Evidence for net renal tubule oxalate secretion in patients with calcium kidney stones

Kristin J. Bergsland,1 Anna L. Zisman,1 John R. Asplin,2 Elaine M. Worcester,1 and Fredric L. Coe1
1Department of Medicine, Nephrology Section, The University of Chicago and 2Litholink Corporation, Chicago, Illinois
Submitted 13 July 2010; accepted in final form 30 November 2010

Bergsland KJ, Zisman AL, Asplin JR, Worcester EM, Coe FL. Evidence for net renal tubule oxalate secretion in patients with calcium kidney stones. Am J Physiol Renal Physiol 300: F311–F318, 2011. First published December 1, 2010; doi:10.1152/ajprenal.00411.2010.—Little is known about the renal handling of oxalate in patients with idiopathic hypercalciuria (IH). To explore the role of tubular oxalate handling in IH and to evaluate whether differences exist between IH and normal controls, we studied 19 IH subjects, 8 normal subjects, and 2 bariatric stone formers (BSF) during a 1-day General Clinical Research Center protocol utilizing a low-oxalate diet. Urine and blood samples were collected at 30- to 60-min intervals while subjects were fasting and after they ate three meals providing known amounts of calcium, phosphorus, sodium, protein, oxalate, and calories. Plasma oxalate concentrations and urine oxalate filtered loads were similar between patients (includes IH and BSF) and controls in both the fasting and fed states. Urinary oxalate excretion was significantly higher in patients vs. controls regardless of feeding state. Fractional excretion of oxalate (FEOx) was >1, suggesting tubular secretion of oxalate, in 6 of 19 IH and both BSF, compared with none of the controls (P < 0.00001). Adjusted for water extraction along the nephron, urine oxalate rose more rapidly among patients than normal subjects with increases in plasma oxalate. Our findings identify tubular secretion of oxalate as a key mediator of hyperoxaluria in calcium stone formers, potentially as a means of maintaining plasma oxalate in a tight range.

Patients with idiopathic calcium nephrolithiasis are known to exhibit a wide range of urine oxalate excretions (1, 5, 7, 34), arising from a mixture of increased production by the liver and increased net gastrointestinal (GI) absorption of oxalate. Increased production by the liver may be due to biological variability (24) or ingestion of oxalate precursors such as animal proteins (11–14, 20, 32). Increased net GI absorption (9, 38) may stem from a low-calcium diet (6, 34), dietary oxalate excess (13, 14), alterations in intestinal flora (18, 31, 36), or from variability in anion transporter function (16), possibly leading to a decreased secretion of oxalate into the GI tract. There is no doubt that even modest upward excursions of urine oxalate can increase urine calcium oxalate (CaOx) supersaturation (7, 25) and promote growth of CaOx stones. In the extreme example of systemic diseases such as enteric hyperoxaluria and primary hyperoxaluria, for instance, massive oxalate excretion is associated with significant nephrocalcinosis and frequent stone formation (15, 26).

Whatever the origin of the increased oxalate, renal oxalate removal must involve oxalate filtration and some component of tubule handling. To date, little is known about how the kidney manages the excretion of oxalate presented for removal. In humans, prior studies of oxalate handling have demonstrated both reabsorption and secretion by the kidney, depending on the choice of subjects and experimental conditions (10, 19, 30). However, it remains unclear whether differences exist between stone formers and healthy controls, and whether these differences exist over the range of oxalate excretions seen in idiopathic stone formers. In such patients, does an increased load of oxalate simply raise the blood oxalate level and therefore filtered load, or do the kidney tubules vary their rates of reabsorption and secretion of filtered oxalate so as to maintain a relatively constant plasma oxalate level despite changing oxalate loads?

Our purpose was to test the most obvious hypothesis: proximal tubule cells have anion exchangers that can drive bidirectional oxalate transport; given that oxalate in the blood poses a potential hazard for tissue crystallizations (43), one might imagine that renal biology has adapted to keep plasma levels from rising via a secretory response when larger than usual amounts of oxalate must be removed from the system. If this were true, then plasma oxalate concentration should be similar among subjects with a wide range of renal oxalate excretion.

