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Effect of vitamin C supplements on urinary oxalate and pH in calcium stone-forming patients

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Background. The contribution of ascorbate to urinary oxalate is controversial. The present study aimed to determine whether urinary oxalate and pH may be affected by vitamin C supplementation in calcium stone-forming patients.

Methods. Forty-seven adult calcium stone-forming patients received either 1 g ($N = 23$) or 2 g ($N = 24$) of vitamin C supplement for 3 days and 20 healthy subjects received 1 g. A 24-hour urine sample was obtained both before and after vitamin C for calcium, oxalate, magnesium, citrate, sodium, potassium, and creatinine determination. The Tiselius index was used as a calcium oxalate crystallization index. A spot fasting morning urine sample was also obtained to determine the urinary pH before and after vitamin C.

Results. Fasting urinary pH did not change after 1 g (5.8 ± 0.6 vs. 5.8 ± 0.7) or 2 g vitamin C (5.8 ± 0.8 vs. 5.8 ± 0.7). A significant increase in mean urinary oxalate was observed in calcium stone-forming patients receiving either 1 g (50 ± 16 vs. 31 ± 12 mg/24 hours) or 2 g (48 ± 21 vs. 34 ± 12 mg/24 hours) of vitamin C and in healthy subjects (25 ± 12 vs. 39 ± 13 mg/24 hours). A significant increase in mean Tiselius index was observed in calcium stone-forming patients after 1 g (1.43 ± 0.70 vs. 0.92 ± 0.65) or 2 g vitamin C (1.61 ± 1.05 vs. 0.99 ± 0.55) and in healthy subjects (1.50 ± 0.69 vs. 0.91 ± 0.46). Ancillary analyses of spot urine obtained after vitamin C were performed in 15 control subjects in vessels with or without ethylenediaminetetraacetic acid (EDTA) with no difference in urinary oxalate between them (28 ± 23 vs. 26 ± 21 mg/L), suggesting that the in vitro conversion of ascorbate to oxalate did not occur.

Conclusion. These data suggest that vitamin C supplementation may increase urinary oxalate excretion and the risk of calcium oxalate crystallization in calcium stone-forming patients.

Vitamin C (ascorbic acid, ascorbate) is an essential micronutrient involved in many biological and biochemical functions, acting as an electron donor or reducing agent in chemical reactions [1].

Key words: vitamin C, ascorbate, oxalate, urinary pH, kidney stone, urinary calculi.

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Humans cannot synthesize vitamin C because they do not have the last enzyme in the biosynthetic pathway [1]. The current recommended dietary allowance (RDA) for vitamin C is 60 mg/day, but it is fairly common to find subjects who because of health fads or the advice of others, take megadoses of vitamin C on a daily basis in addition to the amount provided by their usual diet [2].

Vitamin C can be metabolized to oxalate, which could increase oxalate excretion and hence the risk of calcium oxalate stone formation [3, 4]. Therefore, stone formers are frequently advised to avoid vitamin C supplements.

Most of the oxalate found in the urine is usually formed endogenously as a metabolic end product of glycoxylic acid (50% to 70%) and ascorbic acid (30% to 50%), with minor contributions by gelatin, tryptophan, phenylalanine, tyrosine, aspartic acid, creatinine, and purines [5].

Several studies have been performed in the last 40 years in an attempt to determine the contribution of high-dose vitamin C intakes to urinary oxalate excretion [6–17]. However, the data from various investigations are contradictory, in part because of the difficulties regarding oxalate assay techniques [13–17]. In assays used before 1987, artificial oxalate elevation occurred due to inadvertent ascorbate conversion to oxalate in stored samples, either because of the requirement of heating the urine or because of the use of alkaline eluents [18, 19].

In healthy subjects, three short-term prospective studies conducted in the last 6 years, with appropriate precautions to prevent nonenzymatic conversion of ascorbate to oxalate, have determined the effects of ascorbate intake on oxaluria using doses ranging from 400 mg/day to 4 g/day [14–16]. Levine et al [14] reported a significant increase of 33% in urinary oxalate after 1 g vitamin C supplement, whereas Liebman et al [15] and Auer, Auer, and Rodgers [16] found that ascorbate doses from 2 to 4 g per day did not increase urinary oxalate. In a large epidemiologic study based on completion of dietary questionnaires, Curhan et al [4] did not observe a positive association between consumption of vitamin C supplements and the risk of kidney stones in women.

