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It Is Really Time for Ammonium Measurement

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EDITORIAL

It Is *R*eally *T*ime for *A*mmonium Measurement

In this issue of Advances in CKD, Guest Editors, Jose A. Arruda and Daniel Batlle, have assembled an A-list of clinician-scientists to review the old and new concepts of renal tubular acidosis (RTA). In an era in which dialysis and CKD dominate nephrology clinical practice, is it not nice to encounter something different every now and then? A low-serum venous total CO_2 (referred to as bicarbonate, hereafter), of which the etiology is not readily apparent, is one of the many respites for acid-base mavens of nephrology. Tantalizingly, one is lured toward a diagnosis of RTA, one of the true gems in our field. The opportunity to successfully diagnose one of these fascinating disorders harkens back to the time of Pitts, Lotspeich, Van Slyke, and others, before ensconcing us in a contemporary realm of molecular transporters and genetic discoveries that have verified and illumined these giants' prior work. Collectively, this minireview covers diverse topics including acid-base physiology and pathophysiology, genetics, diagnostic procedures, complications, and management of RTA. It will solidify readers' preexisting knowledge regarding the RTAs and discuss cutting-edge developments in the field.

OVERVIEW OF RTA

The maintenance of normal pH is one of the most important and tightly regulated physiological processes. In the steady state, respiratory ventilation eliminates about 15,000 mmol of carbon dioxide, and the kidneys eliminate 50-100 meq of protons each day to maintain an extracellular $[H^+]$ of ~40 nmol/L. Although the acid elimination requirement is much lower for the kidneys, nonvolatile acid retention, which occurs with RTA, can lead to significant clinical consequences including nephrolithiasis, nephrocalcinosis, dyskalemia, failure to thrive, and reduced growth.

Although genetic etiologies of RTA are uncommon, RTA can be a manifestation of many more common diseases and exposures. In children and young adults, a genetic mutation is a strong possibility. Distal (type 1) RTA can be caused by mutations in the luminal H^+-ATPase or the basolateral Cl^-/HCO_3^- exchanger, and in proximal (type 2) RTA, mutations in the Na^+-HCO_3^- cotransporter or carbonic anhydrase II may be identified. Type-4 RTA can be caused by mutations in the with-no-lysine kinase proteins 1 and 4 (WNK 1 and WNK 4) that produce pseudohypoaldosteronism type 2, also known as Gordon's syndrome, or by mutations of the mineralocorticoid receptor or the epithelial sodium channel (pseudohypoaldosteronism type 1). In adults, autoimmune disorders, particularly Sjögren's syndrome, systemic lupus erythematosus, and rheumatoid arthritis, are common causes of RTA. In addition, RTAs can be a side effect of medications including renin-angiotensin-aldosterone system inhibitors, nonsteroidal anti-inflammatory drugs, calcineurin inhibitors, and carbonic anhydrase inhibitors. Monoclonal gammopathies, hypercalciuric states, heavy metals, and multiple other conditions may cause an RTA.

WHAT BICARBONATE THRESHOLD DEFINES A LOW VALUE?

Although unfortunate from a patient's perspective, the diversity of etiologies of RTA makes it one of the most intellectually challenging diagnostic evaluations. In most cases, a low bicarbonate concentration with a normal albumin-adjusted serum anion gap is the first hint of an RTA. However, the normal bicarbonate concentration range is highly variable across clinical laboratories, which may cause clinicians to overlook an important acid-base abnormality. This significant variability was recently highlighted by Kraut and Madias who reported the normal bicarbonate ranges of 66 clinical laboratories in the United States. They determined that the lower limit of normal ranged from 18 to 25 meq/L, and the upper limit of normal ranged from 26 to 35 meq/L.[1] Hence, a patient with serum bicarbonate of 19 meq/L may not be evaluated for an acid-base disorder if the normal range encompasses this value. To avoid this potential problem, Kraut and Madias proposed that clinical laboratories adopt a normal

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bicarbonate range of 23-30 meq/L based on carefully considered acid-base principles.[1] This lower limit is near the lower limit commonly used in CKD (22 meq/L).[2] Note that this proposed "normal" bicarbonate range does not apply to individuals residing at altitude where the bicarbonate range may be lower than that of sea level, attributable to hypoxemia and hypocapnia. Whether clinical laboratories will adopt a uniform normal bicarbonate range is uncertain. In the meantime, clinicians should consider a serum bicarbonate concentration <23 meq/L as abnormally low, irrespective of the normal range reported by the laboratory.

