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SUMMARY

The formation of various types of kidney stones is strongly influenced by urinary pH. An alkaline pH favours the crystallization of calcium and phosphate containing stones, whereas and acidic urine pH promotes uric acid or cystine stones. The activity of many transport processes involved in calcium, citrate, and phosphate handling are sensitive to changes in systemic or local pH as shown for several phosphate transporters, the citrate transporter NaDC1, or the TRPV5 calcium channel. Defects in urinary acidification (excretion of inappropriately alkaline or acidic urines, respectively) contribute to kidney stone disease. The low excretion of ammonium in patients with metabolic syndrome has been linked to more acidic urine and a higher incidence of uric acid stones. In this state, insulin resistance may reduce ammonium excretion by the proximal tubule. On the other hand, defensive mechanisms may protect from kidney stone formation in conditions such as hypercalciuria where high luminal calcium concentrations stimulate urinary acidification and reduce urinary concentration via a Calcium-sensing receptor resulting in the excretion of acidic and diluted urine. This review will discuss a few aspects that relate to the capacity of the kidney to regulate pH and its impact on the excretion of solutes that participate in the formation or prevention of stones.

INTRODUCTION

Kidney stones are a common health problem in industrialized countries affecting between 2-5 % of the population during lifetime at least once. A considerable percentage of patients, however, experiences recurrent kidney stones with pain, urinary tract infections, and possibly leading to loss of functional renal parenchyma which may ultimately cause even renal insufficiency. Many factors predispose or contribute to the development of kidney stones including genetic variants or mutations, diet, environmental factors, and behaviour (1-3).

Genetic studies in humans and animal models have helped to dissect some pathways that increase the risk to develop kidney stones. Mutations in several enzymes, transporters, receptors or channels have been identified in patients with rare mendelian forms of kidney stones. Many of these mutations cause either an increased excretion of substances that can form crystals or stones, alter the composition of the urine leading to conditions that favour crystal formation, or both. Examples include mutations in the CLC-5 chloride/proton exchanger in Dent's disease, in the chloride/bicarbonate exchanger AE1 (SLC4A1), in the calcium-sensing receptor CaSR, in Bartter's syndrome (mutations in ROMK, NKCC2, ClC-Kb, or Barttin), claudin 16, or the two subunits of the cystine transporter $b^{0,+}$AT-rbAT in cystinuria (SLC7A9-SLC3A2) (1,3-4).

Alterations in urinary pH can be caused by genetic variants or mutations in transport pathways, by life style habits such as specific diets, or metabolic diseases. Inappropriately acidic or alkaline urine affects the solubility of various metabolites and salts. Alkaline urine reduces solubility of calcium phosphate products whereas acidic

urine pH promotes formation of uric acid or cystine containing stones (1-3). Moreover, changes in systemic pH homeostasis as seen in forms of chronic metabolic acidosis alter urine concentrations of substances that contribute to crystal formation (e.g. calcium, phosphate) as well as of substances that may prevent stone formation (e.g. citrate or magnesium).

Here, we will review briefly mechanisms that contribute to stone formation and relate to either systemic acid-base homeostasis or urinary acidification. We will not be able to discuss in detail a large body of literature on other forms and causes of nephrolithiasis, or its treatment.

Local or systemic acidosis alters renal excretion of solutes involved in kidney stone formation

The impact of systemic acid-base homeostasis and/or urinary acidification on the urinary excretion of stone-forming minerals and metabolites has been elucidated on the molecular levels for several substrates. Urinary phosphate excretion increases during metabolic acidosis where urinary phosphate plays an important role as part of the titratable acidity (5-7). The increase in urinary phosphaturia is paralleled by stimulated expression of the intestinal phosphate transporter NaPi-IIb that might contribute to the compensation of renal phosphate losses (8). Moreover, bone releases massive amounts of phosphate which in chronic acidosis favors bone mineral losses (9). Thus, increased intestinal phosphate absorption as well as release of phosphate from bone may contribute to renal phosphate excretion.

Moreover, systemic acidosis and local urinary acidification most likely play an important role by directly affecting phosphate reabsorption in the proximal tubule. It has been discussed whether systemic acidosis increases phosphaturia by reducing the expression of the NaPi-IIa phosphate cotransporter. Ambühl et al. demonstrated reduced mRNA and protein levels in rat kidney (6), whereas we (10) and Villa-Bellosta did not find changes in expression in mouse and rat kidney (11). Moreover, NaPi-IIc and Pit2, two other proximal phosphate transporters, even increase their expression, possibly to compensate for renal phosphate losses. Local pH, however, has a strong impact on the function of brush border membrane phosphate transporters. Acidic pH decreases activity of the two main transporter, NaPi-IIa and NaPi-IIc, whereas Pit2 is stimulated (11), {Amstutz, 1985 #86}{Ravera, 2007 #386}.