There are few studies focusing on the effect of vitamin

C supplementation on oxalate excretion in calcium-stone forming patients [2, 3, 20, 21]. Three studies reported a significant increase from 31% to 100% in urinary oxalate after vitamin C supplement at doses from 0.5 to 2 g per day [2, 3, 20]. On the other hand, Heckers et al [21] found no increase in urinary oxalate in seven calcium stone-forming patients taking 1 g/day vitamin C.

Although it has been suggested that vitamin C has some effect on urinary acidification, reducing the urinary pH, the role of vitamin C as a urinary acidifier is still controversial [22–30].

Since few studies focusing on the effect of vitamin C on urinary oxalate excretion in a population of calcium stone formers have been performed, the objective of the present study was to determine whether urinary oxalate excretion and pH could be affected by vitamin C supplementation in calcium stone-forming patients.

METHODS

Protocol

Forty-seven adult calcium stone-forming patients (24 men and 23 women) participated in the study. Patients with diabetes, hyperparathyroidism, abnormal renal function, or those taking drugs that could affect calcium metabolism were excluded. All patients were referred to the Renal Lithiasis Unit of the Nephrology Division, Universidade Federal de São Paulo, Brazil, and were sequentially enrolled in the study after a diagnosis of renal stone has been established. The diagnosis of stone disease was based on at least one of the following criteria: (*1*) renal colic with confirmed hematuria, (*2*) voiding of a calculus, (*3*) previous surgical or endoscopic removal of stone(s), and/ (*4*) or radiographic (intravenous urography or ultrasonography) evidence of stone(s). A written consent was obtained from all patients and the local Ethics Committee approved the study. A 24-hour urine sample was obtained from the 47 calcium stone-forming patients both before (pre) and after (post) vitamin C supplementation for determination of calcium, oxalate, sodium, potassium, urea, creatinine, magnesium, uric acid, and citrate. Calcium, oxalate, magnesium, and citrate data were used to calculate the risk of calcium oxylate crystallization by the Tiselius index, calculated according to the formula that follows: $1.9 \times \text{calcium}^{0.84} \times \text{oxalate} \times \text{magnesium}^{-0.12} \times \text{citrate}^{-0.22} \times \text{volume}^{-1.03}$ [31]. Patients were instructed to abstain from consuming oxalate-rich and vitamin C–rich foods (a listing of these foods was provided), as well as dairy products for the two 24-hour periods of urine collection (baseline and after vitamin C). The purpose of these measures was to avoid any influence of diet (with respect to oxalate, ascorbate, or calcium intake) on oxalate excretion other than vitamin C supplementation. Patients were then randomly selected to receive either 1 g (500 mg twice a day) or 2 g (1000 mg twice a day) of vitamin C supplement for 3 days. The 24-hour urine sample (post) was collected during day 3 of vitamin C supplementation. A morning spot urine sample was also obtained after a 12-hour fast to determine the urinary pH both at baseline and in the morning following day 3 of vitamin C supplementation in the calcium stone-forming group. Twenty healthy subjects (8 men and 12 women) received 1 g (500 mg twice a day) vitamin C supplement for 3 days and a 24-hour urine sample was obtained from them before and after the supplementation.