Studies to date have been limited by the lack of a reliable plasma oxalate assay, and the normal concentration of plasma oxalate in humans remains controversial (8, 17, 22, 27, 28, 40). We used a sensitive and reproducible measurement of plasma oxalate concentration, which permits assessment of filtered load and net tubule oxalate reabsorption or secretion. We studied hypercalciuric subjects and healthy controls during a three-meal-day on a moderately low-oxalate diet in the General Clinical Research Center (GCRC). Subjects were selected only for hypercalciuria, so urine oxalate levels were neither inclusion nor exclusion criteria. In pursuing this work, we have asked three central questions: 1) do plasma oxalate levels remain constant over a wide range of renal oxalate excretion?; 2) is oxalate secretion specifically related to higher vs. lower oxalate excretion rates within our subjects?; and 3) do stone formers and controls differ from one another?

Methods

Patients and normal subjects. Nineteen hypercalciuric subjects (12 men, 7 women) were compared with 8 normal healthy controls (4 men, 4 women) (Table 1). Subjects were selected for idiopathic hypercalciuria (IH), which was diagnosed by 24-h urine calcium >140 mg/g creatinine. We also studied two male bariatric stone formers (BSF): one had a Roux-en-Y gastric bypass procedure, and the other had a duodenal switch biliopancreatic diversion. Four of the 19 IH subjects and both BSF were hyperoxaluric (24-h urine oxalate ≥45 mg) on self-selected diets (Table 1). Five of the 19 IH subjects and both BSF were hyperoxaluric on the GCRC diet (Table 1). Seventeen of 19 IH patients and both BSF reported passing stones. Stone analysis was reported for 12 IH subjects: 9 had CaOx stones, 2 had stones composed of >65% calcium phosphate (CaP), and 1 had
Table 1. Patients and normal subjects