During acidosis, the urinary excretion of calcium and magnesium is also increased (12). Systemic acidosis downregulates the expression and activity of the TRPV5 calcium channel, expressed in the late distal convoluted tubule and connecting tubule (13). In contrast, it increases expression of the calcium-binding protein calbindin 28k (14). Similar to proximal tubular phosphate transporters, local pH also directly affects the function and recycling of the TRPV5 channel (12,15). Thus, local and systemic acidosis increases urinary excretion of calcium and phosphate and thereby increases the risk for crystallization and stone formation.

Moreover, the excretion of citrate, an important anti-lithogenic metabolite, is decreased during acidosis. Citrate inhibits stone formation by complexing with calcium in the urine, reducing spontaneous nucleation, and thereby preventing growth and agglomeration of crystals (16). Urinary citrate excretion is determined by

the amount of citrate filtered in the glomerulum and the subsequent reabsorption at the level of the proximal tubule by the sodium-dependent citrate cotransporter NaDC-1 expressed in the brush border membrane. Expression and activity of NaDC-1 is increased during metabolic acidosis or potassium depletion thereby lowering urinary citrate levels (17-18).

Uric acid nephrolithiasis and metabolic syndrome: lack of ammonium

Urinary net acid excretion is the product of mechanisms acidifying urine such as Na^+/H^+-exchangers (mainly in the proximal tubule and the thick ascending limb of the loop of Henle) and H^+-ATPases (along the proximal tubule, and mostly in the collecting duct system) and buffers that bind and neutralize protons such as citrate, phosphate, creatinine, and ammonia (7,19-20). Lower urinary concentrations of these buffers can cause more acidic urine which in turn can favor the crystallization of solutes such as cystine or uric acid. Patients with metabolic syndrome develop frequently uric acid kidney stones, which has been thought to be due to metabolic abnormalities increasing uric acid excretion. However, a more careful characterization of patients with metabolic syndrome or type II diabetes revealed an unique combination of normal urinary uric acid levels, rather acidic urine pH and low urinary ammonium levels suggesting that a lack of ammonia buffer may underlie the acidic urine and higher propensity to form uric acid crystals (21-23).

Urine ammonium concentrations are the result of renal ammoniagenesis and the subsequent transport of ammonium along the nephron and its subsequent secretion into urine at the level of the collecting duct (20,24). Ammoniagenesis occurs in the proximal tubule, requires glutamine as substrate, generates bicarbonate

and ammonia, and is closely linked to gluconeogenesis. Glutamine is taken up from blood via the SNAT3 amino acid transporter (25) and metabolized. This process is stimulated during acidosis and may also be regulated by insulin or glucocorticoids (25-28). Subsequently, ammonium is excreted mostly into urine by substituting for protons in the sodium/proton exchanger NHE3. Later, ammonium is partly reabsorbed in the thick ascending limb of the loop of Henle via the NKCC2 cotransporter, accumulated in the interstitium and secreted as ammonia (NH_3) into urine along the collecting duct. The last step, secretion of NH_3 into urine is mediated by the RhCG rhesus protein, a novel NH_3 transporting protein (19-20,29).

Bobulescu and colleagues used obese and diabetic rats to further investigate the mechanisms underlying lower levels of ammonium in patients with metabolic syndrome. They showed accumulation of lipids in the proximal tubule, reduced expression of the Na^+/H^+- exchanger NHE3, and resistance of NHE3 to the stimulatory action of insulin or glucocorticoids (30). In a reverse experiment, Zucker diabetic fatty rats were treated with thiazolidinediones to reduce fat mass which normalized urinary acidification and ammonium excretion (31). Similarly, we used mice fed a high calorie diet to induce obesity and relative insulin resistance. These mice had normal urinary pH and ammonium excretion at baseline but were less able to adapt to an acid load with lower urinary ammonium excretion (Busque and Wagner, unpublished results). We found highly elevated expression of the glutamine transporter SNAT3, and the ammoniagenic enzyme phosphate-dependent glutaminase as well as of the gluconeogenic enzyme phosphoenol pyruvate carboxy kinase (PEPCK) in the proximal tubule. However, the activity of the NHE3 Na^+/H^+ exchanger in the brush border membrane of the proximal tubule was reduced. Taken together all data suggest that obesity and a state of relative or absolute insulin

resistance affect urinary ammonium excretion mostly at the level of the proximal tubule and that dysregulation of NHE3 may play an important role in reducing ammonium excretion. Expression of RhCG as final mediator of ammonia excretion remained normal in obese and insulin-resistant mice.

