Mechanism of Urinary Calcium Regulation by Urinary Magnesium and pH

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ABSTRACT
Urinary magnesium and pH are known to modulate urinary calcium excretion, but the mechanisms underlying these relationships are unknown. In this study, the data from 17 clinical trials in which urinary magnesium and pH were pharmacologically manipulated were analyzed, and it was found that the change in urinary calcium excretion is directly proportional to the change in magnesium excretion and inversely proportional to the change in urine pH; a regression equation was generated to relate these variables ($R^2 = 0.58$). For further exploration of these relationships, intravenous calcium chloride, magnesium chloride, or vehicle was administered to rats. Magnesium infusion significantly increased urinary calcium excretion (normalized to urinary creatinine), but calcium infusion did not affect magnesium excretion. Parathyroidectomy did not prevent this magnesium-induced hypercalciuria. The effect of magnesium loading on calciuria was still observed after treatment with furosemide, which disrupts calcium and magnesium absorption in the thick ascending limb, suggesting that the effect may be mediated by the distal nephron. The calcium channel TRPV5, normally present in the distal tubule, was expressed in Xenopus oocytes. Calcium uptake by TRPV5 was directly inhibited by magnesium and low pH. In summary, these data are compatible with the hypothesis that urinary magnesium directly inhibits renal calcium absorption, which can be negated by high luminal pH, and that this regulation likely takes place in the distal tubule.


The amount of calcium excreted in the urine (calciuria) is tightly regulated by a complex interaction between numerous calcitropic hormones, extracellular volume, acid-base status, and plasma and urinary concentration of various ions.1 Calciuria is a continuous trait in the general population, and hypercalciuria is a major risk factor for nephrolithiasis found in high incidence in the stone former and/or in the osteoporotic populations.2

The first description of an effect of magnesium on calciuria was made in a seminal article by Mendel and Benedict in 1909.3 They observed increased calcium excretion and decreased fecal calcium content when magnesium salts were injected to various animal species. Similar observations were later reported in humans in preeclamptic pregnant women who were treated with magnesium sulfate and in normal individuals.4,5 This effect was attributable to magnesium and not sulfate.6 Attempts have been made to localize the segment where urinary magnesium could mediate its effect on calcium reabsorption in different animal models. Metabolic experi-
ments confirmed that calciuria can be increased by intravenous magnesium infusion in dogs with normal or reduced kidney function, but stop-flow experiments did not precisely identify the segment involved.10,11 Micropuncture experiments in rats infused with magnesium chloride suggested that the loop of Henle maybe involved.10,11 In sum, these studies are compatible with the initial observation of Mendel and Benedict, but the mechanisms by which magnesium increases calciuria remain largely unknown.

Urinary calcium excretion is increased by metabolic acidosis, at least in part by decreasing renal calcium reabsorption.12,13 High dietary animal protein intake decreases urinary pH (by increasing acid load) and increases calcium excretion.14–16 Conversely, urinary alkalinization by potassium alkali decreases urinary calcium.17,18 Recent studies have found that extra- and intracellular protons inhibit the renal epithelial apical calcium channels TRPV5 and TRPV6 within the physiologic urinary and intracellular pH ranges.19–24 These results suggest that acid pH increases calciuria by inhibiting TRPV5/6-mediated calcium reabsorption in the distal nephron. In a heterologous system using sodium current as a surrogate, micromolar concentrations of magnesium have also been shown to inhibit TRPV5.21,25 Here, we report on a collection of translational studies involving humans, rats, and an in vitro experiment showing that calciuria is modulated by both urinary pH and magnesium.

RESULTS

Human Data

Source.

Retrospective data from the University of Texas Southwestern Medical Center Nephrolithiasis Registry from 17 studies17,26–36 that dealt with physiologic and physicochemical effects of various magnesium and potassium salts were categorized into three groups and analyzed. These studies were conducted for a variety of purposes, but they all involved manipulation of urinary magnesium, pH, or both. Table 1 summarizes the characteristics of these studies. In group 1, participants took magnesium oxide (MgO) or citrate (Mg$_3$Citrate$_2$). Participants in group 2 took potassium bicarbonate (KHCO$_3$) or citrate (K$_4$Citrate). Participants in group 3 took potassium magnesium citrate (K$_4$MgCitrate$_2$).