<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Status</th>
<th>Age, yr</th>
<th>Sex</th>
<th>Weight, kg</th>
<th>Height, cm</th>
<th>CCr, ml/min</th>
<th>PreTx UOx</th>
<th>CRC UOx</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normala-d</td>
<td>47</td>
<td>F</td>
<td>86</td>
<td>160</td>
<td>81</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>Normala-d</td>
<td>44</td>
<td>F</td>
<td>55</td>
<td>158</td>
<td>62</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>Normala-d</td>
<td>26</td>
<td>F</td>
<td>68</td>
<td>168</td>
<td>102</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>Normala-d</td>
<td>55</td>
<td>F</td>
<td>67</td>
<td>169</td>
<td>107</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>Normala-d</td>
<td>28</td>
<td>M</td>
<td>66</td>
<td>163</td>
<td>116</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>Normala-d</td>
<td>48</td>
<td>M</td>
<td>80</td>
<td>175</td>
<td>151</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>Normala-d</td>
<td>55</td>
<td>M</td>
<td>88</td>
<td>181</td>
<td>115</td>
<td>2</td>
<td>35</td>
</tr>
<tr>
<td>8</td>
<td>Normala-d</td>
<td>44</td>
<td>M</td>
<td>85</td>
<td>178</td>
<td>110</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>43 ± 6</td>
<td></td>
<td>74 ± 5</td>
<td>169 ± 3</td>
<td>106 ± 9</td>
<td>27 ± 5</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>IHSF</td>
<td>41</td>
<td>M</td>
<td>80</td>
<td>168</td>
<td>139</td>
<td>35</td>
<td>45</td>
</tr>
<tr>
<td>10</td>
<td>IHSF</td>
<td>48</td>
<td>M</td>
<td>102</td>
<td>185</td>
<td>150</td>
<td>85</td>
<td>73a</td>
</tr>
<tr>
<td>11</td>
<td>IHSF</td>
<td>59</td>
<td>M</td>
<td>85</td>
<td>177</td>
<td>137</td>
<td>43</td>
<td>46</td>
</tr>
<tr>
<td>12</td>
<td>IHSF</td>
<td>64</td>
<td>M</td>
<td>89</td>
<td>173</td>
<td>101</td>
<td>33</td>
<td>36</td>
</tr>
<tr>
<td>13</td>
<td>IHSF</td>
<td>66</td>
<td>M</td>
<td>88</td>
<td>185</td>
<td>119</td>
<td>23</td>
<td>30</td>
</tr>
<tr>
<td>14</td>
<td>IHSF</td>
<td>56</td>
<td>F</td>
<td>62</td>
<td>162</td>
<td>88</td>
<td>65</td>
<td>72a</td>
</tr>
<tr>
<td>15</td>
<td>IHSF</td>
<td>55</td>
<td>M</td>
<td>87</td>
<td>168</td>
<td>113</td>
<td>37</td>
<td>41a</td>
</tr>
<tr>
<td>16</td>
<td>IHSF</td>
<td>58</td>
<td>F</td>
<td>58</td>
<td>158</td>
<td>66</td>
<td>25</td>
<td>22*</td>
</tr>
<tr>
<td>17</td>
<td>IH</td>
<td>30</td>
<td>M</td>
<td>75</td>
<td>179</td>
<td>131</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>18</td>
<td>IHSF</td>
<td>44</td>
<td>M</td>
<td>91</td>
<td>181</td>
<td>137</td>
<td>29</td>
<td>33</td>
</tr>
<tr>
<td>19</td>
<td>IHSF</td>
<td>45</td>
<td>F</td>
<td>57</td>
<td>164</td>
<td>103</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>20</td>
<td>IHSF</td>
<td>40</td>
<td>F</td>
<td>82</td>
<td>164</td>
<td>112</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>21</td>
<td>IHSF</td>
<td>27</td>
<td>M</td>
<td>77</td>
<td>174</td>
<td>116</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>22</td>
<td>IHSF</td>
<td>48</td>
<td>M</td>
<td>75</td>
<td>182</td>
<td>127</td>
<td>51</td>
<td>48</td>
</tr>
<tr>
<td>23</td>
<td>IHSF</td>
<td>22</td>
<td>M</td>
<td>93</td>
<td>182</td>
<td>121</td>
<td>32</td>
<td>38</td>
</tr>
<tr>
<td>24</td>
<td>IHSF</td>
<td>52</td>
<td>F</td>
<td>61</td>
<td>158</td>
<td>88</td>
<td>29</td>
<td>33a</td>
</tr>
<tr>
<td>25</td>
<td>IHSF</td>
<td>42</td>
<td>F</td>
<td>68</td>
<td>168</td>
<td>81</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>26</td>
<td>IHSF</td>
<td>44</td>
<td>M</td>
<td>85</td>
<td>182</td>
<td>122</td>
<td>26</td>
<td>30</td>
</tr>
<tr>
<td>27</td>
<td>IH</td>
<td>30</td>
<td>F</td>
<td>103</td>
<td>160</td>
<td>143</td>
<td>45</td>
<td>41*</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>45 ± 3</td>
<td></td>
<td>80 ± 3</td>
<td>172 ± 2</td>
<td>115 ± 5</td>
<td>35 ± 4</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>28</td>
<td>Bariatric</td>
<td>49</td>
<td>M</td>
<td>93</td>
<td>174</td>
<td>91</td>
<td>130</td>
<td>74*</td>
</tr>
<tr>
<td>29</td>
<td>Bariatric</td>
<td>48</td>
<td>M</td>
<td>101</td>
<td>181</td>
<td>119</td>
<td>140</td>
<td>53*</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>49 ± 0.4</td>
<td></td>
<td>97 ± 0.9</td>
<td>177 ± 4</td>
<td>105 ± 14</td>
<td>135 ± 5</td>
<td>64 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE: M, male; F, female; IH, idiopathic hypercalciuria; IHSF, idiopathic hypercalciuria with stone formation. Urine oxalate excretion (UOx) is in mg/24 h measured on the study day (CRC) or, for IH, IHSF, and bariatric subjects in clinical pretreatment (PreTx) 24-hr urine collections. CCr is creatinine clearance in ml/min. Means did not differ significantly among IH, IHSF, and normal subjects in any category. Bariatric subjects were heavier than normal subjects (*P < 0.05) and excreted more oxalate than either IHSF or normal subjects on the CRC day (**P < 0.05, both comparisons). Subject previously reported in Ref. 41. aSubject previously reported in Ref. 3. bSubject is a secretor of oxalate.

One CaOx and one CaP stone. Both BSF had CaOx stones. For five IH subjects, stones were reported as “calcium” by the patient. No subject had diabetes, or any systemic or renal disease, as a cause or consequence of stones. This study was approved by the Institutional Review Board at the University of Chicago (protocol 12881A).

Protocol. Subjects were studied in the GCRC at the University of Chicago over 14 h as previously described (3, 41, 42). Upon admission to the GCRC between 6:00 and 7:00 AM, two 1-h fasting urine samples were collected. Subsequently, three meals were provided, accompanied by hourly urine collections until 3 h after the last meal (14 urine samples total). Matching blood samples were collected hourly, and also every half-hour in the 2 h following meals, for a total of 21 samples.