Effect of acid preservation on urinary oxalate determination

It has been suggested that, for a more reliable urinary oxalate measurement, urine must be acidified in order to ensure the complete dissolution of calcium oxalate crystals, with hydrochloric acid (HCl) being used as a preservative for urine collection. However, when acid is added to the plastic container before urine collection, other parameters such as uric acid, sodium, and potassium cannot be determined in the same urine sample [32]. To test whether the addition of acid after the urine specimen was delivered to the laboratory would interfere with the urinary oxalate results, an additional group consisting of 40 healthy subjects was submitted to two collections of 24-hour urine on different occasions. The first sample was obtained with acid preservation (HCl 6N, 20 mL/L) and the second in a dry plastic container, with HCl added as soon as the urine sample was received at the laboratory. The mean oxalate excretion in these 40 control samples was similar for specimens previously acidified or not (27 ± 14 vs. 29 ± 12 mg/24 hours, $P < 0.05$, Wilcoxon test). Based on these results for the control samples, 24-hour urine samples obtained from the 47 calcium stone-forming patients pre- and post-vitamin C supplement intake were then collected into a dry plastic container, with HCl added as soon as the urine sample was delivered to the laboratory. However, since in vitro conversion of ascorbate to oxalate could be further induced by a nonacid environment, the 47 calcium stone-forming patients and the 20 controls were also asked to repeat the entire protocol, taking vitamin C supplements for 3 additional days, and to collect another 24-hour urine sample on day 3 in a vessel containing HCl.

Effect of EDTA preservation on urinary oxalate determination

It has been suggested that urine should be collected with EDTA for ascorbate stabilization and to inhibit its conversion to oxalate [16, 17, 33, 34]. To assess whether our results could have been biased by an in vitro conversion of ascorbate to oxalate, we performed an additional experiment by collecting a spot urine from 15 control subjects 6 hours after intake of the vitamin C supplement

Table 1. Mean urinary parameters before and after vitamin C supplements in calcium stone-forming patients and healthy subjects

	Calcium stone forming Vitamin C, 1 g ($N = 23$)			Healthy subjects Vitamin C, 1 g ($N = 20$)		Calcium stone forming Vitamin C, 2 g ($N = 24$)		
	Pre	Post	Post[a]	Pre	Post[a]	Pre	Post	Post[a]
Calcium *mg/24 hours*	153 ± 58	169 ± 80	188 ± 102	155 ± 99	159 ± 69	129 ± 44	155 ± 98	157 ± 81
Oxalate *mg/24 hours*	31 ± 12	50 ± 16[b]	49 ± 19[b]	25 ± 13[c]	39 ± 13[b,c]	34 ± 12	48 ± 21[b]	59 ± 19[b]
Sodium *mEq/24 hours*	209 ± 95	203 ± 83	ND	172 ± 57	ND	194 ± 83	197 ± 98	ND
Potassium *mEq/24 hours*	59 ± 25	61 ± 25	ND	56 ± 18	ND	58 ± 23	54 ± 27	ND
Urea *g/24 hours*	20 ± 6	19 ± 9	20 ± 8	20 ± 8	21 ± 9	16 ± 8	22 ± 4	18 ± 9
Creatinine *mg/24 hours*	1413 ± 346	1335 ± 423	1342 ± 481	1262 ± 536	1257 ± 441	1404 ± 404	1354 ± 424	1403 ± 512
Uric acid *mg/24 hours*	608 ± 219	415 ± 168[b]	ND	465 ± 139[c]	ND	484 ± 187	305 ± 109	ND
Magnesium *mg/24 hours*	81 ± 34	81 ± 32	87 ± 37	70 ± 31	73 ± 21	69 ± 28	70 ± 23	91 ± 42[b]
Citrate *mg/24 hours*	347 ± 208	380 ± 246	502 ± 287	432 ± 227	423 ± 225	376 ± 249	331 ± 197	392 ± 219
Tiselius index	0.91 ± 0.65	1.43 ± 0.7[b]	1.41 ± 0.8[b]	0.91 ± 0.46	1.5 ± 0.69[b]	0.98 ± 0.55	1.61 ± 1.05[b]	1.62 ± 1.0[b]
Volume	1997 ± 817	1924 ± 618	1899 ± 578	1250 ± 669	1308 ± 528	1634 ± 565	1649 ± 674	2104 ± 772[b]

X ± SD, [a] Collected under acid preservation. [b] vs. pre; [c] vs. calcium stone-forming $P < 0.05$

(1 g) and dividing the samples into two aliquots, with or without EDTA (final concentration, 0.01 mol/L). The results showed no difference in oxalate concentration between the samples with and without EDTA (28 ± 23 vs. 26 ± 21 mg/L, respectively, $P < 0.05$, Wilcoxon test)

