Does a high concentration of calcium in the urine cause an important renal concentrating defect in human subjects?

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ABSTRACT

The objective of this study was to evaluate the hypothesis that a high concentration of ionized calcium in the lumen of the medullary collecting duct causes an osmole-free water diuresis. The urine flow rate and osmolality were measured in normal human subjects, as well as in patients with a history of nephrolithiasis who excreted more than 5 mmol of calcium per 24 h. There was an inverse relationship between the concentration of calcium in the urine and the 24 h urine volume both in normal subjects and in patients with a history of nephrolithiasis. When the concentration of calcium in the urine was greater than 5 mmol/l, the urine volume was less than 1 litre per day in the majority of subjects. After 16 h of water deprivation, when the concentration of calcium in the urine was as high as 17 mmol/l (ionized calcium 7.4 mmol/l), urine osmolality was 1258 mOsm/kg of water and the urine flow rate was 0.30 ml/min. We conclude that, although a calcium receptor may be present in the lumen of the medullary collecting duct in human subjects, an extremely high concentration of urinary total and ionized calcium does not cause a clinically important defect in the renal concentrating process.

INTRODUCTION

Calcium (Ca) is a critical ion in human physiology. At one extreme, Ca is a principal constituent of bone; at the other, the concentration of ionized Ca is very low in the cytosol, where it is intimately involved in signal transduction, enzyme activity, ion transport and the contraction–relaxation process [1]. New insights into the physiology of Ca have emerged since a receptor for ionized Ca was discovered on the surface of cells [2,3]; this receptor is very sensitive to minor variations in the physiological, ionized concentration of Ca in the extracellular fluid. Its fundamental role is documented for control of the release of parathyroid hormone, and changes in this receptor’s binding capacity and/or affinity for ionized Ca or surrogate cationic ligands, such as magnesium (Mg), may explain many of the findings in certain disease states [4–6].

Riccardi et al. [7] proposed a novel hypothesis to explain why polyuria might develop when the urinary

Key words: antidiuretic hormone, arginine vasopressin, hypercalcaemia, hypercalciuria, loop of Henle, nephrogenic diabetes insipidus, polyuria, urine osmolality, water.

Abbreviations: VP, vasopressin; DDAVP, [1-deamino,8-D-arginine]vasopressin (desmopressin); DI, diabetes insipidus; MCD, medullary collecting duct; mTAL, medullary thick ascending limb.

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The effects of hypercalcemia are shown to the left of the vertical broken line. When the concentration of ionized Ca\(^{2+}\) in plasma rises, Ca\(^{2+}\) binds to its receptor on the basolateral side of the mTAL cells, which leads to inhibition of their ROM-K channels (the major K\(^+\) conductance channel in the luminal membrane of the mTAL of the loop of Henle). With less entry of K\(^+\) into the lumen of the mTAL, there will be less reabsorption of Na\(^+\) and Cl\(^-\) via the Na\(^+\)/K\(^+\)/2Cl\(^-\) co-transporter. In addition, the lumen of the mTAL becomes less positively charged, and this retards the passive reabsorption of Na\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\).

The effects of hypercalciuria are shown to the right of the vertical broken line. If the luminal concentration of ionized Ca\(^{2+}\) rises sufficiently in the MCD, ionized Ca\(^{2+}\) will bind to its receptor in the lumen and impair water reabsorption in the MCD. The effects of hypercalciuria are shown to the left of the vertical broken line. When the concentration of ionized Ca\(^{2+}\) in plasma rises, Ca\(^{2+}\) binds to its receptor on the basolateral side of the mTAL cells, which leads to inhibition of their ROM-K channels (the major K\(^+\) conductance channel in the luminal membrane of the mTAL of the loop of Henle). With less entry of K\(^+\) into the lumen of the mTAL, there will be less reabsorption of Na\(^+\) and Cl\(^-\) via the Na\(^+\)/K\(^+\)/2Cl\(^-\) co-transporter. In addition, the lumen of the mTAL becomes less positively charged, and this retards the passive reabsorption of Na\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\).
Subjects remained on their ambient diet and fluid intake. Stone-formers with a plasma creatinine concentration of greater than 1.3 mg/dl were excluded, as were subjects with a urine pH greater than 6.3 (the latter was to avoid potential errors in the calculation of the concentration of ionized Ca in the urine). Hence a total of 1630 urine samples were included from patients with a positive history of kidney stones and a rate of excretion of Ca that was greater than 200 mg (5 mmol)/24 h. This is the group of subjects most likely to have a concentration of Ca in the urine that, in theory, should bind to the luminal Ca receptor in the MCD. The completeness of each collection was assessed by examining the rate of excretion of creatinine [9].