Diet. The basal 1,800 kcal/day study diet consisted of three isocaloric meals composed of common foods and calculated to provide a total of 1,200 mg each of calcium and phosphorus and 2,000 mg of sodium, evenly distributed among the meals (42). Subjects were stratified to one of three caloric levels (1,800, 2,100, or 2,400 kcal/day) based on an estimate of individual energy needs. The 1,800-kcal base diet provided a total of 92 mg oxalate (personal communication, Dr. Ross Holmes). Subjects were instructed by a dietician to follow a similar diet for 5 days before admission to the GCRC to facilitate dietary equilibration before the study day.

Laboratory measurements. Serum and urine creatinine were measured in our laboratory using a modified Jaffé method on a Beckman DxC 600 analyzer (CREA 442760, Beckman Coulter, Brea, CA). Serum samples were ultrafiltrated using Amicon Ultra-4 filter tubes with a 10-kDa molecular mass cutoff membrane (Millipore, Bedford, MA); creatinine was measured in the ultrafiltrate using the same method as for serum. Urine oxalate was measured on the Beckman analyzer by an enzymatic method utilizing oxalate oxidase (Trinity Biotech, Bray, Ireland).

Plasma oxalate was measured by ion chromatography (IC) using a method similar to that previously reported (16). Before plasma preparation, Amicon Ultra-4 filter tubes were prefreshed as follows: 1 ml 0.1 N NaOH was added to the tube; after 5 min at room temperature, the NaOH was removed by pipetting and replaced with 1 ml deionized water, which was removed and again replaced with 1 ml deionized water; tubes were centrifuged for 15 min at 6,500 rpm at 4°C in a refrigerated centrifuge, and the filtrate was discarded. Blood was drawn into a heparinized Vacutainer tube that was prechilled on ice and kept cold throughout the procedure. After the blood draw, the tube was returned to ice for at least 5 min, and then blood was separated by centrifugation for 5 min at 1,000 g at 4°C. Plasma was removed to a prepared Amicon Ultra-4 filter tube and centrifuged for 20 min at 6,500 rpm at 4°C. The plasma ultrafiltrate was acidified by adding 1 M hydrochloric acid to a final concentration of 50 mM. Samples were stored frozen at −20°C until analysis.

A Dionex DX-500 ion chromatography system (Dionex, Sunnyvale, CA) was equipped with an AG11 guard column and an AS11 analytic column in series, and a 200-µl injection loop. The mobile phase was NaOH at a flow rate of 2 ml/min. Plasma ultrafiltrate was diluted 1:4 with 0.6 M boric acid, pH 2.5 before injection. We detected ion peaks using a conductivity meter with the eluent background conductivity suppressed using an anion self-regenerating suppressor (ASRS 300, Dionex). Chromeleon Software (version 6.80, Dionex) was used to calculate the concentration of oxalate in the plasma.
For the purposes of assay validation, plasma samples from 3 people were run at least 4 times on 1 day and then at least 12 times over 3 separate days to determine intra-assay and interassay variabilities, respectively. For the three subjects, intra-assay coefficients of variation (CV) were 5.4, 2.0, and 1.3%, and interassay CV were 5.3, 12.0, and 15.9%. These levels of precision (mean intra-assay CV of 2.9%; mean interassay CV, 11.1%) were satisfactory according to the Clinical and Laboratory Standards Institute guidelines (EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods). Accuracy of the method was determined by replicate analysis of control sodium oxalate solutions at three concentrations (1, 2, and 4 µM) within the range of the assay standard curve (0.5–5 µM oxalate). A minimum of 16 determinations/concentration were performed over 10 days. The deviation of the mean from the true value (mean percent error) serves as a measure of accuracy; mean values should be within 15% of actual values. The mean percent error for each of the controls we measured was 9.3, 4.6, and 3.4%, which constitutes a satisfactory level of accuracy.

Calculations. Fractional excretion of oxalate was calculated conventionally

$$FEOx = \frac{(UOx \times V)}{(POx \times GFR)}$$

where Uox and Pox are urine and plasma oxalate molarity, V is urine flow rate, and GFR is glomerular filtration rate indexed here as creatinine clearance calculated from ultrafilterable serum creatinine and urine creatinine concentrations. Equation 1 simplifies to

$$FEOx = \frac{(UOx \times UFcr)}{(POx \times Ucr)}$$

where UFcr and Ucr are plasma ultrafilterable and urine creatinines. FEOx contains two separable terms: Uox/(Ucr/UFcr) and Pox. The former is essentially the urine oxalate molarity corrected for water extraction (Adj Uox). If from this term one subtracts Pox, the result is a way of expressing transepithelial oxalate concentration difference, TTOx

$$TTOx = Adj\ UOx - POx$$

Whereas FEOx is the ratio of oxalate filtration to filtered load, TTOx is the minimum concentration difference tubule cells must have produced between plasma and the tubule fluid, and has the units micromoles per liter. Although oxalate is mainly transported in the proximal tubule, TTOx tracks all transport by the renal cells. By correcting for later water removal, we can estimate the magnitude and direction of the changes in luminal oxalate concentration produced by tubule transport. We use FEOx and TTOx in our presentation because, though related, they express somewhat different properties of renal tubule transport.

