups the assigned intervention was initiated within one week from randomisation. Eight patients per group were lost to follow-up. Twelve patients in the standard diet with lemon juice supplementation group withdrew their consent vs. six in the

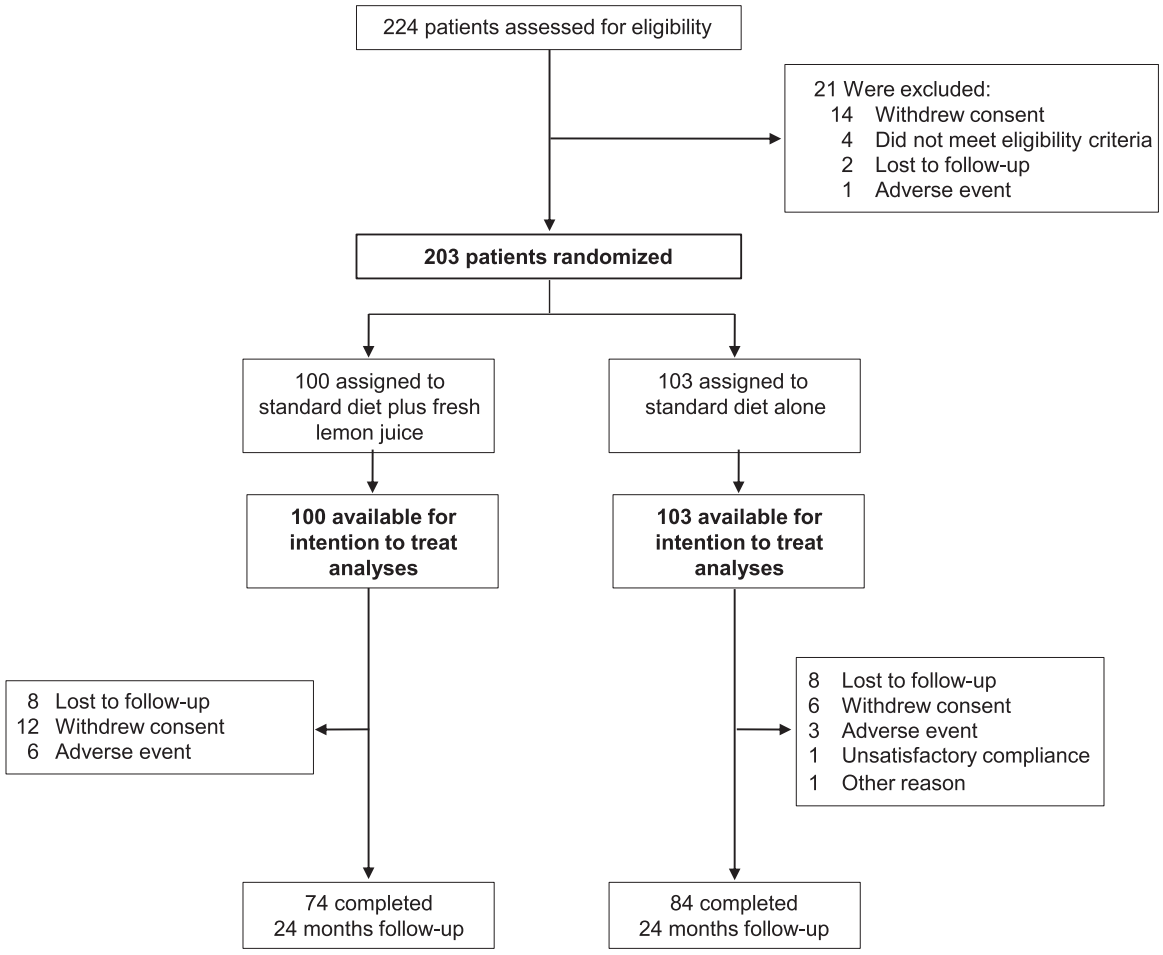


Fig. 1. Trial profile.