METHODS

Urinary parameters

Urinary calcium was determined by atomic absorption spectrophotometry (Perkin-Elmer Atomic Spectrophotometer 290-B, Norwalk, CT, USA), oxalate by an enzymatic reaction using the Sigma Oxalate Diagnostic Kit (Sigma Chemical Co., St. Louis, MO, USA), sodium, and potassium by flame photometry (Celm Fc-130), urea by an enzymatic ultraviolet test, creatinine by Jaffe's method [35], uric acid by an automated colorimetric enzymatic method (ABA VP), and citrate by an enzymatic assay using citrate lyase [36]. The urinary pH was obtained in the second micturition after a 12-hour fast, and determined with a pH meter.

Statistical analysis

Results are reported as mean ± standard deviation (SD). The Wilcoxon test was used to compare the results obtained after the vitamin C supplement to those obtained before the supplement in the same group. The Mann-Whitney test was used to compare the differences between the calcium stone-forming and healthy subject groups. The level of significance was defined as $P < 0.05$.

RESULTS

Mean age (37 ± 14 vs. 37 ± 16 years) and body mass index (27 ± 5 vs. 24 ± 4 kg/m^2) did not differ between calcium stone-forming patients and healthy subjects (data not shown in tables).

Table 1 shows the mean values of urinary calcium, oxalate, sodium, potassium, urea, creatinine, uric acid, magnesium, and citrate and the calcium oxalate crystallization Tiselius index before and after the vitamin C supplements in calcium stone-forming patients and healthy subjects. Calcium stone-forming patients presented significantly higher mean values of urinary oxalate than healthy subjects, both before (31 ± 12 vs. 25 ± 13 mg/24 hours, $P < 0.05$) and after the vitamin C supplements (50 ± 16 vs. 39 ± 13 mg/24 hours, $P < 0.05$). Calcium stone-forming patients also presented higher urinary uric acid than healthy subjects (608 ± 219 vs. 465 ± 139 mg/24 hours, $P < 0.05$). Among healthy subjects, a significant increase in mean urinary oxalate level (39 ± 13 vs. 25 ± 13 mg/24 hours, $P < 0.05$) and in mean Tiselius index (1.5 ± 0.69 vs. 0.91 ± 0.46, $P < 0.05$) was observed after the vitamin C supplement in comparison to baseline, with no changes in the remaining urinary parameters. In calcium stone-forming patients, a significant increase in mean urinary oxalate was observed after supplementation with either 1 g (50 ± 16 vs. 31 ± 12 mg/24 hours) or 2 g (48 ± 21 vs. 34 ± 12 mg/24 hours) vitamin C compared to baseline, representing a 61% and 41% increment in mean oxaluria, respectively. This increase was also observed in urine samples further collected under acid preservation for calcium stone-forming patients taking 1 g (49 ± 19 vs. 31 ± 12 mg/24 hours) or 2 g vitamin C (59 ± 19 vs. 34 ± 12 mg/24 hours). There was a significant reduction of uric acid (415 ± 168 vs. 608 ± 219 mg/24 hours) in calcium stone-forming patients taking 1 g, but this reduction has occurred within normal limits, and was not observed among the patients taking 2 g. Similarly, a significant increase in urinary magnesium (91 ± 42 vs. 69 ± 28 mg/24 hours) in calcium stone-forming patients taking 2 g, but not in these taking 1 g was observed but again, such increase occurred within the range of normal limits. A significant increase in mean Tiselius index was observed after supplementation with either 1 g (0.92 ± 0.65 vs. 1.43 ± 0.70) or 2 g vitamin C (0.99 ± 0.55 vs. 1.61 ± 1.05). The remaining parameters remained unchanged after vitamin C.