Urinary calcium can stimulate urinary acidification

Thus various defects in urinary acidification or deranged systemic acid-base status can affect the excretion and concentration of solutes in urine. Is there a feedback mechanism by which the concentration of urinary solutes can affect urinary pH by other means than changing simply buffering power ? Mice lacking the TRPV5 calcium channel, the major reabsorptive pathway for transcellular calcium reabsorption in the late distal convoluted tubule and connecting tubule, excrete massive amounts of calcium in urine. Moreover, urinary phosphate excretion is also enhanced, possibly due to elevated vitamin D_3 levels and stimulated intestinal phosphate absorption. However, these mice do not show signs of nephrocalcinosis or kidney stone formation (32). The massive increase in urinary calcium und phosphate excretion is paralleled by enhanced diuresis and also a very acidic urine, two factors, that may reduce the risk for the formation of calcium phosphate crystals. Sands and colleagues had shown that the collecting duct expresses luminal Calcium-sensing receptors (CaSR) (33) and that stimulation of the CaSR reduces basal and vasopressin stimulated water absorption via the AQP2 water channel. Consistently, mice lacking TRPV5 had reduced AQP2 water channel expression which may contribute to the higher diuresis. We also tested the hypothesis that high luminal calcium may stimulate urinary acidification by vacuolar type H^+-ATPases expressed

in intercalated cells (34). The activity of these H^+-ATPases is stimulated by a variety of hormones such as angiotensin II, aldosterone or endothelin or by increases in CO_2 or during metabolic acidosis (35-39). Isolated collecting ducts from wildtype and TRPV5 KO mice had similar H^+-ATPase activity. However, high calcium or neomycin stimulated H^+-ATPase activity suggesting that Calcium-sensing receptors or a related sensor may sense luminal calcium concentrations and regulate urinary acidification by stimulating H^+-ATPase activity.

Does increased urinary acidification by H^+-ATPases protect from the formation of kidney stones in mice with hypercalcuria and hyperphosphaturia ? We tested this hypothesis by crossing TPRV5 KO mice with mice lacking the B1 H^+-ATPase subunit. The B1 subunit forms part of the cytosolic H^+-ATPase domain, is selectively expressed in intercalated cells, and is required for maximal urinary acidification (35,37,40-41). Patients with mutations in the B1 H^+-ATPase gene ATP6V1B1 suffer from distal renal tubular acidosis with sensorineural deafness (42). B1 KO mice excrete a more alkaline urine than wildtype mice but do not develop kidney stones. Double KO mice (TRPV5-B1) excreted high amounts of calcium and phosphate but with a more alkaline urine than TRPV5 KO mice. These mice died a few weeks after birth with massive hydronephrosis and kidney stones. Detailed analysis of calcifications and stones demonstrated a composition mainly of phosphate and calcium. Thus, H^+-ATPase mediated urinary acidification prevents the formation of calcium phosphate containing kidney stones in a mouse model of excessive hypercalciuria.

It has been debated whether these protective mechanism can be translated from mouse to man (43). Major differences between hypercalciuric patients and the

TRPV5 KO mouse model may be the level of urinary calcium which is very highly elevated in these mice. Possibly, rather very high calcium concentrations are needed to elicit these protective mechanisms.

Summary

Systemic acid-base status and urine pH have profound effects on the kidneys ability to secrete or reabsorb metabolites and solutes that contribute to the risk of stone formation. The elucidation of molecular mechanisms of transport have provided valuable insights into physiologic processes. Very little is known how changes in systemic or local pH translate into changes in transport functions, whether transport moelcules and ion channels are intrinsically pH-sensitive as suggested for some or whether local or systemic pH sensors trigger regulatory cascades affecting renal function. Nevertheless, some of the mechanisms outlined above contribute to the risk of stone formation in patients but in a large number of patients additional mechanisms must contribute to pathology since many patients with so-called idiopathic hypercalcuria do not display obvious problems in local or systemic pH regulation. In the case of uric acid kidney stones, the discovery that urinary ammonium excretion is deranged and the localization of this defect to the proximal tubule in experimental animal models has deepened our understanding of this particular type of kidney stones.

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