Incomplete dRTA in kidney stone formers: diagnostic performance of furosemide/fludrocortisone testing and non-provocative clinical parameters

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Abstract:	Background and objectives: Incomplete distal renal tubular acidosis is a well-known cause of calcareous nephrolithiasis but the prevalence is unknown, mostly due to lack of accepted diagnostic tests and criteria. The ammonium chloride test is considered as gold standard for the diagnosis of incomplete distal renal tubular acidosis, but the furosemide/fludrocortisone test was recently proposed as an alternative. Due to the lack of rigorous comparative studies, the validity of the furosemide/fludrocortisone test in stone formers remains unknown. In addition, the performance of conventional, non-provocative parameters in predicting incomplete distal renal tubular acidosis has not been studied. Design, setting, participants, and measurements: We conducted a prospective study in an unselected cohort of 170 stone formers that underwent sequential ammonium chloride and furosemide/fludrocortisone testing.

Results: Using the ammonium chloride test as gold standard, the prevalence of incomplete distal renal tubular acidosis was 7.78 %. Sensitivity and specificity of the furosemide/fludrocortisone test FF test were 77 % and 85 %, respectively, yielding a positive predictive value of 30 % and a negative predictive value of 98 %. Testing of several non-provocative clinical parameters in the prediction of incomplete distal renal tubular acidosis revealed fasting morning urinary pH and plasma potassium as the most discriminative parameters. The combination of a fasting morning urinary threshold pH <5.3 with a plasma potassium threshold >3.8 mmolmEq/l yielded a negative predictive value of 98 % with a sensitivity of 85 % and a specificity of 77 % for the diagnosis of incomplete distal renal tubular acidosis.
Conclusions: The furosemide/fludrocortisone test can be used for incomplete distal renal tubular acidosis screening in stone formers, but an abnormal furosemide/fludrocortisone test result needs confirmation by ammonium chloride testing. Our data furthermore indicate that incomplete distal renal tubular acidosis can reliably be excluded in stone formers by use of non-provocative clinical parameters.

Furosemide/Fludrocortisone test and clinical parameters to diagnose incomplete distal renal tubular acidosis in kidney stone formers

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ABSTRACT

Background and objectives: Incomplete distal renal tubular acidosis is a well-known cause of calcareous nephrolithiasis but the prevalence is unknown, mostly due to lack of accepted diagnostic tests and criteria. The ammonium chloride test is considered as gold standard for the diagnosis of incomplete distal renal tubular acidosis, but the furosemide/fludrocortisone test was recently proposed as an alternative. Due to the lack of rigorous comparative studies, the validity of the furosemide/fludrocortisone test in stone formers remains unknown. In addition, the performance of conventional, non-provocative parameters in predicting incomplete distal renal tubular acidosis has not been studied.

Design, setting, participants, and measurements: We conducted a prospective study in an unselected cohort of 170 stone formers that underwent sequential ammonium chloride and furosemide/fludrocortisone testing.

Results: Using the ammonium chloride test as gold standard, the prevalence of incomplete distal renal tubular acidosis was 8 %. Sensitivity and specificity of the furosemide/fludrocortisone test were 77 % and 85 %, respectively, yielding a positive predictive value of 30 % and a negative predictive value of 98 %. Testing of several non-provocative clinical parameters in the prediction of incomplete distal renal tubular acidosis revealed fasting morning urinary pH and plasma potassium as the most discriminative parameters. The combination of a fasting morning urinary threshold pH <5.3 with a plasma potassium threshold >3.8 mEq/l yielded a negative predictive value of 98 % with a sensitivity of 85 % and a specificity of 77 % for the diagnosis of incomplete distal renal tubular acidosis.

Conclusions: The furosemide/fludrocortisone test can be used for incomplete distal renal tubular acidosis screening in stone formers, but an abnormal furosemide/fludrocortisone test result needs confirmation by ammonium chloride testing. Our data furthermore indicate that incomplete distal renal tubular acidosis can reliably be excluded in stone formers by use of non-provocative clinical parameters.

INTRODUCTION

Incomplete distal renal tubular acidosis (dRTA) is a condition characterized by defective urinary acidification capacity in the absence of systemic metabolic acidosis. The entity was first described by Wrong and Davies in 1959 who reported 3 patients that were unable to maximally acidify their urine upon administration of an oral acid load (100 mg [1.86 mmol] ammonium chloride/kg body weight) [1]. Based on this study the one day single dose ("short") ammonium chloride loading test became the "gold standard" for diagnosis [1]. Typically, a pH < 5.3 has been accepted as threshold to rule out dRTA but there has never been a clear consensus on this threshold pH and various other definitions have been employed in the past [2-8]. Using non-uniform definitions and provocative test procedures, a wide range of prevalence of incomplete dRTA from 2 to 21 % have been reported in recurrent stone formers [3, 9, 10].

Provocative urinary acidification testing is not routinely conducted at most stone clinics but most clinics have routine clinical data on their patients with nephrolithiasis. How well do conventional non-provocative clinical parameters perform in the prediction of incomplete dRTA is not known and previous recommendations have been based primarily on expert opinions rather than data [10, 11]. Even when the short ammonium chloride loading test can be performed in some centers, gastrointestinal side effects occur frequently after ammonium chloride ingestion. In 2007, Walsh et al. described an alternative provocation of distal acidification, the furosemide/fludrocortisone test, which is better tolerated than the ammonium chloride loading test [12]. In a study that included 10 patients with nephrolithiasis or nephrocalcinosis (all without dRTA), Viljoen et al. observed a high false positive rate of incomplete dRTA diagnosis with the furosemide/fludrocortisone test compared to the gold standard ammonium chloride test, but fludrocortisone was given 10 h prior to test start and not simultaneously, as described by Walsh et al. [12, 13]. Thus, due to the current lack of

adequately powered well-controlled comparative studies, the validity of the furosemide/fludrocortisone test in uncovering incomplete dRTA in stone formers remains unknown.

To address the issues outlined above, we compared clinical non-provocative parameters and the furosemide/fludrocortisone test results to the standard short ammonium chloride test in an unselected group of stone formers referred to our clinic for metabolic stone work-up.

MATERIALS AND METHODS

Study design and study population

This was a prospective comparative study. Study participants were recruited from stone formers referred to the Division of Nephrology and Hypertension at the Bern University Hospital between September 2012 and June 2016. Inclusion criteria were: i) ≥ 1 kidney stone episodes, ii) age ≥ 18 and iii) written informed consent. Exclusion criteria were: i) active urinary tract infection, ii) intake of medications known to interfere with urinary acidification, iii) pregnancy or lactation. Failure to acidify urinary pH < 5.3 in the ammonium chloride test was considered diagnostic for incomplete dRTA diagnosis. Complete dRTA was defined as presence of a pathological urinary acidification test with a concomitant metabolic acidosis in the morning fasting blood gas analysis (blood pH < 7.35 and blood bicarbonate < 21 mEq/l). Patients with an abnormal urinary acidification test without metabolic acidosis were considered to have incomplete dRTA. No sample size calculation was performed before start of the study because i) the prevalence of idRTA in our referred patients was not known and ii) absence of data with respect to the performance of the furosemide/fludrocortisone test in the diagnosis of idRTA in an unselected cohort of stone formers. The study was approved by the Ethical Committee of the Kanton Bern (approval # 90/12), registered at ClinicalTrials.gov (NCT01690039) and conducted in accordance with the Declaration of Helsinki.