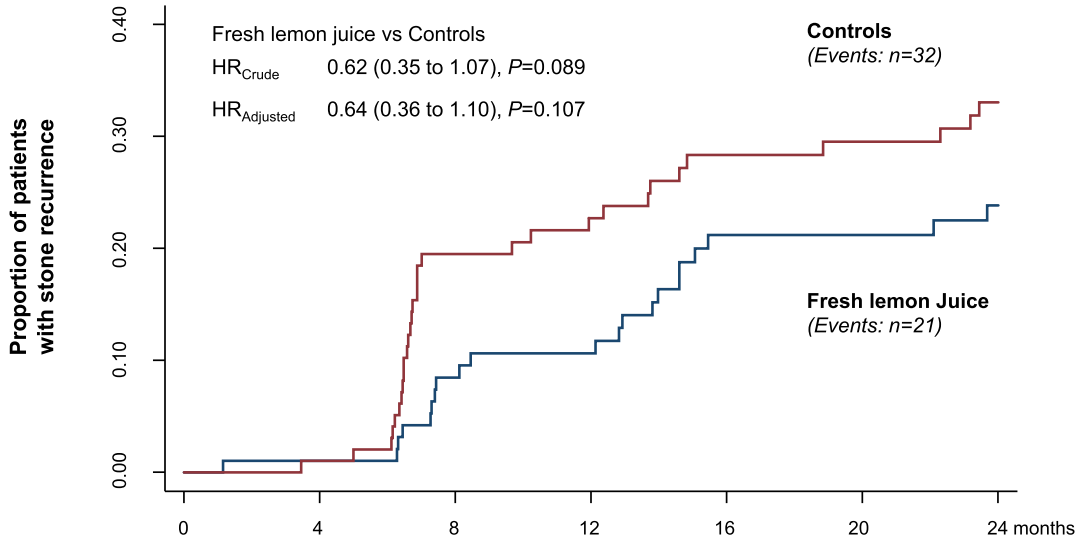
control group. Six patients in the standard diet with lemon juice supplementation vs. three patients in the control group had adverse events. Two additional patients withdrew from the control group because of unsatisfactory compliance or other reasons. Thus, a total of 26 patients withdrew from the standard diet with lemon juice supplementation group (26·0%) and 74 completed the 24-month follow-up period whereas a total of 19 patients withdrew from the control group (18·4%) and 84 completed the follow-up period (Fig. 1). All randomised participants in both study groups were included in intention-to-treat analyses. Included patients were relatively young and normo-weight, with a predominance of males. Slightly more than one third of the whole study population was diabetic at inclusion. Most patients never smoked. Blood pressure and kidney function were in normal range. Main demographic, clinical and laboratory parameters (measured in blood and 24 urine collections), the proportion of patients with normo or hypocitraturia as well as the distribution of pharmacological medications were similar between groups (Table 1).

Stone recurrence

Over two-year follow-up 21 participants in the standard diet with fresh lemon juice supplementation group (21·0%) and 32 in the standard diet group (31·1%) had recurrent stones. Overall, there were 13·6 events per 100 patient-years in the active treatment group and 21·4 events per 100 patient-years in the control group, respectively. After checking for proportionality assumptions (PH test: $p = 0{\cdot}566$) the hazard ratio (95 percent confidence interval) of a recurrence amongst the participants in the standard diet with lemon juice supplementation group as compared with the participants in the standard diet group (Fig. 2) was 0·62 (0·35 to 1·07). This ratio was non-significant ($p = 0{\cdot}089$). At multivariable analyses adjusted by age, sex and citraturia stratum, the hazard ratio of a recurrence was 0·64 (0·36 to 1·10). It was non-significant ($p = 0{\cdot}107$). However, whereas adherence to diet was remarkably good in both treatment groups during the whole study period, patient compliance to lemon juice supplementation progressively declined from 79% at six months, to 68% at one year and 48% at two years after randomisation (Figure