Statistical analysis. ANOVA was performed to test for differences in determinants of oxalate handling between patients and controls during meal periods, and also fasting or in the fed state (averaged over all meals). Since samples were collected at multiple time points within each period, analysis was performed on statistical mean files that were generated to condense the data for each person to one line per period or state. Differences by gender within fasting and fed periods were also compared for all subjects and N. Linear regression was used to assess the relationship between methods of oxalate measurement (ion chromatography vs. enzymatic), and between urine oxalate excretion and plasma oxalate. All statistical calculations were performed using Systat 12 software (Systat Software, Chicago, IL).

RESULTS

Our intent was to study renal oxalate handling in patients and normal subjects among whom we expected a reasonably wide variation of urine oxalate excretion. As noted in the first part of this study and methods, we included two bariatric surgery cases to enlarge our oxalate excretion range; they are shown by separate symbols. Because we analyzed hypercalciuric and bariatric patients together, we use the term patient (P) to represent all noncontrols. As previously noted, our principal question was whether plasma oxalate remains stable over a wide range of urinary oxalate excretion.

Plasma oxalate. Mean plasma oxalate values of P and N overlapped either as individuals (Fig. 1, top left) or as mean values across meal periods (Fig. 2, top left). Plasma oxalate remained stable in both the fasting and fed states (Fig. 1, top left) and across meal periods (Fig. 2, top left).

Oxalate excretion. Oxalate excretion by P, either as individuals (Fig. 1, top right) or as mean values within meal periods (Fig. 2, top right) exceeded that of N (Table 2). Excretion did not vary between the fasting and fed states (Fig. 1, top right) or by meal period (Fig. 2, top right). Values for oxalate filtered load for P and N (Fig. 2, bottom left) overlapped; mean values did not differ. Thus, even though oxalate excretion was significantly greater in P than in N, plasma values did not differ, as noted above. In other words, differences in urinary oxalate excretion were not at the expense of plasma oxalate.

Renal oxalate. Mean FEOx was higher in patients (0.96 ± 0.06, means for all periods ± SE) than in normal subjects (0.68 ± 0.10, P = 0.025) (Fig. 1, bottom left and Fig. 2, bottom right; Table 2). Our alternate approach to tubule handling, TTOx, or the difference between urine oxalate molarity adjusted for water extraction and plasma oxalate molarity, also was higher in P (−0.17 ± 0.19, means for all periods ± SE) than in N (−1.12 ± 0.30, P = 0.014) (Fig. 1, bottom right; Table 2). Despite the higher mean values for FEOx and TTOx in P vs. N, oxalate filtered loads and plasma oxalate did not differ (Fig. 2, left; Table 2). The secretory trait was generally stable, so in most individuals it was either present or not present regardless of feeding state (Fig. 1, bottom). All in all, secretion was evident in 6 of 19 hypercalciuric patients (32%) and in both bariatric patients (8 subjects in all), but not in any normal subjects. There was no difference between men and women in any of the measures in the tables and figures (data not shown).

Because FEOx and TTOx share the core expression of adjusted urine oxalate molarity, they must covary (Fig. 3). Four hypercalciuric subjects and both of the bariatric patients had mean TTOx values > 0, and all six had FEOx values > 1 (Fig. 3, top right quadrant of figure). These six were among the highest in urine oxalate excretion rates, signified by symbol size. Two additional patients had FEOx values > 1, without a positive TTOx value; their oxalate excretions were not remarkably high. All N and the rest of P had modest oxalate excretions and lie in the bottom left quadrant of the graph in Fig. 3. One subject with 30–40 µM/h of oxalate excretion (largest black circle in bottom left quadrant) was a patient who did not secrete oxalate. Overall, secretion segregates with increased urine oxalate excretion, and our two measures of secretion correlate very well. Note that counts from Figs 1 and 3 do not tally, because Fig. 1 is by-meal period and Fig. 3 is the mean for fasting and fed time periods.

