Effect of Potassium Citrate on Calcium Phosphate Stones in a Model of Hypercalciuria

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ABSTRACT

Potassium citrate is prescribed to decrease stone recurrence in patients with calcium nephrolithiasis. Citrate binds intestinal and urine calcium and increases urine pH. Citrate, metabolized to bicarbonate, should decrease calcium excretion by reducing bone resorption and increasing renal calcium reabsorption. However, citrate binding to intestinal calcium may increase absorption and renal excretion of both phosphate and oxalate. Thus, the effect of potassium citrate on urine calcium oxalate and calcium phosphate supersaturation and stone formation is complex and difficult to predict. To study the effects of potassium citrate on urine supersaturation and stone formation, we utilized 95th-generation inbred genetic hypercalciuric stone-forming rats. Rats were fed a fixed amount of a normal calcium (1.2%) diet supplemented with potassium citrate or potassium chloride (each 4 mmol/d) for 18 weeks. Urine was collected at 6, 12, and 18 weeks. At 18 weeks, stone formation was visualized by radiography. Urine citrate, phosphate, oxalate, and pH levels were higher and urine calcium level was lower in rats fed potassium citrate. Furthermore, calcium oxalate and calcium phosphate supersaturation were higher with potassium citrate; however, uric acid supersaturation was lower. Both groups had similar numbers of exclusively calcium phosphate stones. Thus, potassium citrate effectively raises urine citrate levels and lowers urine calcium levels; however, the increases in urine pH, oxalate, and phosphate levels lead to increased calcium oxalate and calcium phosphate supersaturation. Potassium citrate induces complex changes in urine chemistries and resultant supersaturation, which may not be beneficial in preventing calcium phosphate stone formation.

Hypercalciuria is the most common metabolic abnormality observed in patients who form calcium-based kidney stones.1–3 The elevated levels of urinary calcium increase the probability for nucleation and growth of calcium oxalate (CaOx) and/or calcium hydrogen phosphate (CaHPO₄, brushite) crystals into clinically significant kidney stones.1 Calcium phosphate (CaP)—containing stones may be brushite, apatite, or less well defined crystal forms. Patients with idiopathic hypercalciuria (IH), defined as excessive urinary calcium without a demonstrable metabolic cause, generally have normal serum calcium, normal or elevated serum 1,25-dihydroxyvitamin D₃, normal or elevated serum parathyroid hormone (PTH), normal or low serum phosphate, and low bone mass.1,4,5 IH exhibits a polygenic mode of inheritance.4–6

The annual incidence of kidney stones in industrialized nations exceeds 1 per 1000 persons, with a lifetime risk of about 7% in women and about 11% in men.7 After an initial episode of nephrolithiasis, approximately 60%–80% of patients form at least one recurrent stone. Among the strategies to decrease
stone recurrence is the use of potassium citrate (K-cit). Oral citrate would be expected to decrease calcium available for absorption, decrease bone resorption, and increase renal tubular calcium reabsorption, all of which would lower urinary calcium. Increased urinary citrate would sequester urinary calcium preventing the binding to calcium phosphate (CaP) and/or oxalate, potentially leading to a reduction in stone formation. However, oral citrate should also increase urinary pH, which potentially could increase CaP stone formation. In vitro, citrate has direct inhibitory effects on CaOx crystal nucleation and growth (reviewed by Ryall). Thus, it is difficult to predict the precise effects of K-cit on urine supersaturation with respect to the common solid phases of CaP and ultimately stone formation.

In patients, the addition of dietary K-cit appeared to decrease recurrent stone formation. In a randomized, double-blind, placebo-controlled study in humans, K-cit decreased recurrent stone formation. Pearle et al. published a meta-analysis of several treatments for prevention of recurrent stone formation, including thiazide diuretics and citrate. They identified three studies (including Pak et al. and Barcelo et al.) using citrate supplements; stone recurrence decreased in two studies but not in the third. A formal meta-analysis was not possible because one study reported only rate of stone formation and not patient numbers. K-cit treatment has been reported to be effective in patients refractory to thiazide treatment.

