Effect of Citrate on the Urinary Excretion of Calcium and Oxalate: Relevance to Calcium Oxalate Nephrolithiasis

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Studies in 24 recurrent oxalate stone-formers have shown that values for urinary calcium excretion for this group on at-home diets vary significantly (P < 0.001) more than values for creatinine excretions. By placing stone-formers on controlled hospital diets and measuring their calcium excretions, we were able to predict probable outpatient hypercalciuria (> 7.5 mmol/day) with a sensitivity of 95% and a specificity of 95%. In this study, the renal loss of calcium during low-calcium diets was proportional to the absorptive hypercalciuria during high-calcium diets. Calcium loading experiments in fasted stone-formers and normal subjects indicated that citrate, at citrate:calcium molar ratios ranging from 0.12 to 1, stimulated urinary calcium excretion more than did calcium carbonate loading alone. In addition, citrate also significantly (P < 0.05) increased the excretion of urinary oxalate by two normal subjects for a given load of calcium oxalate. Malabsorption of citrate and possibly other hydroxyxycarboxylic acids may thus predispose to oxalate nephrolithiasis by promoting calcium and oxalate absorption.

Patients recurrently forming renal calcium-containing stones often excrete increased amounts of calcium and oxalate and decreased amounts of the hydroxyxycarboxylic acids citrate and ascorbate in their urine (1, 2). We have previously reported evidence (1, 2) that such patients malabsorb orally ingested ascorbate and citrate, resulting in an increased conversion of ascorbate to oxalate in the gut and, subsequently, increased oxalate absorption and urinary excretion. By contrast, normal subjects absorb dietary ascorbate more efficiently than do stone-formers, with only a slight conversion to oxalate (2). Neither stone-formers nor normal subjects convert intravenous ascorbate to oxalate. Their concentrations of urinary oxalate remain unchanged after intravenous ascorbate loading (2). When citrate is given with oral ascorbate, ascorbate absorption is inhibited such that combined ascorbate and citrate loads result in hyperoxaluria in both normal subjects and stone-formers (1). These results suggest that both citrate and ascorbate may be handled by the same carrier of hydroxyxycarboxylic acids in the gut and that the function of this carrier may be impaired in recurrent formers of calcium-containing stones (2–4). Such a mechanism is consistent with the increases in urinary oxalate and the decreases in urinary ascorbate and citrate seen in these patients.

Given that increased oxalate and, to a lesser extent, calcium excretion are important to the etiology of calcium stone disease (5–7), we were interested in determining whether malabsorption of hydroxyxycarboxylic acids could contribute to the hypercalciuria often seen in these patients (1, 5, 8). We present here the effect of anions, particularly citrate, on calcium and oxalate absorption and excretion in normal subjects and in stone-formers. The possible relationship of citrate malabsorption to the hyperexcretion of calcium and oxalate is discussed.

Materials and Methods

Analyses: Urine was collected and analyzed for calcium, oxalate, citrate, ascorbate, and creatinine (1, 2, 9, 10).

Patients: The group of 28 recurrent calcium oxalate stone-formers has previously been described in relation to their stone history and current clinical status (1). None of them had impaired renal function, as evidenced by their concentrations of urea and creatinine in plasma. All had formed more than one mixed calcium oxalate/phosphate stone. Their therapy consisted of careful instructions to avoid foods of high calcium and oxalate content and to maintain a high fluid intake. None of the patients had hyperparathyroidism, enteric hyperoxaluria, vitamin D intoxication, or renal tubular acidosis.

The group comprised 19 men and nine women, ages 21–63 y (mean 43 y). The index of calcium and creatinine variability for 24-h urine collections was calculated as previously described (11):

Index of variability = 

\[
\left( \frac{\text{highest result} - \text{lowest result}}{\text{mean (highest + lowest result)}} \right) \times 100\%
\]

In correlating the excretions of calcium, oxalate, citrate, and ascorbate in 24-h urine specimens collected at home and in correlating the 24-h urinary calcium excretions on low- and high-calcium diets in hospital, we used the product moment correlation (12). In comparing fasting with post-dose urinary excretions of calcium and citrate in serial 24-h urine experiments in normal subjects and stone-formers after oral loads we used the Wilcoxon nonparametric two-sample statistic (13). We used the paired t-test to compare the 2-h urinary oxalate excretions after oxalate + citrate vs oxalate loads given alone.

The 28 normal control subjects were healthy hospital personnel who were taking no medication, equal numbers of men and women, ages 22–55 y (mean, 32 y).

