Hyperoxaluria in idiopathic calcium stone disease: further evidence of intestinal hyperabsorption of oxalate

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Summary

1. Seventeen healthy controls and 63 patients with idiopathic calcium stone disease of the urinary tract were investigated for urinary calcium and oxalate excretion and for $[14C]$oxalate intestinal absorption.

2. Under comparable controlled dietary intake a significant increase in calcium excretion was found in patients with stone disease. Oxalate excretion and $[14C]$oxalate intestinal absorption were mildly but not significantly increased. When patients with stone disease were subdivided into normocalciuric and hypercalciuric subjects, oxalate excretion and $[14C]$oxalate absorption were significantly increased in the latter. There was a significant direct relationship between calcium excretion and both oxalate excretion and $[14C]$oxalate absorption.

3. $[14C]$Oxalate absorption increased significantly in 22 stone-formers when dietary calcium was changed from normal to low.

4. The kinetics of $[14C]$oxalate intestinal absorption showed that the main difference between normocalciuric and hypercalciuric subjects occurred within the first 6 h after the oxalate-labelled meal.

5. These results confirm that mild hyperoxaluria is a frequent feature of idiopathic calcium stone disease even when patients and controls are studied under controlled dietary conditions. Our data are consistent with the hypothesis that hyperoxaluria is secondary to calcium hyperabsorption and is upper intestinal in origin.

Key words: calcium, intestinal absorption, oxalates, urinary calculi.

Introduction

Physicochemical and epidemiological evidence suggests that oxalate plays an important role in urolithiasis [1-4]. The occurrence and pathogenesis of increased urinary oxalate excretion in idiopathic calcium stone disease is controversial. Hyperoxaluria, from mild to moderate, has been reported as a frequent abnormality by some [4-8], whereas others have found urinary oxalate excretion to be normal [9-12].

A number of pathogenetic mechanisms have been proposed to explain this 'mild hyperoxaluria', including high animal protein intake [13], increased renal clearance of oxalate [7], a congenital defect in oxalate metabolism [14], and intestinal hyperabsorption [15]. As calcium stone disease appears to be linked epidemiologically to social conditions [16, 17], it seems reasonable to relate oxalate excretion to exogenous influences such as the animal protein intake. On the other hand, the intestinal hyperabsorption hypothesis is attractive since it relates increased oxalate excretion to hypercalciuria, a frequent finding in calcium stone disease [18-20]. The latter hypothesis, confirmed by some authors in recent studies [4, 21], has been contested by others who claim that only a small percentage of urinary oxalate can be accounted for by intestinal absorption from exogenous sources [22].

The aim of this paper is to report the results of an investigation performed on 80 subjects under controlled dietary conditions to evaluate the occurrence and pathogenesis of hyperoxaluria, with special reference to the intestinal absorption of oxalate.
Materials and methods

Eighty subjects were studied: 17 healthy volunteers (13 males and four females) and 63 patients (44 males and 19 females) who had a recent history of calcium-containing renal stones. Informed consent was obtained from all subjects. Age, body weight, serum calcium concentration, creatinine clearance and immunoreactive parathyroid hormone (IPTH) levels did not differ between the two groups (Table 1). None of the subjects had urinary tract infection at the time of the study. Urinary abnormalities were excluded in the patients by an intravenous pyelogram. Patients with resorptive hypercalciuria, assessed by high values of serum calcium, alkaline phosphatase, IPTH, fasting urinary calcium/creatinine ratio and unsuppressible hypercalciuria, were excluded as well.

After the subjects had collected at least one 24 h urine on a free home diet, they ate a controlled non-synthetic diet containing calcium (21.25 mmol), magnesium (4.8 mmol), sodium (100–120 mmol), phosphate (40 mmol), oxalate (0.7–1.1 mmol), protein (82 g), lipids (71 g), gluclides (183 g), with a calorie content of 7.11 kJ. Patients with resorptive hypercalciuria, assessed by high values of serum calcium, alkaline phosphatase, IPTH, fasting urinary calcium/creatinine ratio and unsuppressible hypercalciuria, were excluded as well.

Fasting blood samples for serum determinations were taken on day 5. In 22 patients the study was repeated on a diet containing only 3.75 mmol of calcium and 6.9 mmol of magnesium, 28 mmol of phosphate, 67 g of proteins, and almost unchanged in the other constituents. Calcium and creatinine were determined by routine methods, IPTH by a radioimmunnoassay (PTHK SORIN, Italy), which uses an antiserum against a COOH-terminal fragment.