Importantly, the severity of hypobicarbonatemia is, in most cases, an unreliable indicator of whether the underlying acid-base disorder is metabolic acidosis or respiratory alkalosis as the bicarbonate concentration may be reduced to a physiological nadir of 10-12 meq/L from chronic respiratory alkalosis, accompanied by astoundingly low PCO_2 levels.[3] Furthermore, estimating urinary ammonium excretion, such as with the urine anion gap (UAG), cannot distinguish between RTA and respiratory alkalosis because ammonium excretion is reduced in both the settings. Thus, a venous, or better yet, arterial or arterialized venous blood gas must be obtained in persons with preserved kidney function to critically determine whether the low bicarbonate level is due to metabolic acidosis or respiratory alkalosis. For those with CKD, it is reasonable to assume that metabolic acidosis is the underlying cause of a low bicarbonate concentration; nevertheless, a blood gas analysis may be warranted depending on the clinical scenario.

EVALUATION OF NORMAL ANION GAP METABOLIC ACIDOSIS

If the clinical presentation is consistent with a normal anion gap metabolic acidosis, a diagnosis of RTA should be considered. Simply described, RTA results from either impaired kidney proton elimination or bicarbonate reclamation. With distal RTA, impaired distal hydrogen secretion leads to acid retention, high urine pH, and low urinary ammonium excretion because of impaired ammonia trapping. Because distal sodium absorption necessitates secretion of a cation to maintain electroneutrality, insufficient hydrogen secretion is offset by an increase in distal potassium secretion. Metabolic acidosis also impairs proximal sodium reabsorption, provoking mild extracellular volume depletion and enhanced aldosterone activity. These factors synergize to produce hypokalemia in distal RTA. In hyperkalemic forms of distal RTA (hypoaldosteronism or voltage dependent), reduced kidney ammoniagenesis from high plasma potassium concentrations reduces ammonium excretion as well, leading to acid accumulation and metabolic acidosis. Finally, bicarbonaturia in proximal RTA is reflected by an increased urine pH > 6.0, until distal bicarbonate resorption can reclaim the filtered bicarbonate load. Net acid retention ensues, whereas urinary ammonium excretion is preserved. Contrastingly, hypokalemia in proximal RTA may result from a generalized impairment of proximal tubule function or a combination of mild volume depletion, hyperaldosteronism, and enhanced distal sodium delivery.

These principles are cornerstones of the diagnostic approach to a normal anion gap metabolic acidosis (Fig 1). The urine pH, serum potassium concentration, and the UAG help the clinician distinguish between the various RTAs and gastrointestinal alkali loss.[4] Perplexed trainees remind us that the diagnostic approach is not so simple. It is easy to comprehend why the urine pH is >5.3 in distal RTA, but the dynamic nature of proximal RTA and hyperkalemic RTA confuses the picture, as the urine pH can be above or below this threshold in these 2 circumstances. The serum potassium concentration may provide some clarity. However, type-1 RTA, which usually presents with hypokalemia, may be accompanied by hyperkalemia.

The UAG sets the stage by signaling whether there is enhanced (negative UAG) or insufficient (positive UAG) kidney ammonium excretion in the setting of a normal anion gap metabolic acidosis. The latter situation is observed in distal RTA and hyperkalemic RTA, whereas the former is seen with loss of alkali from the gastrointestinal tract or kidney (proximal RTA). The concept that the UAG is a surrogate of kidney ammonium excretion frequently puzzles students. After ascertainment, the astute learner will ask the following simplest question: "why do we not just measure urinary ammonium?" The awkward but candid response is that most clinical laboratories do not offer urinary ammonium testing although the test is within the grasp of any clinical laboratory. An enzymatic assay can be performed on 1 mL of urine and is readily and commercially available. The absence of

