EFFECTS OF CALCIUM SUPPLEMENTS ON THE RISK OF RENAL STONE FORMATION IN A POPULATION WITH LOW OXALATE INTAKE

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Abstract. It has been speculated that calcium supplement in subjects with low oxalate intake might increase the risk of calcium stone formation due to an increase in calcium absorption without a significant reduction in oxalate absorption. There have been no human studies addressing specifically the effects of taking calcium supplements in populations whose dietary oxalate is low. This study was conducted to determine the effects of calcium supplements on the risk of calcium stone formation in a population with low oxalate intake. Thirty-two healthy male navy privates, 22.7±1.9 (mean ± SD) years old, who had oxalate intake of less than 1 mmol/day, a serum creatinine of less than 150 μmol/l, and no history of renal stones, participated in the study. Dietary oxalate was controlled to be under 1 mmol/day throughout the study. Twenty-four hour urine collections for the determination of urinary constituents were obtained at baseline and after taking calcium supplements. Detection of calcium oxalate was performed to assess the risk of calcium oxalate stone formation. The urinary excretion of calcium was significantly elevated above baseline values while taking the calcium supplements (3.48±2.13 *vs* 5.17±2.61 mmol/d, $p < 0.05$) and urinary oxalate was significantly decreased when the subjects took calcium supplements compared to the corresponding baseline value (0.13±0.05 *vs* 0.17±0.07 mmol/d, $p = 0.01$). Urinary citrate was significantly elevated when the subjects took calcium supplements compared to the baseline (0.83±0.57 *vs* 0.64±0.39 mmol/d, $p = 0.03$). There was no significant alteration in the activity products of calcium oxalate while taking the calcium supplements (0.54±0.25 *vs* 0.57±0.22, $p = 0.54$). The effect of calcium supplements with meals, for the reduction of the risk of calcium stone formation, was unchanged, even in a population whose oxalate intake is rather low. Taking calcium supplements resulted in a reduction in urinary oxalates and an elevation in urinary citrates. Both alterations in urinary constituents counterbalanced the elevation in urinary calcium which resulted from the calcium supplements.

INTRODUCTION

Increased dietary calcium intake has been shown to reduce urinary oxalate excretion in both healthy patients, and those with nephrolithiasis, due to the combination of calcium and oxalate within the intestine, thereby limiting oxalate absorption (Marshall *et al*, 1972; Bataille *et al*, 1983; Lemann *et al*, 1996; Curhan *et al*, 1997; Liebman and Chai, 1997; Hess *et al*, 1998). However, it has been speculated that calcium supplements in subjects with low oxalate intake might increase the risk of calcium stone formation (Curhan *et al*, 1997) due to an increase in calcium absorption, without a significant reduction in oxalate absorption. Most studies which demonstrated the effect of calcium intake on intestinal absorption of oxalate, were done under oxalate loads, the amount being much higher than the content in a normal diet (Marshall *et al*, 1972; Hess *et al*, 1998). It is uncertain if the effect of calcium supplement on urinary tract stone formation is the same, less, or even opposite in populations whose oxalate intake is low. Moreover, if dietary oxalate content is a major determinant for the effect of calcium supplements on the risk of stone formation, as postulated in a previous study (Curhan *et*

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al, 1997), dietary oxalate content and/or the amount of urinary oxalate should be known before prescription of calcium. It is also uncertain whether the combination of calcium with oxalate in the intestine is the only mechanism for the decrease in the risk of calcium stone formation. A recent animal study demonstrated that calcium oxalate can be absorbed intact, without being dissociated prior to absorption (Hanes *et al*, 1999). Supplementation of calcium also provides an alkaline supplement from the anionic component of the calcium salt to the body. This may lead to an increase in citrate excretion and thereby a reduction in the risk of stone formation (Domrongkitchaiporn *et al*, 2000). We, therefore, conducted this study to determine the effect of calcium supplements on the risk of calcium stone formation in a population whose usual dietary oxalate content was low and to demonstrate possible mechanisms for the reduction in the risk of calcium stone formation.

METHODS

Subjects

Subjects were enrolled from healthy male navy privates who worked as patient assistants at Arpakornkietiwong Hospital, a navy hospital on the Sattahip Navy Base, Chon Buri, Thailand. Dietary records were obtained from all the subjects who participated in this study. Inclusion criteria were: (1) dietary oxalate intake of less than 1 mmol/day, (2) normal renal function (serum creatinine <150 μmol/l), (3) no history of renal stone formation, and (4) no gastrointestinal diseases or malabsorption syndrome. During the study, subjects with the following conditions were excluded from the study: (1) concurrent illness that needed hospitalization or absence from work, (2) developed acute diarrhea or a disease that affected appetite or gastrointestinal absorption of food, (3) failed to follow the protocol strictly. There were 50 navy privates eligible for this study, but only 38 of them participated. Six subjects were excluded after enrollment due to failure to comply with the diet or an inability to follow the study protocol due to military duty during the first week of the study. Only 32 subjects participated throughout the study. Their mean ± SD age, body weight, and height were 22.7±1.9 years, 61.2±7.4 kg, 169.3±3.4 cm, respectively.