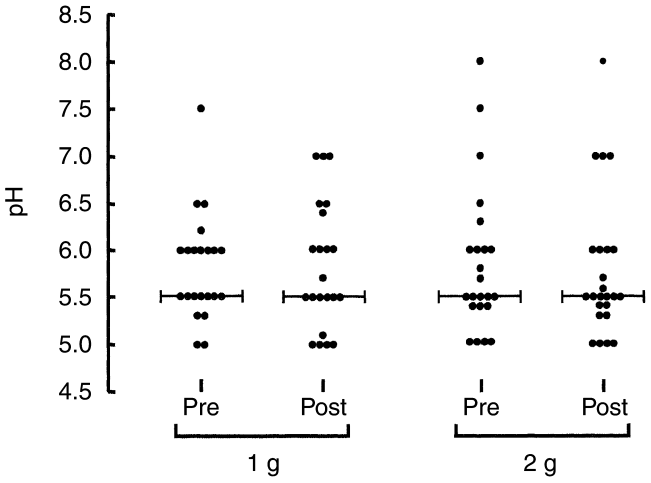

Fig. 1. Urinary pH pre- and post-vitamin C supplements. Mean values are indicated by horizontal bars.

Figure 1 shows that the mean urinary pH values before vs. after1 or 2 g vitamin C supplementation in calcium stone-forming patients were not significantly different.

The distribution of the urinary oxalate values in percentiles is shown in Figure 2A and B.

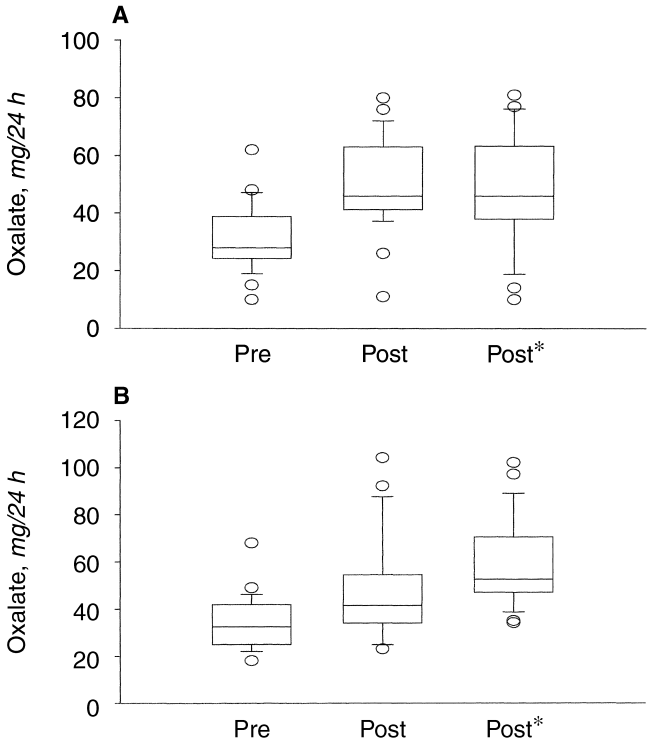

Fig. 2. Box plots of urinary oxalate pre- and post-vitamin C with or without acid preservation in calcium stone-forming patients taking supplements of either 1 gram (*A*) or 2 grams (*B*). The horizontal lines in the box denote the 25th, 50th, and 75th percentile values. The error bars denote the 5th and 95th percentile values. The symbols below the 5th percentile error bar denote the zero and 1st percentile values and the symbols above the 95th percentile error bar denote the 99th and 100th percentile.

DISCUSSION

The assumption that the intake of high doses of vitamin C may be a major causative factor in the formation of renal calcium oxalate stones is old, dating back to the finding that oxalate is one of the metabolic end products of ascorbic acid excreted in the urine [37]. Several studies have been conducted on healthy subjects to examine the effect of vitamin C on urinary oxalate excretion using different doses and periods of supplementation [6–17]. On the other hand, a reduced number of studies has been performed to examine the effect of vitamin C on urinary oxalate in calcium stone-forming patients [2, 3, 20, 21]. However, the data from various investigations are contradictory, in part because of difficulties regarding oxalate assay techniques. Despite newer assays that mitigate the in vitro conversion of ascorbic acid to oxalate, controversy still remains, with some studies suggesting that vitamin C leads to an increase in oxaluria [14], whereas others do not support this observation [13, 15, 16].