Data collection and measurements

Metabolic work-up for stone disease included two 24 h urines on a random outpatient diet. Urine and blood analyses were performed at the Central Laboratory of the University Hospital of Bern, Switzerland using standard laboratory methods. Urine pH in 24 h urines was measured by a S20 SevenEasy pH meter (Mettler Toledo, Greifensee, Switzerland). Estimated glomerular filtration rate (eGFR) was calculated according to CKD-EPI (Chronic Kidney

Disease Epidemiology Collaboration) [28]. Diabetes was defined as reported, treated, fasting glycemia ≥126 mg/dl, or random glycemia ≥200 mg/dl. Hypertension was defined as reported, treated, a mean systolic blood pressure ≥140 mmHg, or a mean diastolic blood pressure ≥90 mmHg.

Ammonium chloride and furosemide/fludrocortisone tests

All participants underwent sequential ammonium chloride and furosemide/fludrocortisone testing, at least 1 week and at most 1 month apart, regardless of 24 h or fasting urinary pH or type of stone, thereby constituting a completely unselected population of calcareous stone formers. The short one day ammonium chloride loading test and the furosemide/fludrocortisone test were performed as previously described [1, 12]. Study participants fasted after midnight and throughout the tests. For the ammonium chloride test, ammonium chloride gelatin capsules (100 mg/kg body weight) were given at 0800 h with water in the presence of the nursing staff over a period of 30 minutes. During the test, fluid intake was ad libitum. Venous blood samples were obtained for chemistry, pH and blood gases at 0800, 1000, 1200 h. Urine was collected hourly from 0800 to 1400 h. For the furosemide/fludrocortisone test, furosemide (40 mg) and fludrocortisone (1 mg) were given at 0800 h with water in the presence of the nursing staff. During the test, fluid intake was ad libitum. Venous blood samples were obtained for chemistry, pH and blood gases at 0800, 1100, 1300 h. Urine was collected hourly from 0800 to 1300 h. Venous blood gas and electrolyte analysis was performed immediately after collection on a ABL800FLEX blood gas analyzer (Radiometer, Thalwil, Switzerland). Urine pH was measured by a S20 SevenEasy pH meter and urinary pCO_2 by an ABL700 blood gas analyzer (Radiometer, Thalwil, Switzerland) immediately after collection.

Statistical analysis

Statistical analyses were conducted using the R software, version 3.2.2 [14]. All statistical tests were two-sided and a *p* value <0.05 was considered as statistically significant. Agreement between the two urinary acidification tests was analyzed by the Bland–Altman method using the R package "BlandAltmanLeh" [15]. ROC analysis was done using the R package "pROC" [16]. The sensitivity-specificity versus nadir urinary pH plot (Fig. 1F) was done by using the R package "OptimalCutpoints" [17]. The associations of the nadir urinary pH with 24 h citraturia and with calciuria were visually assessed by cubic spline functions of the nadir urinary pH and by boxplots stratifying the nadir urinary pH at different thresholds.

RESULTS

Characteristics of study population

Two of the 170 individuals recruited had complete dRTA and were excluded from the final analysis. Baseline characteristics of the remaining 168 participants are depicted in **Table 1**. Median age of the study population was 45 years, 73 % of participants were men. The majority of individuals (76 %) had ≥ 2 symptomatic stone events, stone analysis was available in 83 % of individuals. Baseline blood and urinary biochemistries are depicted in **Suppl. Table 1**.

Phenotype of stone formers according to nadir urinary pH

Thirteen participants (8 %) in the ammonium chloride test and 33 participants (20 %) in the furosemide/fludrocortisone test had a nadir urinary pH ≥ 5.3. Baseline characteristics of stone formers, listed separately by test and nadir urinary pH are depicted in **Table 2**. Stone formers with nadir urinary pH ≥ 5.3 in the ammonium chloride test had a lower BMI (p=0.02). Kidney stones of participants with nadir urinary pH ≥ 5.3 in both test were less likely to contain calcium oxalate but more likely to contain calcium phosphate. None of the stones of participants with nadir urinary pH ≥ 5.3 contained uric acid. Stone formers with nadir urinary pH ≥ 5.3 in the ammonium chloride test displayed a significantly lower plasma potassium and 24 h citrate excretion, while 24 h urea excretion (a measure of daily protein intake), net gastrointestinal alkali absorption (an arithmetic estimate of daily alkali intake) and kidney function were similar in subjects with nadir urinary pH <5.3 or ≥ 5.3 (**Table 3**) [18].

How well do non-provocative parameters perform in predicting incomplete dRTA ?

To this end, we tested the performance of the non-provocative parameters morning fasting urinary pH, plasma potassium, plasma bicarbonate, 24 h citraturia and stone calcium

phosphate content in the prediction of incomplete dRTA, defined by nadir urinary pH ≥ 5.3 in the ammonium chloride test. Second morning fasting urinary pH instead of 24 h urinary pH was chosen since correlation of the nadir urinary pH in the ammonium chloride test was better with the fasting morning urinary pH than the 24 h urinary pH **(Suppl. Fig. 1)**. Receiver operating characteristic (ROC) analysis revealed that a second morning fasting urinary pH and plasma potassium had the highest individual area under the curve (AUC) for the prediction of incomplete dRTA, and a combination of the two parameters - second fasting morning urinary pH and plasma potassium - further increased the AUC **(Suppl. Fig. 2)**. Combination of a fasting morning urinary threshold pH of <5.3 and a plasma potassium threshold of >3.8 mmol/l yielded a negative predictive value of 98 % and a positive predictive value of 24 % for the diagnosis of incomplete dRTA (**Table 4 A**). Use of higher urinary pH threshold caused a significant decrease of specificity, use of a higher plasma potassium thresholds resulted in significantly lower negative predictive values (not shown).