Within-Group Comparison of Urinary Magnesium, pH, and Calcium.

This is summarized in Supplemental Table 1. Urinary magnesium increased significantly during treatment with magnesium salts (groups 1 and 3) but not with potassium alkali (group 2). The rise in urinary magnesium was more pronounced in group 1 than in group 3, likely as a result of dosage effect. Urinary pH rose significantly during treatment with potassium alkali (group 2) and potassium-magnesium citrate (group 3). The rise in pH was comparable between groups 2 and 3 but was minimal and insignificant in group 1. Urinary calcium increased significantly after magnesium oxide/citrate treatment (group 1) and decreased with potassium alkali treatment (group 2). It displayed a slight and statistically insignificant increase after potassium-magnesium citrate treatment (group 3).

Across-Group Comparison of Changes in Urinary Magnesium, pH, and Calcium.

Changes in urinary magnesium were significantly different among all three groups (Supplemental Table 1), with no change in group 2, substantial increase in group 1, and an intermediate change in group 3. The amplitude of changes in urinary pH in groups 2 (potassium alkali) and 3 (potassium-magnesium alkali) were similar and significantly different from the change encountered in group 1 (magnesium). Changes in urinary calcium were all different among the three groups, with an increase in group 1, a decrease in group 2, and no change in group 3.

Dependence of Urinary Calcium on Urinary Magnesium and pH.

A summary of this retrospective analysis is depicted in Figure 1 as a three-dimensional plot of changes in urinary calcium as a function of magnesium and pH. On the basis of the across-group univariate comparisons, the three main groups were distinct from each other. A regression of the change in urinary calcium on the changes in urinary magnesium and urinary pH showed that the relationship could be empirically described by the following equation:

\[ \Delta U_{Ca} \times V = 0.70 + 0.26 \times \Delta U_{Mg} \times V - 2.06 \times \Delta U_{pH} \]

where V stands for the urinary volume. The R$^2$ for the regression was 0.58.

Table 1. Human data retrieved from the University of Texas Southwestern Medical Center Nephrolithiasis Registry

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>MgO or Mg$_3$Citrate$_2$</td>
<td>KHCO$_3$ or K$_4$Citrate</td>
<td>K$_4$MgCitrate$_2$</td>
</tr>
<tr>
<td>No. of trials</td>
<td>4</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Dosage (mEq/d)</td>
<td>46.7 (24.5 to 82.2)</td>
<td>0.0</td>
<td>23.8 (21.0 to 24.5)</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0</td>
<td>60 (40 to 80)</td>
<td>48 (42 to 49)</td>
</tr>
<tr>
<td>Potassium</td>
<td>2.0 (0.9 to 3.0)</td>
<td>2.0 (1.0 to 4.0)</td>
<td>2.6 (1.0 to 3.0)</td>
</tr>
<tr>
<td>Treatment duration (wk)</td>
<td>47 (4 to 21)</td>
<td>89 (5 to 21)</td>
<td>102 (10 to 30)</td>
</tr>
</tbody>
</table>


Calciuria Modulated by Mg and pH

1531
Infused with MgCl₂ is secondary to a fall in parathyroid hormone (PTH), since the magnesium-creatinine or calcium-creatinine ratio (Figure 1A) and also by an increase of the calcium-creatinine ratio. Calcium infusion (CaCl₂) augmented only calciuria and not magnesiuria (Figure 1B). Vehicle alone did not cause any significant change in either magnesium-creatinine or calcium-creatinine ratio. These studies show that the magnesium-induced hypercalciuria can occur independent of PTH.