Urine studies (24-h): All 28 patients were placed on low- and high-calcium diets in the hospital as previously described (11). These diets contained respectively: protein, 80 or 105 g; calories, 2200 or 2100 cal; calcium, 150 or 1000 mg; oxalate, 90 or 150 mg; citrate, 3.7 or 2.2 g; and ascorbate, 150 or 100 mg, as assessed by use of dietary tables (11).

In a separate phase of the study, all patients also collected at least one 24-h urine specimen at home while their diet was controlled, and 24 patients collected multiple 24-h urine specimens at home (mean, four; range, two to 12 collections), which we used to calculate the index of variability. Eighteen patients also collected urine specimens at

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home into divided urine containers (11) for simultaneous measurement of calcium, oxalate, citrate, and ascorbate for the correlation study.

Serial 2-h urine experiments: All subjects were instructed to eat a low-calcium diet the evening before the "load" study meal and to fast for at least 10 h overnight. Early the next day, they emptied their bladders before collecting a 2-h baseline specimen (from −2 to 0 h). At time zero, the loads indicated below were taken with 300 mL of water followed by three 2-h urine collections: after the load, participants were allowed water ad libitum. We gave the following loads on different occasions: 75 g of sucrose; 2 g of citric acid; 2 g of tripotassium citrate + 0.4 g of citric acid; 0.6 g of calcium carbonate + 1.2 g of citric acid; 2.4 g of calcium carbonate (25 mmol of calcium) + 0, 0.6, 1.2, 2.4, or 4.8 g of citric acid (0–25 mmol); 2.4 g of calcium carbonate + 2 g of ascorbic acid; 5.9 g of calcium gluconate; 160 mg of calcium oxalate + 0 or 1 g of citric acid.

Results

The 24-h urinary calcium excretion by 24 recurrent calcium oxalate stone-formers who made between two and 12 collections on their usual diets at home (Figure 1) gave a mean within-patient index of variability for calcium of 60% (SD 30%). This was significantly (P < 0.001) greater than the index of variability for creatinine excretion in the same specimens, 20% (SD 16%), and about the same as that previously reported for oxalate, 61% (SD 36%) (11). Of the 105 calcium estimations made in this group, 52 (49%) exceeded 7.5 mmol per day, the 97th percentile of our normal subjects on their usual diets at home (11, 14, 15).

Eighteen of the stone-formers collected 24-h specimens into divided urine containers for simultaneous measurement of calcium, oxalate, citrate, and ascorbate. For these urines, there was no significant correlation between the excretion of calcium and oxalate (r = 0.10), calcium and ascorbate (r = 0.07), oxalate and citrate (r = 0.03), oxalate and ascorbate (r = 0.39), or citrate and ascorbate (r = −0.25). However, there was a significant correlation between calcium and citrate excretion (r = 0.78, P < 0.01). In contrast, the 28 normal subjects showed no significant correlation between calcium and citrate (r = 0.02). They also showed no significant correlation between calcium and oxalate (r = 0.026).

In an attempt to predict which stone-formers would become hypercalcicuric at times on their usual at-home diets, we performed formal five-day calcium loading tests on all 28 stone-formers in the hospital. The mean calcium excretion on the low-calcium diets (3.75 mmol, 150 mg per day) was 3.52 (SD 1.33) mmol per day and on the high-calcium diets (1000 mg calcium, as gluconate) it was 7.26 (SD 2.63) mmol per day, with 12 patients (42%) exceeding 7.5 mmol per day.

We then divided the stone-formers into two groups, those with a 24-h urinary calcium excretion at home exceeding 7.5 mmol per day (20 patients, termed hypercalcicuric) on at least one occasion and those who consistently excreted <7.5 mmol per day (eight patients, termed normocalcicuric) (11, 15). The values for calcium excretion by these two groups on low (150 mg per day) and high (1000 mg per day) calcium intake overlapped considerably on the low-calcium diet but separated when the calcium intake was increased (Figure 2). If a calcium excretion of 6 mmol per day on the high-calcium diet is used as a cutoff, the positive predictive value of a value exceeding this for probable outpatient hypercalciuria >7.5 mmol per day is 95%, with a sensitivity of 95% and a specificity of 95%, giving an overall test efficiency of 99%.

Figure 3 depicts calcium excretions of stone-formers while on low- and high-calcium diets in the hospital. Patients with the highest calcium excretions on the low-calcium diet gave the highest excretions on the high-calcium diet, resulting in a significant correlation for calcium excretion on the two diets (r = 0.75; P < 0.01). Thus stone-formers with the greatest hyperabsorption of calcium also appear to have the largest renal calcium wasting.