Performance of the FF test in the diagnosis of incomplete dRTA in stone formers

Utilizing the ammonium chloride test as gold standard, sensitivity and specificity of the furosemide/fludrocortisone test were 77 % and 85 %, respectively, yielding a positive predictive value of 30 % and a negative predictive value of 98 % for the diagnosis of dRTA, respectively (**Table 4 B**). Distribution of nadir urinary pH values for both tests are depicted as histograms with kernel density plots in **Figs. 1 A and B**. In both tests, there was no evidence for a bimodal distribution of the urinary pH nadir values. Statistical test comparison revealed a highly significant though only moderately positive linear relationship between the nadir urinary pH values of the two tests (**Fig. 1 C**). The absolute mean-difference plot (Bland-Altman plot) for the two tests is depicted in **Fig. 1 D**. The mean bias was +0.025 pH units, the lower and upper limits of agreement were -0.87 and 0.92, respectively and contained 160 of the 168 data points (95 %), as expected in case of a normal distribution [19, 20]. There was a

tendency to more positive differences at higher pH nadir values, i.e. an increase in bias with magnitude as shown by the positive slopes of linear regression lines in **Fig. 1 D**. The AUC of the ROC analysis was 0.88 (CI 0.79 – 0.97) (**Fig. 1 E**). The sensitivity-specificity *versus* nadir urinary pH plot analysis yielded an optimal threshold nadir urinary pH of 5.19 for the furosemide/fludrocortisone test at a sensitivity and specificity threshold of 85 % and 81 %, respectively, which is close to the currently employed threshold urinary pH < 5.3 (**Fig. 1 F**).

Pooled urine and blood analytes, separated by test and nadir urinary pH are depicted in **Figs. 2 and 3**, respectively. Consistent with previous reports [1, 12], we observed that the nadir urinary pH was achieved at ~3 h in the furosemide/fludrocortisone test and at ~5 h in the ammonium chloride test (**Figs. 2 A, F**). As shown in **Fig. 2 B-E**, participants with a nadir urinary pH ≥ 5.3 in the ammonium chloride test displayed lower hourly urinary sodium, chloride and ammonium excretions while the urinary volume was unaltered. In the furosemide/fludrocortisone test participants with a nadir urinary pH ≥ 5.3 also displayed reduced hourly urinary sodium and chloride excretions, but only in the second half of the furosemide/fludrocortisone test (**Figs. 2 H, I**). In contrast, urinary ammonium excretions in the furosemide/fludrocortisone test were similar in both groups of patients (**Fig. 2 J**) and urinary volumes were higher in participants with a nadir urinary pH ≥ 5.3 (**Fig. 2 G**).

As expected, the administration of ammonium chloride resulted in a large decrease of venous pH and bicarbonate compared to baseline (systemic acid load) (**Figs. 3 A, B**), whereas the administration of furosemide and fludrocortisone caused a small increase of venous pH and bicarbonate compared to baseline (increase of urinary acid excretion) (**Figs. 3 C, D**). During the ammonium chloride and furosemide/fludrocortisone tests, venous pH was not significantly different between participants with nadir urinary pH < 5.3 and ≥ 5.3 at all time points measured. However, venous bicarbonate was significantly higher at baseline and at 4 h during the ammonium chloride test in participants with nadir urinary pH ≥ 5.3 (**Figs. 3 A, B**).

In contrast, venous pH and bicarbonate were not different between participants with nadir

urinary pH < 5.3 and ≥ 5.3 during the furosemide/fludrocortisone test at all time points tested (**Figs. 3 D, E)**.

Is the nadir pH 5.3 for the diagnosis of incomplete dRTA justified ?

Our results indicate that urinary acidification capacity is a continuous trait in stone formers. In addition to alkaline urinary pH, hypocitraturia and hypercalciuria are important prolithogenic factors frequently encountered in dRTA patients. As the next step, we analyzed the association of the nadir urinary pH in the ammonium chloride test with 24 h calciuria or 24 h citraturia. As shown in **Figs. 4 A** and **C**, with increasing nadir urinary pH, citrate excretion decreased and calcium excretion increased. Using the established pH < 5.3 for diagnosis, citraturia was significantly lower in participants with incomplete dRTA (**Fig. 4 B**). However, 24 h calciuria was not different between participants with and without incomplete dRTA at this pH threshold (**Fig. 4 D**). We next performed these analyses at different arbitrarily defined urinary nadir pH thresholds ranging from pH 5.1 to 5.5. As shown in **Suppl. Fig. 3**, a pH < 5.3 was the only threshold pH which allowed a separation of the two groups of participants on the basis of citraturia. In contrast, at all pH thresholds tested, calciuria was not different between the two groups of participants.

DISCUSSION

This is the first controlled study that rigorously compared the performance of the furosemide/fludrocortisone test and non-provocative parameters with the gold standard ammonium chloride test for the diagnosis of incomplete dRTA in stone formers. One important feature of this study compared to previous studies is the fact that we recruited stone formers without preselection into study, i.e. regardless of fasting or 24 h urinary pH, stone type or presence of hypocitraturia or hypercalciuria. The use of unselected patient population renders our results applicable to general kidney stone practices. The results of our study suggest that incomplete dRTA can be reliably excluded by use of the non-provocative parameters which includes second morning fasting urinary pH and plasma potassium. If a provocative test is required, the furosemide/fludrocortisone test has excellent negative predictive value. If incomplete dRTA cannot be excluded by either approach, the diagnosis can then be confirmed by ammonium chloride testing.

24 h urinary pH and prevalence of calcium phosphate containing stones were significantly higher in stone formers with nadir urinary pH $\geq$ 5.3 in the furosemide/fludrocortisone test. Similar results were obtained with the ammonium chloride test, but the difference did not quite reach statistical significance. In contrast, uric acid calculi, typically associated with low urinary pH, were not observed at all in stone formers with incomplete dRTA defined by either tests [21]. This finding corroborates with previous studies that reported an association of a urinary acidification deficit with calcium phosphate-containing stones [22-24]. Interestingly, plasma potassium and 24 h citraturia were lower in stone formers with incomplete dRTA. The lower plasma potassium may at least partially explain the reduction in citraturia via a reduction of proximal tubular pH which is well known in potassium deficiency [25]. The pathogenesis and etiology of the reduced plasma potassium remains unclear, 24 h urinary excretions of sodium and potassium were similar in both groups of patients.

Our surprising observation that stone formers with incomplete dRTA had a higher venous bicarbonate stands in contradiction to a recent study that reported results of urinary acidification testing in 57 individuals with primary Sjögren's syndrome [26]. Patients with primary Sjögren's syndrome and incomplete dRTA had lower venous pH and bicarbonate compared to patients with primary Sjögren's syndrome without incomplete dRTA, but higher venous pH and bicarbonate compared to patients with primary Sjögren's syndrome and complete dRTA. Although confirmation by longer term studies in primary Sjögren's syndrome patients is lacking, such a constellation would be compatible with incomplete dRTA being a "pre-acidotic" *forme fruste* of complete dRTA. In contrast, our finding of an increased venous bicarbonate in individuals with incomplete dRTA suggests that the underlying pathophysiology in stone formers maybe different, as suggested previously [27].