High luminal magnesium can compete with calcium for paracellular reabsorption in the thick ascending limb. We next addressed the role of the thick ascending limb in the magnesium-induced calciuria by disrupting calcium and magnesium absorption with furosemide in this segment. To prevent volume contraction, we preloaded the rats intraperitoneally with 3 ml/100 g body weight 0.45% saline. We next addressed the role of the thick ascending limb in the magnesium-induced calciuria by disrupting calcium and magnesium absorption with furosemide in this segment. To prevent volume contraction, we preloaded the rats intraperitoneally with 3 ml/100 g body weight 0.45% saline. We
injected a single intravenous bolus of 10 mg/kg furosemide at the end of the baseline collection period, just before starting the MgCl₂ perfusion. Figure 3A shows the effect of furosemide on calcium-creatinine ratio. The increase in calcium-creatinine ratio induced by magnesium was not different in amplitude compared with rats that did not receive furosemide, but it peaked earlier after the start of the MgCl₂ perfusion. Furosemide had no effect on the increase in magnesium-creatinine ratio induced by magnesium, as shown in Figure 3B.

When the same furosemide protocol was applied to PTX rats, we still observed an increase of the calciuria after the intravenous magnesium load (Figure 3C), and its peak appeared earlier compared with rats not injected with furosemide. Overall, our data shows that the magnesium-increased calciuria is a phenomenon independent from PTH and is still present despite inhibition of transport at the thick ascending limb. These results point to the distal nephron as the most likely site of magnesium-induced calciuria.

Expression in *Xenopus laevis* Oocytes

Because the distal tubule calcium channel TRPV5 has been shown to be regulated by magnesium and apical pH in absence of calcium as permeant ion,21–25,39 we expressed TRPV5 in *Xenopus laevis* oocytes and measured isotopic calcium uptake at different magnesium concentrations (from 1 to 10 mM) and at two different pH (pH 5 and 8). When compared with pH 8 and 1 mM magnesium,45 calcium uptake was decreased at pH 5 and 1 mM magnesium (Figure 4). At both pH 5 and 8, calcium uptake was significantly inhibited by increasing magnesium concentrations in a concentration-dependent manner, reaching significance at 5 and 10 mM (compared with 1 mM). There was no difference in surface expression of TRPV5 under the conditions of varying pH and extracellular magnesium identical to that of the calcium flux measurements, as assessed by confocal imaging of enhanced green fluorescent protein-tagged TRPV5 (Supplemental Figure 1). These findings indicate that TRPV5 activity assessed by ⁴⁵calcium uptake is inhibited by both high extracellular magnesium concentration and low extracellular pH and is likely mediated by gating of the channel and not by changing number of channels.

**DISCUSSION**

Urinary magnesium and pH are known regulators of calciuria at the whole organism level, but the mechanisms underlying this regulation have not been unveiled yet. With these studies, we are providing several lines of evidence to help decipher this phenomenon. First, in humans, we showed that magnesium-induced calciuria can be influenced by changes in urinary pH. Second, the rat data are compatible with a distal effect of urinary magnesium on renal calcium reabsorption, independent from PTH, pointing out the distal convoluted tubule as the putative location for the magnesium-induced calciuria. Third, the calcium flux assay showed that TRPV5, the apical calcium channel expressed in the distal convoluted and the connecting tubule in the kidney, is...
a strong candidate for this regulatory pathway, because it can be inhibited by both low pH and high magnesium.

Hypercalciuria is a major risk factor for nephrolithiasis. Our results suggest that high urinary magnesium can increase calciuria and potentially increase stone risk. Some have espoused the notion that magnesium is efficacious in preventing stone formation by binding or complexing oxalate in the bowel and urine,26,27,40,41 by inhibiting calcium oxalate crystal formation,26,42,43 and by increasing urinary citrate when given as alkali salt.44 In vitro studies have shown that magnesium is an inhibitor of calcium oxalate crystal growth in artificial, rodent, and human urine but at supraphysiologic concentrations,28,45–50 In our retrospective analysis of human trials, we showed that magnesium supplementation indeed increased calciuria, but this effect was minimized when there was simultaneous alkalization of the urine. One should take caution in any retrospective meta-analysis in that not all variables were vigorously controlled. The impact of magnesium and pH on calciuria can be estimated by a purely empirical equation, but this paradigm will need further studies in a prospective, controlled trial. Nonetheless, the current retrospective analysis of pooled human studies supports the notion of magnesium–pH interaction in regulating calciuria.