The concentrations of sodium (Na\(^+\)), potassium (K\(^+\)), chloride (Cl\(^-\)), Ca, phosphorus, Mg, uric acid and creatinine in urine were measured by standard laboratory methods using a Synchron CX5CE analyser (Beckman Instruments, Brea, CA, U.S.A.). Oxalate and citrate were measured enzymically using oxalate oxidase and citrate lyase respectively. Sulphate was measured by barium precipitation [10], and pH was measured using a glass electrode. The concentration of ionized Ca in the urine was measured using an ion-selective electrode in samples from 21 patients; concentrations in other urine samples were estimated from these measurements using the iterative computer program EQUIL2, which solves simultaneously for all ion-pair interactions [11]. The mean values for the directly measured and calculated values for ionized Ca in the urine were very strongly correlated, as shown in Figure 1 (\(r^2 = 0.97\); \(P < 0.001\)); hence we report the calculated values for ionized Ca in the survey portion of the data.

**Studies with 16 h of water deprivation**

Volunteers (\(n = 14\); age 17–59 years) consumed their usual diets and took no medication. Only subjects who had a concentration of Ca in the urine that exceeded 200 mg/l (5 mmol/l) were included, because they were most relevant to test the hypothesis outlined in Scheme 1. Each subject avoided water ingestion for 16 h, beginning at 20.00 hours on the evening prior to urine measurements (zero time). Subjects voided voluntarily at 08.00 hours the next morning (12 h time point), after which they were given desmopressin acetate [(1-deamino,8-D-arginine)-vasopressin (DDAVP); 0.3 μg/kg body weight] by nasal insufflation; two additional urine samples were provided voluntarily at 2 h intervals after taking DDAVP.

The concentrations of Na\(^+\) and K\(^+\) in these urine samples were determined by flame photometry (Radiometer FLM 3; London Scientific Ltd, London, Ontario, Canada); Cl\(^-\) was determined by electromembranetitration (Chloridemeter CMT 10; London Scientific Ltd); and ionized Ca was measured with an ion-selective electrode (Model 92-20 ionpair; Orion Research Co., Beverly, MA, U.S.A.). Osmolality was measured by freezing-point depression (advanced micro-osmometer model 3MO; Advanced Instruments Inc., Needham Heights, MA, U.S.A.). The pH and partial pressure of CO\(_2\) in urine were measured at 37 \(^\circ\)C with a digital pH/blood gas analyser (Corning 178 blood pH analyser); the concentration of bicarbonate (HCO\(_3\)) was calculated from the pH and partial pressure of CO\(_2\) using a solubility factor of 0.0309 and a pK adjusted for ionic strength [12,13]. Ammonium (NH\(_4\))\(^+\), citrate, creatinine, urea, Ca, Mg, sulphate, uric acid and phosphorus were measured as previously described [14,15].

**RESULTS**

There was an inverse correlation between the concentration of total or ionized Ca in the urine and the 24 h urine volume for normal subjects (Figure 2). For those normal subjects who had concentrations of total and ionized urinary Ca that exceeded 8 mmol/l and 4 mmol/l respectively, the urine volume was less than 1 litre per day. A similar survey was conducted in patients with a history of nephrolithiasis who excreted more than 200 mg (5 mmol) of Ca per day; urines with the highest concentration of Ca corresponded to the lowest urine volumes (Figure 3). Although these patients were not given DDAVP and their urine flow rate may not have been at its nadir, the majority of urine samples with a concentration of total Ca that was greater than 10 mmol/l had a volume that was less than 1 litre per day.

Since individual urine samples throughout the 24 h period might have had a high concentration of Ca and a high flow rate, a second protocol with much shorter collection periods was carried out with 14 normal subjects who excreted less than 200 mg (5 mmol) of Ca per day. In this acute study, DDAVP was administered so that the ability of hypercalciuria to cause a water diuresis of the nephrogenic diabetes insipidus (DI) type...
Figure 2  Relationship between the concentration of calcium in the urine and the 24 h urine volume for normal subjects
For details, see the Methods section. Each point represents the data from one subject. Log–log plots of the concentration of total Ca in the urine against volume (left panel) and of the concentration of ionized Ca in urine against urine volume (right panel) are shown for non-stone-forming subjects. Each point represents a single 24 h urine collection. The solid lines are the linear regressions (left panel, $r^2 = 0.68$, $P < 0.001$; right panel, $r^2 = 0.61$, $P < 0.001$). The urine samples with the highest concentrations of Ca are those with the lowest volumes.