Importantly, our study reveals for the first time that urinary acidification capacity is not a dichotomous but a continuous trait in stone formers. This observation stands in contrast to the initial description by Wrong and Davies and the currently held opinion that incomplete dRTA represents a distinct entity with respect to urinary acidification capacity. Based on urinary citrate excretion, but not based on urinary acidification capacity or calcium excretion, a threshold pH < 5.3 can be justified for the diagnosis of incomplete dRTA.

There are no randomized controlled trials in stone formers with incomplete dRTA. In small studies, treatment with alkali in adult stone formers with incomplete dRTA decreased hypercalciuria, increased citraturia and reduced stone formation [28-30]. In stone formers with incomplete dRTA associated with medullary sponge kidney, alkali therapy also led to a decrease in stone passage and improvement of the associated bone disease [31-33]. Results of these uncontrolled studies indicate that stone formers with incomplete dRTA constitute a unique subset of patients that may benefit from alkali treatment. However, alkali treatment was also effective in the prevention of recurrence in unselected cohorts of patients with calcareous nephrolithiasis [34]. Thus, clearly, longitudinal and interventional trials are needed to

further explore the prognostic and therapeutic relevance of diagnosing incomplete dRTA in stone formers.

DISCLOSURES

DF has served as a consultant for Otsuka Pharmaceuticals. DF has received unrestricted research funding from Novartis, Abbvie and Otsuka Pharmaceuticals. OM served on the Advisory Boards for AbbVie, Allena, Ardelyx, Genzyme-Sanofi, and Triceda.

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REFERENCES

1. Wrong, O, Davies, HE: The excretion of acid in renal disease. *Q J Med,* 28**:** 259-313, 1959.
2. Joshi, A, Gupta, SK, Srivastava, A: Metabolic evaluation in first-time renal stone formers in North India: a single center study. *Saudi J Kidney Dis Transpl,* 24**:** 838-843, 2013.
3. Ito, H, Kotake, T, Suzuki, F: Incidence and clinical features of renal tubular acidosis-1 in urolithiasis. *Urol Int,* 50**:** 82-85, 1993.
4. Osther, PJ, Hansen, AB, Rohl, HF: Screening renal stone formers for distal renal tubular acidosis. *Br J Urol,* 63**:** 581-583, 1989.
5. Gault, MH, Chafe, LL, Morgan, JM, Parfrey, PS, Harnett, JD, Walsh, EA, Prabhakaran, VM, Dow, D, Colpitts, A: Comparison of patients with idiopathic calcium phosphate and calcium oxalate stones. *Medicine (Baltimore),* 70**:** 345-359, 1991.
6. Tannen, RL, Falls, WF, Jr., Brackett, NC, Jr.: Incomplete renal tubular acidosis: some clinical and physiological features. *Nephron,* 15**:** 111-123, 1975.
7. Backman, U, Danielson, BG, Johansson, G, Ljunghall, S, Wikstrom, B: Incidence and clinical importance of renal tubular defects in recurrent renal stone formers. *Nephron,* 25**:** 96-101, 1980.
8. Stitchantrakul, W, Kochakarn, W, Ruangraksa, C, Domrongkitchaiporn, S: Urinary risk factors for recurrent calcium stone formation in Thai stone formers. *J Med Assoc Thai,* 90**:** 688-698, 2007.
9. Wikstrom, B, Backman, U, Danielson, BG, Fellstrom, B, Johansson, G, Ljunghall, S: Ambulatory diagnostic evaluation of 389 recurrent renal stone formers. A proposal for clinical classification and investigation. *Klin Wochenschr,* 61**:** 85-90, 1983.
10. Gambaro, G, Croppi, E, Coe, F, Lingeman, J, Moe, O, Worcester, E, Buchholz, N, Bushinsky, D, Curhan, GC, Ferraro, PM, Fuster, D, Goldfarb, DS, Heilberg, IP, Hess, B, Lieske, J, Marangella, M, Milliner, D, Preminger, GM, Reis Santos, JM, Sakhaee, K, Sarica, K, Siener, R, Strazzullo, P, Williams, JC: Metabolic diagnosis and medical prevention of calcium nephrolithiasis and its systemic manifestations: a consensus statement. *J Nephrol,* 29**:** 715-734, 2016.
11. Arampatzis, S, Ropke-Rieben, B, Lippuner, K, Hess, B: Prevalence and densitometric characteristics of incomplete distal renal tubular acidosis in men with recurrent calcium nephrolithiasis. *Urol Res,* 40**:** 53-59, 2012.
12. Walsh, SB, Shirley, DG, Wrong, OM, Unwin, RJ: Urinary acidification assessed by simultaneous furosemide and fludrocortisone treatment: an alternative to ammonium chloride. *Kidney Int,* 71**:** 1310-1316, 2007.
13. Viljoen, A, Norden, AG, Karet, FE: Replacing the short ammonium chloride test. *Kidney Int,* 72**:** 1163; author reply 1164, 2007.
14. Kimura, S, Zhang, GX, Nishiyama, A, Shokoji, T, Yao, L, Fan, YY, Rahman, M, Abe, Y: Mitochondria-derived reactive oxygen species and vascular MAP kinases: comparison of angiotensin II and diazoxide. *Hypertension,* 45**:** 438-444, 2005.
15. Chamoux, E, Bisson, M, Payet, MD, Roux, S: TRPV-5 mediates a receptor activator of NF-kappaB (RANK) ligand-induced increase in cytosolic Ca2+ in human osteoclasts and down-regulates bone resorption. *J Biol Chem,* 285**:** 25354-25362, 2010.
16. Xue, Y, Karaplis, AC, Hendy, GN, Goltzman, D, Miao, D: Exogenous 1,25-dihydroxyvitamin D3 exerts a skeletal anabolic effect and improves mineral ion homeostasis in mice that are homozygous for both the 1alpha-hydroxylase and parathyroid hormone null alleles. *Endocrinology,* 147**:** 4801-4810, 2006.
17. Nijenhuis, T, van der Eerden, BC, Hoenderop, JG, Weinans, H, van Leeuwen, JP, Bindels, RJ: Bone resorption inhibitor alendronate normalizes the reduced bone thickness of TRPV5(-/-) mice. *J Bone Miner Res,* 23**:** 1815-1824, 2008.