In the rat experiments, all of the animals were on normal chow and most probably magnesium replete. Hence, it is reasonable to assume that these studies examined primarily the effect of acute changes in luminal magnesium. The effect of changes in intracellular magnesium, although unlikely, cannot be ruled out with clearance studies. We attempted to determine the segment of the nephron involved in the magnesium-induced increase in calciuria. Although we did not measure GFR and proximal tubular calcium reabsorption directly, we assume they did not change, on the basis of unaltered endogenous creatinine clearance and studies performed by others.10,11 Previous micropuncture studies in the rat suggested the thick ascending limb as a probable site for this regulation,10,11 but this assumption was never challenged with furosemide to the best of our knowledge. Because disruption of calcium reabsorption in the thick ascending limb by furosemide had little effect on the amplitude of the magnesium-induced increase in calcium-creatinine ratio, we postulate that the majority of the regulation of calciuria by magnesium takes place in the distal convoluted tubule. Magnesium infusion increased urinary calcium excretion in the absence of PTH and in the presence of slight hypocalcemia, even though the effect was less prominent than with intact parathyroids. Inhibition of PTH secretion by magnesium via the calcium-sensing receptor (CaSR) in the parathyroid gland37,38 is unlikely to account for the hypercalciuria. This strengthens a local renal regulation for the rise in urinary calcium after intravenous magnesium.

In the distal convoluted tubule and connecting tubule, calcium is reabsorbed transcellularly through the apical calcium channel TRPV5, complexed in the cytoplasmic compartment by the calbindin system, and extruded to the interstitium by the sodium/calcium exchanger and the calcium ATPase.51 TRPV5 is critical in that process, and its deletion in the mouse leads to pronounced hypercalciuria.43 TRPV5 is regulated by numerous calcitropic hormones but also by its own transported substrate.52 As intracellular calcium concentration rises, TRPV5 open probability decreases drastically. Thus, in electrophysiologic studies, sodium is used as surrogate permeant ion. Using whole-cell configuration, Vennekens et al.21 showed that monovalent sodium currents through TRPV5 were inhibited by increasing magnesium concentration, with K1 in the micromolar range, and that this inhibition was pH dependent. Here, we used calcium as permeant cation of TRPV5 and measured actual calcium flux instead of sodium current in the heterologous X. laevis oocyte expression system. We showed that millimolar concentrations of magnesium inhibits calcium uptake in a pH-dependent manner. This range of concentration is more compatible with urinary magnesium concentrations, usually in the order of 1 to 10 mM.

Taken together, our data support that TRPV5 is an important player in the magnesium-elicited increase of calciuria; however, it does not exclude additional mechanisms for this inhibition of calcium reabsorption.53 The role of the renal CaSR will have to be defined by additional experiments. Magnesium is known to bind to the CaSR and may inhibit calcium reabsorption in the thick limb. In a distal convoluted tubule cell line, Bapty et al.54 showed that CaSR is sensitive to extracellular magnesium in physiologic concentrations.

In conclusion, this study provides a translational approach to a phenomenon described almost one century ago and sheds light on the putative site of action of magnesium on calcium reabsorption and its molecular basis. Speculations about the clinical significance of varying urinary pH in patients on magnesium supplementation await further investigations.

**CONCISE METHODS**

**Human Data Analysis**

Retrospective clinical data were obtained from the University of Texas Southwestern Medical Center Nephrolithiasis Registry using 17 source documents from 12 previous publications that dealt with physiologic and physicochemical effects of various magnesium and potassium salts.17,26–36 Key data retrieved for this communication were 24-h urinary magnesium, pH, and calcium. In seven source documents, all key data had already been published16–29,35; in the remaining 10 source documents, one or more key data had not been previously published.