Citrate has been used after lithotripsy to prevent stone recurrence. In a randomized controlled study, potassium and sodium citrate appeared to reduce the risk of stone recurrence after extracorporeal shockwave lithotripsy or percutaneous nephrolithotomy. In another randomized controlled study, K-cit appeared effective in reducing lower calceal calculi after shockwave lithotripsy. Neither of these studies was placebo controlled. Current recommendations of the American Urological Association include citrate therapy to limit stone recurrence.

However, in all human studies using stone formation as the primary endpoint, patients were chosen for having CaOx stones or calcium lithiasis. Therefore, CaP–stone–forming patients were excluded or would be only a minority of the study participants. No prospective controlled trial has studied the effectiveness of citrate in reducing stone formation specifically in patients with CaP stones, and this remains an important clinical question. Because CaP stone formers are characterized by alkaline urine compared with CaOx stone formers, the effect of citrate on CaP supersaturation and stone formation are difficult to predict. The alkali load would increase urine pH, increasing CaP supersaturation, whereas an increase in citrate and reduction in urinary calcium would lower CaP supersaturation; however, the net effect is uncertain. Given the uncertainty in the effect of citrate on prevention of recurrent CaP stone disease, and the absence of human studies, we used the 95th generation of the genetic hypercalciuric stone–forming (GHS) rats to study the effect of K-cit on CaP stone formation.

The GHS rats were generated by selectively inbreeding Sprague-Dawley rats for increased urinary calcium excretion. When fed a standard, ample calcium diet, each GHS rat now consistently excretes approximately 10-fold more urinary calcium than Sprague-Dawley controls. Like patients with IH, GHS rats have normal serum calcium, increased intestinal calcium absorption and enhanced bone resorption, decreased renal tubule calcium reabsorption, and normal serum 1,25-dihydroxyvitamin D3 levels in addition to decreased bone mineral density. Hypercalciuria is a polygenic trait in GHS rats, as it is in humans. When fed a standard, ample calcium diet, all GHS rats develop kidney stones, which are composed of CaP and ultimately stone formation.

To determine the effects of K-cit on urine solute excretion, supersaturation with respect to the common stone solid phases, and CaP stone formation, GHS rats were fed a normal calcium diet without hydroxyproline with K-cit or potassium chloride (KCl), as control.

## RESULTS

### Urine Solute Excretion

All rats consistently ate their full allotment of food so that solute intake, except for citrate, chloride, and K, was similar for all rats in both groups. K-cit induced a reduction in overall mean urinary calcium (K-cit, 16.1 ± 0.5 mg/d versus KCl, 18.6 ± 0.3 mg/d; P < 0.001), and urinary calcium was reduced with K-cit at 6 and 12 weeks but not at 18 weeks (Figure 1). K-cit led to an increase in both overall mean urinary phosphate (K-cit, 29.1 ± 1 mg/d versus KCl, 20.1 ± 1 mg/d; P < 0.001) and overall mean urinary oxalate (K-cit, 1.11 ± 0.04 mg/d versus KCl, 0.62 ± 0.02, P < 0.001) and the increase in both urinary phosphate and urinary oxalate was observed at each time point (Figure 1).

![Figure 1](image)

**Figure 1.** Urinary calcium (Ca) was decreased and urinary, phosphate (P), and oxalate (Ox) were increased with K-cit. Urine was collected for 24 hours at 6, 12, and 18 weeks to determine solute levels as described in the Concise Methods. Values are mean ± SEM. *K-cit different from KCl, same time period, P < 0.05.
As expected, overall mean urinary citrate was elevated in rats fed K-cit (K-cit, 182±3.4 mg/d versus KCl, 110±2.7 mg/d; P<0.001) (Table 1). K-cit increased the mean overall urinary pH (K-cit, 7.51±0.04 versus KCl, 6.71±0.05; P<0.001). K-cit reduced mean overall urinary NH₄ (K-cit, 0.25±0.01 mmol/d versus KCl, 0.41±0.01 mmol/d; P<0.001). K-cit increased the mean overall urinary sulfate (K-cit, 1.08±0.02 mEq/d versus KCl, 0.86±0.02 mEq/d; P<0.001). Each of these changes was present at each time point. Overall mean urinary uric acid excretion did not differ (K-cit, 1.6±0.1 mg/d versus KCl, 2.0±0.2 mg/d; P=NS).