18. Posada-Ayala, M, Zubiri, I, Martin-Lorenzo, M, Sanz-Maroto, A, Molero, D, Gonzalez-Calero, L, Fernandez-Fernandez, B, de la Cuesta, F, Laborde, CM, Barderas, MG, Ortiz, A, Vivanco, F, Alvarez-Llamas, G: Identification of a urine metabolomic signature in patients with advanced-stage chronic kidney disease. *Kidney Int,* 85**:** 103-111, 2014.
19. Bland, JM, Altman, DG: Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet,* 1**:** 307-310, 1986.
20. Bland, JM, Altman, DG: Comparing methods of measurement: why plotting difference against standard method is misleading. *Lancet,* 346**:** 1085-1087, 1995.
21. Maalouf, NM, Cameron, MA, Moe, OW, Sakhaee, K: Novel insights into the pathogenesis of uric acid nephrolithiasis. *Curr Opin Nephrol Hypertens,* 13**:** 181-189, 2004.
22. Pak, CY, Poindexter, JR, Adams-Huet, B, Pearle, MS: Predictive value of kidney stone composition in the detection of metabolic abnormalities. *Am J Med,* 115**:** 26-32, 2003.
23. Daudon, M, Bouzidi, H, Bazin, D: Composition and morphology of phosphate stones and their relation with etiology. *Urol Res,* 38**:** 459-467, 2010.
24. Dessombz, A, Letavernier, E, Haymann, JP, Bazin, D, Daudon, M: Calcium Phosphate Stone Morphology Can Reliably Predict Distal Renal Tubular Acidosis. *J Urol*, 2014.
25. Moe, OW, Preisig, PA: Dual role of citrate in mammalian urine. *Curr Opin Nephrol Hypertens,* 15**:** 419-424, 2006.
26. Both, T, Hoorn, EJ, Zietse, R, van Laar, JA, Dalm, VA, Brkic, Z, Versnel, MA, van Hagen, PM, van Daele, PL: Prevalence of distal renal tubular acidosis in primary Sjogren's syndrome. *Rheumatology (Oxford),* 54**:** 933-939, 2015.
27. Donnelly, S, Kamel, KS, Vasuvattakul, S, Narins, RG, Halperin, ML: Might distal renal tubular acidosis be a proximal tubular cell disorder? *Am J Kidney Dis,* 19**:** 272-281, 1992.
28. Preminger, GM, Sakhaee, K, Pak, CY: Alkali action on the urinary crystallization of calcium salts: contrasting responses to sodium citrate and potassium citrate. *J Urol,* 139**:** 240-242, 1988.
29. Preminger, GM, Sakhaee, K, Pak, CY: Hypercalciuria and altered intestinal calcium absorption occurring independently of vitamin D in incomplete distal renal tubular acidosis. *Metabolism,* 36**:** 176-179, 1987.
30. Preminger, GM, Sakhaee, K, Skurla, C, Pak, CY: Prevention of recurrent calcium stone formation with potassium citrate therapy in patients with distal renal tubular acidosis. *J Urol,* 134**:** 20-23, 1985.
31. Fabris, A, Bernich, P, Abaterusso, C, Marchionna, N, Canciani, C, Nouvenne, A, Zamboni, M, Lupo, A, Gambaro, G: Bone disease in medullary sponge kidney and effect of potassium citrate treatment. *Clin J Am Soc Nephrol,* 4**:** 1974-1979, 2009.
32. Fabris, A, Lupo, A, Bernich, P, Abaterusso, C, Marchionna, N, Nouvenne, A, Gambaro, G: Long-term treatment with potassium citrate and renal stones in medullary sponge kidney. *Clin J Am Soc Nephrol,* 5**:** 1663-1668, 2010.
33. Higashihara, E, Nutahara, K, Niijima, T: Renal hypercalciuria and metabolic acidosis associated with medullary sponge kidney: effect of alkali therapy. *Urol Res,* 16**:** 95-100, 1988.
34. Fink, HA, Wilt, TJ, Eidman, KE, Garimella, PS, MacDonald, R, Rutks, IR, Brasure, M, Kane, RL, Ouellette, J, Monga, M: Medical management to prevent recurrent nephrolithiasis in adults: a systematic review for an American College of Physicians Clinical Guideline. *Ann Intern Med,* 158**:** 535-543, 2013.

TABLES

Table 1. Baseline characteristics of study population. The available number of participants is indicated for each characteristic. Categorical variables are described by % and continuous variables by their median (IQR; 25^{th}-75^{th} quantile).

Characteristics	N	All participants
Male	123	73%
Age (yr)	168	45 (34-55)
BMI (kg/m^2)	168	27 (24-30)
Current or former smoker		
Yes	75	45 %
Unknown	17	10 %
Hypertension	90	54 %
Diabetes	14	8 %
Number of stone symptomatic events		
1 event	41	24 %
2 events	42	25 %
3 events	33	20 %
≥4 events	52	31 %
Positive family history of kidney stones	85	53 %
Participants with available stone analysis	140	83 %
Kidney stone composition (containing)		
Calcium oxalate	129	77 %
Calcium phosphate	59	35 %
Struvite	6	4 %
Uric acid	10	6 %
Cystine	1	1 %

Table 2. Baseline characteristics according to nadir urinary pH in furosemide/fludrocortisone and ammonium chloride tests. The available number of participants is indicated for each characteristic, stratified for nadir urinary pH. Categorical variables are described by % and continuous variables by their median (25^{th}-75^{th} quantile). Between-group differences were determined by Mann–Whitney U, chi-squared test or Fisher's exact test (if expected number of observations per cell is <5) as appropriate and the corresponding *P* values are indicated.

Characteristics	Furosemide / Fludrocortisone test					Ammonium chloride test				
	N	Nadir pH <5.3	N	Nadir pH ≥ 5.3	*P* value	N	Nadir pH <5.3	N	Nadir pH ≥ 5.3	*P* value
Male	100	74 %	23	70 %	0.77	116	75 %	7	54 %	0.19
Age at first presentation (yr)	135	46 (34-57)	33	42 (31-51)	0.14	155	46 (34-56)	13	38 (31-51)	0.14
BMI at first presentation, kg/m^2	135	27 (24-30)	33	25 (22-29)	0.08	155	27 (24-30)	13	23 (19-27)	0.02
Current or fomer smoker										
yes	57	42 %	18	55 %	0.14	69	45%	6	46 %	0.24
unknown	12	9 %	5	15 %		14	9 %	3	23 %	
Hypertension	76	56 %	14	42 %	0.40	85	55 %	5	39 %	0.40
Diabetes	13	10 %	1	3 %	0.31	14	9 %	0	0 %	0.60
Age at first stone event (yr)	127	33 (26-42)	30	30 (23-38)	0.24	144	33 (26-42)	13	28 (22-34)	0.21
Age at first available stone analysis (yr)	104	45 (33-55)	25	38 (31-50)	0.22	118	45 (34-54)	11	37 (26-49)	0.16
Number of stone symptomatic events										
1 event	32	24 %	9	27 %	0.38	36	24 %	5	39 %	0.19
2 events	33	24 %	9	27 %		38	25 %	4	31 %	
3 events	30	22 %	3	9 %		33	20 %	0	0 %	
≥4 events	40	30 %	12	36 %		48	31 %	4	31 %	
Positive family history of kidney stones	69	53 %	16	52 %	1	77	52 %	8	62 %	0.69
Participants with available stone analysis	113	84 %	29	88 %	0.79	131	85 %	11	85 %	1
Stone composition (containing)										
Calcium oxalate	107	96 %	23	79 %	0.01	122	94 %	8	73 %	0.04
Calcium phosphate	38	34 %	22	76 %	<0.01	52	40 %	8	73 %	0.05
Struvite	3	3 %	3	10 %	0.10	5	4 %	1	9 %	0.39
Uric acid	10	9 %	0	0 %	0.12	10	8 %	0	0 %	1
Cystine	0	0 %	1	3 %	0.21	1	1 %	0	0 %	1

Table 3. Blood and urinary parameters according to nadir urinary pH in furosemide/fludrocortisone and ammonium chloride tests. The available number of participants is indicated for each characteristic, stratified for nadir urinary pH. Variables are described by their median (25^{th}-75^{th} percentile). Between-group differences are determined by Mann-Whitney U test and the corresponding *P* values are indicated. NGIA: net gastrointestinal alkali absorption.