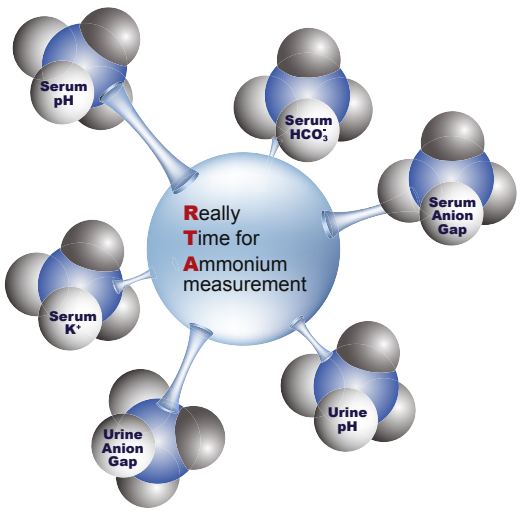

Figure 1. The diagnostic approach to a normal anion gap metabolic acidosis includes several urine and serum tests. The urine anion gap is one of the most important tests, but it merely assesses the robustness of urinary ammonium excretion in the setting of metabolic acidosis. A growing body of literature supports direct urine ammonium measurement in chronic kidney disease, and this measurement can readily be done by clinical laboratories. It is time to measure urine ammonium directly.

urinary ammonium testing may be our fault. Perhaps, we did not advocate strongly enough for ammonium testing's widespread adoption and implementation. To be fair, in cases where an attentive clinician cannot clearly identify the cause of a normal anion gap metabolic acidosis, the UAG may perform quite well as an estimate of the robustness of ammonium excretion. Hence, do we really need urinary ammonium measurements in clinical practice?

THE CASE TO MEASURE AMMONIUM IN CKD

While we survived sans ammonium measurements in cases of normal anion gap metabolic acidosis, results from studies in CKD suggest that urinary ammonium excretion may be a helpful risk-assessment tool. Specifically, lower urinary ammonium excretion was a risk factor for CKD progression and mortality in the African-American Study of Kidney (AASK) Disease and Hypertension and the NephroTest Cohort, a French study of over 1000 adult patients included from 2000 to 2010 with CKD stages 1-4.[5,6] In addition, lower ammonium excretion was associated with 2.5-fold higher risk of developing overt metabolic acidosis in AASK.[5] Estimating ammonium excretion using the standard UAG formula ($UAG = Na^+ + K^+ - Cl^-$) failed to replicate the ammonium findings in AASK. Noteworthy is the observation that there was a direct, not inverse, relationship between UAG and ammonium.[7] Therefore, the standard UAG is not a reliable estimate of ammonium in CKD. Adding urine phosphate and sulfate to the UAG equation demonstrated correlation with ammonium in an inverse direction. This mathematical maneuver better recapitulated the increased risk of CKD progression and death identified through employment of direct ammonium measurement.[7] The results implied that urinary ammonium is a risk factor for poor outcomes in CKD, and the standard UAG does not perform well in this setting, unless phosphate and sulfate are included in the formula. A word of caution, however, this technique requires the measurement of 5 variables, thereby increasing the risk of measurement error and cost.

The urine osmolar gap (UOG) has exhibited excellent correlation with ammonium in normal individuals and diverse settings, including patients with RTA and CKD, with or without metabolic acidosis.[8-10] Consequently, the UOG may provide superior estimation of ammonium excretion in CKD patients compared with the UAG. The difference between the measured and calculated urine osmolality purportedly represents unmeasured urinary osmoles, and ammonium salts are considered as the major component of the UOG. Under these assumptions, one-half of the UOG should roughly correspond to the ammonium concentration. However, results from studies evaluating the correlation between the UOG and ammonium revealed that approximately 10%-20% of individuals had a negative UOG.[8-10] This result is problematically predicated on the assumption that the UOG represents unmeasured osmoles, including ammonium.