K-cit resulted in a slightly lower overall mean urinary sodium (K-cit, 2.16±0.03 mmol/d versus KCl, 2.36±0.04; P<0.001), which was significant at 6 and 18 weeks but not at 12 weeks (Table 1). K-cit resulted in a slightly but significantly lower mean overall urinary K (K-cit, 4.8±0.04 mmol/d versus KCl, 5.3±0.08 mmol/d; P<0.001) that was significant at each time period. As expected, the rats receiving K-cit excreted less chloride than the rats receiving KCl (K-cit, 2.2±0.07 mEq/d versus KCl, 6.4±0.15 mEq/d; P<0.001), which was significant at each 6-week time period. K-cit did not alter the overall mean urine volume (K-cit, 47.3±1.6 ml/d versus KCl, 53.0±2.7 ml/d; P=NS) or the overall mean urinary creatinine (K-cit, 12.85±0.17 mg/d versus KCl, 12.74±0.22 mg/d; P=NS).

**Urine Supersaturation**

K-cit led to a significant increase in the urinary supersaturation with respect to both CaP (K-cit, 8.5±0.6 versus KCl, 4.9±0.2; P<0.001) and CaOx (K-cit, 9.8±0.5 versus KCl, 5.5±0.2; P<0.001) and a reduction of supersaturation with respect to uric acid (K-cit, 0.003±0.0005 versus KCl, 0.022±0.003; P<0.001) (Figure 2). Each of these differences was significant at each time period.

**Serum Levels**

Serum levels of calcium and PTH did not differ between K-cit–fed and KCl-fed rats, while serum phosphate levels were decreased in K-cit–fed rats (Figure 3).

![Figure 2. Urinary supersaturation (SS) of CaP and CaOx were increased and uric acid SS was decreased with K-cit. Urine was collected for 24 hours at 6, 12, and 18 weeks to determine solute levels that were used to calculate relative supersaturation as described in the Concise Methods. Values for relative supersaturation are mean±SEM and are unitless. *K-cit different from KCl, same time period, P<0.05.](image)

**Table 1. Urinary citrate, pH and sulfate were increased while NH₄, chloride, sodium and K were the same or decreased with K-cit compared to KCl**

<table>
<thead>
<tr>
<th>Variable</th>
<th>6 wk</th>
<th>12 wk</th>
<th>18 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KCl</td>
<td>K-cit</td>
<td>KCl</td>
</tr>
<tr>
<td>Urinary citrate (mg/d)</td>
<td>104.2±3.5</td>
<td>175.2±4.6*</td>
<td>99.0±3.4</td>
</tr>
<tr>
<td>Urinary pH</td>
<td>6.85±0.06</td>
<td>7.65±0.03*</td>
<td>6.73±0.08</td>
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<tr>
<td>Urinary NH₄ (mmol/d)</td>
<td>0.43±0.02</td>
<td>0.23±0.03*</td>
<td>0.44±0.01</td>
</tr>
<tr>
<td>Urinary SO₄ (mEq/d)</td>
<td>0.95±0.04</td>
<td>1.17±0.04*</td>
<td>0.90±0.03</td>
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<tr>
<td>Urinary uric acid (µg/d)</td>
<td>2.4±0.1</td>
<td>2.1±0.0</td>
<td>1.7±0.1</td>
</tr>
<tr>
<td>Urinary sodium (mmol/d)</td>
<td>2.3±0.1</td>
<td>2.1±0.1*</td>
<td>2.4±0.1</td>
</tr>
<tr>
<td>Urinary potassium (mmol/d)</td>
<td>5.54±0.14</td>
<td>4.80±0.09*</td>
<td>5.37±0.15</td>
</tr>
<tr>
<td>Urinary chloride (mmol/d)</td>
<td>5.8±0.07</td>
<td>4.80±0.09*</td>
<td>5.9±0.1</td>
</tr>
<tr>
<td>Urine volume (ml)</td>
<td>50.1±3.8</td>
<td>47.9±2.7</td>
<td>53.9±3.1</td>
</tr>
<tr>
<td>Urine creatinine (mg/d)</td>
<td>13.6±0.5</td>
<td>13.6±0.2</td>
<td>12.3±0.2</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±SEM. Urine was collected for 24 hr at 6, 12, and 18 wk to determine solute levels as described in the Concise Methods.