Characteristic	Normal range	Unit	Furosemide / Fludrocortisone test					Ammonium chloride test				
			N	Nadir pH <5.3	N	Nadir pH ≥ 5.3	*P* value	N	Nadir pH <5.3	N	Nadir pH ≥ 5.3	*P* value
Plasma												
Sodium	135 - 145	mEq/L	135	141 (139-142)	33	141 (140-142)	0.95	155	141 (139-142)	13	141 (141-141)	0.25
Potassium	3.5 – 5.0	mEq/L	135	4.0 (3.7-4.2)	33	3.8 (3.6-4.1)	0.06	155	4 (3.7-4.2)	13	3.6 (3.5-3.8)	<0.01
Chloride	97 - 108	mEq/L	134	103 (102-105)	33	103 (101-105)	0.53	154	103 (102-105)	13	102 (102-103)	0.26
Calcium corrected	8.9 – 10.1	mg/dl	133	9.4 (9.1-9.6)	33	9.4 (9.1-9.7)	0.95	153	9.4 (9.1-9.6)	13	9.2 (9-10)	0.89
Phosphate	2.5 – 4.5	mg/dl	135	3.1 (2.7-3.4)	32	3.1 (2.7-3.3)	0.88	154	3.1 (2.7-3.4)	13	3.2 (2.8-3.6)	0.39
Magnesium	1.8 – 2.6	mg/dl	135	2.0 (1.9-2.0)	33	2.0 (1.9-2.0)	0.23	155	2 (1.9-2.1)	13	2 (1.9-2)	0.66
Creatinine	0.6 – 1.4	mg/dl	135	0.9 (0.8-1.0)	33	0.9 (0.8-1.0)	0.74	155	0.9 (0.8-1)	13	0.9 (0.8-1)	0.76
eGFR	>90	ml/min per 1.73 m^2	135	99 (87-109)	33	98 (86-110)	0.95	155	99 (87-109)	13	91 (84-115)	0.61
Urea nitrogen	6 – 20	mg/dl	133	14 (12-17)	33	14 (12-16)	0.80	153	14 (12-17)	13	14 (12-15)	0.51
Uric acid	3 – 7	mg/dl	134	5.5 (4.4-6.5)	33	5.7 (4.7-6.3)	0.68	154	5.5 (4.5-6.5)	13	5.5 (4.0-5.8)	0.35
Alkaline phosphatase	35 - 100	U/L	135	63 (53-75)	33	60 (54-71)	0.60	155	62 (53-73)	13	59 (52-70)	0.73
Urine												
Sodium	50 - 150	mEq/24 h	124	168 (130-212)	32	159 (133-205)	0.71	143	167 (130-213)	13	153 (114-177)	0.26
Potassium	20 - 120	mEq/24 h	123	57 (49-70)	32	68 (41-85)	0.41	142	58 (49-72)	13	58 (33-79)	0.86
Chloride	70 - 250	mEq/24 h	122	151 (121-201)	32	137 (109-191)	0.23	141	149 (121-202)	13	123 (89-163)	0.12
Calcium	100 - 300	mg/24 h	124	221 (145-288)	32	229 (202-305)	0.17	143	222 (151-294)	13	230 (197 -276)	0.41
Phosphate	0.6 – 1.4	g/24 h	124	0.9 (0.7-1.0)	32	0.8 (0.6-1.0)	0.38	143	0.9 (0.7-1)	13	0.8 (0.6-0.9)	0.16
Magnesium	30 - 120	mg/24 h	123	113 (90-148)	32	109 (95-137)	0.76	142	113 (92-146)	13	109 (88-136)	0.56
Creatinine	0.8 - 2	g/24 h	124	1.5 (1.3-1.8)	32	1.5 (1.2-1.7)	0.83	143	1.5 (1.3-1.8)	13	1.4 (1.1-1.7)	0.36
Urea nitrogen	6–14	g/24 h	123	10 (9-13)	32	9 (8-12)	0.28	142	10 (8-13)	13	9 (7-10)	0.14
Uric acid	< 750	mg/24 h	124	527 (414-637)	32	513 (394-617)	0.69	143	534 (413-642)	13	475 (376-549)	0.23
pH	5.8 – 6.2	—	85	5.9 (5.3-6.5)	23	6.4 (5.7-7.0)	0.02	97	5.9 (5.3-6.5)	11	6.3 (5.8-7.0)	0.11
Anion gap	—	mEq/L	133	38 (29-48)	33	38 (31-45)	1.00	153	37 (29-47)	13	39 (33-48)	0.44
Citrate	> 320	mg/24 h	120	455 (307-666)	32	492 (324 -636)	0.97	139	482 (315-657)	13	332 (134-388)	0.02
NGIA	—	mEq/24 h	108	31 (15-44)	30	40 (27-54)	<0.01	126	33 (19-46)	12	38 (21-53)	0.38
Oxalate	20 - 40	mg/24 h	120	22 (16-30)	32	30 (17-33)	0.07	139	23 (16-31)	13	28 (24-33)	0.29

Table 4. Performance of non-provocative clinical parameters (A) and of furosemide/fludrocortisone testing (B) in the prediction of incomplete dRTA in stone formers. A second morning fasting urinary pH < 5.3 and a plasma potassium > 3.8 mEq/L were used as non-provocative thresholds. In parentheses, 95 % CIs are indicated for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

A

Non-provocative parameters pH & K	NH_4Cl-Test: idRTA	NH_4Cl-Test: normal	
idRTA	True Positiv **11**	False Positive **35**	PPV **0.24** (0.13-0.39)
normal	False Negative **2**	True Negative **120**	NPV **0.98** (0.94-1.00)
	Sensitivity **0.85** (0.55-0.98)	Specitivity **0.77** (0.70-0.84)	Prevalence **0.077**

B

FF-Test	NH_4Cl-Test: idRTA	NH_4Cl-Test: normal	
idRTA	True Positiv **10**	False Positive **23**	PPV **0.30** (0.16-0.49)
normal	False Negative **3**	True Negative **132**	NPV **0.98** (0.94-1.00)
	Sensitivity **0.77** (0.46-0.95)	Specitivity **0.85** (0.79-0.90)	Prevalence **0.077**

FIGURE LEGENDS

Figure 1. Comparison of ammonium chloride and furosemide/fludrocortisone test results.