This renal wasting of calcium in our hypercalciuric stone patients was evident when their calcium excretions, measured while fasting, were compared with those of normal subjects (see the −2 to 0 h specimens in Table 1). Hourly calcium excretion declined after the calcium-free load (sucrose), with the stone-formers excreting more calcium at all periods studied than the normal subjects.
Similar values for calcium excretion were obtained in normal subjects after both citric acid and potassium citrate loads (Table 1). The decrease in urinary calcium over the morning has been previously reported and is a component of the diurnal rhythm in calcium excretion (15).

Table 1 summarizes our initial studies on normal subjects and stone-formers of the effect of citric acid on calcium excretion after an oral calcium carbonate load. Apparently, citric acid enhances calcium absorption and excretion from this insoluble calcium salt. Calcium excretion from a 2.4-g calcium carbonate load in the presence of citrate was considerably greater in the stone-formers than in the normal control subjects. The effect of citric acid on calcium absorption was also pronounced when a small calcium carbonate load of 0.6 g was given to the normal subjects (Table 1).

We tested the effect of doses of citric acid ranging from 0.6 to 4.8 g on a 2.4-g calcium carbonate load in normal subjects. The results (Figure 4) show citrate to be maximally effective in stimulating calcium absorption and excretion where present in a molar ratio of 1 citrate per 4 calcium ions or greater. Citric acid loading did not alter urinary citrate excretion appreciably, either in stone-formers or normal subjects (Table 2).

In one normal subject, equimolar calcium gluconate was comparable with citric acid in enhancing calcium excretion. At −2 to 0, 0–2, 2–4, and 4–6 h after the load, calcium excretion from gluconate was respectively 80, 150, 240, and 220 μmol/h as compared with 155, 225, 398, and 290 μmol/h with citric acid. In another normal subject, ascorbate was also effective in promoting calcium absorption (calcium excretions at −2 to 0, 0–2, 2–4, and 4–6 h were respectively 93, 85, 200, and 275 with ascorbate vs 185, 161, 334, and 315 μmol/h with citric acid). The concomitant administration of calcium carbonate with ascorbic acid did not diminish the ascorbate excretion—30.5% of ascorbate was excreted in 24 h with the combined load compared with 19.9–29.3% when ascorbate was given alone (2).

The effect of citrate on urinary oxalate excretion 8 h after an oral dose of calcium oxalate is shown for two normal subjects in Figure 5. In all these specimens, the oxalate excretion after the load was significantly (P < 0.05) increased when citrate was co-administered with calcium oxalate (oxalate + citrate vs oxalate load). By linear extrapolation of the pre-dose oxalate excretions to the 6–8 h post-load excretion, we calculated that, on average, 3.7% or 49.8 μmol of the calcium oxalate load was excreted as oxalate when combined with citric acid, compared with 0.9% or 11.4 μmol when citric acid was not administered. The basal excretion of oxalate, 22 μmol/h, was increased to 33 μmol/h on the combined load.