*K-cit different from KCl, same time period, P<0.05.

Stone Formation

K-cit did not alter the number of stones formed or extent of calcification in the kidneys (Figure 4). All rats formed stones of similar size and density and had similar degrees of calcification within the kidney and collecting system.

Visible stones were dissected from representative kidneys of both groups and analyzed for crystal morphology. The diffraction patterns for all analyzed samples were congruent with the diffraction pattern of biologic apatite, a CaP stone (Figure 5, top). With use of transmission electron microscopy (TEM), all kidney stone crystals had a rod- or needle-like shape, similar to the morphology of biologic apatite crystals (Figure 5, bottom) and unlike the octahedral crystal morphology of CaOx.
DISCUSSION

Hypercalciuria is the most common metabolic abnormality observed in patients who form Ca-based kidney stones.1–3 Most patients with hypercalciuria have IH. The increased excretion of urinary calcium enhances the probability for nucleation and growth of CaOx and/or CaP crystals into clinically significant kidney stones.1

In patients with IH, K-cit is often used to prevent recurrent stone formation.8–13 However, no prospective controlled studies in humans have shown the efficacy of K-cit in preventing recurrent CaP stone formation.21 Citrate has a complex effect on urine solute excretion, and its effect on urine supersaturation with respect to CaP stone formation is difficult to predict. Some studies have shown that CaOx stones may transform into CaP stones over time, and use of citrate therapy may explain this phenomenon.50,51 However, no definitive studies have yet proved or refuted the role of citrate in transformation of CaOx to CaP stone disease.

In this study using GHS rats, the provision of K-cit led to an increase in urine citrate excretion and K-cit also effectively decreased urinary calcium. The mechanism by which K-cit reduces urinary calcium is almost certainly multifactorial. While citrate itself is readily absorbed in the intestine,52 it decreases calcium absorption and urinary calcium excretion.14,15 Increased urinary citrate would also bind urinary calcium, removing the calcium from the pool available for binding with phosphate or oxalate. In addition, citrate metabolism to bicarbonate results in systemic alkalinization, which directly decreases bone resorption53 and increases renal tubule calcium reabsorption, thereby reducing the level of calcium in urine. The decrease in urinary calcium by itself would favorably reduce urinary supersaturation with respect to calcium-containing kidney stones.

As seen in humans, citrate therapy lowered urine calcium and raised urinary citrate and pH in the GHS rat. However, we also found an increase in urinary oxalate, sulfate, and phosphorus and a decrease in serum phosphorus in GHS rats treated with citrate, alterations not previously reported in humans treated with citrate. The binding of intestinal calcium by citrate could decrease intestinal binding of calcium to oxalate, potentially allowing greater absorption of oxalate. In the kidney, oxalate is transported in the proximal tubule.54,55 The murine anion transporter Slc26a6, found in renal proximal tubule and intestine, has specificity for chloride/oxalate exchange.56–58 Knockout of Slc26a6 in mice leads to hyperoxaluria,59,60 hypocitraturia,61 and CaOx stone formation.59 In perfusion experiments the presence of sulfate or bicarbonate inhibited oxalate transport, suggesting competitive
inhibition among these anions.\textsuperscript{55} Co-expression of Slc26a6 and the citrate transporter NaDC-1 indicates that these transporters interact.\textsuperscript{61} NaDC-1 enhanced Slc26a6 transport activity, while Slc26a6 inhibited NaDC-1.\textsuperscript{61} These data suggest a close relationship between oxalate and citrate transport. In this study, we found that administration of K-cit led to a significant increase in urine oxalate excretion, and this increase would increase urinary supersaturation with respect to CaOx.