Relationship of plasma oxalate to renal oxalate handling. Our studies were not designed to explore mechanisms by which tubule transport might respond to oxalate load, but from the core value of adjusted urine oxalate and the independent measurement of plasma oxalate, one can visualize at least the outlines of the process that must occur (Fig. 4). In this figure,
we use not mean values but all values for all subjects. Urine oxalate excretion is gauged by symbol size. Among patients (black circles), adjusted urine oxalate rises steeply with plasma oxalate (tilt of ellipse; regression coefficient $1.026_{1.07}$, standardized coefficient $0.66$, $P < 0.001$), and many points lie above the diagonal line of identity, meaning secretion. Among N (tiny grey circles), the slope was much less ($0.178_{0.04}$, standardized coefficient $0.422$; $P < 0.001$), though significant. Only five points from three of the normal subjects exceeded the line of identity. Of note, the very largest symbols (representing the highest oxalate excretion) overlap with the smaller symbols for any given plasma oxalate value; however, secretion is clearly highly correlated with increased urinary oxalate excretion. To assess the difference in slopes, we constructed a general linear model with adjusted urine oxalate as dependent, and plasma oxalate and subject type (patient vs. normal) as independent variables. The cross product of plasma oxalate by subject type was highly significant ($P < 0.001$). In other words, as plasma oxalate increases, urine oxalate adjusted for water extraction rises more in patients than in normal subjects; we interpret these findings as evidence for physiological protection of plasma oxalate in a tight range.

To date there has been much controversy as to human renal handling of oxalate. In one study using a fasting 2-h urine sample and a single blood measurement, stone formers ($n = 38$) secreted oxalate while normal subjects ($n = 23$) were noted to be reabsorbing oxalate (30). These subjects were on self-selected diets on which information is not available. More recently, a study of normal individuals administered increasing doses of soluble oxalate showed transient increases in serum oxalate, also correlating with increases in urinary excretion (10). Clearance ratios in that challenge study indicate the presence of renal oxalate secretion, which accounted for up to 50% of total urinary oxalate excretion with the highest experimental oxalate load (8 mmol). In a follow-up study of normal

DISCUSSION

Variations in oxalate excretion must arise from differences in oxalate production (24) or net gastrointestinal oxalate absorption (9, 18, 31, 36, 38). Although we selected all but the BSF patients for hypercalciuria, we found that they also differ from the control population with regard to their renal oxalate handling. Despite similarly low daily dietary oxalate intake in the GCRC, patients, most of whom were stone formers, excreted significantly more oxalate throughout the day. This is not surprising, as previous investigators have noted a 20–50% prevalence of at least mild hyperoxaluria in the general stone-forming population (2, 5, 23). Unlike previous studies, however, our data demonstrate a critical role of secretion in accounting for the increased excretion in this population, as approximately one-third of patients were found to be secreting oxalate, with a strong correlation between the highest oxalate excretions and renal tubule secretion. As the plasma oxalate was similar between patients and normal subjects, we interpret these findings as evidence for physiological protection of plasma oxalate in a tight range.
subjects and idiopathic CaOx stone formers with the same protocol, the investigators did not document a difference between normal subjects and stone formers in plasma oxalate, urinary oxalate excretion, and clearance ratios, all of which rose with increasing oxalate loads in both groups (19). This is quite different from our protocol, in which all subjects received a moderately low-oxalate diet, and plasma oxalate remained stable throughout the course of the day. Furthermore, we controlled the diet of these individuals, including all variables that could impact oxalate handling in the kidney, such as magnesium, calcium, sodium, and chloride. Under these strictly controlled conditions, we were able to detect the presence of secretion in a significant number of hypercalciuric subjects in the absence of an increased plasma oxalate or filtered load.

The cause of the secretion in this subset of patients is unclear, but the finding of variable urinary oxalate excretion with stable plasma oxalate levels supports the concept of tight regulation of blood oxalate levels. It is possible that the mechanism to achieve this is via an as yet unidentified enteric