Study protocol

All subjects were on a controlled diet provided by the hospital cafeteria and stayed in the hospital dormitory throughout the study. In phase 1, all the subjects took only the controlled diet provided by the investigators for 1 week. On days 6 and 7 of the first week of taking the controlled diet, the first or baseline of two 24-hour urine collections was obtained from all the subjects. In the second week, the subjects took 1 capsule of 650 mg calcium carbonate immediately after the meal, three times a day, in addition to the controlled diet. An additional two 24-hour urine collections were obtained from all subjects on days 6 and 7 of the second week. All the subjects took the calcium supplements under the inspection of a research nurse, to ensure compliance.

The controlled diet was the usual Thai diet with the following major food compositions: total calories 2,000-2,500 kcal/d, carbohydrate 350 g/d, protein 80 g/d, fat 60 g/d, calcium 10-15 mmol/d, and oxalate < 1 mmol/d. The menu was set in cycle so that during the corresponding days of the study, in the first and second weeks, all the subjects had the same food menu. All the subjects took their food exclusively at the hospital cafeteria. A dietitian at the cafeteria recorded the actual amount of food taken by each subject after he finished each meal. The major food compositions were determined in accordance with Thai food composition tables by a nutritionist (Institute of Nutrition, Mahidol University, 1997). No vitamin or any dietary supplement, apart from what was provided according to the study protocol, was allowed during the study.

Specimen collections and laboratory investigations

Urine samples were collected in plastic containers, using 10 grams of boric acid as a preservative. The urine volume and urine constituents, including sodium, potassium, chloride, urea, uric acid, calcium, magnesium, citrate, oxalate, phosphate, and creatinine, were determined immediately after the 24-hour collection was completed. Creatinine, sodium, potassium, chloride, urea, uric acid, calcium, and phosphate were determined by

autoanalyzer technique, magnesium by atomic absorption spectrometry, citrate by citrate lyase technique (Toftegaard Neitsen, 1976), and oxalate by the HPLC technique (Hagen *et al*, 1993). For the oxalate assay, the inter-assay coefficients of variation averaged 4%, while the recovery of oxalate averaged 93%. The amount of each urinary constituent used for further analysis was derived from the average of the two 24-hour urine collections. The risk of calcium oxalate stone formation was determined by using Tiselius's index (Tiselius, 1991), where the ion-activity product was estimated by an index called AP (CaOx):

$$\text{AP (CaOx) index} = A \times Ca^{0.84} \times Ox^{1.0} \times Mg^{-0.12} \times Cit^{-0.22} \times V^{-1.03}$$

Urinary excretions of calcium (Ca), oxalate (Ox), magnesium (Mg), and citrate (Cit) were expressed in millimoles per collection period, and urine volume (V) in liters. Factor ***A*** was 1.9, depending on the collection time of 24 hours (Tiselius, 1991). The calcium oxalate activity product value calculated by the AP (CaOx) index was highly and positively correlated (r = 0.98) with those obtained by the Equil 2 program (Werness *et al*, 1985). The correlation between the index and stone-forming activity in calcium oxalate stone formers has been demonstrated (Bek-Jensen and Tiselius, 1989; Tiselius, 1989; Tiselius and Sandvall, 1990). It is clinically useful and has been recommended for a routine program of evaluation and follow-up of stone-forming patients (Tiselius, 1996). The upper limit of AP(CaOx) for the Thai population is less than 1.52 (Domrongkitchaiporn *et al*, 2000).

The study protocol was reviewed and approved by the Office of the Sattahip Navy Base Commander, Royal Thai Navy, Thailand. Written informed consents were obtained from all subjects.

Statistical analysis

Data are presented as mean ± SD. Paired Student's *t*-test was applied to determine the differences between two related samples. A p-value of <0.05 was considered statistically significant.

RESULTS

The dietary records demonstrated that all subjects had a dietary intake according to the protocol. There was no significant difference in the major food compositions between baseline and when taking calcium supplements, as shown in Table 1. The urine constituents at baseline and when the subjects took calcium supplements are shown in Table 2. There were only 2 subjects with hypercalciuria at baseline, but 6 subjects developed hypercalciuria after taking calcium supplements. Urinary calcium increased significantly with calcium supplements. While taking calcium

Table 1
Dietary record of major food compositions for subjects at baseline and when taking calcium supplements.