The binding of intestinal calcium by citrate will also allow greater absorption of phosphate. Renal phosphate reabsorption is regulated by the type II Na\textsuperscript{+}-coupled phosphate cotransporter in the proximal tubule, which is suppressed by extracellular acidification.\textsuperscript{62} The systemic and urinary alkalization induced by K-cit would increase renal tubular phosphate reabsorption; however, the increase in PTH, although not statistically significant, might account for the increase in urine phosphate and decrease in serum phosphate, which was found in this study.

Urine sulfate increased in GHS rats fed K-cit. The anion transporter Slc26a2, which demonstrates substrate specificity for sulfate, oxalate, and chloride, is also present in the proximal tubule.\textsuperscript{63} Mutation of SLC26A2 in humans can cause recessive chondrodysplasia, as a result of abnormal sulfate transport.\textsuperscript{63} When expressed in \textit{Xenopus} oocytes, acidic extracellular pH inhibited anion exchange, while acidic intracellular pH activated exchange of extracellular chloride for intracellular sulfate but not chloride or oxalate.\textsuperscript{64} Further studies will be necessary to determine why urinary sulfate increased in the GHS rats fed K-cit.

In this study, the provision of K-cit led to an increase in urinary pH, which will reduce the solubility of CaP. Conversely, the uric acid solid phase, which may be a nidus on which calcium stones form, is more soluble in alkaline urine. K-cit did not alter urinary volume, indicating that any change in urinary supersaturation was not the result of differences in urine volume. K-cit did not alter urinary creatinine excretion, indicating that administration of this drug did not affect muscle mass over this period.

Zerwekh \textit{et al.}\textsuperscript{65} fed Sprague-Dawley rats a low- or high-casein diet, with KCl or K-cit (4 mEq potassium per day) for 8 weeks. Urinary pH did not increase with K-cit. Compared with the low-casein diet+KCl, urinary calcium excretion increased with the high-casein diet and K-cit reduced this increased urinary calcium. K-cit did not alter urinary phosphate or oxalate. In the current study, we used a 3-fold higher dose of K-cit and observed a significant increase in urinary pH, urinary oxalate, and urinary phosphate and a decrease in urinary calcium.

In this study we found that while K-cit effectively decreased urinary calcium it also increased urinary phosphate and oxalate and urinary pH, resulting in an actual increase in urinary supersaturation with respect to the CaP and CaOx solid phases. Despite the increase in CaOx supersaturation, analysis of stones from each group found TEM and diffraction patterns indicating that all of the stones were composed of CaP. Supersaturation of uric acid was decreased with K-cit, but this supersaturation is so low in either case that there is little chance of uric acid stone formation. Stone formation in the GHS rats, which form only CaP stones on this diet, was not altered by K-cit. Of note, the higher supersaturation of CaP in the citrate-treated rats did not lead to an increase in stone formation. This may reflect an increase in urine crystal inhibitory activity due to the higher urinary citrate concentration.\textsuperscript{66} Further studies are necessary to determine whether the provision of K-cit to GHS rats fed hydroxyproline, which causes them to form only CaOx stones, would affect urinary supersaturation or stone formation.

Thus, the provision of K-cit induces complex changes in urine chemistries, some favorable and some deleterious to the goal of decreasing supersaturation with respect to CaP and CaOx. In this model of CaP stone formation, citrate was not effective in reducing stone burden. This study suggests that further studies should be done in human CaP stone formers to determine the effects of K-cit on urine supersaturation with respect to CaP and stone formation.