Figure 3  Relationship between the concentration of calcium in the urine and the 24 h urine volume for patients with a history of nephrolithiasis
For details, see the Methods section. Each point represents the data from one patient. Log–log plots of the concentration of total Ca in the urine against urine volume (left panel) and the concentration of ionized Ca in the urine against urine volume (right panel) are shown for patients with nephrolithiasis. Only urine samples containing $\geq 200$ mg (5 mmol) of Ca per 24 h were included. Each point represents a single 24 h urine collection. The solid lines are the linear regressions (left panel, $r^2 = 0.59$, $P < 0.001$; right panel, $r^2 = 0.52$, $P < 0.001$).

could be evaluated. The range of concentrations of total Ca in the urine from these 14 subjects was from 5 to 17 mmol/l (Table 1, Figure 4). There was no evidence for a low urine osmolality or a high urine flow rate when the concentrations of total and ionized Ca in the urine were at these high values.

We considered the possibility that stone formers may have an abnormal response to the concentration of Ca in the urine in the lumen of the MCD which leads to higher concentrations of Ca in the urine than for normal subjects. To examine this hypothesis, we compared the slopes of the plots of Ca concentration against urine volume using the least-squares statistical model (Figures 2 and 3). There was no significant difference between the stone formers and normal subjects with regard to the concentration of either total or ionized Ca in the urine. Thus there was no support for the hypothesis that stone formers and normal subjects have different responses in renal concentrating ability that are related to the concentration of Ca in the urine.

Additional studies were carried out to evaluate whether the concentration of total Ca in the urine might differ markedly from that of its ionized form, which is the form likely to bind to the Ca receptor in the lumen of the MCD. First, those 24 h urine samples with the highest concentrations of ionized Ca also had the lowest volume (Figure 3). It is notable that the range of concentrations of ionized Ca in the urine was very wide. Secondly, the
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Table 1 Composition of urine samples from normal subjects in which the concentration of Ca was greater than 5 mmol/l

The data are means ± S.E.M. for urine samples that had a concentration of Ca that exceeded 5 mmol/l in a specimen collected after 12–16 h of water deprivation and following administration of DDAVP. Only one urine sample was included for each patient; individual values are shown in Figure 4. The concentration of ionized Ca was measured in samples from eight subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate (ml/min)</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg of water)</td>
<td>959 ± 41</td>
</tr>
<tr>
<td>pH</td>
<td>5.9 ± 0.2</td>
</tr>
<tr>
<td>Conc. (mmol/l)</td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>167 ± 12</td>
</tr>
<tr>
<td>K⁺</td>
<td>53 ± 4</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>173 ± 13</td>
</tr>
<tr>
<td>Total Ca</td>
<td>9.5 ± 0.7</td>
</tr>
<tr>
<td>Ionized Ca²⁺</td>
<td>6.2 ± 0.8</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>7.7 ± 0.7</td>
</tr>
<tr>
<td>Phosphate</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>Urea</td>
<td>431 ± 28</td>
</tr>
<tr>
<td>Creatinine</td>
<td>16 ± 1.4</td>
</tr>
<tr>
<td>Citrate</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>35 ± 7.7</td>
</tr>
</tbody>
</table>

concentration of ionized Ca in the urine was measured for eight of the 14 normal subjects reported in Figure 4, and ranged from 2.5 to 8.8 mmol/l. The concentration of citrate in the urine was relatively constant, and much lower in magnitude than the concentration of total or ionized Ca (Table 1). Hence it is unlikely that urines with a very high concentration of total Ca had a low concentration of ionized Ca due to the formation of Ca–citrate ion complexes.

DISCUSSION

The objective of the present study was to test the hypothesis that a high concentration of Ca in the lumen of the MCD, and thereby in the urine, would cause an important defect in the ability to excrete a very concentrated urine, i.e. induce an VP-resistant type of nephrogenic DI.

There are three types of experiment described in the literature that permit an evaluation of the relationship between the concentration of Ca in the urine and nephrogenic DI. At one extreme are data obtained from in vitro studies in animals. For example, Sands and co-workers [16] perfused isolated inner MCD segments from rats, adding VP to the bath and either 1 or 5 mmol of Ca per litre to the luminal fluid. With the higher luminal concentration of Ca, the permeability for water was significantly reduced and there was no change in the permeability for urea. Although these data support the view that a high concentration of Ca in the urine causes nephrogenic DI in qualitative terms, the decline in water permeability was quantitatively small (33% decrease). This raises the possibility that the residual permeability for water could still be high enough to prevent a major increase in excretion of electrolyte-free water in vivo.