Oxalate was determined by an automated colorimetric reaction with uranium and 4-(2-pyridylazo)resorcinol [24]. Values were corrected for incomplete recovery by a formula previously obtained by a [14C]oxalate dilution method [25].

For the [14C]oxalate measurements urinary aliquots from each collection were supersaturated with the addition of disodium oxalate (50 mmol/l); the precipitation step was performed in a boiling-water bath by the addition of CaCl2.2H2O. After cooling for 2 h oxalate was separated by double centrifugation. The dry calcium oxalate powder was subsequently dissolved in warm hydrochloric acid (1 mmol/l) and 18 ml of standard commercial scintillation fluid was added and the radioactivity measured in a LKB beta counter with quench correction by an external standard ratio. In selected supernatants no significant radioactivity was detected, indicating complete recovery of the labelled oxalate from urine.

Significance of differences was assessed by the Student’s t-test. For skewed values non-parametric tests were used, such as the Mann–Whitney rank sum U-test and the Wilcoxon T-test for matched pairs.

Results

As shown in Table 2, the main difference between normal subjects and stone-formers was in cal-

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**Table 1.** Data for control subjects and patients with stone disease

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 17)</th>
<th>Stone-formers (n = 63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41.6 ± 2.8</td>
<td>42.2 ± 1.3</td>
</tr>
<tr>
<td>Body wt. (kg)</td>
<td>69.6 ± 2.0</td>
<td>66.2 ± 1.1</td>
</tr>
<tr>
<td>Serum calcium (mmol/100 ml)</td>
<td>0.23 ± 0.01</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>Creatinine clearance (ml min⁻¹ 1.73 m⁻² body surface area)</td>
<td>106 ± 3.2</td>
<td>103 ± 2.0</td>
</tr>
<tr>
<td>IPTH (m-units/ml)</td>
<td>3.57 ± 0.33</td>
<td>4.24 ± 0.22</td>
</tr>
</tbody>
</table>

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**Table 2.** Calcium and oxalate urinary excretions and [14C]oxalate intestinal absorption in controls and idiopathic stone-formers

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 17)</th>
<th>Stone-formers (n = 63)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium excretion (mmol/24 h)</td>
<td>4.89 ± 0.35</td>
<td>6.05 ± 0.28</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Oxalate excretion (mmol/24 h)</td>
<td>0.40 ± 0.06</td>
<td>0.45 ± 0.03</td>
<td>N.S.</td>
</tr>
<tr>
<td>[14C]Oxalate absorption (% of oral dose)</td>
<td>14.4 ± 1.4</td>
<td>16.5 ± 1.2</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
TABLE 3. Calcium and oxalate urinary excretion and 14C-oxalate intestinal absorption in controls, normocalciuric stone-formers and hypercalciuric stone-formers

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 17)</th>
<th>Normocalciuric hypercalciuric subjects (n = 34)</th>
<th>Hypercalciuric subjects (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium excretion (mmol/24 h)</td>
<td>4.0 ± 0.06</td>
<td>4.52 ± 0.04</td>
<td>0.84 ± 0.02*</td>
</tr>
<tr>
<td>Oxalate excretion (mmol/24 h)</td>
<td>0.40 ± 0.03</td>
<td>0.38 ± 0.00</td>
<td>0.53 ± 0.01†</td>
</tr>
<tr>
<td>14C-Oxalate absorption (% of oral dose)</td>
<td>14.4 ± 1.4</td>
<td>13.2 ± 1.2</td>
<td>19.9 ± 1.7‡</td>
</tr>
</tbody>
</table>

* P < 0.001 for the difference of hypercalciuric stone-formers vs normals and normocalciuric stone-formers.
† P < 0.005 for the differences of hypercalciuric stone-formers vs normals and normocalciuric stone-formers.
‡ P < 0.001 for the difference of hypercalciuric stone-formers vs normocalciuric and P < 0.05 for hypercalciuric vs controls.

TABLE 4. Significance of differences, assessed by the Mann-Whitney rank sum U-test, in the kinetics of 14C-oxalate intestinal absorption

N.S., Not statistically significant.

<table>
<thead>
<tr>
<th>Intervals after 14C-oxalate administration (h)</th>
<th>Controls vs Normocalciuric patients</th>
<th>Controls vs Hypercalciuric patients</th>
<th>Normocalciuric vs Hypercalciuric patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td>N.S.</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2–4</td>
<td>N.S.</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4–6</td>
<td>N.S.</td>
<td>&lt;0.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>6–8</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>8–12</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>12–24</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>24–48</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Moreover, the differences in oxalate excretion and 14C-oxalate absorption remain the same even if values are corrected for body weight. The pattern of labelled oxalate excretion in the three groups is illustrated in Fig. 1. The differences between hypercalciuric and normal-normocalciuric subjects are significant only in the first 6 h after the oral administration (Table 4).