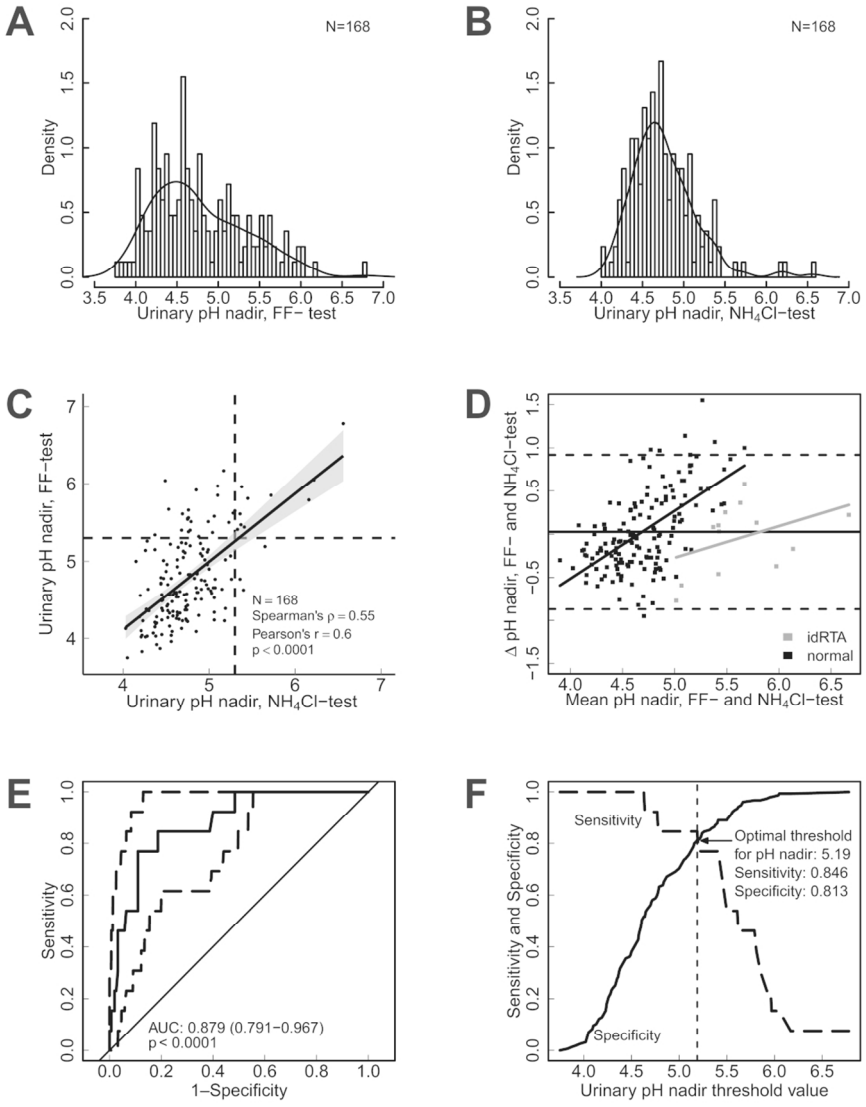
A) Histogram of urinary pH nadir in furosemide/fludrocortisone test with Kernel density plot. B) Histogram of urinary pH nadir in ammonium chloride test with Kernel density plot. C) Association of ammonium chloride test and furosemide/fludrocortisone test nadir urinary pH values. The regression line (solid black line) with 95 % CI (grey area) was created by a linear model. Dashed lines indicate the pH threshold of 5.3 on both axes. D) Bland-Altmann plot with regression lines for participants without (black line) or with incomplete dRTA (grey line). Horizontal dashed lines indicate upper and lower limits of agreement calculated as the 95 % CI around the mean bias (horizontal solid line). E) ROC curve for the furosemide/fludrocortisone test. Dashed lines around the ROC curve indicate the 95% CI of the sensitivity at the given specificity points. The AUC and its 95% CI is indicated. F) Sensitivity-specificity versus nadir urinary pH plot indicate the nadir urinary pH threshold in the furosemide/fludrocortisone test where sensitivity and specificity are simultaneously maximized.

Figure 2. Time course of urinary parameters separated in participants with nadir urinary pH <5.3 and ≥ 5.3. Grey bars indicate nadir urinary pH < 5.3, black bars indicate nadir urinary pH ≥ 5.3. Values shown are medians and IQR. Between group differences were determined by Mann–Whitney U test. *p < 0.05. **p < 0.01. A) Time course of urinary pH in the ammonium chloride test, the dashed line indicates the diagnostic threshold pH 5.3. B) - E) Time course of urinary volume, sodium, chloride and ammonium in the ammonium chloride test. F) Time course of urinary pH in the furosemide/fludrocortisone test, the dashed line indicates the diagnostic threshold pH 5.3. G) – J) Time course of urinary volume, sodium,

chloride and ammonium in the furosemide/fludrocortisone test. Furosemide/fludrocortisone tests lasted 5 h, ammonium chloride tests lasted 6 h.

Figure 3. Time course of blood parameters separated in participants with nadir urinary pH <5.3 and ≥ 5.3. Blood was drawn at baseline and at 2 h and 4 h in ammonium chloride-tests, and at baseline and at 3 h and 5 h in the furosemide/fludrocortisone tests. Grey bars indicate nadir urinary pH < 5.3, black bars indicate nadir urinary pH ≥ 5.3. Values shown are medians and IQR. Between group differences were determined by Mann–Whitney U test. *p < 0.05. **p < 0.01. A) and B) Time course of venous blood pH and venous bicarbonate in the ammonium chloride test. C) and D) Time course of venous blood pH and venous bicarbonate in the furosemide/fludrocortisone test.

Figure 4. Association of nadir urinary pH with 24 h citraturia and calciuria. A) and C) Associations of nadir urinary pH in the ammonium chloride test with 24 h citraturia and with 24 h calciuria, respectively. Black lines in Fig. A and C indicate the cubic spline function of the association. Grey shaded areas represent the 95% CI for the fitted splines. Vertical dashed lines indicate the diagnostic threshold pH 5.3. B) and D) 24 h citraturia and 24h calciuria separated in participants with and without incomplete dRTA. Box plots indicate 25^{th}, 50^{th} and 75th quantile of the distribution of the 24 h excretion for each group. Whiskers above and below the box indicate the 1.5 × IQR: [25^{th} – 1.5×IQR] and [75^{th} + 1.5×IQR], respectively. Between group differences were determined by Mann–Whitney U test.