Table 2. Oxalate excretion and tubule handling

<table>
<thead>
<tr>
<th></th>
<th>Normal-Patient</th>
<th>Fed</th>
<th>Fasting-Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Ox, µM</td>
<td>0.03 (−0.60 to 0.66)</td>
<td>0.14 (−0.59 to 0.87)</td>
<td>−0.11 (−0.90 to 0.68)</td>
</tr>
<tr>
<td>Urine Ox, µM/h</td>
<td>−5.11 (−9.83 to −0.40)*</td>
<td>−7.04 (−12.05 to −2.04)†</td>
<td>−0.79 (−3.79 to 2.20)</td>
</tr>
<tr>
<td>TTOx, µM</td>
<td>−0.69 (−1.25 to −0.13)*</td>
<td>−0.99 (−1.65 to −0.34)†</td>
<td>0.08 (−0.57 to 0.72)</td>
</tr>
<tr>
<td>FEOx</td>
<td>−0.21 (−0.38 to −0.04)*</td>
<td>−0.28 (−0.45 to −0.11)†</td>
<td>0.01 (−0.16 to 0.14)</td>
</tr>
<tr>
<td>Filtered load Ox, µM/h</td>
<td>−1.17 (−5.21 to 2.88)</td>
<td>−1.21 (−4.82 to 2.39)</td>
<td>−1.47 (−5.02 to 2.07)</td>
</tr>
<tr>
<td>Adjusted urine Ox, µM</td>
<td>−0.64 (−1.31 to 0.03)</td>
<td>−0.84 (−1.54 to −0.15)*</td>
<td>0.00 (−0.45 to 0.46)</td>
</tr>
</tbody>
</table>

Values are differences in means with 95% confidence intervals in parentheses. TTOx and FEOx, transepithelial oxalate concentration difference and fractional excretion of oxalate, respectively. Columns 2 and 3 depict changes between normal subjects and patients (including bariatric patients) within fasting and fed states; columns 4 and 5 depict changes between fasting and fed states within normal and patient groups. *Differs from 0, P < 0.05. †Differs from 0, P < 0.01.
stimulus, similar to that seen with phosphorus, for example (4). However, this is less likely as secretion was found to be a stable trait demonstrated in both the fasting and fed states, and no differences were noted with the meal periods. Alternatively, there may be variability in the sensitivity of the anion transporters responsible for oxalate transport, which include several representatives of the soluble carrier family 26 (SLC26). Support for the latter hypothesis comes from evidence of severe hyperoxaluria and nephrolithiasis in mice missing the SLC26A6 transporter (16). Expressed on the apical surface of the proximal tubule, SLC26A6 exchanges chloride for the highest affinity for oxalate (39). Because the same transporter is also expressed in the intestine and the SLC26A6 knockout mice showed high urinary oxalate excretion which fell with decreased dietary oxalate (16), the study supports increased net GI absorption of oxalate (via decreased secretion) as the etiology for hyperoxaluria. Unlike our subjects, however, the knockout mice experienced a rise in plasma oxalate, suggesting that renal elimination solely via the filtered load was insufficient to maintain stable oxalate levels. It is thus plausible that variability in the sensitivity of the renal transporters could be accounting for the secretion demonstrated in our population.

The identification of the secretory trait in only 6 of the 19 nonbariatric patients suggests the possible presence of two distinct groups of calcium stone formers in our study and in the general population of CaOx stone formers. It is important to note that most studies of stone formers in the literature have included heterogeneous patient populations, with variable urinary oxalate excretions. While no studies to date have addressed the potential differences in renal handling between the hyperoxaluric and nonhyperoxaluric CaOx stone formers, some inferences can be made from a loading study of two groups of recurrent CaOx stone formers in which patients that were hyperoxaluric while fasting demonstrated a greater rise in urinary oxalate excretion with an oral oxalate load than those that had normal oxalate excretions at baseline (21). As with the studies of the SLC26A6 knockout mouse, these results were interpreted to support a role for increased net GI oxalate absorption, which could stem from decreased secretion of oxalate into the GI tract. Based on our data, however, it is plausible that the increased urinary oxalate excretion is at least in part due to renal oxalate secretion, and could reflect variability in the oxalate transporters in this population. Interestingly, in the study by Schwille et al. (30) mentioned previously, the investigators noted the presence of the secretory trait only in their patients who were hypercalciuric and not in other calcium stone formers. This is consistent with our findings, although our patients were selected for hypercalciuria, so we cannot draw a comparison. However, these findings lend support to our hypothesis of variability in transporter function as an etiology for secretion. Our group has previously demonstrated abnormalities in proximal tubule mineral handling as an etiology for IH in calcium stone formers (42). As renal oxalate transporters have been localized to the proximal tubule, it is plausible that variability in proximal tubule function accounts for both hypercalciuria and hyperoxaluria.

Unlike previously published work (21, 29, 38), we did not demonstrate an effect of meals on oxalate excretion, as rates of urinary oxalate excretion remained stable throughout the day. This could be due to the relatively low oxalate content of the study diet; however, there is experimental evidence for increased fractional GI absorption of oxalate with lower oxalate intake (13), although this is controversial (37, 44). Notably, the secretory trait in our population of stone formers was stable whether in the fasting or fed state.