The source documents from 17 treatment trials were divided into three groups according to the type of intervention (Table 1): Magnesium oxide (MgO) or magnesium citrate (MgCitrate2; group 1), potassium bicarbonate (KHCO3) or potassium citrate (K3Citrate; group 2), and potassium-magnesium citrate (K3MgCitrate2; group 3). During treatment, participants were kept on a constant metabolic diet in 11 trials17,26–29,33,35 and on instructed diet with a similar intake of...
magnesium, potassium, and calcium in remaining six trials. The 17 source documents represented trials of ≤1 mo in duration. Although we had reported on trials of longer duration, they were not included here because of problems in long-term dietary adherence and concern for development of secondary compensatory physiologic changes. Participants were normal volunteers or patients who had kidney stones and did not have evidence of magnesium depletion or hypomagnesemia, disturbance in acid-base balance, or abnormal serum potassium or calcium concentrations. Endogenous creatinine clearance was >0.6 ml/min per kg. In six trials, participants were normal individuals who had taken hydrochlorothiazide 50 mg/d alone for 1 to 3 wk, together with potassium citrate, potassium-magnesium citrate, or magnesium citrate for 3 wk. Serum magnesium concentration remained unchanged with potassium citrate treatment and increased slightly within normal limits when magnesium salts were co-administered with thiazide. In the remaining 11 trials, only the test potassium or magnesium salts were given.

Group 1 (MgO or Mg₃Citrate₂) comprised two trials with MgO and two with Mg₃Citrate₂ (Table 1). One trial used a high dosage of magnesium (82.2 mEq/d) for 6 d; the remaining trials used a dosage of 24.5 to 40.0 mEq/d magnesium for 2 to 3 wk. Forty-seven recipients received potassium alkali at a dosage of 40 to 80 mEq/d for 1 to 4 wk. Group 3 comprised five trials with K₄MgCitrate₂, and K₃Citrate was given in seven trials. Eighty-nine participants (five to 21 per trial) received potassium alkali at a dosage of 40 to 80 mEq/d potassium and magnesium, and 63.0 to 73.5 mEq/d citrate for 3 wk. Serum magnesium concentration remained unchanged with potassium citrate treatment and increased slightly within normal limits when magnesium salts were co-administered with thiazide. In the remaining 11 trials, only the test potassium or magnesium salts were given.

Clearance Studies in Rats
All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center. Housing and day-to-day care were in accordance with the standards of the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act. Normal and PTX Sprague-Dawley rats were purchased from Charles River (Wilmington, MA), housed in ventilated rooms with 12/12-h dark-light cycles at 22°C, and were ventilated rooms with 12/12-h dark-light cycles at 22°C, and were given free access to regular chow and water. PTX rats drank water supplemented with 1% (wt/vol) calcium citrate to help maintain normocalcemia. Rats (230 to 280 g) were anesthetized with a cocktail of intraperitoneal ketamine, xylazine, and acepromazine (50, 5, and 1 mg/kg body wt, respectively). Femoral veins were identified on both sides, and P50 tubing was used to cannulate the right vein. After a 30-min stabilization period, bladder urine was discarded and baseline urine was collected for 30 min. The intravenous perfusion was then switched to either 5 mM CaCl₂ or 5 mM MgCl₂ in 0.45% saline or maintained at 0.45% saline as the vehicle control, at the same perfusion rate for 1 h. The total calcium or magnesium load was 20 μmol/h per 100 g body wt. At the end of the calcium or magnesium load, perfusion was switched back to 0.45% saline and continued for an additional hour. Urine was collected in 30-min periods. When indicated, furosemide (Furosemide Injection USP; American Regent Laboratories Inc., Shirley, NY) or control vehicle was given as a single bolus (10 mg/kg body wt) after the baseline collection and before the start of the calcium or magnesium load.