**CONCISE METHODS**

**Animals**

The GHS rats were derived from Sprague–Dawley rats (Charles River Laboratories, Kingston, NY) by successively inbreeding the most hypercalciuric progeny of each generation.\textsuperscript{24–27,36–39,49} Eight-week-old male GHS rats from the 95th generation were used in this study.
Experimental Conditions
At the start of the study (day 0), 22 GHS rats were placed in metabolic cages and fed a normal calcium diet, 13 g/d (1.2% calcium; Harlan-Teklad, Indianapolis, IN). K-cit, 4 mmol/d (11 rats), or KCl, 4 mmol/d, as a control (11 rats) was added to the diet to provide equivalent amounts of the anions citrate and chloride. All rats had free access to deionized, distilled water. At 6-week intervals (6, 12, and 18 weeks), urine from each rat was collected for four 24-hour periods. For two collections, urine was acidified with HCl; for another two collections urine was collected in thymol. Collections in thymol were used for pH, uric acid, and chloride, and collections in HCl were used for all other measurements. After 18 weeks, all rats were euthanized and their blood collected, and the kidneys quickly removed. Any animal that ate <10 g of food per day or drank <15 ml of water per day would have been excluded from further analysis; however, all rats met the prespecified criteria during the entire study. The University of Rochester Committee for Animal Resources approved all procedures.

Urine and Serum Chemistries
Urinary calcium, magnesium, phosphate, ammonium, and creatinine were measured spectrophotometrically using a Beckman CX5 Pro autoanalyzer (Beckman Coulter, Brea, CA). Urinary potassium, chloride, and sodium were measured by ion-specific electrodes on the Beckman CX5. Urinary pH was measured using a glass electrode, and citrate, oxalate, and sulfate were measured by ion chromatography using a Dionex ICS 2000 system (Dionex Corp., Sunnyvale, CA). All urine solutes were measured at 6, 12, and 18 weeks, and a mean value for each time period and an overall mean value were calculated. Serum calcium and phosphate were determined colorimetrically (BioVision, Milpitas, CA). Serum PTH was determined by enzyme immunoassay for intact PTH (ALPCO, Salem, NH). We have used all of these methods previously.31,45,47,48,67–69

Urine Supersaturation
With the measured solute excretion, the urinary supersaturation with respect to CaOx, CaP, and uric acid solid phases were calculated using the computer program EQUIL2.70 as we have done previously.28,29,33,34,37,40,43,48,67 Ratios of 1 denote a urine at equilibrium, those >1 denote supersaturation, and those <1 denote undersaturation. We have found excellent correspondence between calculated and experimentally measured saturation in urine and blood and in bone culture medium.31,45,47

Calcification
The kidneys, ureters, and bladder were removed from each rat en bloc, frozen, and imaged in a Faxitron cabinet radiography device (Tucson, AZ) to determine extent of kidney stone formation and calcification. Three observers blinded to treatment scored all radiographs on a scale ranging from 0 (no stones or calcification) to 4 (presence of extensive stones and/or calcification).

Crystallography of Kidney Stones
Stones were dissected from kidneys of both groups of rats and embedded in EMBed 812 resin without fixation or dehydration. Sections 100 nm thick were cut on a Leica Ultracut microtome, placed on Formvar coated grids, and viewed in an FEI Tecnai 20 TEM (FEI Co., Hillsboro, OR). Images and diffraction patterns were collected by an AMT 16000 camera. Diffraction pattern analysis was done by calculating the d-spacings by comparison to a gold pattern collected under the same conditions.

Statistical Analyses
Values were compared by t test with a conventional computer program (Statistica; StatSoft, Tulsa, OK). Values are expressed as mean ± SEM. P ≤ 0.05 was considered to represent a statistically significant difference.

ACKNOWLEDGMENTS
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DISCLOSURES
None.

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