<table>
<thead>
<tr>
<th>Load</th>
<th>n</th>
<th>−2 to 0</th>
<th>0 to 2</th>
<th>2 to 4</th>
<th>4 to 6</th>
<th>6 to 8</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Calcium excretion, μmol/h, mean ± SEM</td>
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<td></td>
<td></td>
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<tr>
<td>Sucrose, 75 g</td>
<td></td>
<td></td>
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<tr>
<td>Normals</td>
<td>3</td>
<td>106 ± 24</td>
<td>76 ± 2</td>
<td>49 ± 8</td>
<td>37 ± 7</td>
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</tr>
<tr>
<td>Stone-formers</td>
<td>5</td>
<td>227 ± 56</td>
<td>181 ± 22</td>
<td>137 ± 42</td>
<td>136 ± 42</td>
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<td>Citric acid, 2 g</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Normals</td>
<td>2</td>
<td>82 ± 23</td>
<td>58 ± 13</td>
<td>46 ± 14</td>
<td>52 ± 22</td>
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<tr>
<td>Potassium citrate, 2 g,</td>
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<tr>
<td>+ citric acid, 0.4 g</td>
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<tr>
<td>Normals</td>
<td>4</td>
<td>92 ± 26</td>
<td>53 ± 5</td>
<td>31 ± 8</td>
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<td>CaCO₃, 2.4 g</td>
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<tr>
<td>Normals</td>
<td>3</td>
<td>197 ± 68</td>
<td>143 ± 31</td>
<td>170 ± 24</td>
<td>159 ± 28</td>
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<tr>
<td>Stone-formers</td>
<td>5</td>
<td>279 ± 72</td>
<td>238 ± 24</td>
<td>314 ± 52</td>
<td>354 ± 70</td>
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<tr>
<td>CaCO₃, 2.4 g, + citric</td>
<td></td>
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<tr>
<td>acid, 4.8 g</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Normals</td>
<td>3</td>
<td>150 ± 22</td>
<td>187 ± 20</td>
<td>383 ± 25</td>
<td>318 ± 14*</td>
<td></td>
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<tr>
<td>Stone-formers</td>
<td>3</td>
<td>303 ± 129</td>
<td>353 ± 149</td>
<td>621 ± 173*</td>
<td>320 ± 184*</td>
<td></td>
</tr>
<tr>
<td>CaCO₃, 2.4 g, + citric</td>
<td></td>
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<td></td>
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<tr>
<td>acid, 1.2 g</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Normals</td>
<td>3</td>
<td>116 ± 9</td>
<td>200 ± 65</td>
<td>316 ± 23*</td>
<td>305 ± 45*</td>
<td>290 ± 46*</td>
</tr>
<tr>
<td>CaCO₃, 0.6 g, + citric</td>
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<tr>
<td>acid, 1.2 g</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normals</td>
<td>3</td>
<td>93 ± 17</td>
<td>124 ± 31</td>
<td>225 ± 18*</td>
<td>206 ± 59</td>
<td>138 ± 25</td>
</tr>
</tbody>
</table>

*Significantly (P < 0.05) more than in the −2 to 0 h collection.
Discussion

The 24-h urinary calcium excretion by the 24 recurrent calcium stone-formers was highly variable (Figure 1). This variability was not ascribable to inaccuracies in the specimen collection, because calcium excretion was about three times more variable than creatinine excretion in the same urines. All 28 patients studied were normocalciuric on low-calcium diets in the hospital, but hypercalciuria could be provoked in half of them when dietary calcium intake was increased (Figures 2 and 3). This variation of calcium excretion with diet has been well documented (15, 16) and suggests further that the commonly performed collection of a single 24-h urine specimen from a subject on an uncontrolled diet is an insensitive test to use in the diagnosis of hypercalciuria.

Using a cutoff value of 6 mmol per day for 24-h calcium excretion on the 1000 mg per day in-hospital calcium diets, we have obtained a positive predictive value of 95% for outpatient hypercalciuria (where this is defined as a urinary calcium excretion of >7.5 mmol per day on at least one occasion as an outpatient). It is noteworthy that the optimum discriminatory level for the inpatient test is only 6 mmol per day, and this may reflect the fact that, in this test, calcium gluconate was given, whereas, in a normal diet, phosphate and citrate are the predominant anions.

There is significant correlation ($r = 0.75$; $P < 0.01$) between calcium excretion on the low- and high-calcium diets (Figure 3). Those patients who best absorbed calcium from the high-calcium diets also had the greatest calcium loss on the low-calcium diets. Indeed, some of our patients would have been in negative calcium balance even if they absorbed all the 3.75 mmol of calcium per day that was in the low-calcium diet. This suggests us that a sustained high flux of calcium through the kidney may attenuate the calcium conservation mechanisms in the renal tubules (15, 16). This would explain the higher calcium excretions during fasting (Table 1) that we found in stone-formers as compared with normal subjects, which has been previously reported (1, 5, 8). Such a conservation mechanism would be superfluous when the kidney is being flooded with calcium, and two days may have been too short a time on the low-calcium diets for the conservation mechanism to recover, because adaptation takes at least four weeks (16). Whether or not the hyperexcretion of calcium by stone-formers is an adaptation to cope with hyperabsorption of calcium is not currently known and would require long-term controlled dietary studies.

A diurnal rhythm for urinary calcium excretion has previously been demonstrated, and this persists even when food intake is controlled and evenly spread over the 24-h period (15). Both the stone-formers and the normal subjects showed higher urinary calcium excretions early in the morning, which progressively declined as the subjects were ambulant during the morning (Table 1), and this also was true when either citric acid or sucrose was given. This diurnal variation suggests that the measurement of calcium on a low-calcium diet over a 24-h period should be more accurate than preprandial urine collections as a means of assessing the magnitude of any renal leakage of calcium.