	Overall *(n = 203)*	Standard diet plus fresh lemon juice *(n = 100)*	Standard Diet Alone *(n = 103)*
Demographic/clinical characteristics			
Age, *years*	44·9 ± 12·9	43·0 ± 13·2	46·7 ± 12·4
Male sex, *n (%)*	128 (63·1)	67 (67·0)	61 (59·2)
Patients with diabetes mellitus, *n (%)*	74 (36·5)	36 (36·0)	38 (36·9)
Patients with hypocitraturia, *n (%)*	32 (15·8)	16 (16·0)	16 (15·5)
Patients with normocitraturia *n (%)*	171 (84·2)	84 (84·0)	87 (84·5)
Smoking status, *n (%)**			
Never smoked	114 (58·2)	59 (60·2)	55 (56·1)
Former smoker	38 (19·4)	16 (16·3)	22 (22·4)
Current smoker	44 (22·4)	23 (23·5)	21 (21·4)
Clinical features			
BMI, *Kg/m^2*	24·98 ± 4·36	24·45 ± 4·14	25·49 ± 4·53
Systolic blood pressure, *mmHg*	117·1 ± 13·1	116·6 ± 13·7	117·5 ± 12·6
Diastolic blood pressure, *mmHg*	72·4 ± 9·0	72·2 ± 9·0	72·6 ± 9·0
MAP, *mmHg*	87·3 ± 9·9	87·0 ± 10·0	87·6 ± 9·8
Pulse pressure, *mmHg*	73·0 ± 10·4	72·8 ± 10·1	73·2 ± 10·7
Blood parameters			
Creatinine, *mg/dL*	0·85 ± 0·15	0·87 ± 0·15	0·84 ± 0·15
Urea, *mg/dL*	32·72 ± 7·72	32·93 ± 8·31	32·53 ± 7·12
Sodium, *mEq/L*	141·04 ± 2·00	141·18 ± 1·92	140·91 ± 2·07
Potassium, *mEq/L*	4·20 ± 0·32	4·19 ± 0·32	4·20 ± 0·32
Phosphate, *mg/dL*	3·25 ± 0·53	3·23 ± 0·55	3·26 ± 0·52
Calcium, *mg/dL*	9·38 ± 0·37	9·41 ± 0·38	9·34 ± 0·36
pH	7·34 ± 0·04	7·34 ± 0·03	7·34 ± 0·04
PTH, *pg/mL*	53·84 ± 29·63	55·53 ± 36·53	52·24 ± 21·26
Uric acid, *mg/dL*	5·21 ± 1·10	5·14 ± 1·09	5·27 ± 1·11
Base excess	2·09 ± 1·59	2·31 ± 1·76	1·89 ± 1·40
Urinary parameters			
Volume, *mL/24h*	2299·0 ± 836·3	2275·0 ± 797·4	2322·8 ± 876·5
Citrate, *mg/24h*	623·09 ± 360·35	613·05 ± 402·75	632·94 ± 315·01
Calcium, *mg/24h*	228·64 ± 107·75	225·83 ± 104·98	231·35 ± 110·78
Oxalate, *mg/24h*	37·70 ± 21·67	37·40 ± 19·75	38·00 ± 23·48
Magnesium, *mg/24h*	86·61 ± 30·82	85·76 ± 32·49	87·45 ± 29·22
Phosphate, *mg/24h*	853·78 ± 294·25	817·88 ± 272·44	888·29 ± 311·21
Urea, *g/24h*	23·20 ± 7·58	22·55 ± 7·31	23·82 ± 7·81
Uric acid, *mg/24h*	529·52 ± 185·47	514·90 ± 184·66	543·57 ± 186·07
Sodium, *mEq/24h*	160·91 ± 64·94	156·33 ± 57·18	165·18 ± 71·44
Sulphate, *mM/24h*	19·22 ± 7·59	18·46 ± 7·06	19·96 ± 8·03
Creatinine, *g/24h*	1·59 ± 0·51	1·58 ± 0·52	1·61 ± 0·51
pH	6·09 ± 0·56	6·14 ± 0·51	6·04 ± 0·60
Calcium, *mg/kg/24h*	3·16 ± 1·34	3·20 ± 1·37	3·12 ± 1·32
Patients with medications, *n (%)*			
ACE inhibitors and/or ARB	27 (13·3)	12 (12·0)	15 (14·6)
Beta-blockers	15 (7·4)	7 (7·0)	8 (7·8)
Calcium-channel blockers	6 (3·0)	4 (4·0)	2 (1·9)
Diuretics	1 (0·5)	1 (1·0)	0 (0·0)
Potassium citrate supplementation	28 (13·8)	13 (13·0)	15 (14·6)
- Hypocitraturic patients	27 (84·4)	12 (75·0)	15 (93·8)
- Normocitraturic patients	1 (0·6)	1 (1·2)	0 (0·0)