Blood was drawn at baseline, at the end of the calcium or magnesium load, and at the end of the experiment from the left femoral vein. Urinary calcium, magnesium, and creatinine levels were analyzed by the Mineral Metabolism Core Laboratory of our center. Calcium and magnesium were assayed by atomic absorption using a Varian SpectrAA 220 instrument, and creatinine was assayed using the Roche kinetic alkaline picrate kit run on a Roche (Indianapolis, IN) COBAS MIRA autoanalyzer. Plasma sodium, calcium, magnesium, albumin, and creatinine were measured by the Core Laboratory of our center using a Beckman CX9ALX (Beckman Coulter, Fullerton, CA).

Expression in X. laevis Oocytes
Stage 5 to 6 oocytes were surgically removed from X. laevis females, isolated by collagenase digestion, and kept at 19°C in modified Barth’s saline (MBS; 90 mM NaCl, 1 mM KCl, 0.41 mM CaCl₂, 0.33 mM Na₂CO₃, and 0.82 mM MgSO₄) supplemented with penicillin (100 U/ml) and streptomycin (100 mg/ml). Capped cRNA was prepared from 1 μg of BambH1-linearized cDNA of the rabbit TRPV5 cDNA with the T7 mMessage mMACHINE kit (Ambion Inc., Austin, TX). Complementary RNA integrity was visualized on ethidium bromide–stained agarose gel for quality and quantified by spectrophotometry. Five nanograms of cRNA (in 50 nl of water) or equimole of water for control was injected per oocyte with a Nanoject II injector (Drummond Scientific Co., Broomall, PA).

After incubation in regular MBS for 48 h and in MBS without calcium 6 h before the assay, uptake of ⁴⁵Ca was performed for 1 h at room temperature with gentle occasional shaking. Uptake solution contained 90 mM NaCl, 0.1 mM CaCl₂, 1 mg/ml ⁴⁵CaCl₂, 1 to 10 mM MgCl₂ as indicated, and 10 mM HEPES (pH 5 or 8 as indicated). It is noteworthy that in the complete absence of magnesium, there was a large and variable background calcium uptake attributable to some endogenous magnesium-sensitive calcium conductances. After extensive washing (6×) with ice-cold stop solution (in mM: 90 NaCl, 0.5 CaCl₂, 1 MgCl₂, 5 HEPES, 1.5 L-aconitase, oocytes were dissolved in 200 μl of 10% SDS and counted individually in scintillation liquid on an LS 3801 Beckman Counter (Beckman Instruments). The data are presented as mean ± SEM of 28 to 35 oocytes from three different experiments and were normalized to the mean obtained from uptake performed at pH 5 and 1 mM MgCl₂.

For semiquantitative assay of surface TRPV5, oocytes were injected with either water or enhanced green fluorescent protein-TRPV5 cRNA and subjected to a regular calcium uptake assay (1 h) but without ⁴⁵Ca (only cold). Oocytes were immediately fixed in 3% PFA, washed in PBS, frozen in liquid nitrogen, and cut at 6-μm sections on a cryostat. Sections were thawed, dried, and rehydrated directly in the mounting medium. Images were acquired on a Leica (Heerbrugg, Switzerland) SP5 confocal microscope using an argon laser and the same setup gain and offset for each condition (magnification ×20). Water-injected oocytes gave just a negligibly faint halo of autofluorescence.
Statistical Analysis
We performed univariate within-group and across-group evaluations of changes in urinary calcium, magnesium, and pH as well as a multivariate assessment of the relationship of those three variables. The within-group analysis used paired t test. The univariate across-group analysis of mean changes was accomplished with a one-way ANOVA followed by a post hoc comparison of the mean changes using the Student-Newman-Keuls multiple range test. A linear regression of the change in urinary calcium onto the associated changes in urinary magnesium and pH was used to capture the relationship of those three variables. The statistical analyses were implemented in the SAS 9.1 statistical software package (SAS Institute, Cary, NC). For each statistical test, an observed significance (P value) <0.05 was considered to be statistically significant. In all analyses, observations were weighted by the number of participants per trial.

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DISCLOSURES

None.

REFERENCES


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