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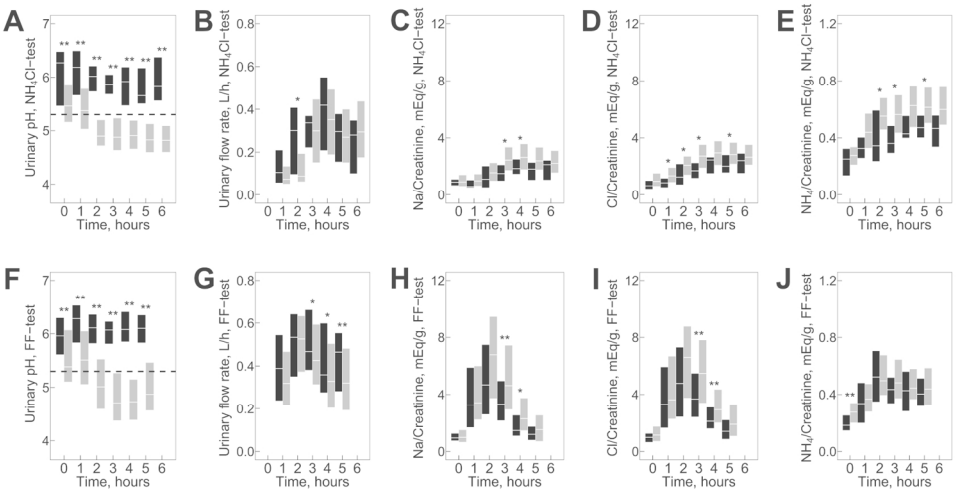

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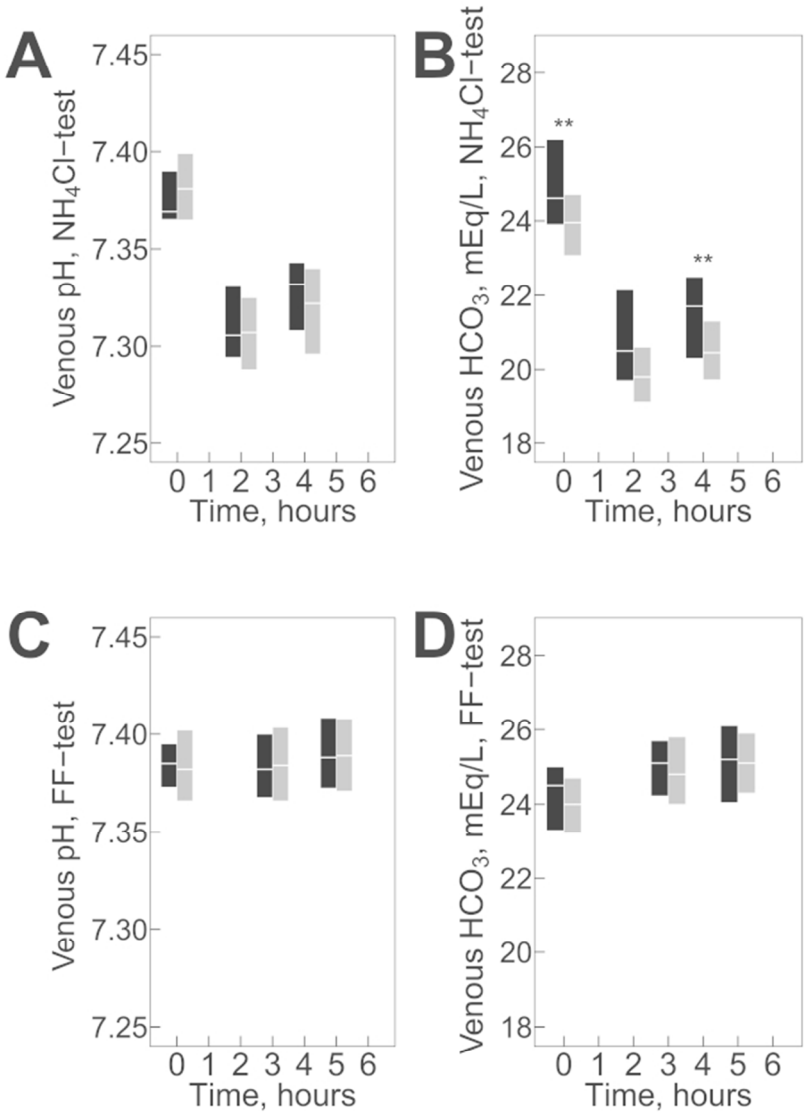

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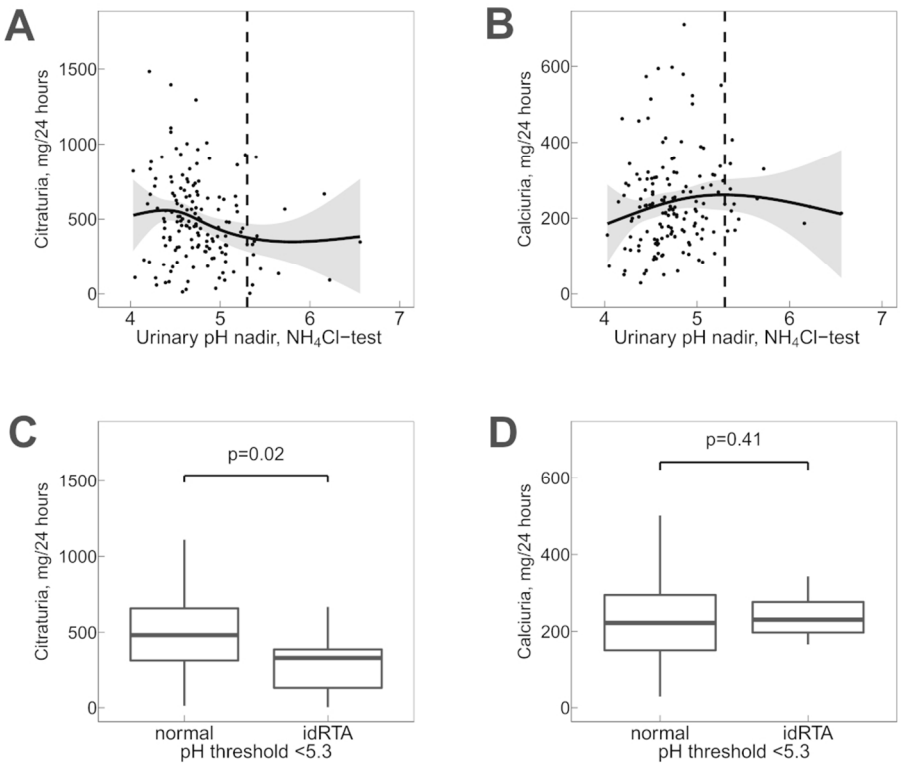

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