***Table 1*: Patient characteristics at baseline in the study group as a whole (Overall) and according to treatment group.**
Data are mean ± SD or numbers (percentages). * Information not available for 2 patients on standard diet plus fresh lemon juice and for 5 patients on standard diet alone. Abbreviations: ACE, Angiotensin converting enzyme; ARB, angiotensin receptor blocker; BMI, Body-mass index; MAP, mean arterial pressure; PTH, parathyroid hormone.



Patients at risk (censored)

Fresh lemon Juice	100 (0)	96 (3)	85 (4)	80 (3)	65 (6)	61 (4)	56 (3)
Controls	103 (0)	97 (5)	77 (2)	71 (3)	61 (4)	60 (0)	57 (0)

Fig. 2. Kaplan-Meier curves for the primary endpoint of stone recurrence over two-year follow-up.

Kaplan-Meier curves show the proportion of patients in the fresh lemon juice supplementation group and in the control group who reached the primary endpoint of stone recurrence over two-year follow-up. Hazard ratios (HRs) and 95% confidence intervals are crude and adjusted for age, sex and citraturia stratum. The number of patients at risk is shown in the bottom table. Blue line, fresh lemon juice supplementation group; red line, control group.

S1). When the follow-up was closed at one year after randomisation, when adherence to lemon juice supplementation was still at 68%, we found that ten participants in the standard diet with lemon juice supplementation group (10%) and 22 in the standard diet group (21·4%) had recurrent stones. After checking for proportionality assumptions (PH test: $p = 0{\cdot}616$) the hazard ratio of a recurrence amongst the participants in the standard diet with lemon juice supplementation group as compared with the participants in the standard diet group was 0·43 (0·20 to 0·89) (Fig. 3). The ratio reached the statistical significance ($p = 0{\cdot}028$). Similar findings were obtained when analyses were adjusted by age, sex and citraturia stratum [hazard ratio 0·45 (0·20 to 0·93), $p = 0{\cdot}036$)].

Other outcomes

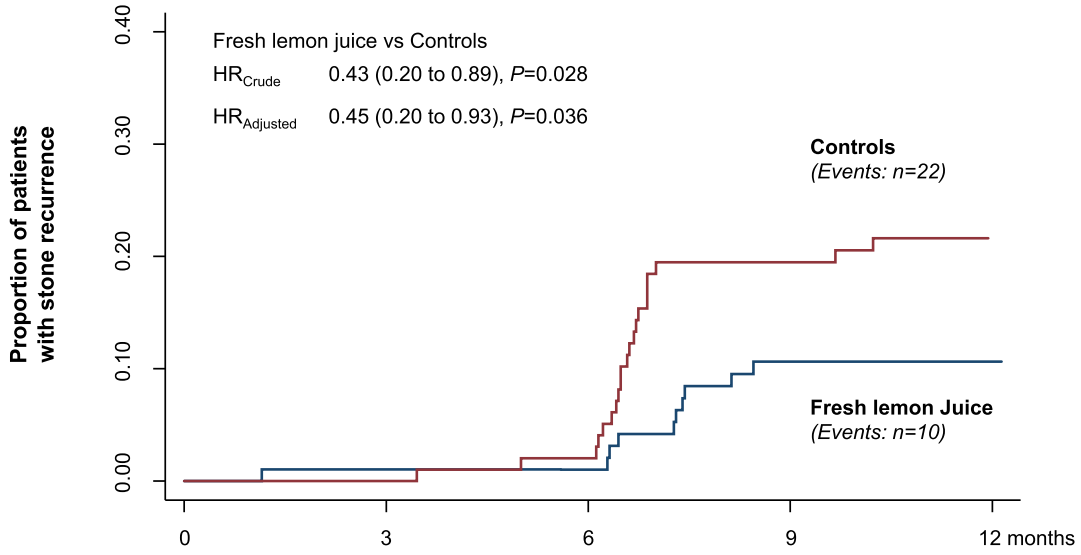
At six months after randomisation 24-hour urinary sodium excretion decreased by 8·60±65·68 mEq/24-h in the standard diet with lemon juice supplementation group and increased by 3·88±64·78 mEq/24-h in the control group (Table S2). Thus, changes in 24-hour urinary sodium excretion significantly differed ($p = 0{\cdot}031$) between treatment groups at six months after randomisation (Table 2). This difference was lost at subsequent visits. After randomisation, changes in 24-hour urine volume, pH or 24-hour urinary excretion in any other considered solute, including sulphate and calcium, never differed between groups at any considered visit (Table 2, Table S3).