In subjects given a fixed load of calcium carbonate, the increase in urinary citrate after citric acid loading did not correlate with and was inadequate to account for the increased calcium excretion (Table 2). Further, no increase in urinary calcium was seen when citric acid was given alone.

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Table 2. Calcium and Citrate Excretions in Urine after Citrate Loading*  

<table>
<thead>
<tr>
<th>Collection time, h</th>
<th>Calcium</th>
<th>Citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normals</td>
<td>S/Formers</td>
</tr>
<tr>
<td>0-2</td>
<td>-91 ± 64</td>
<td>42 ± 69</td>
</tr>
<tr>
<td>2-4</td>
<td>66 ± 95</td>
<td>150 ± 98</td>
</tr>
<tr>
<td>4-8</td>
<td>303 ± 13b</td>
<td>81 ± 46</td>
</tr>
</tbody>
</table>

*Mean ± SEM, μmol per millimole of creatinine, for three subjects in each group. Results for each subject are calculated as the excretion on the “citric acid (4.8 g) day” – “baseline day.” Calcium carbonate (2.4 g) was given on both days.

bSignificantly ($P < 0.05$) increased over baseline.
calcium the containing (5, acids although effective of out mens applicable absorption collection already (Table mmol dietary tion calcium.
renal (7). The highly a 7). We have also shown that citric acid appears to amplify calcium absorption (Figure 4) from calcium carbonate, an insoluble calcium salt, and that calcium absorption proceeds at about half the maximum rate when citric acid is present in a molar ratio of ~1:8 with calcium. The approximate ratio of citrate to calcium in cow’s milk is of this order, 1:5 (17), so it is possible that malabsorption of citrate by the hydroxy-carboxylic acid uptake pathway could lead to higher intraluminal citrate concentrations and to greater calcium absorption in stone-formers. The results also suggest that a high dietary intake of calcium and citrate, if maintained in normal subjects, could lead to hypercalciumia, because the peak rate of calcium excretion exceeds 312 mmol/h (7.5 mmol per day) between 2 and 6 h after a calcium citrate load (Table 1). The possibility of a relationship between a large dietary intake of citrate and calcium nephrolithiasis has already been indicated by recent findings. Normally hydroxaluric stone patients became consistently normo-oxaluric on controlled citrate, oxalate, and ascorbate intakes (11, 18), and a positive association between urinary stone disease and the dietary intake of citrate-containing carbonated beverages has been reported (19).

When calcium absorption is to be assessed after a single oral calcium load, we would suggest collecting urines between 2 and 4 h after the load rather than the 0–4 h collection commonly recommended (20, 21), because calcium absorption is maximal at least 2 h after a load (Figure 4). It is also necessary to standardize the anion used, because reference intervals applicable to one anion may not be applicable to others. Also the time of day that the test is performed is important if the effect of diurnal rhythms is to be controlled. For these reasons, we prefer to assess calcium absorption by our patients by analyzing 24-h urine specimens collected for three days, while the subject is on a diet containing 1 g of elemental calcium per day, and is in the hospital. This procedure, though time consuming, smooths out the effects of circadian rhythms and both allows control of the anion content and predicts with a high degree of accuracy which of our patients will be hypercalciumic as outpatients. We find that a cutoff value of 6 mmol per day when calcium gluconate is used as the calcium source is effective in predicting which patients will excrete >7.5 mmol of calcium per day as outpatients. Little additional information is obtained from studies with low-calcium diets, although they may help in the assessment of any overt renal leakage of calcium resulting from other clinical conditions.

The calcium oxalate loading experiment indicated, as expected, poor oxalate absorption and excretion because of the insolubility of this salt (22–24). In contrast, citric acid taken with calcium oxalate enhanced oxalate excretion significantly. Citrate, and possibly other hydroxy-carboxylic acids (21, 25–27), therefore have an important role in calcium nephrolithiasis in that they enhance ascorbate-induced oxalogenesis (1) as well as absorption and excretion of calcium and oxalate. In addition, these hydroxy-carboxylic acids, some of which are hypo-excreted in the urine in calcium stone-formers (1, 2), are inhibitors of stone formation and aggregation (28). With this combination of factors it is highly probable that calcium stone formation will occur (5, 7).

In conclusion: a primary defect in hydroxy-carboxylic acid absorption gives rise to a series of metabolic disturbances, all of which give an increased predisposition to calcium oxalate stone formation.

We thank the John P. Kelly Research Foundation for a grant supporting this project.

References