Food compositions	At baseline	Taking calcium supplements
Protein (g/d)	83.92 ±12.38	88.01 ±10.75
Carbohydrates (g/d)	352.65 ±65.42	316.90 ±57.08
Fat (g/d)	66.55 ±11.52	61.30 ±9.09
Calcium[a] (mmol/d)	14.76 ±2.32	11.35 ±1.81
Total calories (kcal/d)	2,041.7 ±270.2	2,172.6 ±270.3

[a]Amount of calcium intake contributed by the calcium supplement was not included in the dietary record.

Table 2
Urinary constituents and AP (CaOx) at baseline and during calcium supplementation with meals.

Urine constituents	Baseline (mmol/d)	Calcium supplement (mmol/d)
Creatinine	16.85 ±6.15	14.50 ±8.69
Urea	223.06 ±58.22	254.04 ±108.20
Uric acid	3.06 ±0.97	2.84 ±1.80
Calcium	3.48 ±2.13	5.17 ±2.61[a]
Phosphate	19.64 ±8.08	19.49 ±8.74
Sodium	147.3 ±67.5	166.2 ±65.08
Potassium	21.0 ±7.1	26.6 ±13.28
Chloride	178.4 ±73.0	201.3 ±73.3
Magnesium	3.60 ±1.20	3.80 ±1.20
Oxalate	0.17 ±0.07	0.13 ±0.05[a]
Citrate	0.64 ±0.39	0.83 ±0.57[a]
Volume (l/d)	1.66 ±1.07	1.57 ±0.78
AP (CaOx)	0.54 ±0.25	0.57 ±0.22

[a]$p < 0.05$ compared to corresponding baseline value.

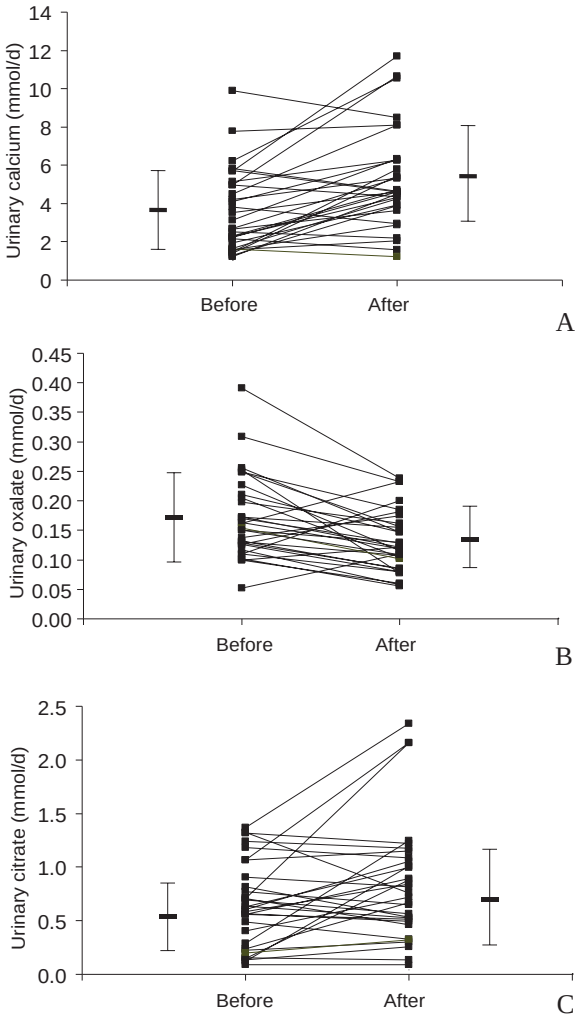

Fig 1–Urinary excretion of A) calcium, B) oxalate and C) citrate for individual patients at baseline and when taking calcium supplements. The cross line and error bar represents a mean ± SD for the entire group.

supplements, urinary oxalate was significantly decreased ($p < 0.05$), whereas urinary citrate was significantly elevated ($p < 0.05$) above the baseline value. Both AP(CaOx) at baseline and during calcium supplementation was far below the upper limits of normal. There was no significant alteration in AP(CaOx) from the corresponding baseline value when taking calcium supplements. Urinary excretion of calcium, citrate, and oxalate for individual subjects at baseline and when taking calcium supplements is shown in Fig 1 and

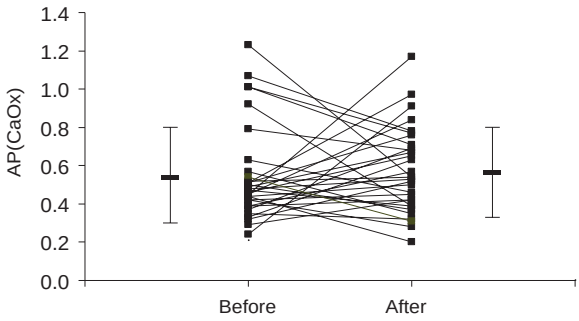

Fig 2–AP (CaOx) for individual patients at baseline and when taking calcium supplements. The cross line and error bar represents a mean ± SD for the entire group.

the AP(CaOx) is shown in Fig 2.