Safety

Throughout the whole study there were only two patients (1·0%) who had serious adverse events, both were in the control group: one with a melanoma of the skin and one with a cerebral aneurysm rupture (Table 3). Patients with non serious adverse events were similarly distributed between treatment groups with the exception of patients with gastrointestinal disorders who were more frequent in the standard diet with lemon juice supplementation group and patients with kidney and urinary tract disorders who tended to be more frequent in the control group. Overall, 45 patients (22·2%) had gastrointestinal disorders: 37 (37·0%) in the lemon juice supplementation group and eight (7·8%) in the control group ($p<0{\cdot}001$). In particular, there were 21 patients with gastric pyrosis/heartburn and eight with epigastralgia in the lemon juice supplementation group as compared to one with gastric pyrosis/heartburn ($p<0{\cdot}001$) and three with epigastralgia ($p = 0{\cdot}130$) in the control group. Two of the 37 patients with lemon juice supplementation who had gastrointestinal disorders

Urinary variable	Baseline		6 months		1 year		2 years	
	Standard diet plus fresh lemon juice	Standard diet alone	Standard diet plus fresh lemon juice	Standard diet alone	Standard diet plus fresh lemon juice	Standard diet alone	Standard diet plus fresh lemon juice	Standard diet alone
Volume, *mL/24h*	2275 ± 797	2323 ± 877	2352 ± 813	2457 ± 930	2292 ± 741	2475 ± 800	2211 ± 719	2290 ± 840
Citrate, *mg/24h*								
- overall	613·05 ± 402·75	632·94 ± 315·01	646·89 ± 558·68	634·75 ± 323·37	590·06 ± 269·65	642·02 ± 294·44	590·86 ± 264·22	621·24 ± 282·36
- with normocitraturia	687·86 ± 395·52	709·15 ± 279·30	707·92 ± 590·54	696·25 ± 314·68	629·01 ± 254·17	688·20 ± 291·21	617·47 ± 257·03	660·30 ± 277·23
- with hypocitraturia	220·31 ± 95·23	223·31 ± 117·58	333·60 ± 100·18**	331·06 ± 148·57*	380·31 ± 262·45	428·44 ± 206·15**	457·83 ± 270·37*	428·71 ± 229·91*
Calcium, *mg/24h*	225·83 ± 104·98	231·35 ± 110·78	233·23 ± 112·25	251·03 ± 114·88*	236·30 ± 104·71	235·43 ± 118·51	228·76 ± 118·48	222·10 ± 117·59
Oxalate, *mg/24h*	37·40 ± 19·75	38·00 ± 23·48	41·53 ± 17·80	37·95 ± 17·92	42·58 ± 14·25*	44·54 ± 26·47	37·75 ± 17·10	39·70 ± 17·45
Magnesium, *mg/24h*	85·76 ± 32·49	87·45 ± 29·22	88·55 ± 33·29	93·56 ± 31·80	86·53 ± 28·23	90·37 ± 33·07	82·84 ± 32·75	81·73 ± 34·20
Phosphate, *mg/24h*	817·88 ± 272·44	888·29 ± 311·21	851·77 ± 295·79	906·24 ± 310·82	861·11 ± 290·15	864·57 ± 278·89	838·17 ± 282·93	840·02 ± 297·93
Urea, *g/24h*	22·55 ± 7·31	23·82 ± 7·81	24·23 ± 8·74	25·32 ± 8·03*	23·73 ± 7·51	24·62 ± 7·90	22·91 ± 7·38	24·75 ± 11·49
Uric acid, *mg/24h*	514·90 ± 184·66	543·57 ± 186·07	536·04 ± 166·61	569·57 ± 182·18	562·26 ± 211·99	594·08 ± 206·72*	553·72 ± 166·99	566·77 ± 189·56
Sodium, *mEq/24h*	156·33 ± 57·18	165·18 ± 71·44	149·24 ± 54·92#	168·74 ± 61·05	159·49 ± 56·77	170·22 ± 68·24	158·94 ± 61·81	160·51 ± 51·75
Sulphate, *mM/24h*	18·46 ± 7·06	19·96 ± 8·03	19·30 ± 8·05	19·87 ± 7·55	19·49 ± 8·62	19·87 ± 8·82	18·49 ± 8·31	18·19 ± 7·37
Creatinine, *g/24h*	1·58 ± 0·52	1·61 ± 0·51	1·68 ± 0·57	1·71 ± 0·54*	1·67 ± 0·53	1·68 ± 0·56	1·59 ± 0·46	1·66 ± 0·53
pH	6·14 ± 0·51	6·04 ± 0·60	6·13 ± 0·56	6·00 ± 0·57	6·07 ± 0·48	6·06 ± 0·53	6·04 ± 0·55	5·96 ± 0·51
Calcium/body weight, *mg/kg/24h*	3·20 ± 1·37	3·12 ± 1·32	3·31 ± 1·37	3·42 ± 1·57**	3·25 ± 1·36	3·08 ± 1·43	3·04 ± 1·45	3·08 ± 1·63