The second category of experimental data was obtained in human subjects given an acute infusion of Ca to raise the concentration of Ca in plasma to 15 mg/dl (3.75 mmol/l) [17]. These subjects had a larger urine volume when hypercalciuria was present. Notwithstanding, although the urine flow rate was high, the urine osmolality was never less than the plasma osmolality. This high urine flow rate was largely the result of an increase in the rate of excretion of Na⁺ and Cl⁻ (rise in Na⁺ excretion from 100 to 536 µmol/min). Therefore an important reason for the high urine flow rate accom-

Figure 4 Effect of concentration of calcium in the urine on renal concentrating ability in human subjects given DDAVP

Urine flow rate (right panel) and osmolality (left panel) are plotted against urine Ca concentration. For details, see the Methods section. Each point represents the data from one of the 14 normal human subjects.
panying acute hypercalcaemia and hypercalciuria was the very high rate of excretion of \( \text{Na}^+ \) and \( \text{Cl}^- \), which may reflect the effects of hypercalcaemia in the medullary thick ascending limb (mTAL) in the loop of Henle (Scheme 1).

The third category of data was obtained from studies in patients with a chronic elevation in the concentration of Ca in the urine. The report of Zeffren et al. [18] is very important because 12 patients were studied, the concentration of \( \text{Na}^+ \) in plasma was reported, the urine osmolalities before and after VP administration were available, and they provided a longer-term follow-up. These data were again not consistent with a nephrogenic DI-type of lesion, because all but one of these patients had hyponatraemia rather than a tendency to hypernatraemia, a urine/plasma osmolality ratio of greater than 1, and a small further rise in urine osmolality when VP was administered. In the single patient who had data consistent with a diagnosis of nephrogenic DI (hypernatraemia and a urine/plasma osmolality ratio of less than 1 when AVP was administered), these findings persisted after the hypercalcaemia and hypercalciuria were reversed.

There have been many studies in animals in which hypercalcaemia and/or hypercalciuria was/were associated with a larger volume of urine (e.g. see [19–24]). In all of these studies, the increased urine volume was not associated with a urines osmolality that was excessively low; rather, an electrolyte-induced osmotic diuresis was present, consistent with the effects of hypercalcaemia acting on the mTAL (Scheme 1). Overall, the net effects of hypercalcaemia on the mTAL are similar to those of a loop diuretic. Therefore one can anticipate the excretion of a large volume of urine containing appreciable quantities of \( \text{Na}^+ \) and \( \text{Cl}^- \).

**Perspectives**

Although a Ca receptor faces the lumen of the MCD, extremely high urinary concentrations of total and ionized Ca did not compromise the renal concentrating process to a measurable extent (Figures 2–4). Rather, polyuria can be explained by the actions of hypercalcaemia on the mTAL of the loop of Henle, which lead to both a lower medullary interstitial osmolality and a higher distal delivery of \( \text{Na}^+ \) and \( \text{Cl}^- \). Nevertheless, can the hypothesis outlined in Scheme 1 be rescued? Clearly the portion in the MCD is the component of the hypothesis that is in jeopardy. A high degree of complex formation of Ca with citrate is a possible explanation as to why nephrogenic DI was not present. However, this explanation is not likely, because the concentration of ionized Ca was high and the concentration of total Ca was much greater than that of citrate (Table 1). Moreover, the excretion of citrate declines markedly in patients with metabolic acidosis [25,26], a condition that is not associated with nephrogenic DI. Further, although it might be logical for a high pH to attenuate the effects of urinary Ca on water permeability in the MCD (higher proportion of \( \text{HPO}_4^{2-} \)), this was not supported by our data (Table 1; Figures 2–4).

Perhaps there are other ways by which an elevated luminal concentration of Ca could increase the urine volume by a small, but significant, amount and thereby lessen the risk of Ca stone formation. Two points are relevant in this context. First, one need only achieve a small increase in urine volume to reduce the risk of this precipitation reaction. In more detail, if the urine flow rate doubled within the oliguric range, for example from 0.3 to 0.6 ml/min, the Ca \( \times \) oxalate ion product would decrease by 4-fold \((0.5 \times \text{concentration of Ca}) \times (0.5 \times \text{concentration of oxalate})\). Secondly, the volume of urine will increase if the rate of excretion of osmoles rises at a given urine osmolality. The two major categories of osmoles to consider are electrolytes (\( \text{Na}^+ \) and \( \text{Cl}^- \) for the most part) and urea. Since the reabsorption of urea in the inner MCD is enhanced during oliguria owing to the actions of VP [27], it would be theoretically possible to have a small increment in urine flow rate when the concentration of Ca in the urine is high if the net response of the binding of ionized Ca to its luminal receptor diminished the permeability of the inner MCD to urea. Consistent with this speculation is the observation of Gowrishankar et al. [28], who demonstrated that urea did not approach a diffusion equilibrium between the lumen of the inner MCD and the papillary interstitial compartment when the rate of excretion of electrolytes was low. Having urea as an ‘effective’ osmole in this setting would prevent an even lower urine volume [28,29] and thereby a higher concentration of Ca in the urine (Scheme 1).

**REFERENCES**

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