When calcium excretion is plotted both against oxalate excretion and 14C-oxalate absorption, direct significant relationships are found: the relation between urinary calcium and urinary oxalate is \( y = 0.037 \times + 0.217 \), \( r = 0.384 \), degrees of freedom = 78, \( P < 0.001 \). The relation between calcium excretion and 14C-oxalate intestinal absorption, both corrected for body weight, is \( y = 1.428 \times + 0.117 \), \( r = 0.38 \), degrees of freedom = 59, \( P < 0.01 \).

[14C]Oxalate intestinal absorption under normal and low calcium intakes in 22 stone-formers is shown in Table 5. The decrease in calcium excretion is associated with a significant increase of [14C]oxalate absorption.
Discussion
The rate of intestinal absorption of oxalate in normal subjects estimated by our radionuclide method was not significantly different from that given in previous reports [23, 27]. These results probably overestimate the absorption of dietary oxalate, but they provide more reproducible values than those from non-radionuclide methods, because of the greater reliability of $^{14}$C-oxalate measurements. Moreover, dietary oxalate intake during these tests is normal [28], thus avoiding unphysiological dietary loads of oxalate.

The mean value of oxalate excretion in normal subjects here reported is slightly increased as compared with that from previous reports [5, 6, 29]. Indeed, in this series three normal subjects were included who showed high values of oxalate excretion. However, in a previous study [8] we had found that oxalate excretion, determined by the described method, was high to a similar extent in some of the control subjects investigated under free home diet. So it was decided to maintain these mildly hyperoxaluric subjects in the control group.

Therefore the most striking difference between normal subjects and stone-formers, in this study, was in calcium excretion, which was significantly increased in the latter. Oxalate excretion was mildly but not significantly increased in the stone-former group. If the above high values of oxalate excretion are excluded from the control group, the difference becomes significant ($P < 0.05$) if compared with the mean value for the stone-formers on the whole, but it remains non-significant if compared with the normocalciuric patient subgroup.

These small changes cannot be secondary to differences in body weight, urine volume or dietary intake, since all these variables were the same in normal subjects and patients. The difference between normals and stone-formers, and within the patients when grouped as normocalciuric or hypercalciuric, were related to calcium excretion; the higher the levels of calcium excretion, the higher the values of oxalate excretion and $^{14}$C-oxalate absorption. As patients with secondary hypercalciuria, namely of the resorptive type [26], have been excluded from this study, the increased calcium excretion is likely to be, in part, intestinal in origin [30–32]. Our studies are consistent with the hypothesis that the bowel, probably in its upper portion, is the site where the association between calcium and oxalate excretion takes place. Thus:

(a) $^{14}$C-oxalate intestinal absorption was significantly increased when calcium intake was lowered;

(b) the difference in $^{14}$C-oxalate excretion in hypercalciuric patients is accounted for by an increased absorption in the first 6 h after the labelled meal and occurs therefore in the proximal portion of the small bowel, which is the site of selective hyperabsorption of calcium in idiopathic hypercalciuric subjects [33];

(c) in a group of enteric hyperoxaluric patients, who underwent similar investigations [34], the striking difference in $^{14}$C-oxalate absorption from that of the controls was due to an increase in the first 6 h and from 6 to 12 h after the oral meal. We suggest that this sustained increase is an expression of hyperabsorption of oxalate in the lower portions of the bowel. This delayed peak was not observed in our idiopathic hypercalciuric patients (Fig. 1).

In conclusion, the results of our study agree with those which report an increased oxalate excretion in renal calcium stone-formers. Among the pathogenetic mechanisms proposed to explain this mild increase, those which relate hyperoxaluria to exogenous influences seem so far more attractive. The hypotheses which relate hyperoxaluria to endogenous causes like metabolic defects in oxalate metabolism or renal leak of oxalate, need further experimental tests to be convincingly supported. On the other hand the hypothesis that high animal protein intake can cause the mild increase observed in stone-formers studied under their free home diet, has the advantage in linking oxalate, one of the main risk factors in the genesis of renal stones, to affluence, which seems to correlate with the prevalence of this disease.

In the present study the animal protein intake was fixed at equal amounts in all the subjects considered. Therefore absorptive hypercalciuria seems to be the main causative factor in these hyperoxaluric patients. Our data add further evidence to the hypothesis that in these patients a mechanism somewhat similar to that observed in enteric hyperoxaluria leads to a mild increase in urinary oxalate excretion.

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References
Hyperoxaluria and kidney stone disease