***Table 2*: Urinary variables at baseline and during the study period in the two treatment groups.**
Data are mean ± SD. *p<0·05 **p<0·01 vs baseline. #p<0·05 ##p<0·01 vs standard diet alone by ANCOVA.



Patients at risk (censored)

Fresh lemon Juice	100 (0)	96 (3)	94 (2)	82 (3)	80 (2)
Controls	103 (0)	98 (5)	96 (0)	76 (3)	71 (2)

Fig. 3. Kaplan-Meier curves for stone recurrence over one-year follow-up.

Kaplan-Meier curves show the proportion of patients in the fresh lemon juice supplementation group and in the control group who had recurrent stones over one-year follow-up. Hazard ratios (HRs) and 95% confidence intervals are crude and adjusted for age, sex and citraturia stratum. The number of patients at risk is shown in the bottom table. Blue line, fresh lemon juice supplementation group; red line, control group.

permanently withdrew the study treatment. Overall, 69 patients had renal and urinary tract disorders: 28 (28·0%) in the lemon supplementation group and 41 (39·8%) in the control group ($p = 0·103$). There were 24 patients with renal colic with or without stone expulsion and three patients with macrohematuria in the lemon supplementation group as compared to 26 patients with renal colic and six with macrohematuria in the control group. Moreover, seven patients in the lemon supplementation group had pyelic stones as compared to ten in the control group. Five patients on lemon juice supplementation as compared to four controls required lithotripsy (Table 3).

Discussion

Results of the present study show that a standard recommended diet with normal calcium content but reduced amounts of salt and animal proteins [5] integrated with 60 mL twice daily of fresh lemon juice and a standard diet without lemon juice supplementation are similarly effective in reducing the risk of recurrent stones in patients with calcium oxalate nephrolithiasis. Results were most probably biased by the progressively declining patient adherence to recommended lemon juice supplementation during the two-year follow-up period. Indeed, when the follow-up was closed at one year after randomisation - when patient adherence was still at 68 percent - standard diet with lemon juice supplementation was found to be more protective against stone recurrence than standard diet without supplementation. Treatment effect was significant even after statistical adjustments for three predefined potential risk factors such as age, sex and citraturia stratum. Baseline characteristics of study patients, including urine volume, urine pH and all considered urinary solutes concentration at baseline, were similar between treatment groups and all patients were recommended the same standard diet.[5] Thus, study findings were unlikely confounded by an unbalanced distribution of risk factors for stone recurrence in the two study groups.