DISCUSSION

In this study we demonstrated that calcium supplements with meals did not increase the risk of calcium stone formation. Taking calcium supplements with meals resulted in a reduction in urinary excretion of oxalate and concomitant elevation in urinary citrate. Both factors counterbalanced the effect of elevated urinary calcium. There was no significant alteration in the activity products for calcium oxalate stones.

The combination of calcium and oxalate in the gastrointestinal tract resulting in a reduction of oxalate absorption has been postulate as the major mechanism for reduced urinary oxalate excretion. It has been speculated that calcium supplement in subjects with low oxalate intake might increase the risk of calcium stone formation (Curhan *et al*, 1997) due to an increase in calcium absorption without a significant reduction in oxalate absorption. If this is the case, a knowledge of dietary oxalate content might be required before prescription of calcium supplements. Our findings do not support this postulation. The oxalate content in our subjects was low compared to what had been reported in other studies done in western populations (Lemann *et al*, 1996; Tiselius, 1996; Holmes *et al*, 2001). The average oxalate intake ranges from 1 to 2.5 mmol in the Western population, whereas it was less than 1 mmol in our subjects. This was confirmed by the low urinary excretion of oxalate at baseline.

The amount of urinary oxalate, as well as other urine constituents found in our study, was in the range of what was reported in other studies in the Thai population (Sriboonlue *et al*, 1991). Although the study was performed with a controlled diet, the dietary content and the food provided by the dietitian were the usual Thai diet. Based on the findings of our study and Hess' study, in which oxalate intake was supraphysiologic (Hess *et al*, 1998), the effect of calcium supplements with the meal was unchanged; regardless of the oxalate content in the diet.

It should be pointed out that the dietary calcium of our subjects was low compared to the Western diet. Calcium supplements should further reduce gastrointestinal oxalate absorption. It is uncertain whether the beneficial effect of taking calcium with the meals would be the same or less in populations with higher dietary calcium. For populations with high calcium intake, the amount of calcium in the gastrointestinal tract may maximally combine with the intestinal oxalate. Any addition of calcium to the diet might not further reduce intestinal oxalate absorption, but might cause hypercalciuria and an elevation in AP(CaOx).

The calcium supplements also increased urinary excretion of citrate. This resulted from the intestinal absorption of carbonate, the anion that accompanies the calcium salt. Carbonate provides an alkaline supplement to the body, leading to an elevation in urinary citrate excretion (Domrongkitchaiporn *et al*, 2000). It should be pointed out that the baseline urinary citrate in our subjects was relatively low compared to levels reported in studies from Western countries (Minisola *et al*, 1981). The levels found among our subjects were in the range of other studies in the Thai population (Tungsanga *et al*, 1992; Nimmannit *et al*, 1996). Urinary excretion of citrate in the Thai population, either in normal subjects or in stone formers, is relatively low (Tungsanga *et al*, 1992; Nimmannit *et al*, 1996). No studies have been reported to explain the low urinary citrate in the Thai population. Difference in diet may be an explanation for this.

There were limitations in our study. Firstly, it should be noted that our subjects had no history of stone formation. Calcium handling may differ between subjects who do and do not form stones (Lemann, 1992). Secondly, all the subjects in this study were male. Thirdly, the diet and the baseline urinary findings may be different from other populations. Extrapolation of our findings to other populations should be done with caution. Calcium supplements in our study were taken with meals. Therefore, the findings from this study can not be applied to other regimens, *eg* at bedtime or on an empty stomach. Fourthly, our findings did not exclude the possibility that taking calcium supplements could induce an acute elevation in urinary calcium excretion and lead to an elevation in AP(CaOx), especially a few hours after ingestion. This may increase the risk of stone formation, rather than decrease it. A long-term, prospective cohort study to determine the incidence of renal stone formation in a population that takes calcium supplements should be conducted to confirm our findings.

In conclusion, the effects of calcium supplements with meals for the reduction of the risk of calcium stone formation is unchanged in a population whose oxalate intake is low. Taking calcium supplements results in a reduction in urinary oxalate and an elevation in urinary citrate. Both alterations in urinary constituents counterbalance the elevation in urinary calcium resulting from the calcium supplementation.

ACKNOWLEDGEMENTS

The determination of urinary electrolytes and magnesium by Miss Chatuporn Ruang-raksa was highly appreciated.

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