The two BSF in our study demonstrated markedly elevated urinary oxalate excretion on self-selected diets, which was halved with the administration of the low-oxalate GCRC diet. This is in line with the extreme and variable hyperoxaluria after urinary oxalate excretion with an oral oxalate load than those that had normal oxalate excretions at baseline (21). As with the studies of the SLC26A6 knockout mouse, these results were interpreted to support a role for increased net GI oxalate absorption, which could stem from decreased secretion of oxalate into the GI tract. Based on our data, however, it is plausible that the increased urinary oxalate excretion is at least in part due to renal oxalate secretion, and could reflect variability in the oxalate transporters in this population. Interestingly, in the study by Schwille et al. (30) mentioned previously, the investigators noted the presence of the secretory trait only in their patients who were hypercalciuric and not in other calcium stone formers. This is consistent with our findings, although our patients were selected for hypercalciuria, so we cannot draw a comparison. However, these findings lend support to our hypothesis of variability in transporter function as an etiology for secretion. Our group has previously demonstrated abnormalities in proximal tubule mineral handling as an etiology for IH in calcium stone formers (42). As renal oxalate transporters have been localized to the proximal tubule, it is plausible that variability in proximal tubule function accounts for both hypercalciuria and hyperoxaluria.

Unlike previously published work (21, 29, 38), we did not demonstrate an effect of meals on oxalate excretion, as rates of urinary oxalate excretion remained stable throughout the day. This could be due to the relatively low oxalate content of the study diet; however, there is experimental evidence for increased fractional GI absorption of oxalate with lower oxalate intake (13), although this is controversial (37, 44). Notably, the secretory trait in our population of stone formers was stable whether in the fasting or fed state.

The two BSF in our study demonstrated markedly elevated urinary oxalate excretion on self-selected diets, which was halved with the administration of the low-oxalate GCRC diet. This is in line with the extreme and variable hyperoxaluria after
bariatric surgery that has been previously described by our group (1) and others (26, 33). Notably, this is the first report documenting renal oxalate secretion as a mechanism for the hyperoxaluria seen in BSF. While we were only able to include two BSF in our protocol, the magnitude of the decline in oxalate excretion with a low-oxalate diet raises the possibility that the transporters in the gut or the kidney are upregulated by high baseline oxalate intake in the BSF population. However, in this case we would have anticipated a drop in blood oxalate, as the transporters would be overactive for the low degree of oxalate intake in our protocol. Clearly, this is an area that requires more investigation.

Our work has a number of limitations. First, we did not measure GI absorption of oxalate, so we do not know whether differences in GI absorption of oxalate between IH and N were present. GI absorption has been reported to be greater in IH (9, 38), although this is controversial (19). Second, we did not ask our subjects to complete food diaries for 5 days before admission, so it is possible that there was significant variability in the pretest diet. This could impact sensitivity of the GI and renal transporters and manifest as secretion. However, the low-oxalate/high-calcium diet in our protocol would limit the effect of GI absorption in our subjects. Furthermore, based on the pre-GCRC 24-h urine sodium excretions (42), we know that there were no differences in compliance between P and N, so it is unlikely that the detected differences in FEOX and TTOX are secondary to dietary differences. Similarly, epidemiological evidence showed no difference in oxalate ingestion on self-selected diets between idiopathic stone formers and controls (35). We also are unable to comment on the bacterial colonization status of our subjects, as this was not studied. Even so, none of these could affect our major finding of overlapping plasma oxalate between P and N.

In summary, to our knowledge, this is the first study to document renal oxalate secretion as a critical mediator of the increased oxalate excretion in hyperoxaluric idiopathic calcium stone formers. Our work suggests that plasma oxalate is maintained reasonably constant under varying conditions of oxalate excretion via changes in renal reabsorption or secretion. Further studies are necessary to determine the signaling responsible for these phenomena and the mechanisms involved in the communication between the multiple organ systems involved in oxalate homeostasis.

ACKNOWLEDGMENTS

The authors thank the patients and normal subjects for participating and the nursing staff of the University of Chicago GCRC for expert assistance.

GRANTS

This publication was made possible by National Institutes of Health (NIH) Grant P01 DK56788 and by Grant U1L RR024999 to the University of Chicago GCRC from the National Center for Research Resources (NCRR), a component of the NIH, and NIH Roadmap for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