Twenty-four hour urinary sodium excretion tended to decrease in treated patients and to increase in controls. These trends significantly differed between treatment groups at six month after randomisation. However, as for the primary outcome, between-group difference was lost on longer follow-up, in parallel with decreasing patient adherence to lemon juice supplementation. Finding that between-group differences in sodium excretion were consistent with differences in event rate, was consistent with evidence that changes in urinary sodium excretion may have a role in the risk of stone recurrence in patients with calcium oxalate nephrolithiasis.[15,16] These data could be taken to suggest that

	Standard diet plus Lemon Juice *(n = 100)*	Standard Diet alone *(n = 103)*	*P value*
Any adverse event	64 (64·0%)	54 (52·4%)	0·118
Cardiac disorders	0 (0·0%)	2 (1·9%)	0·498
Congenital, familial and genetic disorders	1 (1·0%)	0 (0·0%)	0·493
Endocrine disorders	2 (2·0%)	1 (1·0%)	0·618
Gastrointestinal disorders	37 (37·0%)	8 (7·8%)	<0·001
- Nausea	2 (2·0%)	1 (1·0%)	0·618
- Epigastralgia	8 (8·0%)	3 (2·9%)	0·130
- Gastric pyrosis/heartburn	21 (21·0%)	1 (1·0%)	<0·001
- Constipation/diarrhoea	4 (4·0%)	0 (0·0%)	0·058
- Dental erosion, hypersensitivity	2 (2·0%)	0 (0·0%)	0·241
- Other	8 (8·0%)	3 (2·9%)	0·130
Infections and infestations	3 (3·0%)	6 (5·8%)	0·498
Injury, poisoning and procedural complications	2 (2·0%)	1 (1·0%)	0·618
Metabolism and nutrition disorders	1 (1·0%)	2 (1·9%)	1·000
Musculoskeletal and connective tissue disorders	2 (2·0%)	4 (3·9%)	0·683
Neoplasms benign, malignant and unspecified[#]	1 (1·0%)	3 (2·9%)	0·621
Nervous system disorders°	1 (1·0%)	2 (1·9%)	1·000
Psychiatric disorders	1 (1·0%)	0 (0·0%)	0·493
Renal and urinary disorders	28 (28·0%)	41 (39·8%)	0·103
- Renal colic*	24 (24·0%)	26 (25·2%)	0·872
- Pyelic stones	7 (7·0%)	10 (9·7%)	0·614
- Macrohematuria	3 (3·0%)	6 (5·8%)	0·498
- Dysuria	1 (1·0%)	4 (3·9%)	0·369
- Renal/parapyelic cyst	1 (1·0%)	5 (4·9%)	0·212
- Endoscopic ureterolithotripsy	3 (3·0%)	2 (1·9%)	0·680
- Shock wave litotripsy	2 (2·0%)	2 (1·9%)	1·000
- Other	4 (4·0%)	2 (1·9%)	0·440
Reproductive system and breast disorders	1 (1·0%)	2 (1·9%)	1·000
Respiratory, thoracic and mediastinal disorders	1 (1·0%)	0 (0·0%)	0·493

***Table 3*: Number (%) of patients with at least one adverse event (AE) according to treatment arm by MedDRA System Organ Classification (SOC).**
Chi-square test. Including two serious adverse events: Dorsal Melanoma[#] and Cerebral Aneurysm Rupture°. *with or without stone expulsion.

the protective effect (if any) of lemon juice supplementation against stone recurrence could be explained by citrate-induced reduction in urinary sodium excretion. Consistently with previous reports[11,17] lemon juice supplementation did not translate into increased 24-hour urinary citrate excretion. This finding could be explained by evidence that up to 90 per cent of citrate filtered by the renal glomeruli is reabsorbed by the renal tubuli.[18] Citrate is mainly reabsorbed as the divalent anion in the proximal tubule by a sodium-coupled mechanism. However, as the luminal pH increases citrate can be predominantly reabsorbed as trivalent anion.[19] Thus, enhanced delivery of citrate to renal tubuli could translate into enhanced tubular reabsorption of citrated-coupled sodium. We speculate that this could explain why in our study lemon juice supplementation was not associated with enhanced citrate excretion, but rather with decreased sodium excretion in treated patients as compared to controls. We hypothesise that reduced urinary sodium concentration should result in decreased urine saturation which in turn should exert a protective effect against stone recurrence.