In the present study, a significant increase of 61% and 41% was observed in mean urinary oxalate after supplementation with 1 or 2 g vitamin C, respectively, in calcium stone-forming patients. The lack of a further increase on urinary oxalate after 2 g vitamin C may be ascribed to a saturable transport mechanism leading to a reduced relative absorption capacity with increasing intakes of the compound [5, 38]. Our findings are in accordance with Tiselius et al [20] who observed a 48% increase in oxaluria following a dose of 1 g per day of ascorbic acid in four calcium stone-forming patients and with Chalmers, Cowley, and Brown [3] who observed a 79% increase in mean urinary oxalate after 2 g vitamin C in 17 calcium stone-forming patients. Differences regarding the magnitude of urinary oxalate increase between the present data and these other studies may be ascribed to different oxalate assays and duration of supplementation. Urivetzky, Kessaris, and Smith [2], using the same assay as ours, also observed increases of 38% and 107% in oxaluria following doses of 1 g and 2 g per day in 15 calcium stone-forming patients, respectively. Conversely, no increase in urinary oxalate after a single dose of 1 g vitamin C was detected by Heckers et al [21], who regrettably did not indicate the assay used for urinary oxalate determination.

In the present series, the significant increase in urinary oxalate observed even in the urine specimens collected in a gallon containing acid preservative minimizes the possibility that the increase in oxaluria after vitamin C was due to the in vitro nonenzymatic conversion of ascorbate to oxalate since HCl reduces the urinary pH to values around 1. According to Auer, Auer, and Rodgers [16], the possibility that urinary oxalate measurements may be

falsely elevated by the presence of high urinary ascorbate must be considered. However, Liebman et al [15] reported that whereas 2 g vitamin C supplement produced increments in mean urinary ascorbate concentration in six healthy subjects ranging from 100 to 540 mg/L, the increases in urinary oxalate were less than 1.0 mg/L, suggesting that urinary oxalate data did not appear to be confounded by the potential interference of ascorbate.

It has been suggested that the addition of disodium EDTA stabilizes ascorbate in urine and inhibits its conversion to oxalate [16, 17, 33, 34]. However, when EDTA is added to the container before urine collection, other parameters such as calcium and sodium cannot be determined in the same urine sample. Therefore, to assess whether the addition of EDTA could prevent the in vitro conversion of ascorbate to oxalate, we performed ancillary analyses in spot urine obtained from 15 healthy volunteers with or without EDTA after the intake of the vitamin C supplement and found no difference between them. These findings minimize the possibility that our oxaluria results were due to an in vitro conversion of ascorbate to oxalate.

In the present study, calcium stone-forming patients presented significantly higher mean values of urinary oxalate than the healthy subjects both at baseline and after the vitamin C supplements. Higher baseline values of urinary oxalate among stone formers when compared to normal volunteers have also been observed by other investigators [39–41], including previous studies by our group [42]. It is possible that this increase in oxaluria results from lower levels of intestinal colonization by *Oxalobacter formigenes*, an oxalate-degrading bacterium leading to less oxalate degradation in the intestinal lumen, as suggested by Sihdu et al [43].

Some studies have reported that ascorbic acid reduces urinary pH [23–25, 29, 30], whereas others have found the agent to be ineffective as a urinary acidifier [22, 26–28]. Some of these conflicting results may be ascribed to the type of urine collection, usually consisting of 24-hour urine samples, which are subjected to diet interference and eventual bacterial contamination. In the present study, urinary pH measured after a 12-hour fast (except for vitamin C supplement intake) in the second micturition showed no significant change from baseline values. These findings are in agreement with other investigators [22, 28].

CONCLUSION

In conclusion, the intake of vitamin C supplements of 1 or 2 g per day may produce a significant increase in urinary oxalate, elevating the risk of calcium oxalate crystallization. As a consequence, patients with a history of stone disease should be discouraged from taking a vitamin C amount exceeding the recommended daily allowance. Vitamin C does not seem to be an efficient urinary acidifier in calcium stone-forming patients.

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