In a study of kidney transplant recipients, there was no correlation between the UOG and ammonium excretion ($r = 0.01$), and 40% of samples (28 of 70) had a negative UOG.[11] Similar findings were observed in another study evaluating the correlation between calculated and measured urine osmolality in 5 European cohorts. A large proportion of individuals in that study had a higher calculated than measured osmolality. For example, the mean calculated osmolality was 7 mOsm/L higher than mean measured osmolality in the largest of these cohorts ($n = 2305$).[12] The reason urine-calculated osmolality exceeded its measured counterpart may stem from incomplete dissociation of sodium and potassium ions from their respective accompanying anions. For example, in sodium chloride solutions with osmolality in the physiologic range of serum, the osmotic coefficient (φ) of sodium chloride is 0.93. The φ also decreases with increasing osmolality and is affected by other constituents in the solution, making it difficult to predict what fraction of sodium chloride is dissociated in complex solutions such as urine.[13,14] Hence, doubling the concentrations of sodium and potassium overestimates their true contributions to urine osmolality and mathematically produces a negative UOG. Thus, the UOG must be cautiously interpreted with this concept at the top of mind and may not represent a reliable indicator of unmeasured osmoles, including ammonium.

Thus, ammonium excretion appears to be a marker of poor outcomes in CKD, and commonly used estimates equally poorly evaluate risk of progression. Therefore, the case to incorporate urinary ammonium measurements into routine clinical practice to assess risk in CKD is fortified. Urinary ammonium assessment may also facilitate therapeutic decision-making in CKD. It is plausible that a patient with normal serum bicarbonate concentration but low ammonium excretion might benefit from alkali before the onset of overt metabolic acidosis. Nonetheless, this approach requires further investigation.

Another situation in which knowledge of ammonium excretion may be valuable is in those with calcium-phosphate stones. In this setting, a potassium citrate dose that reduces daily ammonium excretion by one-half to two-third has been advocated to inhibit apatite nucleation and growth while minimizing the risk of increasing urine pH and calcium-phosphate supersaturation.[15] If urinary ammonium is indeed a good predictor of poor outcomes in CKD and/or can help guide therapeutic decision-making, more clinical laboratories may be inclined to perform urine ammonium testing. Some clinical laboratories (Litholink, Chicago, IL, and Mayo Clinic, Rochester, MN) in the United States currently measure urine ammonium concentration primarily for the metabolic evaluation of urolithiasis. Other laboratories could follow suit by measuring ammonium from an aliquot of urine diluted 1:100 using the standard, auto-analyzer plasma ammonia assay.[16] More frequent calls for evaluation of urinary ammonium during evaluations of a normal anion gap metabolic acidosis, CKD risk assessments, and nephrolithiasis can and should start now.

RTA AND GLOMERULAR FILTRATION RATE DECLINE

The adverse effects of RTA on potassium, calcium stone disease, and growth in children are well known. Whether RTA is associated with a more rapid decline of glomerular filtration rate (GFR) is unclear. The notion is credible because acid retention, hypokalemia, and nephrocalcinosis, individually and collectively, contribute to kidney injury and tubulointerstitial fibrosis. Prior studies have not clearly demonstrated an increased risk of GFR decline in RTA[17] although a recent study of 89 individuals with distal RTA showed that approximately one-third had CKD (defined as eGFR < 90 mL/min per 1.73 m^2). A few demonstrated a rapid rate of GFR decline after puberty.[18] These findings require confirmation but support the tenet that RTA patients are at risk for ongoing dissipation of kidney function. This may be due to a variety of reasons, including kidney acid retention, aggravated by high-protein diets, as delineated recently by Goraya and colleagues.[19]

SUMMARY

Since their conceptions, the UAG and UOG have served the vital purpose of informing nephrologists about the robustness of urinary ammonium excretion in the setting of metabolic acidosis. The UAG is more commonly determined and often confirms what is suspected clinically. The UOG may be a helpful adjunct, particularly in cases of toluene poisoning, diabetic ketoacidosis, or pyroglutamic acidosis. In these situations, the UOG will be appropriately increased, whereas the UAG may be positive, despite robust ammonium excretion, because of the presence of unmeasured urinary anions. However, the intrinsic chemistry of urinary solutions and presence of unmeasured urinary anions deny UAG analyses the precision of direct ammonium measurement. With the availability of highly esoteric laboratory tests, it is silly that we estimate urinary ammonium excretion when we have the capacity to directly measure it. It is "really time for ammonium" measurement in our clinical laboratories.

Il meglio è nemico del bene (The better is enemy of the good).—Orlando Pescetti, *Proverbi italiani* (1603)

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