Lemon juice supplementation was safe, but was not well tolerated. Indeed, it was associated with an excess incidence of gastrointestinal disturbances, in particular gastric pyrosis. Conceivably, in addition to treatment costs, also gastrointestinal disturbances contributed to the progressively decreasing patient adherence to lemon juice supplementation observed during the study period. Notably, poor adherence could be a major limitation to the use of lemon juice as a protective intervention against stone recurrence also in everyday clinical practice. Conversely, good compliance to reduced animal protein intake was confirmed by low urinary sulphate excretion in both study groups. According to urinary excretion, also dietary calcium intake was relatively low whereas adherence to salt restriction appeared

to be less satisfactory. Finding that urinary oxalate excretion never differed between groups throughout the whole study period was consistent with similar fruit intake in both treatment arms.

The major study limitation was poor patient adherence to lemon juice supplementation. In most cases this limitation was explained by investigator's inability to freely supply lemon juice to study patients. In a substantial proportion of cases, however, poor adherence was also explained by gastrointestinal symptoms associated with lemon juice intake. Independent of the reason, poor adherence, however, faithfully reflects what might happen in everyday clinical practice, which enhances the generalizability of the findings of this pragmatic trial to real world. Notably, all study patients were available for intention-to-treat analyses and 158 of the 203 randomised patients (77·8%) completed the planned two years of follow-up. This high retention rate was a major strength of the study along with close patient monitoring and careful end point evaluation.

In conclusion, in patients with calcium oxalate nephrolithiasis, fresh lemon juice supplemented to a standard diet with normal calcium content but reduced amounts of sodium and animal proteins appeared to exert some protective effect against stone recurrence as compared to the same standard diet but without lemon juice supplementation. Treatment effect, however, was largely mitigated by progressively decline patient adherence during the study period and emerged only at explorative, post-hoc analyses restricted to the first year of follow-up. Thus, our findings are not robust enough to recommend oral juice supplementation in everyday clinical practice, but provide novel useful information to inspire more effective strategies to improve patient adherence in future trials aimed to further test the hypothesis that oral juice supplementation might decrease the risk of stone recurrence in patients with calcium oxalate nephrolithiasis.

Declaration of interests

G.R. reported personal fees from Akebia Pharmaceuticals Inc, Alexion Pharmaceuticals, BioCryst Pharmaceuticals Ins, AstraZeneca and Janssen Research & Development LLC, as well as speaker honorarium/travel reimbursements from Boehringer Ingelheim, Menarini Ricerche Spa and Silence Therapeutics. All the other authors declare that they have nothing to disclose.

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Contributors

PR, MRC and GR had the original idea; PR wrote the original version of the manuscript; MC elaborated the iconography and contributed to manuscript finalisation; AP, TP and GAG performed the statistical analyses; SR performed all echography evaluations; PB contributed to imaging recording and interpretation; GI prescribed the diet based on normal calcium content, but with reduced intake of salt and animal proteins; DV and OD monitored the study; MT contributed to data analysis; MRC and GN identified, treated and monitored study participants and contributed to data recording; AP handled the raw data and takes responsibility for their fidelity; PR and GR elaborated the final version of the manuscript, all authors critically revised the final version, GR took the responsibility for the submission for publication. No medical writer was involved.

Data sharing statement

Sharing of individual participant data with third parties was not specifically included in the informed consent of the study, and unrestricted diffusion of such data may pose a potential threat of revealing participants' identities, as permanent data anonymisation was not carried out (patient records were instead de-identified per protocol during the data retention process). To minimize this risk, individual participant data that underlie the results reported in this article will be available after three months and up to five years from article publication. Researchers shall submit a methodologically sound proposal to Dr. Annalisa Perna (annalisa.perna@marionegri.it), head of the Laboratory of Biostatistics of the Department of Renal Medicine of the Istituto di Ricerche Farmacologiche Mario Negri IRCCS. To gain access, data requestors will need to sign a data access agreement and obtain the approval of the local ethics committee.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2021.101227.

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